1-1-1988

Effects of adrenocortical steroids on paraventricular hypothalamic knife cut-induced obesity.

Sue Anne Assimon
University of Massachusetts Amherst

EFFECTS OF ADRENOCORTICAL STEROIDS ON PARAVENTRICULAR HYPOTHALAMIC KNIFE CUT-INDUCED OBESITY

A Dissertation Presented

by

SUE ANNE ASSIMON

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 1988

Department of Psychology

EFFECTS OF ADRENOCORTICAL STEROIDS ON PARAVENTRICULAR HYPOTHALAMIC KNIFE CUT-INDUCED OBESITY

A Dissertation Presented

by

SUE ANNE ASSIMON

Approved as to style and content by:

Richard M. Gold, Chairperson of Committee

John W. Donahoe, Member

George N. Wade, Member

Gordon A. Wyse, Member

Seymour M. Berger, Chairperson
Department of Psychology

DEDICATION

This dissertation is dedicated to my parents for their love, generosity, and caring, which allowed me to arrive at the opportunity to pursue this endeavor, and for their instilling in me the perseverance and strength required to complete it.

ACKNOWLEDGEMENTS

I wish to express my appreciation to my dissertation committee for serving in this capacity. I am grateful for their patience, input, and time. Special mention goes to Dick Gold for his service as my advisor, and as chair of this doctoral committee. The feedback he has provided and the concern he has shown during my years at UMASS are recognized with gratitude. I thank John Donahoe for his advise and helpful discussions during the course of this doctoral work, and my graduate work. His insights were of value, and keen sense of humor was always greatly appreciated. I extend thanks to George Wade for accompanying his feedback and criticisms with exceptional tolerance and understanding. Acknowledgement is also due George for generously allowing the use of his laboratory supplies and equipment which enabled me to execute aspects of this work. I commend Gordon Wyse for his contributions, and extreme tolerance throughout this dissertation process. I am thankful for his gracious treatment when I showed up at his office after long intervals without contact, and his cooperation when I made requests of his time typically with short notice.

I wish also to acknowledge many fellow graduate students for making my time in graduate school a more pleasant experience than it would otherwise have been. Regards go to Vivian Dorsel, Karla Drewsen, Joan Hamilton, Hank Heller, Dave Palmer, Beth Powell, Anita Sylvan,

Laurie Teraspulsky, and Sandra Woods, among others. Along with many enlightening discussions and helpful exchanges, their friendship, encouragement, concern, and humor were appreciated.

Thanks are bestowed Jay Alexander for his expertise in making the figures and for cheerfully making the many changes subsequently requested. I am also grateful for his patience with respect to the financing of his work, and for his general helpfulness during my days in Tobin. Thanks are also due Alison Player for consenting to type the tables and figure captions, and for doing so in such a cooperative and competent manner.

Finally, I wish to recognize my beloved siblings who had nothing whatsoever to do with the completion of this dissertation, but who would never let me live down going without mentioning them here. I would, however, like to take this rare opportunity to acknowledge them for other meaningful contributions they have made to me. First, I express my thanks for their friendship, interest, and candor, and their constant in humor and laughter. Although not contributing to this formal educational process, they, and their characteristically unrelenting feedback, provided me with an education of another kind, and for this I praise them. From them, along with my parents, I learned the value and the importance of respect for the individual, and of many fundamental aspects of life, living, and constructive interrelating. During a formative

time, they created an atmosphere which allowed the exchange and expression that encouraged thought, trenchant objectivity, and honest self-analysis, along with growth and maturity. I have come to see in my adulthood that this was a unique and invaluable opportunity and one that affirmed and shared in my development as a person. I am indebted to them for this experience. And, I am glad I chose to learn and to benefit from it.

ABSTRACT

EFFECTS OF ADRENOCORTICAL STEROIDS
ON
HYPOTHALAMIC PARAVENTRICULAR KNIFE CUT-INDUCED OBESITY

MAY 1988

SUE ANNE ASSIMON, B.A., COLGATE UNIVERSITY

M.S., UNIVERSITY OF MASSACHUSETTS

Ph.D., UNIVERSITY OF MASSACHUSETTS

Directed by: Professor Richard M. Gold

The present investigation examined whether the obesity seen subsequent to bilateral parasagittal knife cuts (KC) alongside the PVN in the rat can be attributed to diminished adrenal cortical and/or adrenal medullary activity. Specifically, it examined the effects of the administration of corticosterone (CORT), the natural glucocorticoid of the rat, and dexamethasone (DEX), a synthetic glucocorticoid documented to enhance catecholamine (CA) biosynthesis in the adrenal medulla, upon feeding and body weight.

In Experiments 1 and 2, the effects of the administration of CORT and of DEX on the obesity produced by PVN knife cuts were compared. CORT and DEX differentially influenced regulatory responses. CORT did not alter the body weight gain or food intake of sham-operated or PVN KC rats. In contrast, DEX attenuated weight gain in both PVN KC and brain intact rats, and at times decreased food intake. In KC rats exposure to DEX

was associated with greatly elevated blood glucose levels and sometimes with glycosuria.

In Experiment 3, the nature of the suppressive effects of DEX was explored further. The role of the adrenal medulla and of pituitary ACTH were examined. The adrenal medulla did not prove to be the major site at which DEX acts to suppress body weight and food intake. However, the participation of adrenal medullary CAs in DEX-induced effects on glucose homeostasis in KC adrenal intact rats was indicated. DEX's inhibition of pituitary ACTH was not revealed as contributing to the altered ingestive responses produced by DEX. Thus, the involvement of other sites in DEX's suppressive effects was suggested.

Because supplements of CORT or ACTH did not block the hypothalamic obesity syndrome, deficits in the availability of circulating CORT or in pituitary ACTH did not emerge as causing the overeating and obesity that follow PVN KCs. Definitive conclusions about the relationship between the obesity induced by PVN KCs and altered adrenomedullary CA activity could not be drawn from this work. Direct measures of adrenal medullary functions are needed.

Finally, this work revealed that the glucocorticoids CORT and DEX markedly differ in their effects on body weight, food intake, growth, and blood glucose levels. Possible explanations for the dissimilar effects of these glucocorticoids were addressed.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

ABSTRACT viii

LIST OF TABLES xi

LIST OF FIGURES xiii

Chapter

I. INTRODUCTION 1

General Experimental Focus 2
Adenohypophysial Connections of the PVN 3
Extrahypophysial Interconnections of the PVN 13
PVN Projections and Their Relationship to the Adrenal Gland 26
Adrenal Secretions. 29
Overview 32

II. EXPERIMENT I 37

Methods 37
Results 43
Discussion 56

III. EXPERIMENT II 63

General Methods 63
Results 68
Discussion 90

IV. EXPERIMENT III 99

General Methods 100
Results 104
Discussion 115

V. GENERAL DISCUSSION 124

REFERENCES 131

LIST OF TABLES

Table		Page
1.	A Summary of the Status of the Pituitary-Adrenal System of Rats After Surgical Disruption of the PVN	9
2.	A Summary of Pituitary Responses Seen Subsequent to Bilateral Adrenalectomy in PVN Knife-Cut and Sham-Operated Rats	12
3.	A Comparison of the Responses Elicited by Rats After Receiving Parasagittal Knife Cuts Alongside the PVN or Central Injections of CRF	22
4.	Mean Body Weight Change Per Day (g ± SEM) of Sham-Operated and PVN Knife-Cut Rats Briefly Administered 0.1 mg/kg DEX in Conjunction with Their Chronic Treatments of No Hormone (CON) or CORT During Experiment One	50
5.	Mean Daily Food Intake (g ± SEM) of Sham-Operated and PVN Knife-Cut Rats Briefly Administered 0.1 mg/kg DEX in Combination with Their Chronic Treatments of No Hormone (CON) or CORT During Experiment One	51
6.	Mean Absolute Body Weight Change (g ± SEM), Mean Rate of Weight Change (g/day ± SEM), and Mean Daily Food Intake (g ± SEM) Exhibited By Sham-Operated and PVN Knife-Cut Rats Exposed to No Hormone (CON) or CORT Treatments During Phase Two of Experiment One	54
7.	Mean Blood Glucose Levels (mg/dl ± SEM) at the Conclusion of Experiment One in Sham-Operated and PVN Knife-Cut Rats After Chronic Administration of No Hormone (CON), CORT, or DEX	55
8.	Mean Naso-Anal Lengths (mm ± SEM) of Rats Prior to Brain Surgery and 36 Days After Receiving Sham or PVN Knife Cut Surgery and Chronic Treatments of No Hormone (CON), CORT, or DEX Including Subgroups That Experienced the Addition of DEX to CON or CORT for a Brief Period	57

Table		Page
9.	Mean Obesity Indices (± SEM) of Rats Prior to Brain Surgery and 36 Days After Receiving Sham or PVN Knife Cut Surgery and Chronic Treatments of No Hormone (CON), CORT, or DEX Including Subgroups That Experienced the Addition of DEX to CON or CORT for a Brief Period	58
10.	Mean Intake (ml ± SEM) of a 2% Saline Solution Provided Sham-Operated and PVN Knife-Cut Rats Exposed to No Hormone (CON), CORT, or DEX During Phase C of Experiment Two	84
11.	Mean Blood Glucose Levels (mg/dl ± SEM) of Sham-Operated and PVN Knife-Cut Rats Treated with No Hormone (CON) or Different Doses of CORT or DEX Across Phases 2A-D of Experiment Two	85
12.	Mean Adrenal Gland Weights (g ± SEM) for Sham-Operated and PVN Knife-Cut Rats Exposed to No Hormone (CON), 16 mg/kg CORT or 0.2 mg/kg DEX at the Conclusion of Experiment Two	87
13.	Mean Naso-Anal Lengths (mm ± SEM) and Mean Obesity Indices (± SEM) for Sham-Operated and PVN Knife-Cut Rats Chronically Exposed to No Hormone (CON), CORT, or DEX During Experiment Two	89
14.	Mean Daily Water Intake (ml ± SEM) of Sham-Operated and PVN Knife-Cut Rats with Intact or Demedullated (DEM) Adrenal Glands During Each Phase of Experiment Three	113
15.	Mean Blood Glucose Levels (mg/dl ± SEM) of Sham-Operated and PVN Knife-Cut Rats with Intact or Demedullated (DEM) Adrenal Glands During Experiment Three	114

LIST OF FIGURES

Figure		Page
1.	Diagrammatic representation of the major afferent and efferent neural pathways associated with the PVN of the hypothalamus (PVH) of the rat from Sawchenko and Swanson (162)	15
2.	Diagrammatic representation of the paraventricular hypothalamic-pituitary-adrenal axis, and the paraventricular extrahypophysial projections that control both the parasympathetic and the sympathetic divisions of the autonomic nervous system	25
3.	Mean body weight change of sham-operated and PVN KC rats treated with no hormone (CON), 0.1 mg/kg DEX, or a moderate (MCORT) or high (HCORT) dose of CORT for 18 days after brain surgery (Phase One of Hormone Exposure)	45
4.	Mean daily food intake of sham-operated and PVN KC rats described in Figure 3 during Phase One (Days 1-18) and Phase Two (Days 18-48) of Hormone Exposure	48
5.	Mean body weight change of sham-operated and PVN KC rats treated with either no hormone (CON), or 4, 8, 16, or 32 mg/kg CORT across Phases A - D, respectively, or 0.05, 0.1, 0.2 mg/kg DEX and 0.2 mg/kg DEX plus 5 U/kg insulin across Phases A - D, respectively	70
6.	Mean daily food intake of sham-operated and PVN KC rats treated with either no hormone (CON), or 4, 8, 16, 32 mg/kg CORT across Phases A - D, respectively, or 0.05, 0.1, 0.2 mg/kg DEX across Phases A - C, respectively	72
7.	Mean daily water intake of sham-operated and PVN KC rats treated with either no hormone (CON), or 4, 8, 16, and 32 mg/kg CORT across Phases A - D, respectively, or 0.05, 0.1, 0.2 mg/kg DEX across Phases A - C, respectively	74

LIST OF FIGURES (Continued)

Figure		Page
8.	Mean body weight change of sham-operated and PVN KC rats treated with 0.2 mg/kg DEX alone in Phase C, followed by treatments of this 0.2 mg/kg DEX dose in conjunction with 5 U/kg insulin in Phase D and 8 U/kg insulin in Phase E	81
9.	Mean daily food intake of sham-operated and PVN KC rats treated with 0.2 mg/kg DEX alone in Phase C, followed by treatments of this 0.2 mg/kg DEX dose in conjunction with 5 U/kg insulin (I) in Phase D and with 8 U/kg insulin (I) in Phase E	82
10.	Mean body weight change of sham-operated and PVN KC rats with intact or demedullated (DEM) adrenal glands and treated with no hormone (CON) or 0.1 mg/kg DEX in each phase (A - C) with the addition of ACTH (8 U, 2 x day) during Phase B	106
11.	Mean daily food intake of sham-operated and PVN KC rats with intact or demedullated (DEM) adrenal glands and treated with no hormone (CON) or 0.1 mg/kg DEX in each phase (A - C) with the addition of ACTH (8 U, 2 x day) during Phase B	110

C H A P T E R I

INTRODUCTION

The medial hypothalamus has long been recognized as playing an integral role in the regulation of feeding and body weight. Within this area, the paraventricular nucleus (PVN) is now distinquished as focal in the control of ingestive and metabolic processes. Both surgical and pharmacological manipulations which serve to inactivate the PVN promote eating and weight gain. For instance, medial parasagittal hypothalamic lesions (36, 45, 89) and knife cuts (43, 44, 48, 49, 76), which either disrupt this nucleus or its connections, produce chronic increases in food intake and body weight. The PVN thus appears to inhibit feeding. Injections of nor epinephrine (NE) or nor adrenergic agonists into the medial hypothalamus (92, 93, 113, 154) or even the cerebral ventricles (131) of the rat reliably stimulate a feeding bout. The PVN is the most effective and most sensitive injection site (87, 88, 90, 92). This point is further substantiated by the finding that discrete PVN lesions prevent the feeding behavior typically elicited by central NE injections (96).

Recently, attempts have been made to integrate the acute feeding response seen after injections of NE into the PVN and the long-term food intake and body weight changes seen after surgical interruption of the PVN. When central infusions of NE are made 4 times a day (99), or

chronically in a continuous or pulsatile fashion (102), rats demonstrate increases in total daily food intake and body weight gain across the series of days that NE exposure is continued.

Along with being recognized as a site that plays an integral role in ingestive processes, the PVN is responsible for the modulation and integration of neuroendocrine and of specific visceral secretory functions (158, 162, 185, 188).

General Experimental Focus

The major neuronal projection systems associated with the PVN govern the expression of the functions of this nucleus. One projection system mediates the activity of the pituitary-adrenal system via paraventricular projections to the median eminence. Another system is comprised of the neural interconnection between the PVN and catecholaminergic and autonomic sites in the myelencephalon and spinal cord and mediates gustatory, visceral, and autonomic responses. Each of these paraventricular projection systems influences the adrenal glands. The former influences the activity of the adrenal cortex via adrenocorticotropic hormone (ACTH) release from the anterior pituitary, while the latter influences the adrenal medulla via autonomic sites in the spinal cord.

The present investigation examined the role of the adrenal cortex and adrenal medulla and their secretions in

the obesity that results after disconnection of the PVN. It specifically examined the effects upon feeding of the administration of corticosterone, the natural adrenal glucocorticoid of the rat, and dexamethasone, a synthetic glucocorticoid that also significantly alters catecholamine (CA) biosynthesis in the adrenal medulla.

Hyperphagia and obesity was induced by bilateral parasaggittal retracting wire knife cuts alongside the PVN. This allowed reference to and comparison with the existing literature that deals with this type of experimentally-induced obesity.

The remainder of this chapter is a survey of the literature associated with the neuroanatomical and functional characteristics of the PVN and of related findings associated with the medial hypothalamic area. The discussion focusses on the relationship between each projection systems of the PVN and ingestion.

Adenohypophysial Connections of the PVN

Neuroanatomy

Many studies have implicated the medial hypothalamus in the control of the pituitary-adrenal system. Recent work characterizes the PVN as the nucleus within this area primarily responsible for modulating the activity of this neuroendocrine system. The PVN contains the cell bodies of the neurons that possess the 41-amino-acid peptide Corticotropin Releasing Factor (CRF) (164,

168, 190). Several immunocytochemical studies locate a large population of immunoreactive CRF cell bodies in the parvocellular division of the PVN (11, 119, 120, 139, 165). CRF immunopositive efferent fibers project from the PVN to the external lamina of the median eminence (11, 120, 139, 168) where they terminate on the primary portal capillaries (40, 119).

The hypophysiotropic role of CRF is supported by the experimental conditions under which enhanced immunocytochemical staining for CRF is seen. In normal intact rats minimal immunopositive staining of CRF neuronal perikarya is found without pretreatment with colchicine (139, 165, 168). Colchicine, which blocks axonal transport, increases CRF-immunostaining in the PVN (11, 119, 165, 168, 190). This enhancement is accompanied by decreased CRF staining in the median eminence (11, 119). This indicates that CRF is synthesized in the PVN and transported by axoplasmic flow to terminals in the ME. The responsiveness of CRF to feedback inhibition is demonstrated in adrenalectomized and in hypophysectomized rats. The loss of negative feedback from the pituitary-adrenal system in these rats is accompanied by an enhanced immunostaining of CRF cells (120, 139, 165, 168, 190).

Direct evidence confirming the role of CRF in the secretion of ACTH has been obtained using live animals. The increase in ACTH levels induced by exogenously-applied

CRF, by ether stress, or by the loss of negative feedback associated with adrenalectomy is substantially attenuated by antibody antiserum to CRF (151, 152). It has also been shown that a circadian rhythmicity in CRF-like immunoreactivity in the hypothalamus leads and parallels the circadian rhythmicities of ACTH and corticosterone (125).

Feeding

Some evidence suggests that the mediation of consummatory behaviors by the PVN may relate to this site's influence on the function of the pituitary-adrenal system. Hypothalamic hyperphagia and obesity are altered by removal of the pituitary or the adrenals. The obesity produced by lesions of the VMH (which destroy fibers of passage from the PVN) is prevented by adrenalectomy (26, 70). This effect appears to be due to the loss of adrenal steroids, because corticosterone replacement reinstates the hyperphagia and weight gain characteristic of VMH-lesioned animals (26). The feeding response induced by the central infusion of NE also relies on the presence of pituitary and adrenal glands. Hypophysectomy abolished the feeding, but not drinking, behavior exhibited after centrally-applied NE (88, 91, 98, 154). The consummatory response elicited by PVN injections of NE or clonidine is attenuated by prior adrenalectomy (10, 91, 98, 153). In each case the behavioral deficits are reversed by corticosterone replacement therapy. In addition, the

hormone-reinstated food intake response is positively correlated with the level of circulating corticosterone made available (91, 98, 153).

Circadian Rhythms

On a 12:12 light:dark cycle the diurnal variations of adrenocortical secretions, food intake, and activity in the rat coincide (32, 78, 79). Peak ACTH and corticosterone levels occur just prior to the onset of dark (32, 78, 79, 205), the phase in which ad libitum-fed rats typically consume most of their food (8, 68, 150, 175) and are the most active (35, 78). A range of evidence suggests that the periodicities of these rhythms are intricately interrelated. For example, under restricted feeding regimens rats shift their diurnal corticoid rhythms such that the peak in corticosterone levels occurs just prior to the times of meal presentation (32, 78, 90, 124). Under conditions of prolonged food deprivation, circadian rhythms for activity and corticosterone are not present (32, 72). Under conditions of constant light or constant dark, food intake, activity, and corticosterone rhythms are not synchonized and are seen to free-run in accordance with the endogenous rhythm of each (32, 77). However, under constant light or dark, food can serve as a zeitgeber for corticosterone and activity rhythms because both of these rhythms can be entrained to a discrete food presentation schedule (32, 128).

The medial hypothalamus has been implicated as an anatomic substrate of these interrelated circadian rhythmicities. Disruption of the PVN or the VMH abolishes or attenuates the 12:12 diurnal rhythms normally associated with feeding (8, 32, 64, 68, 174, 175), activity (32, 37, 41, 64, 66), and corticosterone (9, 32, 80, 174). The obesity produced by this manipulation has been attributed at least in part to the elevated daytime food intake along with the accompanying loss of nighttime activity. In addition, the periodicity documented for the feeding response mediated by adrenergic receptors in the PVN appears to be dependent on circulating corticosterone, because adrenalectomy suppresses the food intake induced by NE or clonidine more during the nighttime, when corticosterone levels are normally high, than the daytime, when corticosterone levels are normally low (10).

Disruption of Hypothalamo-Adenohypophysial Connections

The PVN emerges as the key neural substrate for the regulation of both the hypothalamo-pituitary-adrenal axis and consummatory behavior. It has been well-established that disruption of the PVN results in profound changes in food intake and body weight gain (eg. 48, 97, 174). In recent years, a range of work has also revealed that disruption of the PVN produces changes in the functioning of the hypophysial-pituitary-adrenal system (eg. 107, 108, 109). Since the focus of the present investigation is to examine how the changes in feeding behavior seen after

parasagittal knife cuts made alongside the PVN relate to the function of the adrenal gland, it is useful to examine the nature of adenohypophysial-adrenal functions subsequent to interruption of the PVN.

First, disruption of the PVN in rats reduces adrenal weight (107, 109), attenuates the amplitude of circadian ACTH and corticosterone rhythms (67, 107, 174), and lower circulating corticosterone levels (67, 109, 174). However, some laboratories do not find changes in basal corticosterone levels (107, 108).

Stress response tests, a procedure commonly utilized to assess the functioning of the hypothalamus-pituitary-adrenal axis, have been performed on rats that have experienced either electrolytic lesions of the PVN or isolation of the PVN by a rotating wire knife. Animals exposed to either of these procedures show a diminished capacity to respond to stress. Disruption of the PVN results in a transient reduction in the increments in plasma corticosterone levels that typically accompanies exposure to a stress (see Table 1). This diminished corticosterone stress response is present 1 week (108, 109) and 3 weeks (67) after receiving PVN surgery but not after 4 weeks (107, 108, 109).

Both pituitary ACTH content and ACTH release are also influenced by deletion of the PVN (see Table 1) . Bruhn et.al. (27) found that at approximately one week after

TABLE 1

A SUMMARY OF THE STATUS OF THE PITUITARY-ADRENAL SYSTEM OF RATS AFTER SURGICAL DISRUPTION OF THE PVN

		Short-term	Long-term
CORT:	stress response plasma levels	attenuated*	normal
ACTH:	pituitary content	elevated	------
	stress-free plasma levels	normal	normal
	stress response plasma levels	diminished	normal
	IV CRF-stimulated plasma levels	greatly elevated	------
	CRF-stimulated release *in vitro*	------	elevated

Short-term = represents data collected 1-4 weeks after surgery.
Long-term = represents data collected 5 or more weeks after surgery
* = assessments made with respect to values obtained from rats that received only sham hypothalamic surgery

surgery PVN-lesioned rats have a 50% elevation in the pituitary concentration of ACTH-like immunoreactivity. This did not influence ACTH release under stress-free conditions, because the basal levels of plasma ACTH ofPVN-operated rats did not differ from control rats at 6 days (27), 3 weeks (67), and 6 weeks (107, 108) after surgery. In contrast, when rats are presented an ether stress (27), or a high level lapatomy-intestine traction stress (108) 6 days after PVN surgery, the stress-induced release of plasma ACTH is diminished 45 to 85% of controls. However, when PVN-lesioned rats are given IV ovine CRF at this time, they exhibit a dramatic 125-328% enhancement of plasma ACTH levels as compared to sham-operated controls (27). These results suggest an enhanced sensitivity to CRF after disruption of the PVN. Indeed, by 6 weeks post-surgery rats with PVN knife cuts exhibit the same increase in plasma ACTH levels in response to stress as do control rats (108). Similarly, 6 weeks after transection of the PVN, an elevated release of ACTH is found in response to CRF in an <u>in vitro</u> incubated anterior pituitary preparation (108). In sum, although the response of rats with PVN lesions or rotating wire knife cuts to acute stresses, as measured by plasma corticosterone and ACTH levels, may recover over time, the underlying functional status of the neuroendocrine axis of these animals is not the same as intact rats.

This point is also suggested by the results of work

from other procedures utilized to assess the responsiveness of the hypothalamo-pituitary-adrenal system. For example, transection of the PVN inhibits the compensatory adrenal hypertrophy that typically follows unilateral adrenalectomy (110).

The loss of negative feedback by bilateral adrenalectomy is characteristically accompanied by increases in ACTH levels under both in vivo and in vitro conditions (107, 108, 109, 202). However, rats that undergo adrenalectomy 8 days after rotating wire knife cuts of the PVN do not exhibit enhanced plasma ACTH levels (109). Even when the adrenals are removed 6 weeks after the PVN cuts, there is no compensatory increase in plasma ACTH, or in basal or CRF-stimulated in vitro ACTH release from anterior pituitary quarters (108) (see Table 2).

A dichotomy appears to exist between pituitary ACTH responses to stress and to adrenalectomy after destruction of the PVN. As Makara (107, 108) suggests, it appears that long-term PVN-lesioned or -knife-cut rats can compensate and respond to a brief challenge such as acute stress, but not to a sustained stimulus to ACTH release such as adrenalectomy. It has not, however, been determined whether PVN-operated rats could maintain the stress-induced release of ACTH or corticosterone if the stress was continued over long periods of time. Because the consummatory responses of animals with PVN lesions or

TABLE 2

A SUMMARY OF PITUITARY RESPONSES SEEN SUBSEQUENT TO BILATERAL ADRENALECTOMY IN PVN KNIFE-CUT AND SHAM-OPERATED RATS

		Short-term		Long-term	
		Sham	KC	Sham	KC
ACTH:	plasma levels	inc*	no inc	inc	no inc
	basal release *in vitro*	---	---	inc	no inc
	CRF-stimulated release *in vitro*	---	---	inc	no inc

Short-term = represents responses seen when adrenalectomy is performed approximately 1 week after sham or PVN knife cut (KC) surgery.

Long-term = represents responses seen when adrenalectomy is performed 6 weeks after sham or PVN KC surgery.

* inc = increase

knife cuts are typically studied over extended periods of time, which includes these animals encountering various and repeated stressors in their daily lives, this is a relevant issue. The findings suggests that the demonstration of normal basal and stressed levels of corticosterone in short-term tests may not in itself represent an uncompromised hypothalamus-pituitary-adrenal system. Overall, it appears that the interruption of CRF-ACTH connections can disturb the capacity and response of the pituitary-adrenocortical system.

Extrahypophysial Interconnections of the PVN

Neuroanatomy

In addition to its parvocellular projections to the median eminence which serves the anterior pituitary, the PVN possesses two other major types of neuroanatomical connections. One is the classical magnocelluar neurohypophysial projection, and the other class consists of neural parvocellular connections to other sites within the brain and spinal cord (158, 186, 187, 188).

Most of the projections of the magnocellular division of the PVN are to the posterior pituitary (186, 187, 188, 209). The magnocellular neurosecretory system is principally involved in the homeostasis of water balance. It is not considered in itself to play a major, or direct, role in feeding.

The parvocellular division of the PVN has extensive

bidirectional connections with the brainstem and the spinal cord (see Figure 1). These include a number of interconnected noradrenergic brainstem cell groups (158, 162, 186, 187, 188). These noradrenergic pathways relay visceral sensory information to the PVN. The PVN also contains parvocellular neurons that project to the locus coeruleus (LC) and to preganglionic cell groups of the autonomic nervous system (ANS) in the dorsal vagal complex (DVC) and the interomediolateral column (IMLC) of the spinal cord (105, 162, 163, 179, 184, 186, 188). These projections mediate autonomic responses associated with both the parasympathetic and the sympathetic divisions of the ANS (158, 162, 187).

Feeding

The localization of a 41-amino acid CRF peptide, considered the primary corticoliberin, to cells of the PVN has served to focus the study of the feeding responses mediated by the PVN toward an examination of the pituitary-adrenal axis. However, the neural projections between the PVN and brainstem also seem to contribute to the regulation of ingestion.

The overeating and obesity seen subsequent to the disruption of PVN neurons appear to be the greatest when a substantial interruption of the hindbrain projection fibers to and/or from the PVN is involved. Several reports have revealed that the PVN lesions that are the most effective in producing hyperphagia and body weight

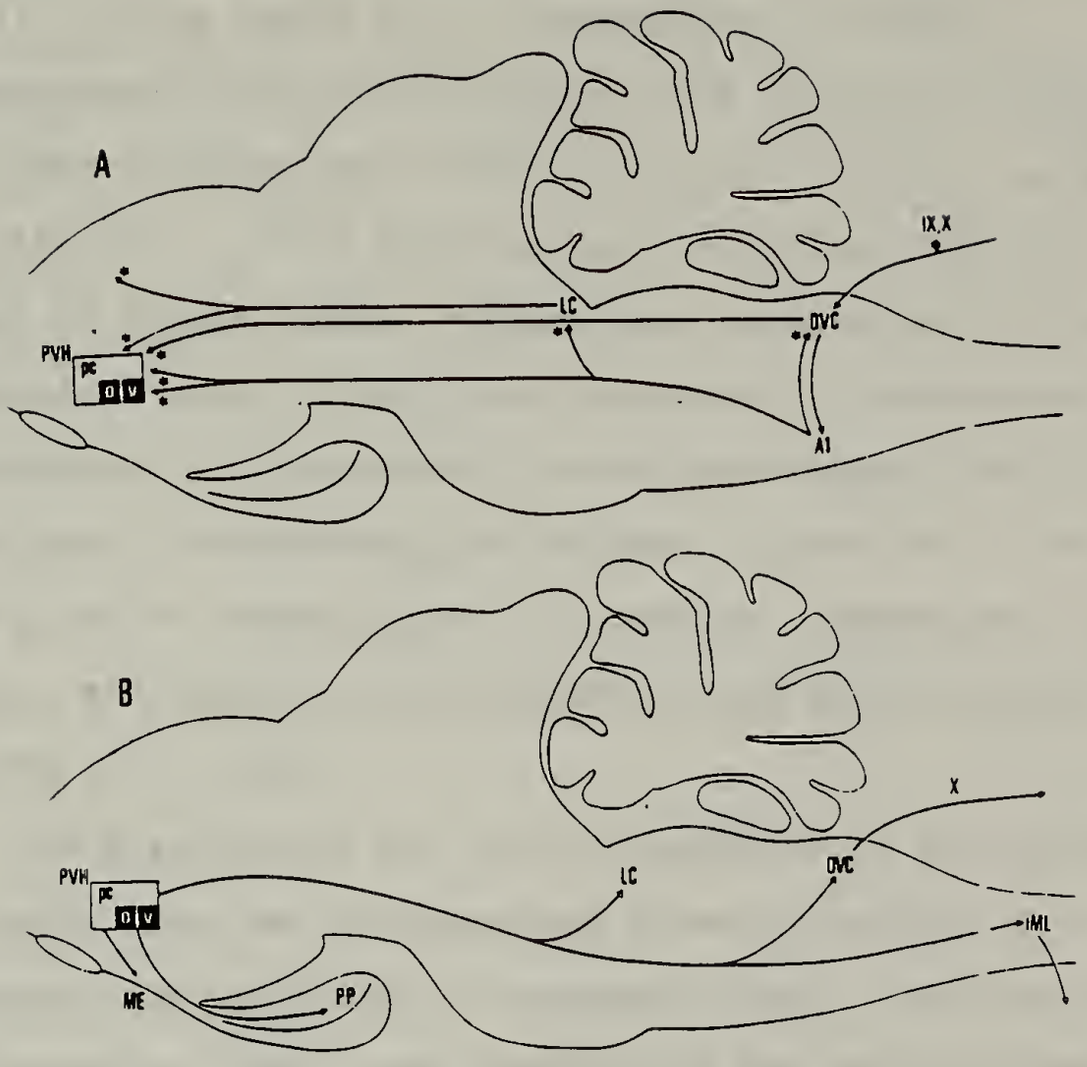


Figure 1. Diagrammatic representation of the major afferent and efferent neural pathways associated with the PVN of the hypothalamus (PVH) of the rat from Sawchenko and Swanson (162).

A. A summary of the organization of noradrenergic (NAc) inputs to the PVN, and the interconnections among the cell groups that give rise to these projections.

B. A summary of the major outputs of the PVN.

Principal Abbreviations: ME, median eminence; PP, posterior pituitary; LC, locus coerulus; DVC, dorsal vagal complex; IML, intermediolateral cell column; A1, NAc cells in A1 cell group; IX and X, glossopharyngeal and vagal cranial nerves, respectively.

gain are lesions that damage the caudal aspect of the PVN (2, 97, 171 as noted in 2). Furthermore, coronal hypothalamic knife cuts increase food intake and weight gain when cuts are made caudal, but not rostral, to the PVN (48, 172). This localization coincides with the routes of direct neural connections between the PVN and autonomic nuclei of the lower brainstem as determined by anterograde and retrograde tracing techniques. The cells of origin of descending projections in particular, but also sites of termination of ascending projections to some degree, are found to concentrate in the caudal aspect of the PVN (163, 188).

The hyperphagia and obesity produced by hypothalamic PVN knife cuts can be prevented or even reversed by complete subdiaphragmatic vagotomy (159). The branch of the abdominal vagus most responsible for this attenuation is the coeliac branch (160) which innervates the pancreas and part of the small intestine. These findings indicate the involvement of the parasympathetic division of the ANS and thus, the participation of autonomic nuclei in this syndrome.

The evidence accumulated to date points to the disruption of efferent descending projections of the PVN rather than afferents to this nucleus as key in the hypothalamic obesity syndrome. Damage to noradrenergic afferents results in hypophagia and reduced body weight (94, 135). Similarly, the destruction of noradrenergic

innervation to the PVN with the neurotoxin 6-hydroxy-dopamine causes decrements in food intake (5, 149). In contrast, increases in food consumption and body weight follow the destruction of PVN efferents as they course through the midbrain or thalamic periventricular gray regions en route to the DVC (204). In addition, the role of descending caudal outputs of the PVN is established for the eating elicited by central injection of noradrenergic agonists (111, 161, 204).

In the present investigation paraventricular fibers were cut bilaterally alongside the PVN. These parasagittal knife cuts sever medio-laterally oriented axons leaving or entering the PVN (2, 3, 48). After travelling to the lateral hypothalamus, PVN efferent projections turn caudally and/or ventrally. Work using asymmetrical knife cut and/or lesion techniques (47, 48, 172, 173) or staining procedures (105, 173, 184) suggest that the longitudinal fiber pathway that is relevant to the expression of paraventricular hypothalamic obesity courses through the basolateral hypothalamus into the midbrain, taking a path over the substantia nigra in the pontine tegmentum as it travels to sites in the caudal hindbrain.

Over the years, investigators have put forth the notion that the obesity syndrome associated with damage to the medial hypothalamus is a reflection of an alteration in the functioning of the ANS (15, 16, 56, 103, 144, 158).

They suggest that this damage results in an increase in parasympathetic activity together with a decrease in sympathetic activity, which encourages increased food intake and weight gain. A range of experimental tests of this hypothesis, particularly with respect to disruption of the VMH, have been performed (15, 31, 146, 159, 160, 170). But the findings of these studies have rendered discrepant conclusions. In light of the evidence that PVN efferents innervate preganglionic cell groups and relay nuclei of the ANS, it seems more plausible that the PVN is the medial hypothalamic site that influences the activity of the ANS and plays a role in the obesity that develops after PVN knife cuts.

Extrahypophysial CRF System

Several authors have proposed the existence of another major CRF system which is for the most part anatomically and functionally distinct from the paraventriculo-infundicular CRF projection system (116, 118, 141, 167). The primary functions of this system are posited to involve the central control of both divisions of the ANS and the mediation of a variety of autonomic responses.

The physiological state associated with this CRF system is the converse of the one thought to be responsible for the hypothalamic obesity syndrome. As determined by the measurement of a number of physiological responses, the central administration of CRF decreases

parasympathetic outflow and increases sympathetic outflow.

CRF acts within the CNS, independently of its effects on ACTH (21, 24), to induce changes in sympathetic nervous system (SNS) activity. The administration of CRF into the intracerebral ventricles of rats produces a rapid and prolonged elevation of plasma epinephrine (E) and NE levels (23, 24). The ganglionic blocker, chlorisondamine, antagonizes these CRF-induced increases in peripheral CA concentrations (24), and centrally-applied CRF receptor antagonists suppress stress-induced elevations of plasma E (21). Finally, increases in oxygen consumption also accompany the central, but not peripheral, infusion of CRF (23).

CRF also influences the autonomic control of gastric acid secretion (GAS). Microinfusions of CRF into the PVN (59, 193) or the cisterna magna (192) of the rat produces a dose-dependent and long-lasting inhibition of basal and pentagastrin-stimulated GAS. The inhibition of GAS following intracisternal (ICT) injections persists after hypophysectomy (192), which indicates that it is independent of the hypophysiotropic actions of CRF. Support for the contribution of the parasympathetic nervous system (PNS) in this response exists. Vagal activation is established as critical in the GAS response elicited by pylorus ligation, ICT thyrotropin-releasing hormone infusions, and IV pentagastrin injections. Central

CRF suppresses the secretion of gastric acid typically stimulated under each of these experimental conditions (192, 193). Moreover, prior vagotomy reduces the inhibitory action of CRF on GAS (192, 193). The ability of CRF to act within the brain to suppress GAS also appears to encompass the activity of the sympathetic division, because prior adrenalectomy, administration of ganglionic or alpha-adrenergic blockers, or transection of the spinal cord at the cervical C5 level, attenuates the suppression of GAS by ICT CRF (193). A complete blockade of the inhibition of GAS by CRF is seen in rats after vagotomy plus adrenalectomy (192). Finally, support for the physiological basis of this response is provided by the finding that the stress-induced release of endogenous CRF decreases GAS (115, 169, 193).

Changes in peripheral metabolism are also produced by the central administration of CRF. Intracerebrally-applied CRF elevates plasma glucagon (24), and plasma glucose in a dose-related manner (21, 23, 24, 25). The hyperglycemia stimulated by CRF is not related to this peptide's tropic actions on the pituitary gland, because hypophysectomy did not prevent it (24). Evidence suggests this elevation in glucose is at least partially mediated by the enhanced levels of peripheral CAs that also accompany the infusion of CRF (21, 24).

In addition to influencing the vegetative state of an animal, the central application of CRF can modify

behavior. It increases locomotor activity (23, 73, 183), enhances the behavioral effects of novelty (17, 18, 73), and increases grooming (17, 18, 73, 129, 183). These behavioral enhancements are exhibited in hypophysectomized rats (73, 129), but not when CRF is injected peripherally, nor when an inactive CRF analog is administered (183). CRF also influences food intake. CRF applied ICV decreases food intake under a number of test conditions (17, 18, 54, 73, 101, 129). Deprivation-induced feeding is suppressed when CRF is injected into the PVN but not other brain nuclei (75). The suppression of nocturnal feeding seen after CRF injections exists after hypophysectomy (129) but not after adrenalectomy (54), and is not reinstated by corticosterone replacement (54). As would follow, ICV infusions of CRF did not significantly reduce food intake, except at a high dose, in adrenal demedullated rats (54). Taken together, these results indicate that CRF's suppression of eating is not dependent on the pituitary-adrenocortical axis. The influence of centrally-applied CRF on ingestion appears to result from the changes it produces in the ANS---particularly in the SNS.

The responses elicited upon activation of autonomic circuitry within the CNS by CRF generally oppose the responses produced by interruption of the PVN (see Table 3). Converse to the responses elicited by central injections of CRF, knife cuts alongside the PVN increases

TABLE 3

A COMPARISON OF THE RESPONSES ELICITED BY RATS AFTER RECEIVING PARASAGITTAL KNIFE CUTS ALONGSIDE THE PVN OR CENTRAL INJECTIONS OF CRF

TYPE OF RESPONSE	PVN KNIFE CUTS	CENTRAL CRF
Food intake	↑	↓
Locomotor Behavior	↓	↑
Grooming Behavior	↓	↑
Gastric Acid Secretion	↑	↓
Plasma Glucose Levels	↓*	↑
Plasma Glucagon Levels	↓	↑
Plasma E Levels	↓	↑
Plasma NE Levels	varies	↑
Energy Expenditure	↓	↑

↑ = represents an increase ↓ = represents a decrease

* = represents glucose levels just following KC surgery and prior to ad libitum access to food.

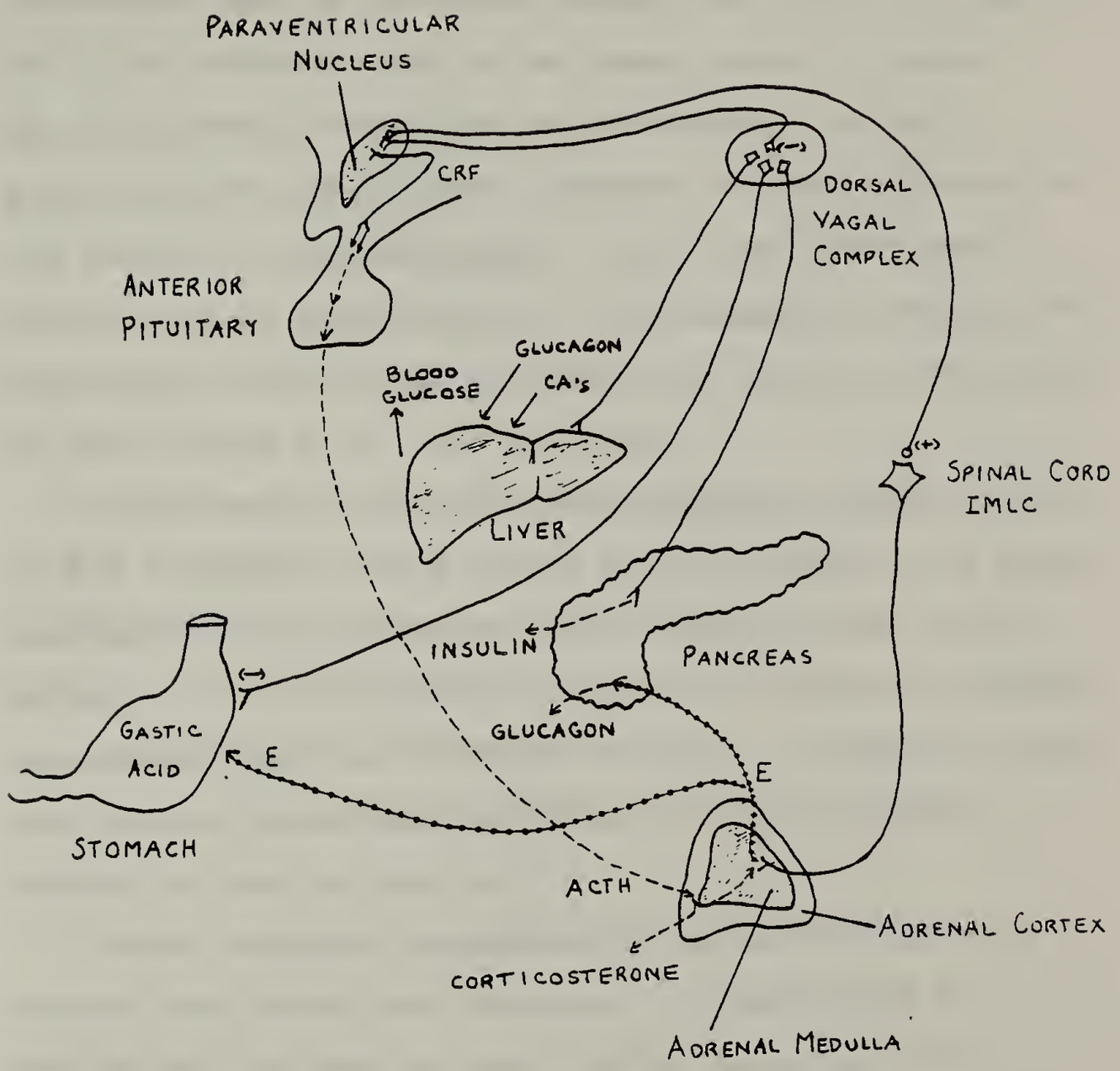
food intake (eg. 48), decreases in activity (37), decreases grooming behavior (personal observation), and increases GAS (159). The latter indicates an alteration in the status of PNS activity. Contrary metabolic and sympathetic responses are also seen. Within a short time after PVN surgery, plasma glucose falls (6, 50, 65), plasma glucagon falls, and plasma E is greatly diminished, as is the E/NE ratio (50). Rats with PVN knife cuts also have a diminished capacity for thermogenesis (31, 61).

Since the constellation of responses influenced by the activation of autonomic circuitry and by knife cuts along the PVN parallel each other, similar physiological systems may underlie both set of responses (see Figure 2).

The results from a range of neuroanatomical and electrophysiological work lend additional support to this idea. First, some CRF-immunoreactive (ir) labeled cells in the parvocellular division of the PVN give rise to extrahypophysial descending autonomic projections (164). Double-labelling techniques reveal a substantial number of long direct projections to autonomic sites in the brainstem and the sympathetic IMLC of the spinal cord originate from PVN cells and stain for oxytocin, vasopressin, and neurophysin (163, 179, 184, 188, 209). These neuropeptides have not emerged as being significant in the mediation of autonomic responses (20), but CRF has been shown to coexist with all three of these peptides in the PVN (71, 116, 164, 165, 166). Next, CRF-ir has been

Figure 2. Diagrammatic representation of the paraventricular hypothalamic-pituitary-adrenal axis, and the paraventricular extrahypophysial projections that control both the parasympathetic and the sympathetic divisions of the autonomic nervous system.

Principal Abbreviations: CRF, corticotropin release factor; ACTH, adrenocorticotropic hormone; E, epinephrine; IMLC, intermediolateral column; CAs, catecholamines.


PARAVENTRICULAR NUCLEUS
CRF
DORSAL VAGAL COMPLEX
ANTERIOR PITUITARY
GLUCAGON
BLOOD GLUCOSE
CA's
LIVER
SPINAL CORD IMLC
INSULIN
PANCREAS
GLUCAGON
GASTIC ACID
E
E
STOMACH
ACTH
ADRENAL CORTEX
CORTICOSTERONE
ADRENAL MEDULLA

demonstrated in cells of the LC, and cells and fibers of the nucleus of the solitary tract (NST), dorsal motor nucleus of the vagus (DMNV), the central nucleus of the amygdala, and the laminae of the thoracic and lumbar levels and IMLC of the spinal cord (116, 136, 137, 141, 190). In addition, CRF-ir has been located in other neuroanatomical sites known to be relevant to the processing of visceral and gustatory information such as the sensory glossopharyngeal, vagal, and hypoglossal nuclei, the nucleus ambiguus, the parabachial nucleus, the trigeminal nucleus, and the medullary regions that contain CA cell groups (116, 136, 137, 167).

Furthermore, CRF alters electrophysiological activity in the brainstem. CRF given by ICV injections or by local microapplication activates neural firing in the LC (201). In turn, electrostimulation of the LC in the cat activates the adrenal medulla releasing CAs (42). In addition, CRF and oxytocin have been identified within the adrenal medulla in the rat (62, 141).

These findings strengthen the assertion that the obesity seen after the disruption of the PVN can be attributed, at least in part, to the severing of PVN caudal efferent projections.

PVN Projections and Their Relationship to the Adrenal Gland

Two major projections of the PVN share a common target---the adrenal gland.

Caudal projections from the PVN innervate autonomic cell groups in the IMLC of the spinal cord which are the source of preganglionic sympathetic fibers to the adrenal medulla. Disruption of the PVN or its hindbrain projections are posited to diminish the activity of these splanchnic nerves.

CRF-positive paraventriculo-infundibular neural projections are primary in the control of the pituitary-adrenal axis. In turn, interruption of the PVN or its hypophysial projections reduces the responsiveness of the pituitary-adrenocortical system. Studies that examine the integrity of the hypothalamo-dpituitary system typically focus on the status of the adrenal cortex: circulating corticosterone levels, and the influence of circulating corticosterone levels on peripheral and central sites. Examination of the relationship between the hypothalamic-pituitary-adrenal system and ingestion also focus on these aspects of adenocortical function. Indeed, this work has revealed that circulating corticosterone levels play a significant role in the expression of consummatory responses (26, 32, 70, 97) and the mediation of a range of metabolic processes (63, 122, 140).

However, these lines of investigation tend to neglect another significant component of the function of the adrenal cortex. In addition to secreting corticosterone into the blood, the adrenal cortex influences the secretions of the adrenal medulla. Any manipulation which

compromises the responsiveness of the adrenal cortex can indirectly influence the status of the adrenal medulla (4, 30, 39, 83, 207, 208).

The adrenal gland consists of an outer cortical region where glucocorticoids are synthesized, an inner medullary region that contain chromaffin cells where CAs are synthesized, and a portal vascular system that allows undiluted adrenocortical venous blood to perfuse the medulla. The regulation of CA biosynthesis in the adrenal medulla is affected both by the activity of the splanchnic nerves and by the concentration of glucocorticoids in the medulla. The neural and humoral factors jointly modulate the enzymes responsible for the synthesis of medullary CAs and influence the proportions of E and NE produced.

The effects of the neural and humoral inputs on CA synthesis in the adrenal medulla have been illustrated in a range of work. Experimentally-induced increases in the enzymes involved in the biosynthesis of CA, tyrosine hydroxylase (TH), dopamine B-hydroxylase (DA-B-OH), and phenylethanolamine-N-methyl transferase (PNMT), can be abolished by transecting the splanchnic nerves to the adrenal (39, 83, 134, 197, 198). Hypophysectomy, which eliminates corticotropic stimulation of the adrenal cortex, also reduces the activity of CA biosynthetic enzymes in the adrenal medulla (4, 30, 39, 83, 197, 207, 208). This effect is reversed by exogenous ACTH (4, 30,

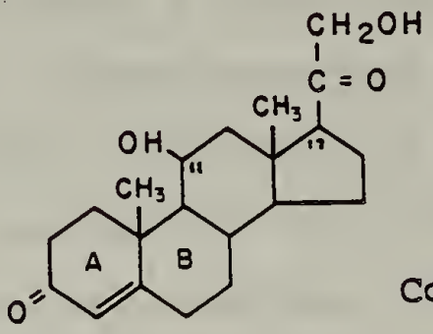
39, 83, 207, 208) or the synthetic glucocorticoid DEX (4, 39, 83, 197, 207, 208).

Adrenal Secretions

The adrenal gland is responsible for the production and release of a multitude of humoral substances. As mentioned the catecolamines NE and E are synthesized and released by the adrenal medulla. Also a range of steroid hormones are manufactured from a common precursor, cholesterol, within the cells of the adrenal cortex and are secreted into the blood supply. The glucocorticoids, which influence intermediary metabolism, are one major class of adrenocortical steroid hormone. This group of hormones consists of cortisol (or hydrocortisone), cortisone, and corticosterone. Another major class of adrenocortical steroids are the mineralocorticoids. They contribute to the regulation of fluid-electrolyte balance. The principal mineralocorticoid that circulates is aldosterone, but small amounts of deoxycorticosterone is also formed and released. Lastly a group of steroids having androgenic or estrogenic activity is also manufactured by the adrenal cortex. Testosterone is produced in small amounts. Moderately active male sex hormones called adrenal androgens are also liberated. These include dehydroepiandrosterone, androstenedione, and 11-B-hydroxyandrostenedione. The female sex hormones of progesterone and estrogen are secreted but only in minute

quantities.

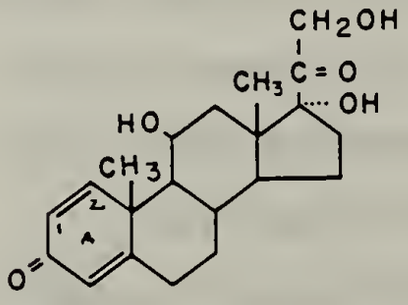
The predominant naturally occurring glucocorticoid in the rat is corticosterone (CORT). The molecular structure of this corticosteroid follows:



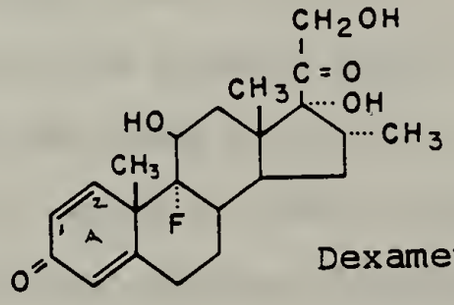
Corticosterone

It is derived from the basic steroid molecule which consists of four carbon rings fused together with three six-membered rings and a fourth five-membered ring.

In the present investigation the effects of CORT are compared to those of a synthetic analogue of this steroid, dexamethasone (DEX). DEX is a modification of the synthetic adrenocortical compound prednisolone. The molecular structure of each of these synthetic glucocorticoids is represented as follows:



Prednisolone



Dexamethasone

(9 ∝ Fluoro-16 ∝ methyl-prednisolone)

The primary difference between these synthetic steroid analogues and the natural adrenocorticoid CORT is a 1,2-double bond in the A ring of the basic steroid 4 ring nucleus, and the -OH in the C-17 position.

Although glucocorticoids are primarily considered for their glucose-promoting effects, they do often also possess some salt-retaining mineralocorticoid potency. This is the case for corticosterone. However the structural modifications in the composition of DEX noted above contributes to the enhancement of the glucocorticoid potency of DEX but essentially eliminates its mineralocorticoid effects.

For the most part CORT and DEX are used interchangeably as glucocorticoid agents. However, over time some difference in their actions and in the effects they produce have been evidenced.

In addition to its traditional classification as a glucocorticoid agent, DEX is established as an activator of CA biosynthetic enzymes, particularly PNMT, in the adrenal medulla (143, 207, 208). DEX appears to have a greater capacity to influence the adrenal medulla in this manner than exogenously-applied CORT does (207).

The negative feedback action of CORT and DEX also differs to some extent. CORT has greater binding affinity to hypothalamic and limbic sites than to the anterior pituitary (34, 147), while the converse holds for DEX (33, 34, 148). CORT also exhibits a greater capacity to inhibit

CRF at the hypothalamic level than ACTH release at the pituitary level (156, 157). Again the reverse is true for DEX (148, 156, 157).

A survey of the findings available to date reveals that the body weight of rats is affected differently by these corticosteroids. Several reports indicate that the administration of DEX results in decreases in body weight (7, 104, 140, 178). Another prednisolone-based glucocorticoid, methylprednisolone, is also found to diminish the body weight gains of normal and VMH-lesioned rats (52, 53). However, corticosterone given to normal rats does not affect body weight (140, 178) and in some instances appears to potentiate body weight gain (57, 106).

The significance of the disparities that do exist between CORT and DEX and the role they play in the responses seen herewithin are addressed throughout this investigation.

Overview

The present investigation assessed whether the obesity seen subsequent to bilateral parasaggital knife cuts alongside the PVN can be attributed to alterations in adrenal cortical and/or adrenal medullary activity.

Review and Rational

The PVN and its projections are integral in controlling the pitutary-adrenocortical system and in

modulating the activity of the ANS.

CRF-positive paraventriculo-infundibular projections link the PVN to two aspects of the function of the adrenal cortex. By its control of adrenocorticotropic stimulation of the adrenal cortex, the PVN regulates the level of circulating CORT, and also indirectly mediates the intraadrenal supply of CORT flowing to the adrenal medulla. The latter adrenocortical effects, in turn, is significant to the activity of the adrenal medulla as it is one factor that regulates CA synthesis in the adrenal medulla. Support for this is derived from the decline in adrenomedullary TH, DA-B-OH, and PNMT activity and E content produced by hypophysectomy and its reversal by ACTH (4, 39, 83, 85, 207, 208).

Paraventricular projections to autonomic sites in the caudal aspect of the CNS also impose on the function of the adrenal gland. Via these efferents, the PVN modulates the SNS which includes the sympathetics innervating the adrenal medulla. Neural stimulation of the adrenal medulla via splanchnic nerve fibers is the other major factor contolling adrenomedullary CA production. Activation of adrenal medullary CA biosynthesis is prevented by adrenal denervation and by cutting sympathetic nerves to the superior cervical ganglia (4, 39, 83, 197, 198).

Thus, the PVN emerges as a unique neuroanatomical site which influences the activity of the adrenal cortex

and medulla. These influences include a functional relationship with the two factors that modulate adrenomedullary CA synthesis.

It follows that knife cuts alongside the PVN of the hypothalamus should affect the function of both parts of the adrenal. As documented in previous sections, disconnection of the PVN reduces the responsiveness of the pituitary-adrenocortical system and diminishes sympathetic activation of the adrenal medulla. Hence, the manufacture of CAs in the adrenal medulla would be altered by interruption of the PVN and/or its projections.

The studies herewithin explored the possibility that KC-induced alterations in adrenal secretions contribute to the obesity that results after transection of the PVN. CORT and DEX were employed to test this possibility. If the obesity produced by PVN KCs relates to insufficiencies in the levels of circulating CORT available, CORT should ameliorate the obesity. If, however, PVN KC-induced obesity is instead linked to deficits in the production and balance of available adrenomedullary NE and E, then only DEX treatments should attenuate the propensity for weight gain in KC rats. The synthetic steroid DEX is capable of reinstating the medullary CA biosynthetic enzyme activity in rats with disrupted adrenocortical and neural connections to the adrenal medulla as it can activate these enzymes in hypophysectomized and adrenal denervated rats (eg. 4, 197, 208).

The administration of physiological or even low pharmacological doses of CORT does not replicate the effects of endogenous glucocorticoid on the adrenal medulla. Exogenously-administered CORT, which primarily supplies the peripheral blood, does not restore CA activity in the adrenal medulla after hypophysectomy (208). ACTH, which enhances both the concentration of glucocorticoids perfusing the adrenal medulla and circulating in the periphery, does (83, 207, 208). Enhancements in adrenal medullary CA enzyme activity, particularly PMNT, are produced by DEX (198, 207, 208) indicating this synthetic glucocorticoid actually represents endogenous CORT's action at the adrenal medulla more accurately than does exogenous CORT itself.

Experiments

In Experiment 1 and 2, the effects of the administration of CORT and of DEX on the obesity produced by PVN knife cuts were compared.

The steroids were administered to rats that still possessed their adrenals. This contrasts the approach typically taken in endocrinology studies. The traditional paradigm involves the removal of a gland followed by hormone replacement therapy. This approach was not utilized in these studies for several reasons. The adrenal is a multi-structural, -functional gland that modulates the levels of glucocorticoids, mineralocorticoids and

adrenomedullary CAs. The replacement of glucocorticoids alone following adrenalectomy would not restore all three aspects of adrenal function. Thus the responses subsequently seen would not solely relate to the action of glucocorticoids. Furthermore, removal of the adrenals subsequent to severing CRF fibers, which is already associated with deficits in adrenocortical functioning, would introduce a confusing redundancy. Thus it would not serve as a cogent argument for the participation of the adrenals in the PVN obesity syndrome. To assess the role of the adrenal medulla in paraventricular hypothalamic obesity, it was necessary to use animals possessing adrenals. This permitted the induction of adrenomedullary CA activity by DEX. There is no known surgical technique for removal of the adrenal cortex that will leave the medulla intact and innervated.

In Experiment 3, the effect of DEX on appetitive responses was further examined. To determine whether DEX acts on appetite primarily via the adrenal medulla , DEX was administered to knife cut and sham-operated rats after adrenal demedullation.

C H A P T E R I I

EXPERIMENT I

In Experiment 1, rats with bilateral knife cuts alongside the PVN received chronic treatments of CORT, or DEX, or the control treatment. The purpose of this study was to determine if CORT, the rats' natural glucocorticoid, or DEX, a synthetic glucocorticoid, affect the body weight and food intake of these rats.

Methods

Sixty adult female Charles River CD albino rats were maintained in stainless steel hanging wire cages under a 12:12 light:dark cycle and were provided unlimited access to Purina Laboratory Chow (# 5001) pellets and tap water.

Phase One of Hormone Exposure

One day prior to surgery the rats were divided into groups balanced for initial body weight. Naso-anal lengths in millimeters (mm) were measured using a "stretch" technique as described previously (50). The animals then received either a moderate corticosterone dose (MCORT), a high corticosterone dose (HCORT), dexamethasone (DEX), or control treatments consisting of no hormone (NH) or cholesterol (CH).

Corticosterone (Sigma Chemical Corp., St. Louis, MO.) was administered as a solid pellet implanted

subcutaneously in the nape of the neck. Pellets were formed of molten solid hormone alone, the 100% corticosterone pellet (HCORT dose), or of hormone and cholesterol combined to form a 50% corticosterone pellet (MCORT dose). Cholesterol pellets served as control implantations. Details of the procedure involved in making these pellets are found in (121). The HCORT and MCORT pellets supplied respectively an average dose of 1.58 $\pm$ 0.20 mg CORT/day and 0.52 $\pm$ 0.03 mg CORT/day (or ~6.2 mg/kg and ~2.0 mg/kg with respect to initial body weights).

Animals treated with DEX (Sigma Chemical Corp., St. Louis, MO.) received it via their drinking water. The day prior to surgery all DEX-treated rats had access to 1 ug/ml of DEX in their drinking bottles. After surgery, DEX was diluted to a concentration that allow each rat to consume approximately 0.1 mg/kg/day (approximately 0.71 ug/ml for knife cut rats and 0.34 ug/ml for sham-operated rats). The DEX dosage for each rat was adjusted daily for the first six days after surgery, thereafter every third day. It was calculated respective to the previous 1 day or 3 day average intake of water, and the current body weight. These adjustments corrected for the large differences in water intake that typically exist between sham-operated and hypothalamic knife cut animals. The 0.1 mg/kg dose was administered through Day 18 post-surgery (Phase One of Hormone Exposure). The protocol after Day 18

is described separately.

Stereotaxic brain surgery was performed with the rats under ether anesthesia and consist of bilateral parasagittal knife cuts alongside the PVN using a retracting wire knife as detailed in (48). Briefly, the parasagittal knife cuts were $\pm$ 0.9 mm from the midline, extended between 5 and 8 mm anterior to the interaural line, and 3 mm dorsal from the base of the brain. Sham surgery excluded extension of the retractable wire knife.

To summarize, the experimental groups were as follows: sham-operated rats treated with either no hormone (SH-NH, n=5), cholesterol (SH-CH, n=5), DEX (SH-DEX, n=8), MCORT (SH-MCORT, n=6), or HCORT (SH-HCORT, n=6); and knife cut-operated rats treated with no hormone (KC-NH, n=5), cholesterol (KC-CH, n=5), DEX (KC-DEX, n=8), MCORT (KC-MCORT, n=6), or HCORT (KC-HCORT, n=6). No differences resulted between the NH or CH groups within surgery manipulations, so these groups were collapsed (SH-CON, n=10 and KC-CON, n=10).

On the day of surgery the rats were switched from Purina Laboratory Chow pellets to Purina Mouse Chow (#5015) pellets which provide a higher percentage of fat (11%) in the diet. This diet promotes excessive weight gain in PVN KC rats (46). Body weight, water intake, and food intake corrected for spillage were measured.

After approximately 18 days of DEX exposure, a few

KC rats exhibited a rapid elevation in water intake and began gnawing (which consisted of eating and/or spilling) large amounts of food, which are indicative of a diabetic condition. At this time urine samples were analyzed for glycosuria using Tes-tape Glucose Enzymatic (Eli Lilly & Co.) test strips.

Phase Two of Hormone Exposure

After 18 post-surgery days additional dosage and hormone manipulations were performed.

First, employing the principles of an ABA design the SH-DEX and KC-DEX groups were switched from 0.1 mg/kg/day DEX to a 0.01 mg/kg/day maintenance dose for the period from Day 18 to Day 39. This manipulation provides information to confirm whether the lower weight gain seen in KC-DEX rats was due to inadequate brain surgery, and to determine whether the reduced weight gain under DEX was permanent or transitory. The maintenance dose was needed rather than no drug because DEX treatment atropies the adrenal cortex via negative feedback inhibition of ACTH. At Day 39, all rats were returned to their original dose of DEX. This step also provides a test of whether the DEX effects were coincident only with the initial 'dynamic' post-surgery weight gain.

Second, 2-3 rats from each CON or CORT treatment group were given 0.1 mg/kg/day DEX via their drinking water in addition to their CH or CORT pellet implants. The combined hormone treatment was employed to determine

whether the suppressive effects of DEX occurred by the suppression of endogenous CORT secretion. On Day 27 these SH-MCORT, -HCORT and KC-MCORT, -HCORT rats were removed from the DEX-treated water and returned to tap water until the end of the experiment.

On Day 36 post-surgery, naso-anal lengths were taken again on all animals. While subjects were still under ether anesthesia from this procedure, CORT pellets were replaced and CH pellets were removed.

Blood Glucose Samples

At the end of the experiment (Days 48-51), blood samples were collected prior to 'lights off'. Samples were taken from the tip of the tail into microhematocrit tubes and centrifuged. The serum samples were stored in microcentrifuge tubes, and frozen. Samples were assayed for glucose with a YSI Model 23A Glucose Analyzer (Yellow Springs Instrument Co., Yellows Springs, Ohio) using a glucose oxidase method.

Naso-Anal Lengths and Obesity Indices

The Lee Obesity Index (86) was computed for each animal from naso-anal length and body weight measures taken just prior to surgery and again approximately 36 days after surgery. The formula for calculation of obesity indices is $(10^4) \frac{\sqrt[3]{\text{Body weight (g)}}}{\text{Naso-anal length (mm)}}$.

Surgical Verification

Two measures were utilized to verify the functional accuracy of the knife cuts.

In the initial 24 hour period after receiving parasagittal knife cuts animals had to exhibit food intake at least 20% in excess of preoperative rates.

Examination of the brain of KC rats was performed by making a series of coronal sections of formalin fixed brains with a scalpel or razor blade and inspecting the brain sections under a 20X Zeiss operating microscope (OpMi-9, West Germany). Our laboratory has found that in order to obtain rapid weight gains, knife cuts must be alongside the PVN, must extend to the base of the brain, and must be between 0.6 and 1.2 mm from the midline (48).

Data for animals which do not meet both the behavioral and anatomical criteria were excluded from the analyses. Based on these criteria a single rat from the KC-CON group was excluded. Also omitted were the data from a KC-DEX rat that contracted a respiratory infection and was discovered upon autopsy to have an abdominal abscess.

Data Analysis

Data matrices were established and stored in the IDAP system (Interactive Data Analysis Package, University of Massachusetts, Amherst). IDAP system commands were utilized to perform one- and two-way ANOVA, and comparison tests such as the multiple range Duncan test. BMDP4V and BMDP2V program packages (University of California, L.A.)

were utilized to perform 2-way ANOVA and 2-way repeated measures ANOVA analyses where appropriate.

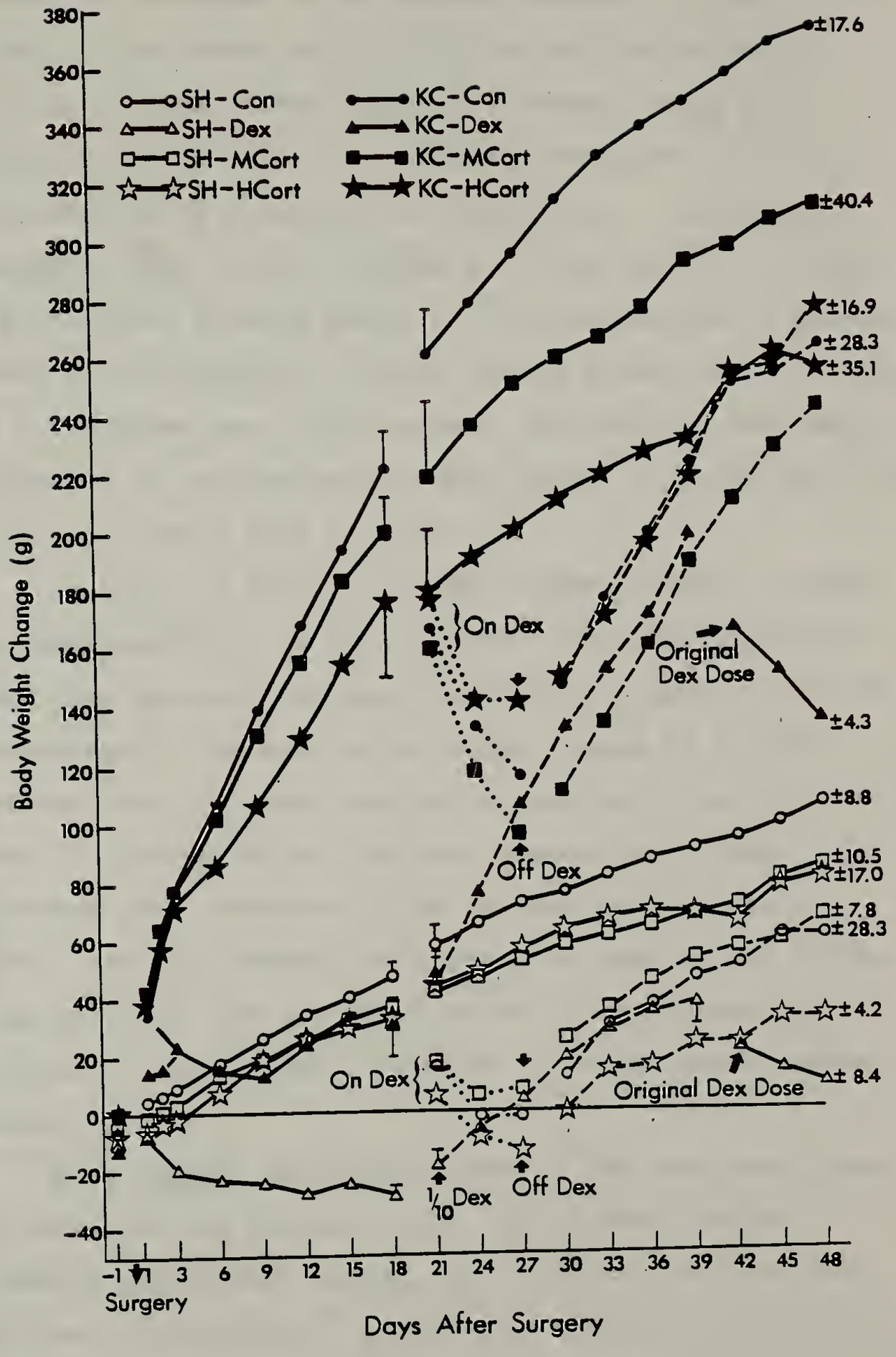
Results

Phase One of Hormone Exposure

Body weight. A repeated measures analysis of variance (ANOVA) of the cumulative body weight change over the first 18 post-surgery days revealed significant main effects for surgery ($F(1,49)=473.3$, $p<.05$) and hormone ($F(3,49)=80.8$, $p<.05$) and a significant surgery by hormone interaction ($F(3,49)=13.9$, $p<.05$). The within varible of body weight change across days was also significant ($F(7,343)=339.5$, $p<.05$) and interacted with both hormone ($F(21,343)=46.5$, $p<.05$) and surgery factors ($F(7,343)=136.6$, $p<.05$).

Chronic exposure to CORT via implanted pellets did not substantially alter body weight. The cumlative body weight change of sham-operated rats treated with either the 50CORT or 100CORT pellets did not differ significantly from that of the sham-operated controls (Day 18, Figure 3). The data suggest that exposure to CORT has a modest inhibitory effect on the weight gain induced by PVN KCs. By Day 18, KC rats exposed to HCORT had gained significantly less cumulative weight than KC rats treated with CON (multiple range Duncan comparison test, $p<.05$). The response of KC rats exposed to the smaller MCORT dose was intermediate, but not significantly different from

Figure 3. Mean body weight change of sham-operated and PVN KC rats treated with no hormone (CON), 0.1 mg/kg DEX, or a moderate (MCORT) or high (HCORT) dose of CORT for 18 days after brain surgery (Phase One of Hormone Exposure). Between Days 21 - 48 (Phase Two of Hormone Exposure), these rats experienced either the continuation of their original CON or CORT treatment only (solid lines), the combination of their original CON or CORT treatment plus 0.1 mg/kg DEX for nine days (Days 18 - 27, dotted lines) followed by exposure to only CON or CORT (Days 27 - 48, dashed lines), or the reduction of their DEX dose to 0.01 mg/kg for three weeks (Days 18 - 39, dashed lines) followed by the reinstatement of the original 0.1 mg/kg DEX dose (Days 39 - 48, solid lines). See Tables 4 - 6 for the number of animals in the subgroups in Phase Two.


SH - Con
SH - Dex
SH - MCort
SH - HCort
KC - Con
KC - Dex
KC - MCort
KC - HCort
±17.6
±40.4
±16.9
±28.3
±35.1
On Dex
Original Dex Dose
±4.3
±8.8
Off Dex
±10.5
±17.0
± 7.8
± 28.3
±4.2
± 8.4
On Dex
Original Dex Dose
1/10 Dex
Off Dex
Body Weight Change (g)
Surgery
Days After Surgery

either of the other two KC groups. However, it is difficult to accept the validity of this experimental finding as much of the difference between these group means is attributable to one HCORT-treated KC rat which exhibited an 45 g weight loss around Day 6. Furthermore, comparing only KC rats treated with CON, MCORT, or HCORT, a significant hormone factor did not emerge from a one-way ANOVA of the cumulative weight change across Phase 1. Also no differences were found between the absolute body weight change of KC rats exposed to CON, MCORT, or HCORT over the last 3 or last 6 days of Phase 1.

In striking contrast to the minimal effects of CORT, the administration of DEX inhibited the excessive weight gain that typically accompanies PVN KC surgery. By Day 18 post-surgery, the cumulative weight change of KC rats treated with 0.1 mg/kg DEX was significantly less than all other KC groups ($p<.05$) and was comparable to sham-operated rats treated with CON or with either dose of CORT. DEX also greatly diminished the body weight of the sham-operated rats beginning on Day 3 post-surgery and continuing through Day 18 (versus all other sham-operated groups, $p<.05$).

Food intake. An overall ANOVA of the mean food intake of rats over the initial 18 day experimental period revealed significant surgery ($F(1,50)=468.8$, $p<.05$) and hormone ($F(3,50)=20.9$, $p<.05$) main effects along with a significant surgery by hormone interaction ($F(3,50)=7.0$,

$p<.05$). The within variable effect, mean food intake across days, was significant ($F(7,350)=9.8$, $p<.05$) and also interacted with the surgery ($F(7,350)=7.3$, $p<.05$), but not the hormone, factor (Figure 4).

A small but statistically significant reduction in total mean daily food intake emerged between KC treated with HCORT and the KC rats given no hormone ($p<.05$). This parallels the differences in body weight noted. However, a repeated measures ANOVA of mean daily food intake across Phase 1 comparing only KC rats exposed to CON or MCORT or HCORT did not reveal a significant main effect for hormone. The food intake of sham-operated rats was not influenced by CORT at either dose.

DEX treatments produced a 74% reduction in the hyperphagia of KC rats. Though the amount of food consumed by DEX-treated KC rats was significantly less than eaten by the other KC groups ($p<.05$), it was still more than consumed by the sham-operated groups ($p<.05$). Exposure to DEX did not alter the average quantity of food consumed by sham-operated rats during Phase 1, but the DEX-treated sham-operated rats did tend to eat less food than other sham-operated rats early in the treatment phase. This trend is compatible with the significant main effect for hormone treatment seen in a one-way repeated measures ANOVA that compares the main daily food intake of all sham-operated groups across Phase 1 ($F(3,26)=5.81$, $p<.05$).

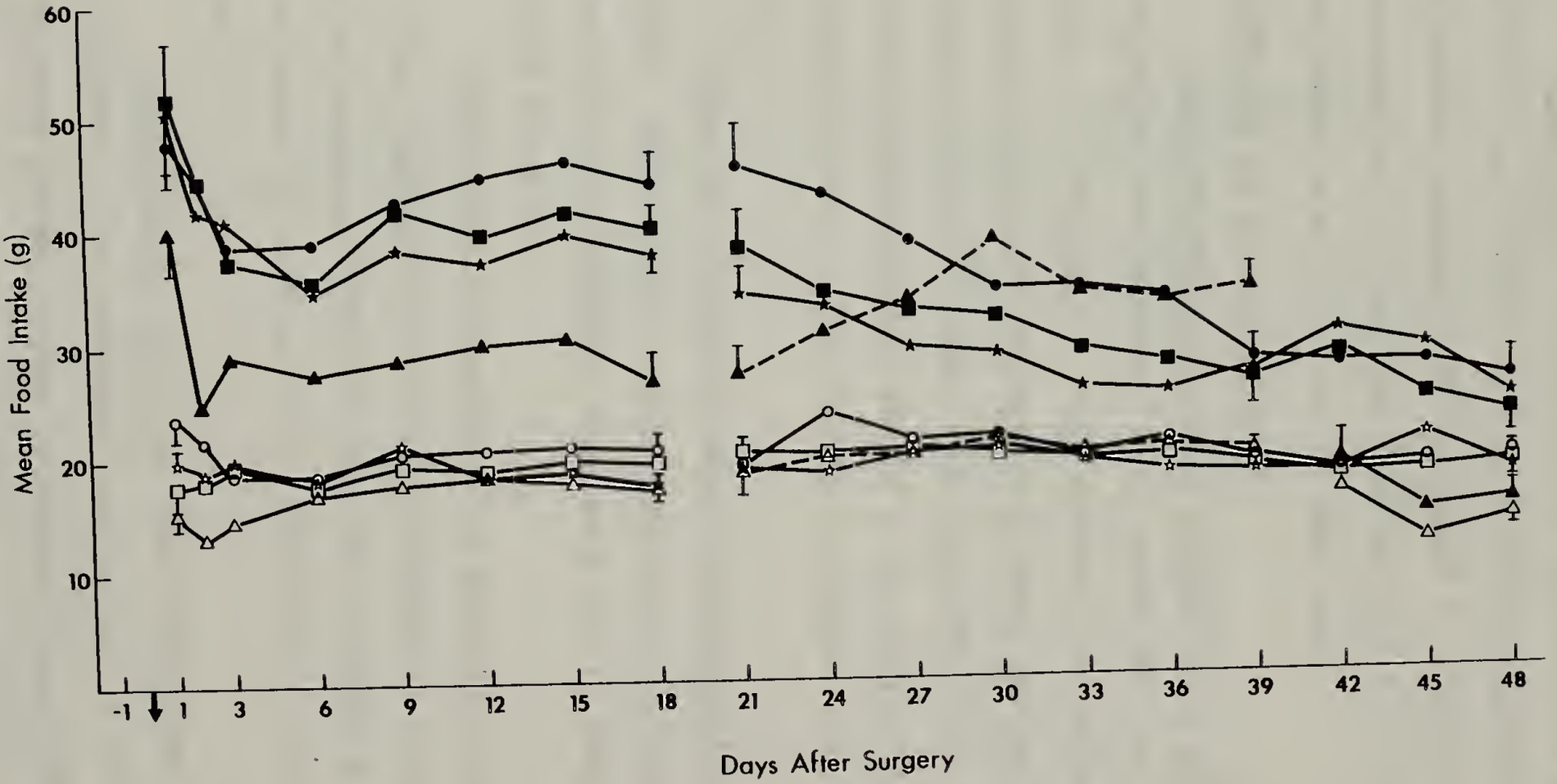


Figure 4. Mean daily food intake of sham-operated and PVN KC rats described in Figure 3 during Phase One (Days 1 - 18) and Phase Two (Days 18 - 48) of Hormone Exposure. Symbols are as indicated in Figure 3.

Urinalysis. Urinalysis for glucose was performed on subjects on or about Day 18 at the conclusion of phase 1 of hormone exposure. The presence of glucose in urine was detected only in KC rats treated with 0.1 mg/kg DEX. It was found in 4 of 7 rats in this group.

Phase Two of Drug Exposure

Combined administration of CORT plus DEX. When subgroups were exposed to 0.1 mg/kg DEX via the drinking water in addition to their initial CON, MCORT, HCORT hormone treatment, decreases in body weight and food intake similar to those seen with DEX alone were observed in both sham-operated and KC rats, despite the availability of CORT (Figure 3, dotted lines; Table 4 and 5).

When DEX treatments were discontinued nine days later, rapid gains in food intake and body weight were seen in all subgroups (Figures 3 and 4, dashed lines; Table 4 and 5).

DEX produced glycosuria in several KC rats but never in any sham-operated rats. In addition, KC rats treated with exogenous CORT plus DEX did not show a greater incidence of this condition than KC-CON rats treated with DEX.

Reduction in DEX dose. The reduced DEX dosage was coincident with increased body weight in both KC and sham-operated groups (Figure 3, dashed lines). The rate of weight gain of KC rats while treated with 0.01 mg/kg DEX

TABLE 4

MEAN BODY WEIGHT CHANGE PER DAY (g ± SEM) OF SHAM-OPERATED AND PVN KNIFE-CUT RATS BRIEFLY ADMINISTERED 0.1 mg/kg DEX IN CONJUNCTION WITH THEIR CHRONIC TREATMENTS OF NO HORMONE (CON) OR CORT DURING EXPERIMENT ONE

	N	3 Days Before DEX (Original Hormone Treatment Only)	9 Days of DEX Exposure + Original Hormone Treatment	3 Days After DEX (Original Hormone Treatment Only)
SHAM:				
CON	3	+1.8 ± 0.5	-4.5 ± 0.3	+4.7 ± 0.7
MCORT	2	+1.7 ± 1.0	-3.3 ± 0.6	+6.2 ± 1.2
HCORT	2	+0.8 ± 0.2	-3.8 ± 0.0	+4.5 ± 0.5
KNIFE CUT:				
CON	3	+7.7 ± 1.1	-9.3 ± 0.5	+10.1 ± 5.7
MCORT	1	+3.3 ± 0	-8.9 ± 0	+5.0 ± 0
HCORT	2	+7.0 ± 1.3	-6.4 ± 1.0	+2.5 ± 2.5

CON represents the combined control treatments of exposure to no hormone (NH) or 100% cholesterol (CH) pellet implanted subcutaneously.
CORT given as solid pellet implanted subcutaneously; HCORT represents a pellet made of 100% corticosterone, and MCORT represents a pellet made of 50% corticosterone, 50% cholesterol. See text for their approximate dosages.
The CON and CORT conditions described hold for subjects noted on Tables 4 through 9.

TABLE 5

MEAN DAILY FOOD INTAKE (g ± SEM) OF SHAM-OPERATED AND PVN KNIFE-CUT RATS BRIEFLY ADMINISTERED 0.1 mg/kg DEX IN COMBINATION WITH THEIR CHRONIC TREATMENTS OF NO HORMONE (CON) OR CORT DURING EXPERIMENT ONE

	N	3 Days Before DEX (Original Hormone Treatment Only)	9 Days of DEX Exposure + Original Hormone Treatment	3 Days After DEX (Original Hormone Treatment Only)	1 Day After DEX (Original Hormone Treatment Only)
SHAM:					
CON	3	18.8 ± 2.1	15.1 ± 1.3	17.9 ± 1.7	20.0 ± 1.6
MCORT	2	17.4 ± 3.1	14.9 ± 0.01	18.6 ± 1.3	19.0 ± 0.02
HCORT	2	15.3 ± 1.4	12.7 ± 0.5	14.5 ± 1.9	18.9 ± 0.1
KNIFE CUT:					
CON	3	38.4 ± 3.9	18.0 ± 2.8	23.5 ± 6.5	26.7 ± 2.7
MCORT	1	32.9 ± 0	20.2 ± 0	19.7 ± 0	24.4 ± 0
HCORT	2	40.4 ± 1.9	15.7 ± 2.1	10.1 ± 4.5	19.5 ± 1.0

was 8.1 ± 0.7 day. This is reminiscent of the weight gain of the KC-CON rats which had been 12.4 ± 0.7 g/day from brain surgery to Day 18 and 5.5 ± 0.6 g/day between post-surgery Days 18 to 39. Sham-operated rats rebounded to gain weight at a rate of 3.1 ± 0.4 g/day when treated with the 0.01 g/day maintenance dose of DEX. This compares with the 2.6 ± 0.3 g/day and the 1.9 ± 0.2 g/day weight gain of sham-operated CON-treated rats between Day 1-18, and 18-39, respectively.

A significant elevation in mean daily food intake was seen in DEX-treated KC rats upon switching to the lower DEX dose (Figure 4, dashed lines) such that their intake did not significantly differ from any other KC group (KC-DEX rats, Mean Daily Food Intake: Day 1-18, 28.7 ± 1.6g; Day 18-39, 32.9 ± 1.8 g; correlated $t(6)= 4.07$, $p<.05$). The amount eaten by DEX-treated sham-operated rats did not differ from other sham-operated treatment groups during Phase 2, and it was slightly higher than the average amount consumed by this group when administered 0.1 mg/kg DEX in Phase 1 (SH-DEX rats, Mean Daily Food Intake: Day 1-18, 17.1 ± 0.7g; Day 18-39, 19.8 ± 0.9 g; correlated $t(7)=4.20$, $p<.05$).

On Day 21, 3 days after the switch to the maintenance DEX dose, glucose was detected in the urine of 2 of 7 rats in the KC-DEX group, but was not revealed in this group at any subsequent time through Day 39. Its presence was not seen in any other treatment group from Day 18 to 39.

Reintroduction of the initial DEX dosage, 0.1 mg/kg, to DEX-treated groups on post-surgery Day 39 decreased both the body weight and food intake of KC and sham-operated rats (Figure 3 and 4).

Glycosuria was noted in almost all KC rats, but no sham-operated rats, treated with 0.1 mg/kg DEX during this final experimental period, Days 39-48.

Continued exposure to CORT. The remaining sham-operated and KC rats were maintained on their original treatments of no hormone (CON), MCORT, or HCORT through Day 48.

No profound changes in the weight gain response of KC or sham-operated rats were seen with the continued exposure to CORT during Phase 2. Although not readily apparent in Figure 3 depicting cumulative body weight gain, the absolute change and the rate of change in body weight of rats treated with CON, MCORT, HCORT within each surgery overlapped (Table 6).

Blood Glucose

Long-term exposure to neither CORT nor DEX altered the blood glucose levels of sham-operated rats (Table 7). The blood glucose values of the KC groups receiving CORT or CON were slightly, sometimes significantly, higher than for sham-operated groups. DEX treatments, however, caused a marked elevation in blood glucose in KC rats. The mean level of serum glucose of KC rats treated with 0.1 mg/kg DEX was 3.3-3.6 times greater than any other experimental

TABLE 6

MEAN ABSOLUTE BODY WEIGHT CHANGE (g ± SEM), MEAN RATE OF WEIGHT CHANGE (g/day ± SEM), AND MEAN DAILY FOOD INTAKE (g ± SEM) EXHIBITED BY SHAM-OPERATED AND PVN KNIFE-CUT RATS EXPOSED TO NO HORMONE (CON) OR CORT TREATMENTS DURING PHASE TWO OF EXPERIMENT ONE

		Phase Two, Post-surgery Days 21-48		
	N	Absolute Weight Change	Rate of Weight Change	Mean Daily Food Intake
SHAM:				
CON	7	53.3 ± 4.2	1.8 ± 0.1	19.9 ± 0.9
MCORT	4	47.0 ± 9.8	1.6 ± 0.3	18.7 ± 1.1
HCORT	4	42.0 ± 15.0	1.4 ± 0.5	18.9 ± 0.9
KNIFE CUT:				
CON	6	140.0 ± 14.3	4.7 ± 0.5	33.5 ± 2.1
MCORT	4	109.3 ± 22.6	3.6 ± 0.8	29.4 ± 2.2
HCORT	4	104.5 ± 29.3	3.5 ± 1.0	28.3 ± 2.4

TABLE 7

MEAN BLOOD GLUCOSE LEVELS (mg/dl ± SEM) AT THE CONCLUSION OF EXPERIMENT ONE IN SHAM-OPERATED AND PVN KNIFE-CUT RATS AFTER CHRONIC ADMINISTRATION OF NO HORMONE (CON), CORT, OR DEX

	All Rats	Excluding Rats Experiencing 9 Days of DEX plus CON or CORT
SHAM:		
CON	100.8 ± 1.9(10)	101.8 ± 2.3(8)
MCORT	101.8 ± 2.5(6)	103.3 ± 2.9(4)
HCORT	101.0 ± 5.4(6)	107.5 ± 5.6(4)
DEX	102.3 ± 2.3(7)	--------
KNIFE-CUT:		
CON	108.7 ± 4.3(9)	108.7 ± 4.3(6)
MCORT	107.4 ± 6.5(5)	109.8 ± 7.8(4)
HCORT	108.0 ± 5.8(6)	116.3 ± 3.9(4)
DEX	362.3 ± 11.6(6)*	--------

(#) - number of subjects on which mean ± SEM based.

* - a group that differs from all other treatment groups, $p < .05$

group.

Naso-anal Length and Obesity Indices

Because the rats underwent a series of experimental treatments prior to these measurements, the obtained values represent a gross assessment of the effects of experimental treatments on NA and OBI. Diminished linear growth was seen in both sham-operated and KC subjects chronically treated with DEX, but not in those treated only with CORT (Table 8). Even the subgroup (n=2) of rats that experienced a brief nine day exposure to DEX in combination with their long-term CON or CORT treatments were shorter than counterparts never exposed to DEX.

Regardless of hormone treatment, KC groups had higher obesity indices than sham-operated groups (Table 9). Glucocorticoid treatments tended to lower obesity indices, affecting KC rats more than sham-operated rats. Exposure to DEX diminished obesity indices the most, followed by exposure to HCORT.

Discussion

This experiment reveals that the glucocorticoids CORT and DEX differ in their effects on the consummatory responses of PVN KC rats. The hyperphagia and excessive body weight gain of rats receiving KCs was not substantially altered by the CORT within a wide dose range, 0.5 to 1.6 mg/day. Neither did CORT at these doses influence the body weight or food intake of sham-operated

TABLE 8

MEAN NASO-ANAL LENGTHS (mm ± SEM) OF RATS PRIOR TO BRAIN SURGERY AND 36 DAYS AFTER RECEIVING SHAM OR PVN KNIFE CUT SURGERY AND CHRONIC TREATMENTS OF NO HORMONE (CON), CORT*, OR DEX° INCLUDING SUBGROUPS THAT EXPERIENCED THE ADDITION OF DEX TO CON OR CORT FOR A BRIEF PERIOD

		DAY 36:		
	Pre-Surgery	All Rats	Only Experienced Original CON or CORT	Subgroups-Experienced Addition of DEX for 9 days
SHAM:				
CON	210.2 ± 1.9(10)	224.6 ± 1.9(10)	226.4 ± 2.3(7)	220.2 ± 0.4(3)
MCORT	214.2 ± 2.1(6)	224.1 ± 2.7(6)	228.1 ± 1.5(4)	217.5 ± 0.7(2)
HCORT	212.5 ± 1.6(6)	224.3 ± 2.2(6)	226.4 ± 2.5(4)	220.0 ± 2.5(2)
DEX	215.1 ± 1.4(8)	219.9 ± 2.4(8)	--------	--------
KNIFE CUT:				
CON	212.7 ± 1.4(9)	224.1 ± 1.1(9)	225.0 ± 1.2(7)	221.0 ± 0.0(2)
MCORT	216.8 ± 2.2(6)	227.6 ± 3.4(5)	229.5 ± 3.6(4)	220.0 ± 0.0(1)
HCORT	213.2 ± 2.4(6)	226.4 ± 1.5(6)	227.6 ± 2.0(4)	224.0 ± 0.0(2)
DEX	213.7 ± 2.2(6)	220.0 ± 1.8(6)	--------	--------

(#) - number of subjects on which mean ± SEM based.
* - see text for dosage and administration protocol for CORT.
° - DEX-treated subjects provided 0.1 mg/kg/day DEX from Day 1-18, and 0.01 mg/kg/day from Day 18-39 including Day 36.

TABLE 9

MEAN OBESITY INDICES (± SEM) OF RATS PRIOR TO BRAIN SURGERY AND 36 DAYS AFTER RECEIVING SHAM OR PVN KNIFE CUT SURGERY AND CHRONIC TREATMENTS OF NO HORMONE (CON), CORT*, OR DEX° INCLUDING SUBGROUPS THAT EXPERIENCED THE ADDITION OF DEX TO CON OR CORT FOR A BRIEF PERIOD

		DAY 36:		
	Pre-Surgery	All Rats	Only Experienced Original CON or CORT	Subgroups- Experienced Addition of DEX for 9 days
SHAM:				
CON	303.0 ± 2.3(10)	306.4 ± 2.7(10)	310.1 ± 2.4(7)	297.7 ± 4.6(3)
MCORT	297.2 ± 2.2(6)	302.7 ± 1.4(6)	302.9 ± 2.0(4)	302.2 ± 1.8(2)
HCORT	299.4 ± 2.4(6)	299.4 ± 4.0(6)	304.9 ± 3.0(4)	288.5 ± 0.5(2)
DEX	294.8 ± 2.4(8)	294.9 ± 3.2(8)	--------	--------
KNIFE CUT:				
CON	298.9 ± 1.3(9)	365.4 ± 5.5(9)	370.1 ± 5.7(7)	348.8 ± 7.8(2)
MCORT	293.2 ± 1.3(6)	349.6 ± 5.7(5)	353.2 ± 5.9(4)	335.5 ± 0.0(1)
HCORT	298.5 ± 3.0(6)	343.6 ± 4.1(6)	346.7 ± 5.0(4)	337.5 ± 6.7(2)
DEX	296.0 ± 3.6(6)	339.5 ± 3.0(6)	--------	--------

Symbols are as indicated in Table 8.

rats. This held even when exogenously-applied CORT was available continuously for 48 days. In contrast, DEX attenuated the weight gain and the enhanced food intake subsequent to KCs alongside the PVN. Exposure to DEX also decreased the weight of sham-operated rats, but without dramatic reductions in food intake.

The suppression by DEX of the hyperphagia and obesity coincident with KC surgery was not permanent. When the dose of DEX was reduced by a factor of 10, a rapid increase in body weight and an elevation in food intake were observed. However, the rate of body weight gain of KC rats treated with maintenance 0.01 mg/kg DEX (8.1 g/day) did not reach the magnitude seen in controls after KC surgery (12.1 g/day) nor did the food intake of the DEX-treated KC group equal the levels seen in other KC subjects immediately after surgery. This may be due to residual effects of DEX, to the later point in time after surgery at which this manipulation occurred, or to the 0.01 mg/kg dose of DEX.

The diminished weight gain produced by DEX in sham-operated rats was also transitory. Interestingly, the compensatory body weight response of sham-operated rats upon reduction of the DEX dose occurred without a dramatic increase in food intake. This suggests that DEX's influence on body weight is not tied to food intake alone, but may encompass underlying metabolic changes.

The suppressive effects of DEX on the ingestive

responses of KC rats were not limited to the initial or 'dynamic' post-surgery weight gain. DEX administered when the weight gain began to plateau still yielded decrements in body weight and food intake.

The treatment of KC rats with 0.1 mg/kg DEX was associated with glycosuria. This condition could not be attributed solely to DEX because glycosuria was never seen in DEX-treated sham-operated rats. Glycosuria also didn't necessarily relate to the administration of exogenous glucocorticoids per se, because it was not seen in KC rats on CORT. Furthermore, the amount of urinary glucose in KC rats was not enhanced by simultaneous CORT and DEX. Thus, the emergence of this condition is tied to elements related to KCs alongside the PVN and the glucocorticoid DEX. It also appears to be a function of elevated circulating glucose levels as only DEX-treated KC rats had greatly elevated levels of plasma glucose. The glycosuria disappeared in the DEX-treated KC rats with the change from the 0.1 to 0.01 mg/kg dose of DEX indicating this condition was reversible. Reversibility has been recognized previously as a characteristic of steroid-induced diabetes (100).

The excretion of calories that accompanies exposure to DEX may contribute to the reduced weight and naso-anal lengths of KC rats, but cannot account for these characteristics in DEX-treated sham-operated rats. Thus

other aspects of DEX's action must also contribute.

The central finding of this experiment was that the glucocorticoid CORT and DEX differentially influence the regulatory responses of rats after KC or sham brain surgery. Several factors may account for this outcome. First, CORT possesses mineralocorticoid properties, while DEX doesn't (60, 55, 63, 122). The absence of mineralocorticoid effects by the steroid DEX could have contributed to decreases in body weight and food intake. However when DEX was administered jointly with CORT, which would contribute mineralocorticoid effects, the suppressive effects of DEX were still seen. This suggests that an absence of the mineralocorticoid properities were not crucial.

Other differences between CORT and DEX in their type or site of action may explain the incongrous results produced by these steroids. For example, the site(s) at which each steroid produces the greatest negative feedback action differs (eg. 156, 157). CORT and DEX have also been noted to differ in the potency of their glucocorticoid function (eg. 60, 63, 122, 195). comparison to exogenously-applied CORT, DEX better replicates the action of endogenous intraadrenal corticosterone on CA synthesis in the adrenal medulla (208). It is this latter disparity in action that was postulated to be of significance in examining PVN KCs and the functional status of the adrenal gland. Each of these disparities between CORT and DEX will

be further addressed in subsequent sections.

Last, the differing routes of administration employed for CORT and DEX in this study may account for their contrasting regulatory responses. This possibility was tested in Experiment 2.

C H A P T E R I I I

EXPERIMENT II

In Experiment 1, the administration of DEX, but not CORT, attenuated the excessive weight gain and hyperphagia typically seen subsequent to knife cuts alongside the PVN. The purpose of Experiment 2 was to verify and to further characterize the results obtained in Experiment 1.

The present experiment first tried to establish that CORT's inability to block PVN knife cut-induced obesity was not peculiar to the subcutaneous route of administration employed for CORT. In Experiment 2 both hormones was administered via the drinking water. This also allowed the steroids to be supplied in an approximation of the rats' circadian rhythm.

Experiment 2 also included the administration of these steroids in incremental dose phases to demonstrate dose-response relationships.

General Methods

Thirty adult female 200-240 g Charles Rivers CD albino rats were individually housed in hanging stainless steel wire cages and exposed to 12:12 hour light:dark conditions. Purina Laboratory Chow (# 5001) pellets were available ad libitum until the time of surgery. After surgery rats were switched to ad libitum Purina Mouse Chow (# 5015) pellets. Drinking water was available without

restriction.

Hormone treatment began one day prior to surgery. Dexamethasone (DEX) or corticosterone (CORT), or a no hormone control (CON) (vehicle only) was given via the drinking water. Rats within each hormone treatment group received either sham operations or bilateral symmetric parasagittal retracting wire hypothalamic knife cuts as described in Experiment 1. The stereotaxic coordinate for the KC surgery was A8.2 mm (anterior to the earbars); L9.0 mm; V7.0mm. From this point, the wire knife was extended caudally, except in the sham manipulation, and the assembly was lowered approximately 3 mm until it contacted the base of the skull. At the conclusion of the experiment, one rat from the KC-DEX treatment group was determined to have a misplaced KC and was excluded from the experimental analyses.

Each hormone was dissolved in 100% ethanol. Daily water intakes and body weights were monitored. The hormone doses were adjusted daily to the scheduled doses. Doses were calculated with regard to the previous days' water intake and the current body weight. The drinking water solution was made by micropipetting the appropriate volume of a concentrated stock ethanol-hormone solution into the appropiate volume of fresh water.

Experimental Phases A-D

The experimental groups were exposed to successive dosage increments. Each dose was given for 6-7 days. In

accordance with the relative potencies of these steriods, the dose series of CORT administration was 4, 8, 16, 32 mg/kg/day (Phase A, B, C, D, respectively) and that of DEX was 0.05, 0.1, 0.2 mg/kg/day (Phase A, B, C, respectively). In addition to the measurement of body weight and water intake, rats' food intake was determined with adjustments for food spillage.

Treatment with glucocorticoids, particularly DEX, can produce a glycosuric condition. KC animals treated with DEX appear to be especially susceptible. To better establish the factors associated with the presence and the pattern of this condition, the presence of glucose in the urine was closely monitored in this experiment. Urinalyses for glucose were performed on the third and last day of each dose exposure phase. To collect a urine sample, an animal was removed from its home cage and placed in a clean, empty wire mesh cage which rested over a clean piece of cellophane plastic wrap. Typically within 3-10 minutes, an animal urinated providing a testable sample on the cellophane. If a considerable amount of time passed without obtaining a sample, to encourage urination, a subject was handled briefly. Urine glucose content was measured using Diastix Reagent Strips (Ames Division, Miles Laboratory, Indiana).

Blood Glucose Samples

On day 6-7 of each hormone dose exposure phase, blood

samples were taken from restrained, unanesthesized rats. Beginning 1/2 hour into the light phase, animals were taken from the animal room into an outer room. Here blood samples were drawn from the tip of the tail into a microcentrifuge tube. The tubes were then be centrifuged, and plasma serum samples were separated and frozen for later analysis. Plasma serum was measured for for blood glucose levels.

Saline Test

Several factors suggest that DEX may influence the status of the fluid and/or electrolyte balance of an animal to produce its effects. In contrast to CORT which possesses mineralocorticoid properities in addition to its glucocorticoid actions, DEX possesses no mineralocorticoid properities. Also, at higher doses DEX suppresses the adrenal cortex which might compromise endogenous mineralocorticoid secretion. Finally, knife cuts alongside the PVN disrupt vasopressin neurons which would also compromise water conservation. This surgery in combination with DEX treatment may therefore synergistically disturb the water and /or electrolyte balance of an animal.

To examine the possible contribution of these factors, a saline solution exposure test was performed. This test was conducted from the second to the fifth day of the dose phase when rats were treated with either 0.2 mg/kg DEX or 16 mg/kg CORT or the control, CON. In addition to their drinking water bottles, the rats were

given a 2% saline solution. A strong salt-tasting solution was used so that rats which were not in need of salt for homeostatic balance would avoid it; whereas, rats in need of sodium would exhibit an affinity. Intakes of the saline and water solutions were measured daily.

Experimental Phase D-E

Insulin test. In the present experimental protocol the occurrence of glycosuria tended to be unique to KC DEX-treated rats. This manipulation was performed to study the responsiveness of this hormone-induced condition to insulin.

The subjects utilized were the SH- and KC-DEX treatment groups from the previous phases, A-C. These two groups were maintained on 0.2 mg/kg/day of DEX, which was a continuation of their 7 day exposure to this dose in Phase C. The rats received daily subcutaneous injections of long-acting Lente insulin zinc suspension (E. R. Squibb & Sons, Inc., Princeton, N. J.). Insulin was administered just prior to lights off at a dose of 5 U/kg for 6 days, followed by a dose of insulin of 8 U/kg for 6 days. On the final day of exposure to each dose, blood samples were obtained for glucose analysis. Body weight, food intake, and water intake were recorded. Urinalyses for the presence of glucose were performed.

Adrenal Gland Weights

At the end of the experimental testing period, the adrenal glands of all subjects were removed, dissected of

peripheral fat tissue, blotted, and weighed to the nearest 0.001 g. Prior to this procedure, animals experienced at least 9 days of exposure to their respective hormone treatments at the dosage levels of 16 mg/kg CORT, 0.2 mg/kg DEX, or the CON water control.

Naso-anal Lengths and Obesity Indices

When rats were under ether anesthesia upon removal of their adrenals their final naso-anal lengths were measured. The final obesity indices of the rats were determined.

Results

Phase A

In treatment phase A, the doses of DEX and CORT were respectively 0.05 mg/kg and 4 mg/kg.

Body weight. Analysis of variance of the absolute and the percentage body weight change during this phase reveals a significant main effect for surgery (Absolute: $F(1,23)=382.9$, Percentage: $F(1,23)=300.0$, $p<.05$) and hormone treatments (Absolute:$F(2,23)=9.06$, Percentage: $F(1,23)=6.9$, $p<.05$) (Figure 5).

The body weight gain of neither KC nor sham-operated rats was altered by exposure to CORT. In contrast, DEX attenuated weight gain. For the sham-operated rats this difference was not statistically significant and may reflect an extreme response of one rat. A significant reduction was seen in both the absolute and the percent

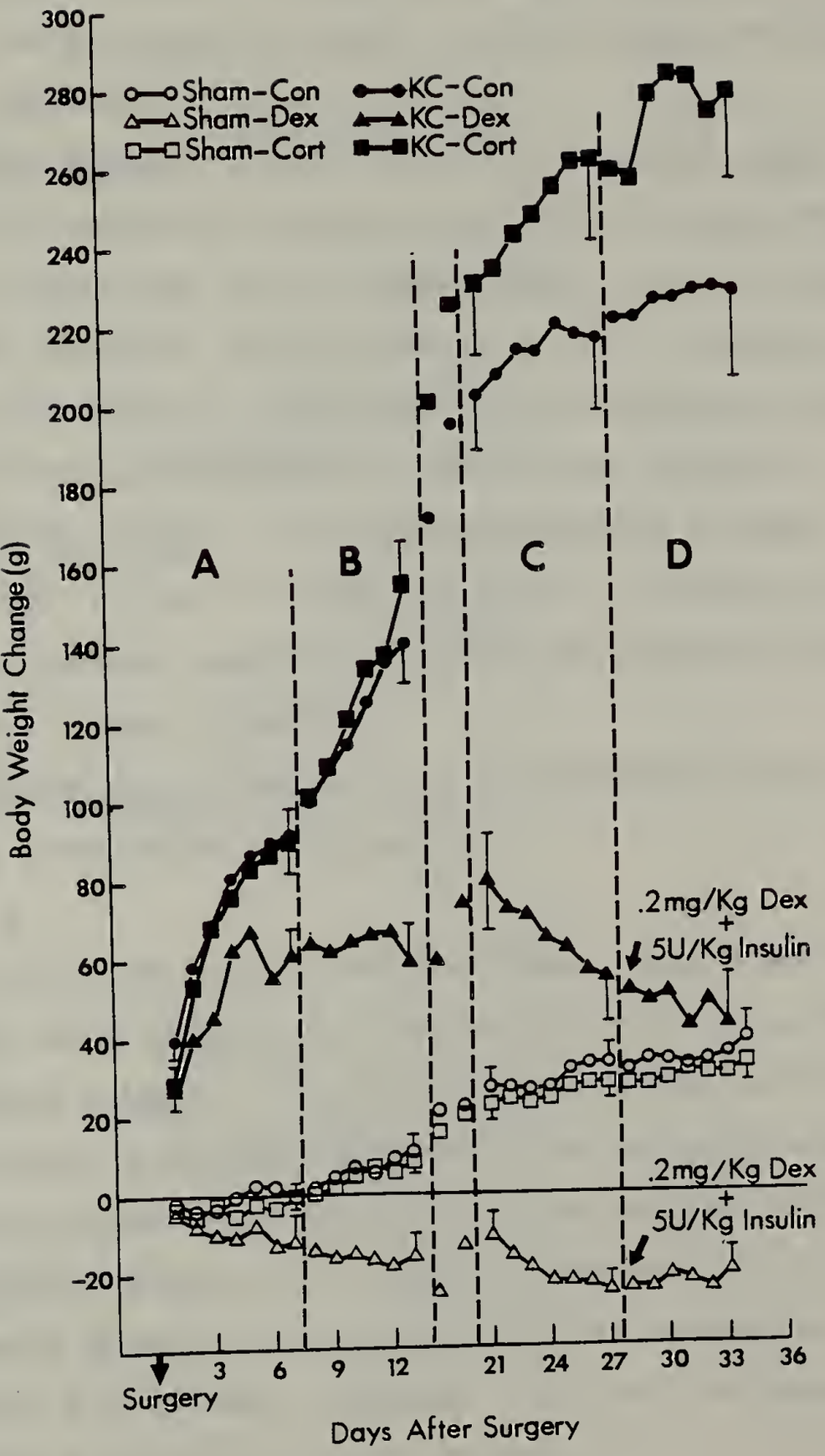

Sham-Con
Sham-Dex
Sham-Cort
KC-Con
KC-Dex
KC-Cort
A
B
C
D
.2mg/Kg Dex
+
5U/Kg Insulin
.2mg/Kg Dex
+
5U/Kg Insulin
Surgery
Body Weight Change (g)
Days After Surgery
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
3
6
9
12
21
24
27
30
33
36

Figure 5. Mean body weight change of sham-operated and PVN KC rats treated with either no hormone (CON), or 4, 8, 16, and 32 mg/kg CORT across Phases A - D, respectively, or 0.05, 0.1, 0.2 mg/kg DEX and 0.2 mg/kg DEX plus 5 U/kg insulin across Phases A - D, respectively.

body weight gain of PVN KC rats treated with 0.05 mg/kg DEX (Duncan, $p<.05$). These rats gained approximately 66% of the weight gained by CORT- or CON-treated rats after PVN KC surgery.

Food intake. During the first treatment phase the mean food intake of rats with PVN KCs was significantly greater than that of rats experiencing only sham brain surgery (Surgery: $F(1,23)=529.0$, $p<.05$). Exposure to the 0.05 mg/kg DEX or 4 mg/kg CORT had no significant effect on the food intake of any surgery group (Figure 6).

Water intake. PVN KC surgery produced a large elevation in water intake (Surgery: $F(1,23)=68.9$, $p<.05$). Neither hormone treatment produced any additional changes in water intake (Figure 7).

Urinalysis. Glucose was not detected in the urine of any rats during this phase.

Phase B

During the second treatment phase the doses of CORT and DEX were doubled to 8 mg/kg CORT and 0.1 mg/kg DEX.

Body weight. A significant main effect for surgery and hormone treatment, along with a significant surgery by hormone interaction, resulted for body weight in Phase B (Absolute - Surgery: $F(1,23)=98.3$, Hormone: $F(1,23)=46.3$, Surgery x Hormone: $F(2,23)=15.3$, $p<.05$; Percentage - Surgery: $F(1,23)=64.3$, Hormone: $F(2,23)=43.6$; Surgery x Hormone: $F(2,23)=7.0$, $p<.05$) (Figure 5).

During this phase the weight gain of PVN KC rats

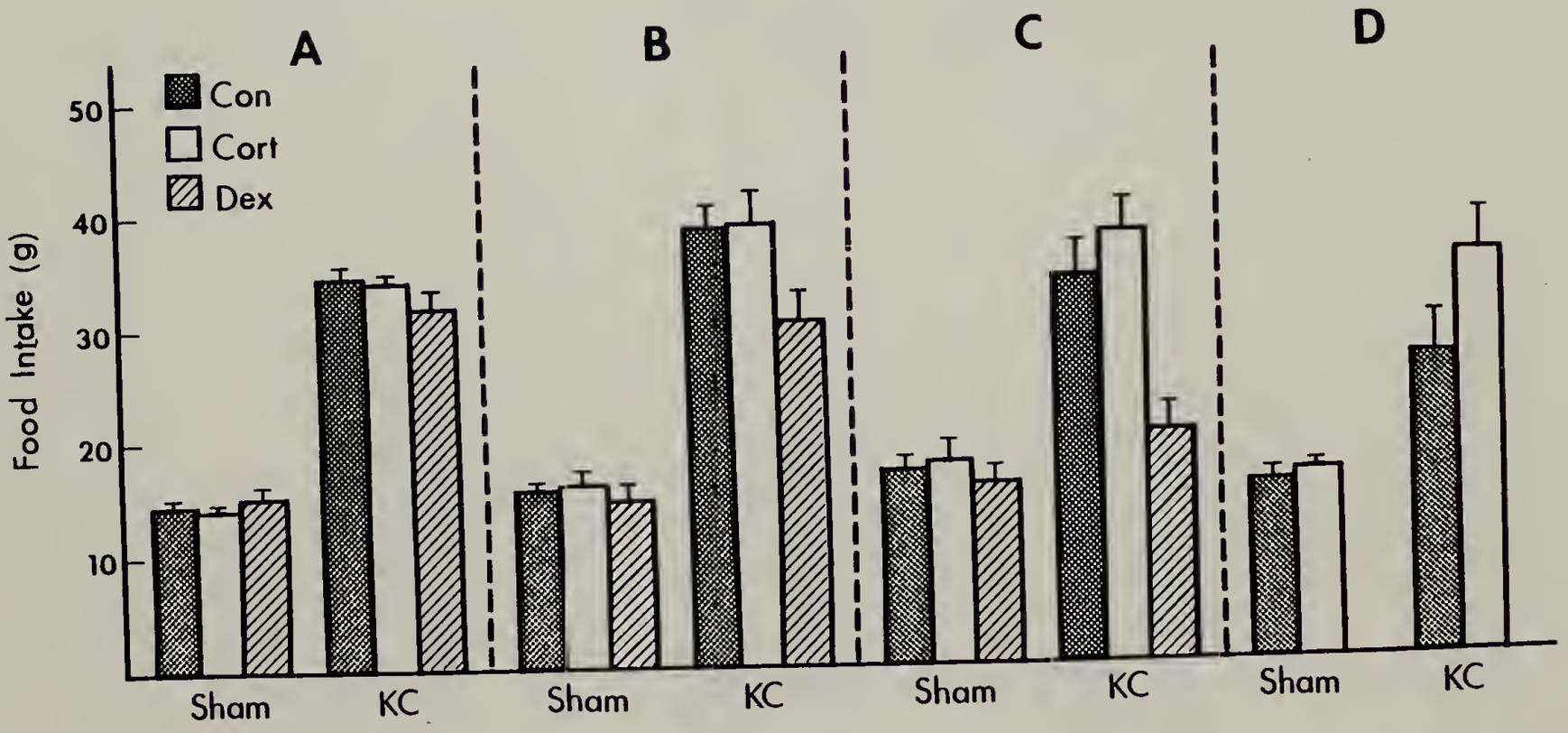
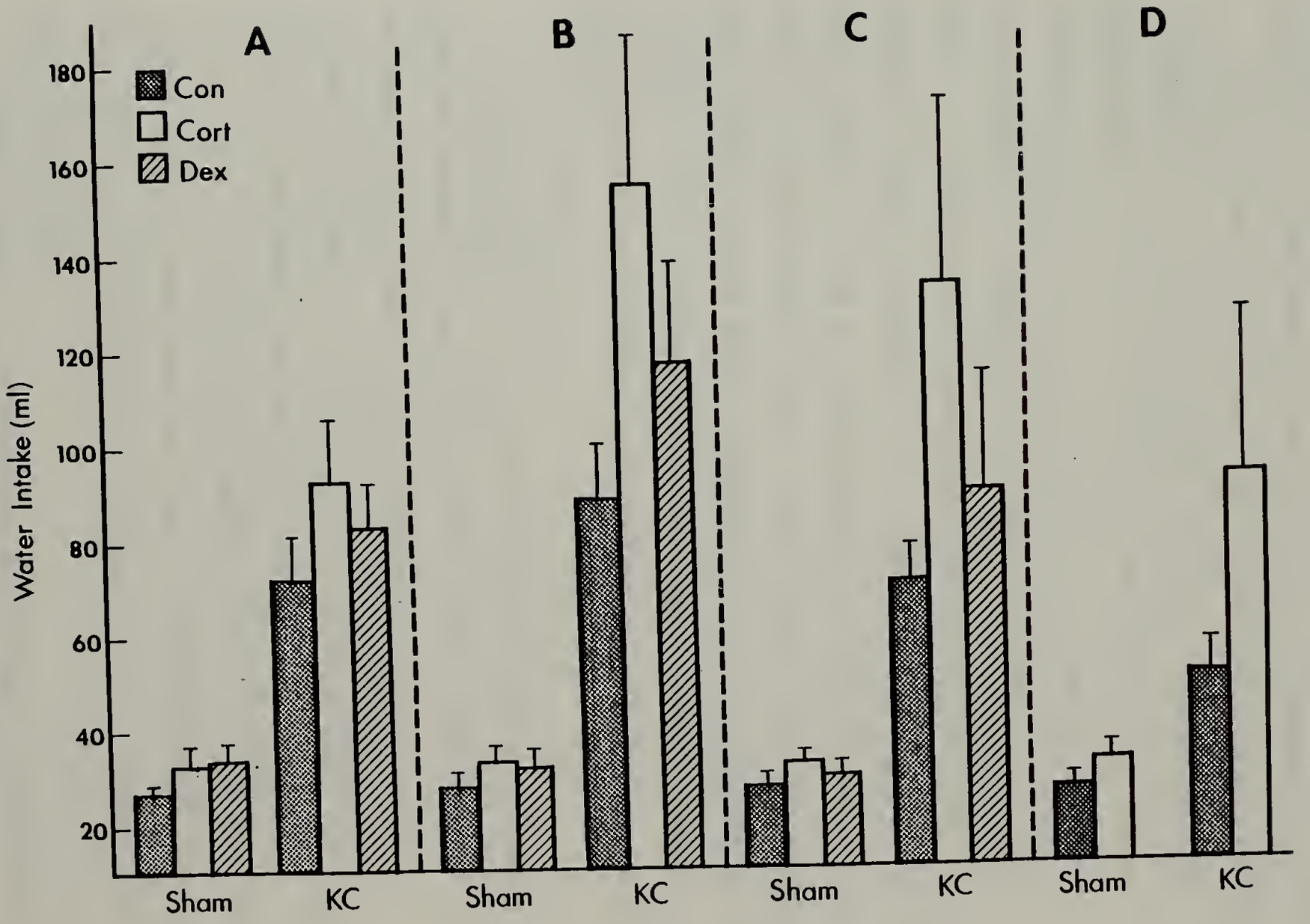


Figure 6. Mean daily food intake of sham-operated and PVN KC rats treated with either no hormone (CON), or 4, 8, 16, and 32 mg/kg CORT across Phases A - D, respectively, or 0.05, 0.1, 0.2 mg/kg DEX across Phases A - C, respectively.

Figure 7. Mean daily water intake of sham-operated and PVN KC rats treated with either no hormone (CON), or 4, 8, 16, and 32 mg/kg CORT across Phases A - D, respectively, or 0.05, 0.1, 0.2 mg/kg DEX across Phases A - C, respectively.


A
B
C
D
Con
Cort
Dex
Water Intake (ml)
180
160
140
120
100
80
60
40
20
Sham
KC
Sham
KC
Sham
KC
Sham
KC

exposed to DEX declined to match that of sham-operated rats treated with CORT or CON. The weight gain of sham-operated rats exposed to DEX was also suppressed ($p<.05$).

PVN KC rats on CON or CORT continued to gain weight in excess of all other groups ($p<.05$). Although CORT treatments did not influence the weight gain of sham-operated rats, PVN KC rats exposed to 8 mg/kg CORT significantly outgained the vehicle-treated KC group ($p<.05$). This holds when weight gain is expressed as absolute weight change or as a percent. The potentiated weight gain of PVN KC rats exposed to CORT continued into the first five days of the 'break' period ($p<.05$), a time when hormones were still available. But in the following 2 days in this 'break' period when no hormones were administered the KC-CORT and KC-CON groups did not differ in their weight gain.

Food intake. During this second treatment phase (post-KC surgery days 7-14), PVN KC rats continued to consume significantly more food than sham-operated subjects (Surgery: $F(1,23)=180.9$, $p<.05$) (Figure 6). Hormone treatments influenced food intake (Hormone: $F(2,23)=3.6$, $p<.05$). DEX-treated rats with PVN KCs ate significantly less than the CON- or CORT-treated with PVN KCs ($p<.05$), but they still overate ($p<.05$, versus sham-operated groups).

Water intake. PVN KC rats continued to consume more water than sham-operated rats (Surgery: $F(1,23)=53.9$,

$p<.05$). KC rats treated with CORT drank significantly more water than KC rats treated with vehicle ($p<.05$), but not more than KC rats treated with DEX (Figure 7). In turn, the water intake of DEX-treated rats with PVN KCs was not significantly different from that of PVN KC controls.

Urinalysis. Glycosuria was detected in the last three days of this phase in 3 of 4 PVN KC rats treated with DEX. The amount of glucose present in urine on any given day ranged from +1/4 % (2 rats) to +1 % (1 rat) which represent concentrations of 250 mg/dL and 1000 mg/dL, respectively. No glucose excretion was detected in DEX-treated sham-operated rats nor any rats treated with CON or CORT.

Phase C

In treatment phase C, experimental groups treated with CORT received a dose of 16 mg/kg, and those treated with DEX received a dose of 0.2 mg/kg.

Body weight. A significant main effect for the surgery and hormone conditions emerged from the analysis of the absolute (Surgery: $F(1,23)=10.6$, Hormone: $F(2,23)=21.6$, $p<.05$) and the percentage (Surgery: $F(1,23)=6.4$, Hormone: $F(2,23)=38.7$, $p<.05$) body weight changes during this phase. A significant surgery X hormone interaction was present for absolute body weight change (Surgery x Hormone: $F(2,23)=3.9$, $p<.05$).

Exposure to 0.2 mg/kg DEX produced a steady decrease in the body weights of PVN KC and sham-operated rats that was significantly different from all other groups ($p<.05$).

As depicted in Figure 5, a significantly higher mean cumulative weight gain was seen in PVN KC rats treated with CORT than in KC rats in the CON treatment group in Phase C ($p<.05$).

Food intake. Analysis of variance of the mean food intake during Phase C revealed a significant main effect for surgery ($F(1,23)=63.2$, $p<.05$) and hormone ($F(2,23)=9.0$, $p<.05$) treatment conditions in addition to a significant interaction ($F(2,23)=6.7$, $p<.05$) (Figure 6).

During Phase C, post KC surgery days 21-27, both CON- and CORT-treated rats with PVN KCs were still hyperphagic ($p<.05$). In contrast, the food intake of PVN KC rats exposed to DEX was suppressed such that it was comparable to the intake of the sham-operated controls. Neither of the hormones administered during this phase altered the food intake of sham-operated rats.

Water intake. The water intake response of rats during this phase parallel the pattern seen in the previous treatment phase (Figure 7). The water intake of CORT-treated rats with PVN KCs was greater than that of CON-treated KC rats ($p<.05$) but not DEX-treated rats.

Urinalysis. Only PVN KC rats ($n=4$) administered 0.2 mg/kg DEX exhibited glycosuria. Glucose was present in their urine intermittently throughout this phase in

concentrations ranging from 0 to 2% (or 2000 mg/dL). This included one DEX-treated KC rat that was never glycosuric and one that excreted a slight 1/10 % (or 100 mg/dL) concentration of glucose on only one day.

Phase D

In treatment phase D, the dose of CORT administered was increased to 32 mg/kg. The results of the experimental manipulation performed on DEX-treated rats during this period are discussed below.

Body weight. At the end of Phase D the mean cumulative weight gain of PVN KC rats treated with 32 mg/kg CORT was significantly greater than the weight gain exhibited by PVN KC rats receiving the CON treatment ($p<.05$). However, a comparison of the actual or the percent changes in weight that took place over this phase did not reveal a significant difference between these PVN KC groups. The body weight response of sham-operated rats was not influenced by exposure to the 32 mg/kg CORT dose (Figure 5).

Food intake. From day 27-34 after PVN KC surgery which constitutes Phase D of hormone treatment, rats with PVN KCs still ate more than rats that underwent sham brain surgery (Surgery: $F(1,23)=37.4$, $p<.05$) (Figure 6). Whereas exposure to this pharmacological dose of CORT did not alter the food intake of sham-operated rats, PVN KC rats treated with 32 mg/kg CORT consumed significantly

more food than their control counterparts ($p<.05$).

Water intake. Only PVN KC rats exposed to CORT drank in excess of sham-operated groups in Phase D ($p<.05$) (Figure 7).

Urinalysis. In this last phase, glucose was never detected during urinalysis. Even the administration of the pharmacological 32 mg/kg dose of CORT did not produce glycosuria.

Phase D-E

Sham-operated and KC groups treated with DEX received a dose of 0.2 mg/kg DEX during treatment phases D and E. This hormone treatment was combined with insulin at a dose of 5 U/kg/day in Phase D and of 8 U/kg/day in Phase E.

Insulin test. An overall analysis of variance revealed that insulin influenced the body weight responses produced by 0.2 mg/kg DEX (Absolute change: $F(2,6)=37.4$, Mean change: $F(2,6)=17.0$, $p<.05$).

The administration of 5 or 8 U/kg/day of long-acting insulin during Phase D-E arrested the weight loss seen in sham-operated rats exposed to 0.2 mg/kg DEX (Bonforroni t-test, adjusted family-wise error rate $<.017$), but it did not reinstate a substantial weight gain in these rats (Figure 8). In contrast, the food intake of sham-operated rats treated with 0.2 mg/kg DEX was not significantly altered by insulin (Figure 9). These rats ate the same amount whether treated with DEX alone or with 5 U/kg insulin plus DEX. However, the amount eaten in the 5

versus 8 U/kg treatment phases did differ (Bonforroni adjusted t-test). During this test period, sham-operated rats treated with DEX increased their water intake (data not shown) when insulin was administered in conjunction with DEX (Bonforroni adjusted t-test). Finally urine glucose was not detected in sham-operated rats when DEX and insulin treatments were combined.

The decreases in body weight observed in KC rats exposed to 0.2 mg/kg DEX were not significantly altered by the addition of insulin at 5 or 8 U/kg. However, the data suggest that insulin treatments may mitigate the DEX-induced decreases in the body weight of KC rats to some degree as some relevant group comparisons bordered on statistical significance (Bonforroni adjusted t), and a repeated measures analysis of variance revealed a significant change between the phases of the insulin test ($F(2,2)=21.2$, $p<.05$). Neither the food intake nor the water intake (data not shown) of KC rats treated with 0.2 mg/kg DEX was significantly influenced by the administration of 5 or 8 U/kg/day insulin. Lastly, analysis of urine glucose revealed that insulin injections did not alleviate the glycosuria seen in KC rats exposed to 0.2 mg/kg DEX. Insulin may indeed potentiate this condition.

Saline Test

The consumption of the 2% saline solution presented

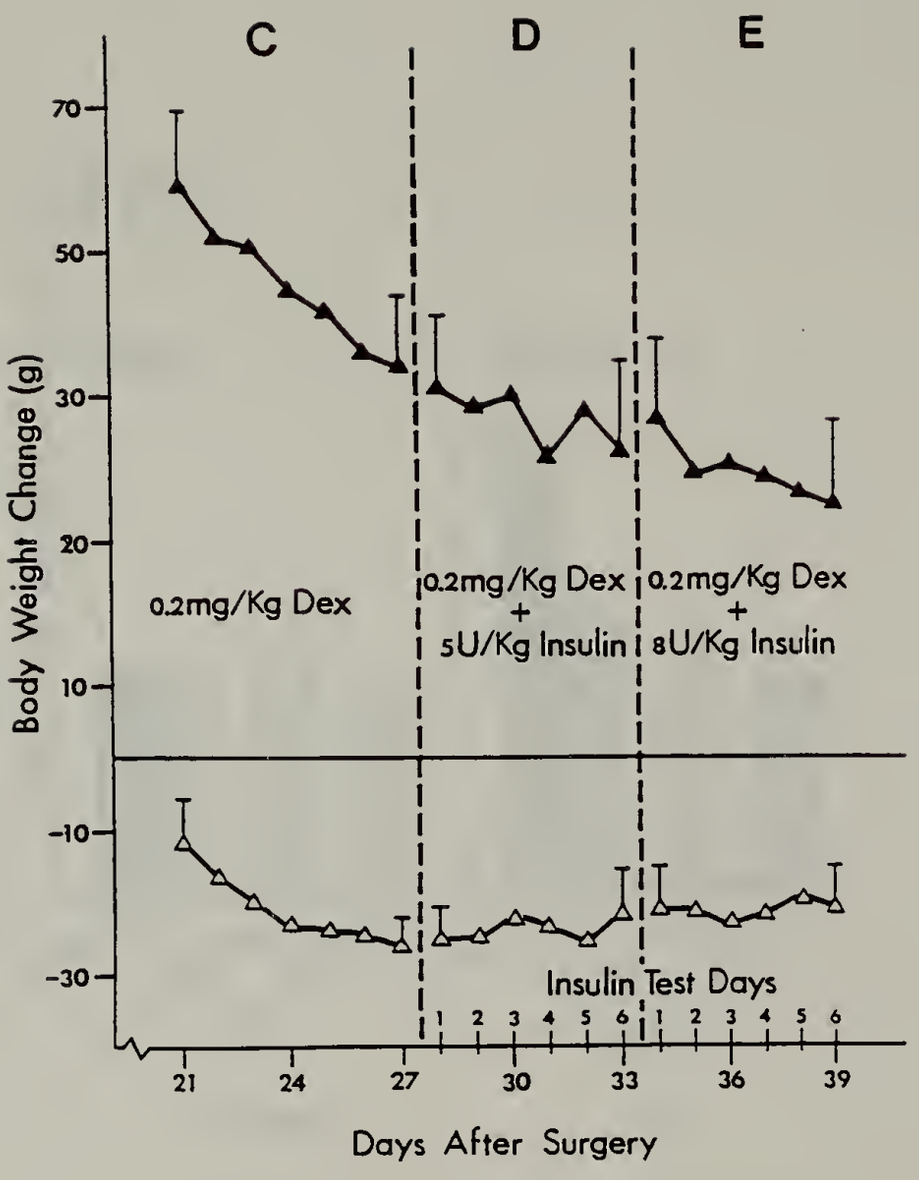


Figure 8. Mean body weight change of sham-operated and PVN KC rats treated with 0.2 mg/kg DEX alone in Phase C, followed by treatments of this 0.2 mg/kg DEX dose in conjunction with 5 U/kg insulin in Phase D and with 8 U/kg insulin in Phase E. Symbols are as indicated in Figure 5.

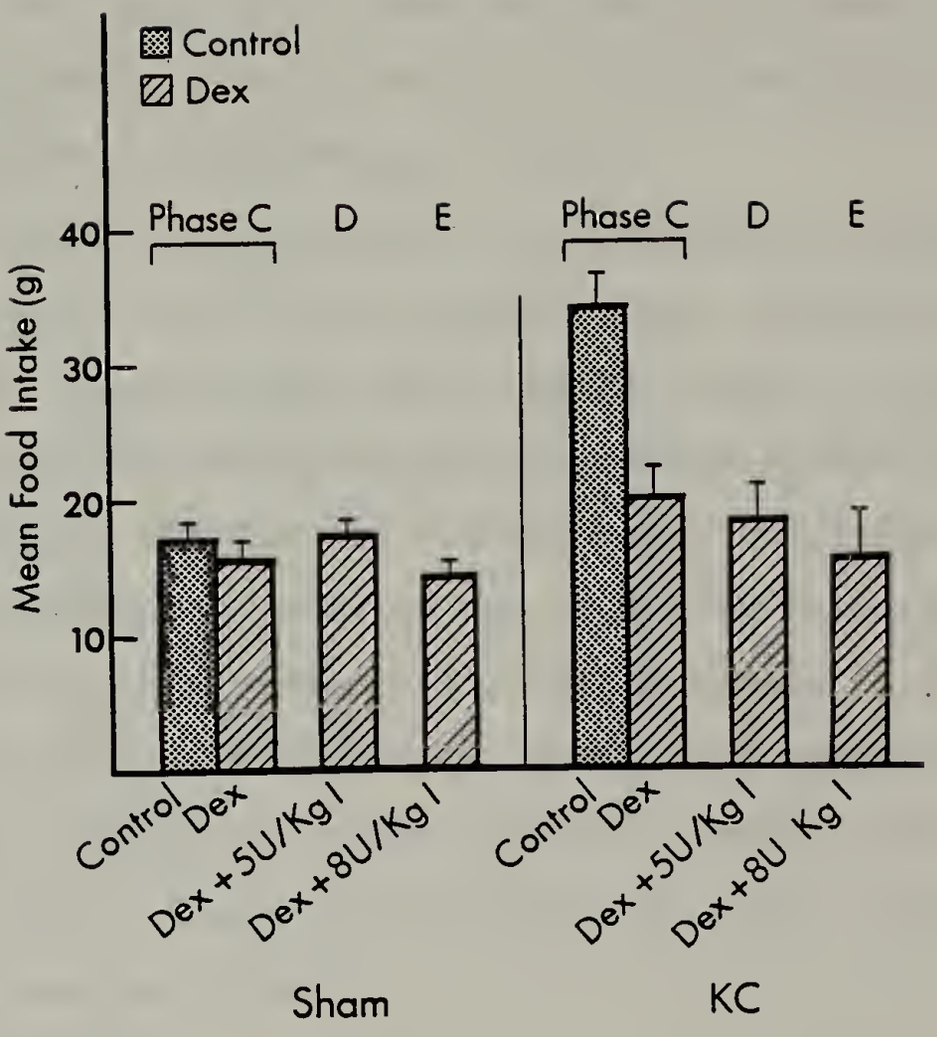


Figure 9. Mean daily food intake of sham-operated and PVN KC rats treated with 0.2 mg/kg DEX alone in Phase C, followed by treatments of this 0.2 mg/kg DEX dose in conjunction with 5 U/kg insulin (I) in Phase D and with 8 U/kg insulin (I) in Phase E. Mean daily food intake values for sham-operated and KC rats exposed to no hormone (Control) during Phase C included for comparisons.

during Phase C did not significantly differ between experimental groups (Table 10). Although KC rats provided DEX tended to consume less of the saline solution than other rats did, this trend was not significant as determined by a Duncan multiple range comparison test or a nonparametric Mann-Whitney U test.

This provides evidence that exposure to these glucocorticoids did not significantly disrupt electrolyte balance, particularly with respect to Na+. It also suggests that the differing mineralocorticoid properties of CORT and DEX did not contribute to the different body weight responses produced upon their administration. There appears to be a tendency for saline intake to shift in a counter-intuitive direction. CORT, with some mineralocoid action, yielded the highest saline intakes, while DEX, which has virtually no mineralocoid action, yielded the lowest saline intake.

Blood Glucose

A slight elevation in blood glucose was seen in sham-operated rats treated with DEX or with the high 16 and 32 mg/kg doses of CORT (Table 11).

Exposure to pharmacological doses of CORT did not markedly alter the serum glucose levels of PVN KC rats, but the availability of exogenous CORT maintained the slightly potentiated glucose levels of KC rats beyond the initial 2-3 weeks after KC surgery.

At all doses DEX dramatically enhanced the amount of

TABLE 10

MEAN INTAKE (ml ± SEM) OF A 2% SALINE SOLUTION PROVIDED SHAM-OPERATED AND PVN KNIFE-CUT RATS EXPOSED TO NO HORMONE (CON), CORT, OR DEX DURING PHASE C OF EXPERIMENT TWO

	SALINE INTAKE
SHAM:	
CON	12.4 ± 4.5
CORT	17.7 ± 6.5
DEX	11.2 ± 2.9
KNIFE CUT:	
CON	12.7 ± 2.2
CORT	23.4 ± 11.1
DEX	5.2 ± 1.4

TABLE 11

MEAN BLOOD GLUCOSE LEVELS (mg/dl ± SEM) OF SHAM-OPERATED AND PVN KNIFE-CUT RATS TREATED WITH NO HORMONE (CON) OR DIFFERENT DOSES OF CORT OR DEX ACROSS PHASES 2A - D OF EXPERIMENT TWO

	Phase 2A (4 mg/kg CORT or 0.05 mg/kg DEX)	Phase 2B (8 mg/kg CORT or 0.1 mg/kg DEX)	Phase 2C (16 mg/kg CORT or 0.2 mg/kg DEX)	Phase 2D (32 mg/kg CORT or [0.2 mg/kg DEX + 5 U/kg Insulin])
SHAM:				
CON	82.8 ± 4.3	84.0 ± 3.9	87.6 ± 4.4	85.8 ± 3.2
CORT	81.0 ± 5.5	84.4 ± 2.8	95.5 ± 4.6	96.6 ± 5.5
DEX	93.8 ± 1.0	95.0 ± 6.2	110.2 ± 10.9	[93.0 ± 7.3]
KNIFE CUT:				
CON	103.7 ± 5.8	100.8 ± 8.0	96.2 ± 5.7	89.0 ± 5.1
CORT	103.3 ± 4.7	99.4 ± 6.3	107.8 ± 6.9	105.8 ± 5.0
DEX	182.3 ± 42.6*	281.1 ± 105.8*	409.5 ± 134.4*	[330.8 ± 51.7]*

* - a group that differs from all other treatment groups within a phase, $p < .05$

serum glucose in KC rats. This DEX-induced increase was dose-related and reached as high as four times the glucose levels of KC controls. As with glycosuria, there was large individual variability within the DEX-treated KC group.

Adrenal Gland Weights

At the conclusion of the experiment and prior to the removal of their adrenal glands, rats were exposed to a 16 mg/kg dose of CORT, or a 0.2 mg/kg dose of DEX, or CON for approximately 9 days. Since other hormonal manipulations preceeded this exposure, it serves best only as a general assessment of how the dosages of glucocorticoids used here influenced the adrenal glands (Table 12).

Significant surgery and hormone treatment effects were found in the ANOVA for the weights of the right adrenal gland (Surgery: $F(1,21)=11.2$, Hormone: $F(2,21)=27.8$, $p<.05$) and the left adrenal gland (Surgery: $F(1,21)=7.9$, Hormone: $F(2,21)=19.0$, $p<.05$), and combined weight of these glands (Surgery: $F(1,21)=9.8$, Hormone: $F(2,21)=23.9$, $p<.05$).

KCs alongside the PVN alone significantly reduced adrenal gland weights ($p<.05$). Exogenous treatments with glucocorticoids also caused adrenal atrophy. The smallest adrenal glands were seen in KC rats exposed to DEX or to CORT, and in sham-operated rats exposed to DEX ($p<.05$). The reduction in adrenal size produced by CORT alone is comparable to that produced by KCs alone ($p<.05$).

TABLE 12

MEAN ADRENAL GLAND WEIGHTS (g ± SEM) FOR SHAM-OPERATED AND PVN KNIFE-CUT RATS EXPOSED TO NO HORMONE (CON), 16 mg/kg CORT OR 0.2 mg/kg DEX AT THE CONCLUSION OF EXPERIMENT TWO

	ADRENAL WEIGHTS		
	RIGHT GLAND	LEFT GLAND	GLANDS COMBINED
SHAM:			
CON	0.032 ± 0.0050*	0.032 ± 0.0057[3]	0.064 ± 0.0105
CORT	0.023 ± 0.0009[2]	0.025 ± 0.0015[2,3]	0.048 ± 0.0025
DEX	0.010 ± 0.0003[1]	0.010 ± 0.0002[1]	0.020 ± 0.0005
KNIFE-CUT:			
CON	0.023 ± 0.0018[2]	0.023 ± 0.0026[2]	0.046 ± 0.0043
CORT	0.010 ± 0.0020[1]	0.011 ± 0.0017[1]	0.021 ± 0.0038
DEX	0.009 ± 0.0005[1]	0.009 ± 0.0008[1]	0.018 ± 0.0013

* - a group that differs from all other treatment groups, $p < .05$

1 or 2 or 3 - groups labelled with similar numerical values represent treatment groups that are similar to each other, but differ from all other treatment groups, $p < .05$.

Naso-anal Lengths and Obesity Indices

Naso-anal length (NA) and obesity index (OBI) measures were determined after rats were exposed to 16 mg/kg CORT, 0.2 mg/kg DEX, or CON for several days (Table 13). Because the rats underwent a series of experimental treatments prior to these measurements, the obtained values represent a gross assessment of the effects of experimental treatments on NA and OBI.

Chronic exposure to DEX, but not CORT, reduced NA length. Each DEX-treated group was significantly shorter than all other experimental groups ($p<.05$) with sham-operated rats being attenuated more than KC rats by DEX ($p<.05$).

Analysis of variance of OBI reveals a significant interaction effect (Surgery x Hormone: $F(2,20)=20.0$, $p<.05$). The obesity indices of sham-operated rats treated with either CORT or DEX, and KC rats treated with DEX were comparable to the normal weight of SH-CON control rats. KC rats treated with CON and those treated with CORT had elevated OBI values ($p<.05$). The extent of the elevation seen in these two groups was similar, but the mean OBI value of CORT-treated KC rats may not be accurately represent this group. Because two of the heaviest KC rats treated with CORT died prior to the determination of their obesity indices, their data could not be included in the calculation of this mean.

For the DEX-treated KC rats, the correction for

TABLE 13

MEAN NASO-ANAL LENGTHS (mm ± SEM) AND MEAN OBESITY INDICES (± SEM) FOR SHAM-OPERATED AND PVN KNIFE-CUT RATS CHRONICALLY EXPOSED TO NO HORMONE (CON), CORT, OR DEX DURING EXPERIMENT TWO

	NASO-ANAL LENGTHS	OBESITY INDEX
SHAM:		
CON	227.2 ± 2.3	292.8 ± 7.9
CORT	224.4 ± 1.6	293.5 ± 1.7
DEX	210.5 ± 1.0*	289.6 ± 3.6
KNIFE CUT:		
CON	225.0 ± 2.1	364.7 ± 6.8+
CORT	225.0 ± 0	363.7 ± 3.4+
DEX	217.3 ± 0.95*	296.4 ± 6.0

* - differs from all other treatment groups, $p < .05$

+ - differs from all other treatment groups, but not each other, $p < .05$

shortened body length inherent in the OBI calculation still resulted in less obesity than was observed for the CON- and CORT-treated KC groups.

Discussion

In the present study CORT and DEX were administered via the same route of administration. The response to the agents was similar to that seen in Experiment 1. This indicates that the differential response elicited by these hormones in Experiment 1 is not due to the route of administration.

DEX suppressed body weight at most doses used and food intake at some of them, whereas CORT retarded neither weight gain nor feeding. The synthetic glucocorticoid DEX is typically cited as having anywhere from 5 to 80 times the potency of CORT (60, 63, 122, 195). The potency difference between these hormones does not appear to be responsible for the differential responses produced by them in this study. Even when 32 mg/kg CORT was administered, which is 640 times the lowest (0.05 mg/kg) effective DEX dose given, it did not attenuate body weight, food intake, or water intake, nor did it ever produce glycosuria. In fact, our data suggest that high doses of CORT potentiate the weight gain of PVN KC rats, as the cumulative weight gain of CORT-treated KC rats exceeded all other KC groups. An elevation in food intake was seen as well in KC rats exposed to the highest dose of

CORT. Others have also found a potentiation of body weight gain and food intake in rats following hydrocortisone and corticosterone injections (57, 106).

In the sham-operated rats of the present study, the administration of CORT at any dose, even high pharmacological doses, did not alter body weight or ingestive behavior. These sham-operated rats had reduced adrenal weights which indicates the negative feedback action of CORT was present.

A range of evidence clearly indicates that knife cuts alongside the PVN disturb the capacity and response of the pituitary-adrenocortical system (see Chapter I). This is reflected by measures such as reduced adrenal weight (107, 109, this study), diminished levels of circulating corticosterone (67, 109, 174), and lack of compensatory increase in ACTH after adrenalectomy (108, 109). What is unclear is whether and how deficits in the responsiveness of the adrenocortical system contribute to the obesity syndrome seen after disruption of the PVN. In the present experiment, compensating for the adrenocortical insufficiency by supplementing rats with CORT did not diminish the obesity that resulted after KCs alongside the PVN. This finding suggests that the limitations in the availability of circulating CORT that result from severing CRF-positive paraventriculo-infundibular neural projections do not directly underlie knife cut-induced obesity.

However, the presence of basal levels of glucocorticoid hormone appears to be necessary for the development of hypothalamic obesity because the elimination of CORT via adrenalectomy prevents the obese condition, while replacement therapy with CORT reinstates it (26, 70). Taken together, this suggest that circulating CORT plays a permissive, but not causative, role in the expression of hypothalamic obesity.

In contrast, DEX curtailed body weight gain at a range of dosages. The lowest (0.05 mg/kg) dose of DEX attenuated the post-surgery weight gain of PVN KC rats by approximately one-third without attenuating the body weight of sham-operated rats. The reduction in weight gain produced by 0.05 mg/kg DEX was not due to decreased food consumption because food intake was not altered at this dose even though plasma glucose levels were augmented. Neither could it be attributed to the loss of calories in the urine, as glycosuria was not detected during this phase.

As a result, KC rats treated with 0.05 mg/kg DEX had a lower feed efficiency than KC rats treated with CORT or with CON (KC-DEX, -CORT, -CON: 0.064$\pm$ 0.007, 0.087 $\pm$ 0.005, 0.087 $\pm$ 0.004 g weight gain/kcal eaten). This suggests an increased energy expenditure, most likely an increase in the activity of the sympathetic nervous system. The ability of DEX to enhance CA, particularly E,

synthesis in the adrenal medulla may account for its effects on body weight. Conversely, a compromise of adrenal medullary function may contribute to the weight gain seen after KCs alongside the PVN.

As the dose of DEX was increased to 0.1 and 0.2 mg/kg, reductions in body weight gain increased in knife cut rats and emerged in sham-operated rats. The weight loss observed in sham-operated rats at these higher doses was not accompanied by declines in food intake nor by excretion of glucose. The low feed efficiency in these sham-operated rats again is consistent with potentiation in sympathetic and/or adrenomedullary activity.

In contrast, the diminished body weights of PVN KC rats during the higher doses of DEX treatment were accompanied by decreases in food intake, elevated blood glucose levels, and varying degrees of glycosuria. Clearly, the relation between DEX treatment and body weight in KC rats is encumbered by the occurrence of glycosuria. However, this condition contributed minimally at the 0.1 mg/kg DEX dose, being more prevalent at the 0.2 mg/kg DEX.

The glycosuria that occurred upon exposure to DEX was again seen only in rats with PVN KCs. The condition was not ameliorated by insulin, indicating that it is extrapancreatic in origin. Glycosuria or so called 'steroid diabetes' has been noted previously with the administration of glucocorticoids (55, 63, 100, 195).

This condition tends to occur in humans predisposed to a diabetic-like state when they are treated with glucocorticoids (195). Glycosuria has also been documented after distruction of the hypothalamic paraventricular area in rabbits and cats that are not even treated with glucocorticoids (6, 28) and in VMH KC rats fed a palatable diet high in fat and sugar (29). Thus, its occurrence only in KC rats in this study may stem from other physiological changes in carbohydrate metabolism associated with the disruption of the PVN (eg. obesity) that act synergistically with DEX.

Being a glucocorticoid, DEX is considered to increase blood glucose by a range of metabolic processes including hepatic glucose production, lipolysis, and hyperglycemic hormones such as glucagon (19, 60, 100, 193). Although not readily acknowledged, another source of DEX's glucose-promoting effects may lie in its activation of sympathoadrenal CAs. This is also significant in light of the fact that glucocorticoids potentiate the effects of other hyperglycemic hormones such as CAs and glucagon (100, 38).

Extreme hyperglycemia induces hyperinsulinemia which can in turn decrease glucose tolerance. Insulin secretion in response to glucose in <u>in vitro</u> pancreas preparations is reported to vary depending on the particular glucocorticoid hormone present (100). The addition of

CORT inhibits glucose-induced insulin secretion while DEX does not. As elevated insulin levels are also characterisitic of rats experiencing disruption of the PVN (50, 170, 176), one can speculate that the combination of DEX treatments and KC surgery may foster a state of insulin resistance which would encourage high circulating glucose levels and glycosuria. On the other hand, Lenzen (100) suggests that increased insulin concentrations are not fully involved in the precipitation of steroid diabetes and indicates that the emergence of this condition is "critically dependent on the functional reserve of the endocrine pancreas". Perhaps KCs alongside the PVN somehow also alter this aspect of pancreatic function.

Differences also exist in the inhibitory action of DEX and CORT at hypothalamic and pituitary sites. When given at lower doses, CORT acts at the hypothalamus reducing CRF, while DEX acts at the anterior pituitary suppressing ACTH (156, 157). This difference could underlie the incongruous consummatory responses these steroids produce. Negative feedback at both the hypothalamic and anterior pituitary levels occurs with relatively large doses of CORT or of DEX (157). This dual feedback inhibition most likely takes place at the higher doses of CORT used in the present study. However, for DEX to inhibit both hypothalamic CRF and pituitary ACTH after a 6 hour infusion period, amounts 5 to 10 times greater

than the highest doses used here were required (157). How this relates to the chronic DEX exposure used in this study cannot be certain, but it suggests that DEX acted only at the anterior pituitary. Yet, in Experiment 1, when rats were administered CORT in addition to DEX, no deviations from the typical suppressive effect of DEX were seen even though the hypothalamus, along with the pituitary, was inhibited. In sum, the dissimilarities in the regulatory responses of rats treated with CORT versus DEX still remained whether one or both of the primary feedback sites along the adrenocortical axis are inhibited. This suggests the differential affinity of these steroids at the hypothalamus and pituitary is not critical to their effects on body weight and food intake.

Another aspect of the action of CORT and of DEX is their mineralocorticoid properties. CORT has some salt-retaining effects, while DEX doesn't. However, in the present study when rats had access to 2% saline solution, no statistical differences in saline intake emerged between groups and the trends that occurred were in a direction counter to that predicted. However, Simpson et. al. (178) also found an increase in saline intake with successive injections of CORT and no change upon exposure to DEX. This suggests that the difference in the mineralocorticoid properties, at least with respect to Na+, possessed by these two hormones did not play a

significant role in the divergent ingestive patterns produced.

Another difference between these hormones is important to note. In both sham-operated and KC rats, chronic exposure to DEX, but not CORT, reduced linear growth. Attenuated growth has previously been revealed after long-term treatment with some glucocorticoids (52, 63, 140). Conversely, elongated body length has been noted following PVN KCs (47, 48, 49).

One of a number of properties of DEX may have yielded this effect on growth. Negative feedback inhibition by DEX on pituitary ACTH (156, 157) and B-endorphin (58) release has been demonstrated. While the pituitary content of growth hormone is normal in DEX-treated rats (140), DEX's inhibitory action may include depression of pituitary growth hormone secretion. However, in seeming paradox, Wehrenberg and colleagues (203) have shown that chronic DEX treatments enhance the pituitary growth hormone response to intravenously-applied growth hormone-releasing factor (hp GRF-44) in vivo. From this, along with other evidence, these investigators suggest that one way DEX attenuates growth is by depressing the CNS pathways that regulate normal growth hormone secretion. However, the exogenous addition of GH did not reverse the diminished body lengths produced by long-term exposure to the adrenocorticoid, methylprednisolone (53). The inhibitory effects of DEX on somatic growth may relate to

this steroid's actions in the periphery. In augmenting the production of glucose, glucocorticoids promote mobilization of tissue protein, transport of derived amino acids to the liver, and decrease utilization of amino acids in muscle and fat (19, 60, 100, 194). DEX, more than CORT, may encourage the use of proteins for glucose production at the expense of linear growth.

The present results reveal that marked differences in the response to CORT versus DEX occur, and that the results obtained with one glucocorticoid cannot be extended to another.

The next experiment examined whether the dissimilarities between these hormones may be attributed specifically to the action of DEX at the adrenal medulla.

C H A P T E R I V

EXPERIMENT III

In Experiment 3, the nature of DEX's effects on rats with PVN KCs was explored further. The steroid hormone DEX acts at several sites. After hypophysectomy (4, 39, 83, 197, 207, 208) or adrenal denervation (4, 143, 197) DEX restores the activity of the enzymes (TH, DA-B-OH, PNMT) that synthesize CAs in the adrenal medulla. This includes the reinstatement of medullary CA responses to stress (4, 39, 83), and to peripheral injections of 6-hydroxy-dopamine (197). In comparison to exogenously administered CORT, DEX has a greater capacity to induce CA biosynethic enzyme activity in the adrenal medulla (208). DEX is an especially potent enhancer of medullary PNMT activity (143, 207, 208). The action of DEX at the adrenal medulla may account for the ability of this steroid to reduce the body weight gain of PVN KC rats. To determine whether DEX acts via the adrenal medulla to produce the responses observed in Experiments 1 and 2, DEX was administered to rats that underwent PVN KC or sham brain surgery after the removal of adrenal medullae (experimental phase 3a).

The primary site of the negative feedback action of DEX is the anterior pituitary. DEX exhibits a higher binding affinity to anterior pituitary cells (34, 148), and a greater degree of inhibition of ACTH release at the pituitary level (156, 157), than does the natural

glucocorticoid CORT (147, 156, 157) To determine whether the responses produced by DEX are due to its inhibition of pituitary ACTH, KC and sham-operated rats with intact or demedullated adrenals were given ACTH treatments in combination with DEX (experimental phase 3b).

General Methods

Forty adult 250 gram female albino Charles Rivers CD rats were singly housed in stainless steel cages under a 12:12hr light:dark illumination cycle. Purina Laboratory Chow pellets (#5001) were available ad libitum until the time of brain surgery. Thereafter the rats were maintained on Purina Mouse Chow pellets (#5015). Free access to water was provided throughout the experiment.

Bilateral adrenal demedullation surgery was performed on half the rats. Under ether anesthesia the adrenal glands were located through bilateral incisions in the dorsal side of the body wall. Each gland was exposed by gently teasing away surrounding fat tissue with care taken to keep the blood supply to the gland intact. On each side, the adrenal was punctured and slit at the posterior pole of the gland with a #12 scalpel. The adrenal medullae was squeezed out and removed. The remaining cortical tissue and surrounding fat was then be returned to the body cavity.

All other subjects received control surgery which consisted of identical procedures excluding incisions of

the adrenal glands and extrusion of the medullae.

For 4 postoperative days rats had continuous access to a 0.85% saline solution in addition to water. This allowed rats to compensate for temporary deficits in minerocorticoid secretion that may exist after surgical insult to the adrenal cortex. Animals were monitored for persistent excessive intake of the saline solution and poor health upon removal of the saline. No rats exhibited these behaviors suggesting none suffered from inadequate adrenocortical functions as a result of adrenal surgery.

Seven to twelve days after adrenal demedullation or sham demedullation half the rats from each of these groups received bilateral parasagittal retracting wire knife cuts alongside the PVN. The other half of each group underwent sham brain operations. Hypothalamic knife cut surgery was executed as described in Experiments 1 and 2.

DEX treatment began one day prior to brain surgery. Rats treated with DEX were given a 0.1 mg/kg/day dose via the drinking water. It was administered as in Experiment 2.

To summarize, a 2 x 2 x 2 design yielded eight experimental groups as follows: knife cut (KC) or sham knife cut; and demedullation (DEM) or sham demedullation (ie. adrenal intact); hormone (DEX) or no hormone (CON), (n=5).

Experimental Phases

In the initial treatment phase (3a) DEX or no hormone (CON) was administered for 8 days following knife cut or sham KC surgery.

In the next treatment phase (3b) ACTH injections were combined with the DEX or the CON condition. From Day 9 to Day 13 after brain surgery, all rats were injected just after lights 'on' and just prior to lights 'off' with 8 U of the ACTH gel Cortigel-40 (Savage Laboratories, Melville, New York). Following ACTH treatment, rats were continued on DEX or CON alone for about 1 week (Phase 3c). Thereafter, rats were withdrawn gradually from DEX.

Body weight, water intake, and food intake accounting for spillage were measured throughout each phase of this experiment.

Urinalysis for glucose was performed periodically. In this experiment, a urine sample was collected by placing a clean piece of cellophane plastic wrap under the home cage and waiting for a testable sample. While samples were collected, water bottles were absent from the cages. Food was removed from rats that tended to eat during the day. Urine glucose content was measured using Diastix Reagent Strips.

Blood Glucose Samples

Blood samples for glucose determination was taken at the end of the initial DEX exposure phase (Day 8) and at the end of the ACTH treatment phase (Day 14). The protocol

that was used for collection and storage of samples was described in Experiment 2.

Surgical Verification

At the conclusion of the experiment rats were assessed for completeness of adrenal demedullation under Nembutal anesthesia. Using a dorsal approach, the adrenals were located, isolated, and then examined under a 20X Zeiss operating microscope for evidence of medullary tissue. A sham brain-operated and a KC rat were considered to have incomplete demedullations and were eliminated from the experiment.

Rats with PVN KCs were perfused, and brains were removed and stored in a formalin fixation solution. Criterion utilized to verify the accuracy of PVN KC surgery were as indicated in Experiment 1 and resulted in two rats being dismissed as subjects.

Because the accuracy of the adrenal demedullation and the PVN KC surgery of a KC/DEM rat was each debatable, this subject was also removed from the study. Finally, a KC/DEX rat died during the experiment removing it from consideration. The loss of several subjects in this study unfortunately left the experimental treatment groups small in size. This should be kept in mind while assessing the data and its discussion.

Results

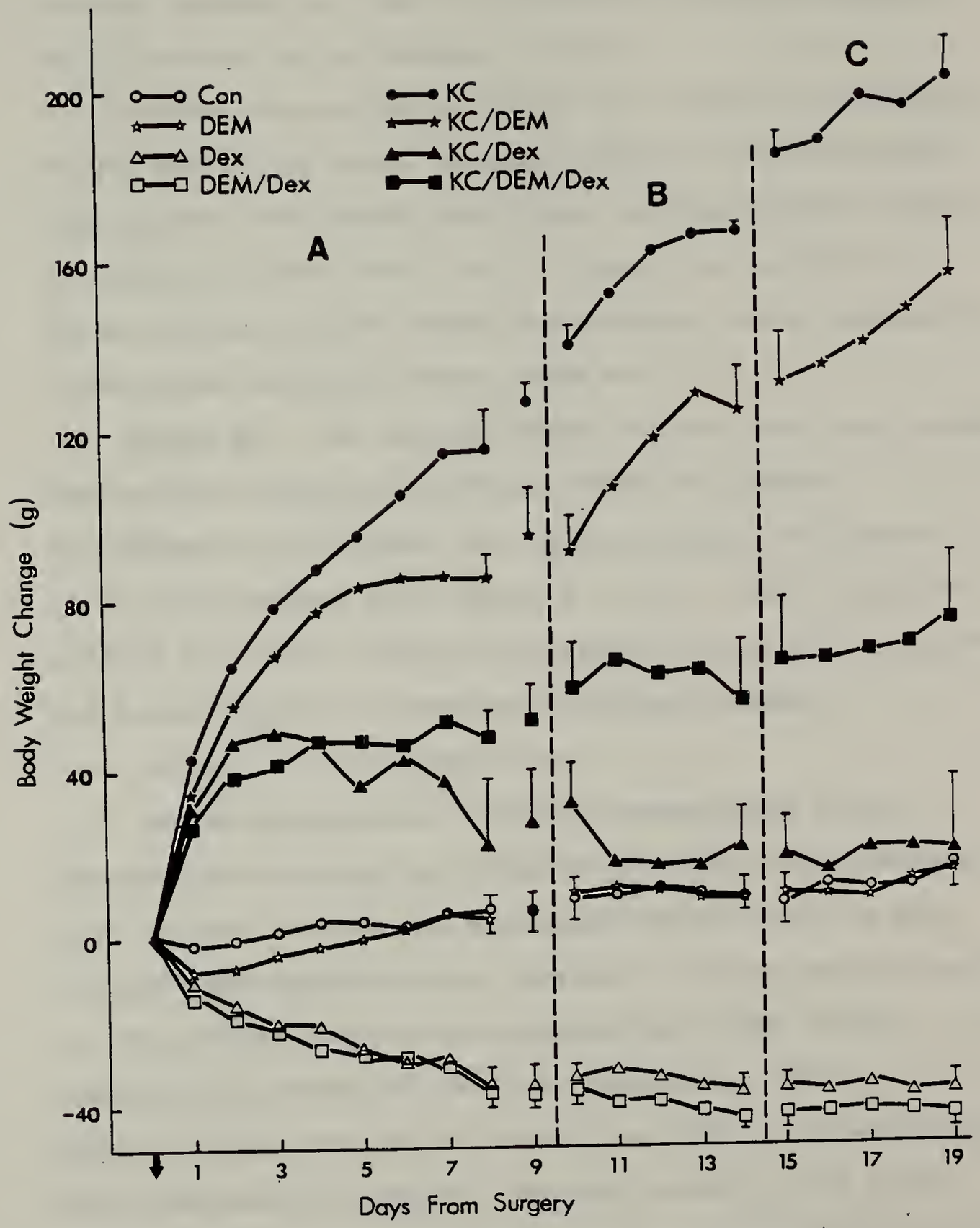
Body Weight

Phase 3a. A 2 x 2 x 2 ANOVA of the cumulative weight change over the initial experimental phase revealed a significant main effect for the KC surgery ($F(1,26)=306.2$, $p<.05$) and the hormone ($F(1,26)=136.3$, $p<.05$) conditions but not for the adrenal surgery. There were also significant two-way interaction effects for brain surgery by hormone ($F(1,26)=12.3$, $p<.05$), and hormone by adrenal surgery ($F(1,26)=10.8$, $p<.05$) in addition to a significant three-way interaction between brain surgery by hormone by adrenal factors ($F(1,26)=11.0$, $p<.05$) (Figure 10).

As in the preceding experiment, chronic DEX treatments suppressed body weight. In sham brain-operated rats, prior adrenal demedullation did not alter the suppressive effects of DEX on body weight. Adrenal demedullation alone failed to influence the body weight gain of vehicle-treated sham brain-operated rats.

DEX also attenuated the weight gain typically seen subsequent to PVN KC surgery (KCs, Hormone: $F(1,11)=51.9$, $P<.05$). This attenuation effect was not prevented by adrenal demedullation. However, there was an interaction between adrenal demedullation and DEX over time (KCs, Hormone x Adrenal x Days: $F(7,77)=5.01$, $p<.05$). The weight gain of DEX-treated adrenal demedullated KC rats versus DEX-treated adrenal intact KC rats diverged after Day 6 at which time the the body weight curve of the demedullated

Figure 10. Mean body weight change of sham-operated and PVN KC rats with intact or demedullated (DEM) adrenal glands and treated with no hormone (CON) or 0.1 mg/kg DEX in each phase (A - C) with the addition of ACTH (8 U, 2 x day) during Phase B.


Con
DEM
Dex
DEM/Dex
KC
KC/DEM
KC/Dex
KC/DEM/Dex
A
B
C
200
160
120
80
40
0
-40
Body Weight Change (g)
1
3
5
7
9
11
13
15
17
19
Days From Surgery

rats flattened while that of adrenal intact rats was further reduced by DEX (KCs, Hormone x Adrenal Surgery: $F(1,11)=11.4$, $p<.05$; Duncan, $p<.05$).

Adrenal demedullation alone also slowed the excessive weight gain of KC rats. Demedullated KC vehicle-treated rats gained less weight over Phase 3a than adrenal intact KC vehicle-treated rats ($p<.05$). Again the effects of demedullation on body weight was delayed, more substantial differences emerging between Days 6-9.

Phase 3b. An overall ANOVA for the mean body weight change upon exposure to ACTH in Phase 3b yielded significant main effects for brain surgery ($F(1,26)=27.4$, $p<.05$) and hormone ($F(1,26)=42.4$, $p<.05$), and significant effects for brain surgery by hormone ($F(1,26)=18.7$, $p<.05$) and brain surgery by hormone by adrenal surgery ($F(1,26)=6.5$, $p<.05$) (Figure 10).

The weight gains of vehicle-treated sham brain-operated groups were not affected by ACTH. ACTH treatments also did not reverse the suppressed body weight of DEX-treated sham-operated rats. However, further weight loss in DEX-treated sham brain-operated rats with intact adrenals was curtailed by ACTH. These rats lost significantly less weight during the ACTH treatment period than previously (Phase 3a, Day 4-8: -3.55 ± 0.31 g/day versus Phase 3b: 0.02 ± 0.61 g/day; correlated $t(4)=5.116$, Bonforroni adjusted familywise error rate, $p<.0167$) such that their weight change was not different from that of

either sham brain-operated group not exposed to DEX. Similar results were not obtained in DEX-treated brain intact rats with demedullated adrenals in response to ACTH.

Attenuated body weight gains were still seen in both KC groups treated with DEX when ACTH was presented in Phase 3b (KCs, Hormone: $F(1,11)=26.8$, $p<.05$). However, while a further decline in weight occurred in the adrenal intact KC group under the combined DEX and ACTH treatment, no change in weight was seen in the demedullated KC group. This found DEX-treated KC rats with demedullated adrenals outweighting DEX-treated KC rats with intact adrenals in Phase 3b ($p<.05$).

The introduction of ACTH also did not significantly alter the rate of weight gain of KC groups not treated with DEX (Phase 3a vs Phase 3b vs Phase 3c, n. s.). However, it appeared to enhance the weight gain of the adrenal enucleated KC rats as this group did not differ from adrenal intact KC rats in absolute weight gain in Phase 3b as they did in Phase 3a.

Phase 3c. A 2 x 2 x 2 ANOVA of the mean body weight change over Phase 3 revealed significant effects for the hormone ($F(1,26)=14.7$,$p<.05$) and brain surgery factors ($F(1,26)=14.8$, $p<.05$), and for the brain surgery by hormone interaction ($F(1,26)=5.6$, $p<.05$) (Figure 10).

Only having a brief five day post-ACTH phase makes the outcome of this phase difficult to interpret. All

groups exhibited similar rates of weight change in this phase as in the previous phase when ACTH was presented. This suggests that the weight change in Phase 3b may relate to time from surgery and not to ACTH effects. But residual ACTH effects may have been present in Phase 3c and instead may have been reflected in the response of some groups during this phase.

The diminished level of weight gain obtained by adrenal intact KC rats exposed to DEX plus ACTH in Phase 3b continued into Phase 3c even though ACTH was withdrawn. In contrast to DEX-treated adrenal intact KC rats, the weight gained by DEX-treated demedullated KC rats was comparable to vehicle-treated KC groups in Phase 3c.

Food Intake

Phase 3a. A 2 x 2 x 2 ANOVA of the quantity of food consumed during Phase 3a revealed a significant main effect for KC surgery ($F(1,26)=380.6$, $p<.05$) and a significant brain surgery by hormone interaction ($F(1,26)=6.7$, $p<.05$) (Figure 11).

In brain intact rats, exposure to DEX attenuated food intake of both adrenal intact and demedullated groups (SHs, Hormone: $F(1,15)=87.4$, $p<.05$). However, regardless of their hormone treatment or adrenal condition, all KC groups exhibited a similar elevation in food intake subsequent to brain surgery. This contrasted the food intake response seen in DEX-treated KC rats in earlier

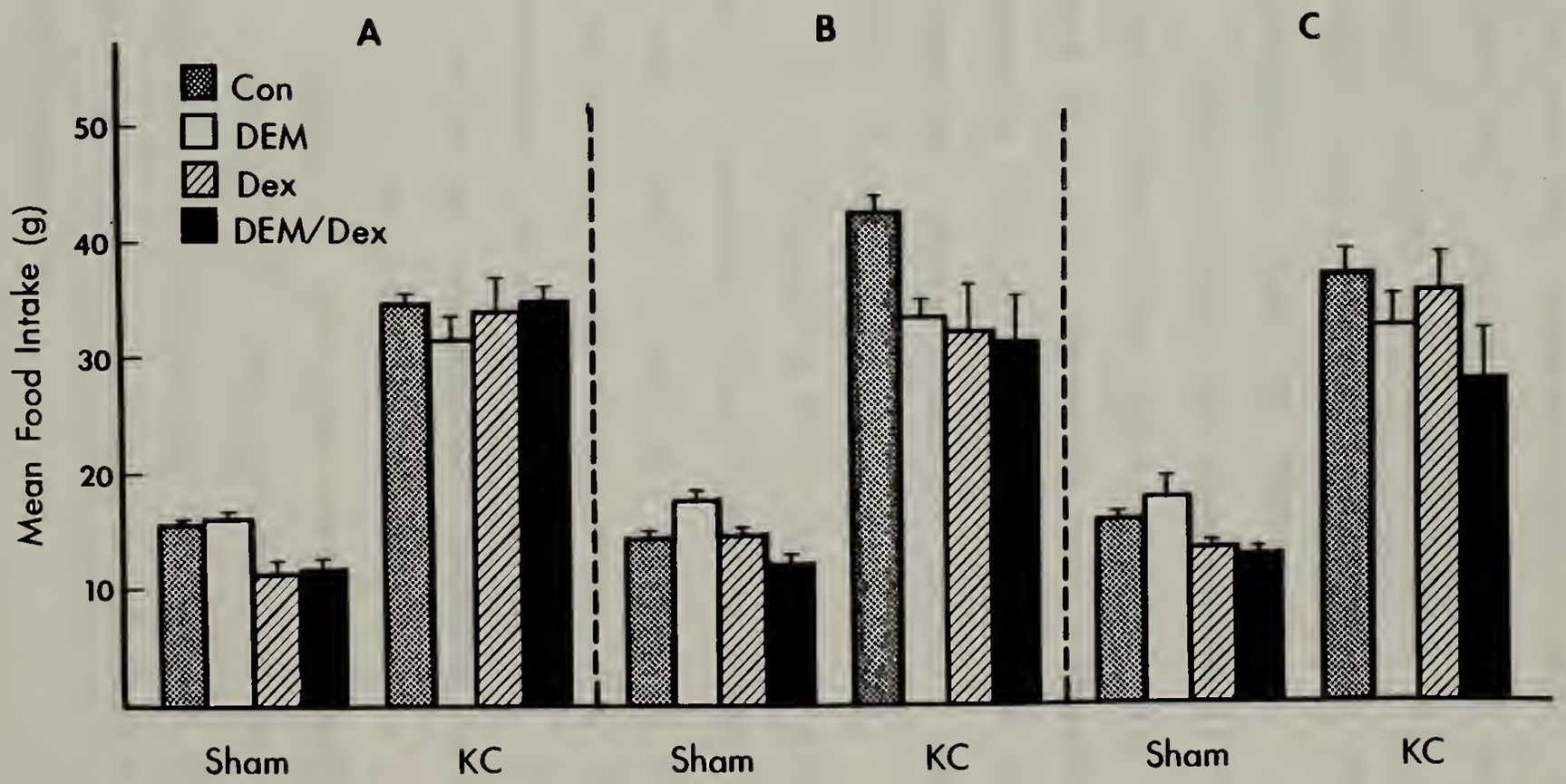


Figure 11. Mean daily food intake of sham-operated and PVN KC rats with intact or demedullated (DEM) adrenal glands and treated with no hormone (CON) or 0.1 mg/kg DEX in each phase (A - C) with the addition of ACTH (8 U, 2 x day) during Phase B.

experiments.

Phase 3b. Significant brain surgery ($F(1,27)=246.7$, $p<.05$) and DEX treatment ($F(1,27)=12.5$, $p<.05$) main effects and a significant 3-way brain surgery x hormone x adrenal surgery interaction effect ($F(1,27)= 7.5$, $p<.05$) emerged from a 2 x 2 x 2 ANOVA of the mean amount of food consumed over Phase 3b (Figure 11).

ACTH countered the suppression of feeding by DEX in adrenal intact sham brain-operated rats (SH/CON vs SH/DEX: Phase 3a, $p<.05$; Phase 3b, n.s.). Whereas, demedullation prevented this effect of ACTH on food intake (SH/DEM vs SH/DEM/DEX, Phase 3a and 3b, $p<.05$). (SHs, Hormone x Adrenal Surgery: $F(1,15)=19.4$, $p<.05$).

An elevation in food intake was still exhibited by each KC treatment group under in Phase 3b. The intake of adrenal intact KC rats not exposed to DEX was enhanced by ACTH (Duncan, $p<.05$ versus other KC groups in Phase 3b; Bonforroni correlated t, adjusted EF, $p<.0167$ versus Phase 3a) while the consummatory response of the other KC groups was not significantly altered.

Phase 3c. With the removal of ACTH, the amount of food consumed by sham-operated rats was again as seen in Phase 3a. The potentiated food intake of adrenal intact KC controls subsided in Phase 3c confirming its mediation by ACTH (Figure 11).

Water Intake

Neither exposure to DEX nor prior adrenal

demedullation altered the water intake of sham-operated rats. The addition of ACTH (Phase 3b) also had no impact (Table 14).

As typically found, KC rats drank significantly more water per day than sham-operated rats(Phase 3a, Brain Surgery: $F(1,26)=33.62$, $p<.05$). The water intake of KC rats proved to be sensitive to DEX and adrenal surgery (Brain Surgery x Hormone: $F(1,26)=4.52$, $p<.05$; Brain Surgery x Adrenal Surgery: $F(1,26)=10.4$, $p<.05$). Exposure to DEX radically elevated the already high water intake of KC rats with intact adrenals without influencing the quantity of water ingested by DEX-treated KC rats with demedullated adrenals ($p<.05$).

ACTH (Phase 3b) failed to reverse the polydipsia of any KC groups. Indeed, introduction of ACTH tended to augment the already elevated water intake of the adrenal intact KC rats, but not of the demedullated KC rats. However, this change did not reached statistical significance.

Blood Glucose

In sham brain-operated rats, DEX and/or ACTH slightly enhanced circulating glucose levels (Table 15). Prior demedullation tended to block this enhancement.

PVN KC surgery alone caused a small increase in serum glucose which was slightly but not significantly diminished by demedullation. The main effect was that DEX

TABLE 14

MEAN DAILY WATER INTAKE (ml ± SEM) OF SHAM-OPERATED AND PVN KNIFE-CUT RATS WITH INTACT OR DEMEDULLATED (DEM) ADRENAL GLANDS DURING EACH PHASE OF EXPERIMENT THREE. RATS EXPERIENCED EXPOSURE TO EITHER NO HORMONE (CON) OR 0.1 mg/kg DEX THROUGHOUT EACH EXPERIMENTAL PHASE IN ADDITION TO ACTH TREATMENTS (8 U, 2xday) DURING PHASE 3B

	N	Phase 3A (Day 1-8)	Phase 3B (Day 10-14, +ACTH)	Phase 3C (Day 15-17)
SHAM:				
CON				
INTACT	5	28.2 ± 2.3	27.4 ± 2.2	24.5 ± 2.8
DEM	4	34.2 ± 3.8	33.3 ± 2.4	32.5 ± 2.1
DEX				
INTACT	5	30.2 ± 1.7	33.5 ± 2.3	31.8 ± 2.1
DEM	5	29.9 ± 1.9	34.0 ± 3.5	36.9 ± 4.3
KNIFE-CUT:				
CON				
INTACT	4	87.7 ± 7.8	107.9 ± 16.1	88.7 ± 6.4
DEM	4	57.6 ± 5.5	59.6 ± 5.2	56.4 ± 7.0
DEX				
INTACT	4	178.1 ± 48.2*	302.6 ± 87.7*	290.3 ± 90.6*
DEM	3	64.3 ± 11.2	63.9 ± 11.4	59.1 ± 9.0

* - a group that differs from all other treatment groups within a phase, $p < .05$

TABLE 15

MEAN BLOOD GLUCOSE LEVELS (mg/dl ± SEM) OF SHAM-OPERATED AND PVN KNIFE-CUT RATS WITH INTACT OR DEMEDULLATED (DEM) ADRENAL GLANDS DURING EXPERIMENT THREE. RAT EXPERIENCED NO HORMONE (CON) OR 0.1 mg/kg DEX IN EACH PHASE WITH THE ADDITION OF ACTH (8 U, 2 x day) DURING PHASE 3B

	N	Phase 3A	Phase 3B (+ ACTH)
SHAM:			
CON			
INTACT	5	94.2 ± 3.1	105.4 ± 2.8
DEM	4	92.8 ± 0.5	101.3 ± 2.9
DEX			
INTACT	5	106.4 ± 4.5	107.4 ± 4.0
DEM	5	97.4 ± 5.1	93.0 ± 4.0
KNIFE-CUT:			
CON			
INTACT	4	118.2 ± 7.8	111.6 ± 5.3
DEM	4	104.3 ± 7.6	106.8 ± 3.2
DEX			
INTACT	4	583.0 ± 18.5*	714.0 ± 194.1*
DEM	3	110.7 ± 8.7	107.0 ± 4.5

* - differs from all other treatment groups within the same phase, $p < .05$

produced an elevation five times normal (!!?) in the blood glucose of KC rats with intact adrenals. ACTH increased blood glucose even more. In contrast, this dramatic hyperglycemia was entirely blocked by the prior demedullation of KC rats.

Urinalysis

No urine glucose was found any sham brain-operated rats at any point during Experiment 3.

In concert with the water intake and blood glucose values, glycosuria was observed in DEX-treated KC rats with intact adrenal glands. By Day 2 post-KC surgery, urine glucose in amounts ranging from +1/4 (250 mg/dl) to +1 (1000 mg/dl), was detected in 3/4 of the rats in this group. By Day 7, urine glucose concentrations of +1 (1000 mg/dl) were seen in all rats in the adrenal intact KC/DEX group. At the end of experiment 3b (Day 13-14), urine glucose values had elevated to +2 (2000 mg/dl) in 3/4 of these rats suggesting that their glycosuria was potentiated by ACTH. Glycosuria was never detected in any of the other three KC groups.

Discussion

This experiment attempted to eludicate the underlying nature of the suppression of food intake and body weight by DEX seen in the preceding experiments.

The posited role of the adrenal medulla in mediating the suppressive effects of 0.1 mg/kg DEX was examined.

Support for this notion was mixed. Experiment 3 revealed that the adrenal medulla participates in DEX's effects on glucose homeostasis. Yet, the role of this site in DEX's attenuation of body weight was not obvious.

In sham brain-operated rats removal of the adrenal medullae did not prevent the suppression of body weight or food intake by DEX. This suggests that DEX does not act via the adrenal medulla to produce its effects in these rats. In contrast, in KC rats removal of the adrenal medullae did partially protect the rats from the suppression of weight gain by DEX. Severe hyperglycemia, polydipsia, and glycosuria were exhibited by DEX-treated KC rats with intact adrenals in this study. Thus, the lower cumulative weight gain of these adrenal intact KC rats likely reflects the urinary caloric loss. The altered glucose homeostasis that produced this condition indicates that adrenal medullary CAs potentiate the action of DEX as adrenal demedullation prevented the glycosuria. The involvement of other sites in DEX's suppressive effects is also suggested. Demedullated KC rats treated with DEX gained less cumulative weight than KC groups never exposed to DEX, although by experimental phase 3c DEX-treated demedullated KC rats were gaining weight at a rate comparable to the latter KC groups.

DEX induces PMNT in the adrenal medulla and more so in rats with compromised neural or humoral inputs to this tissue (eg. 68, 203, 204). It is plausible that

adrenomedullary E causes the elevated glucose levels in DEX-treated adrenal intact rats, as E produces hyperglycemia by a range of metabolic processes including gluconeogenesis and glycogenolysis and the mobilization of fuels (eg., lipolysis) to synthesize glucose (19, 60, 195). Also glycosuria has been noted to accompany the oversecretion of CAs (eg. pheochromocytoma, CA-secreting tumor) (195). ACTH's potentiation of glucose levels here also appears to be adrenal medullary CAs.

Additional insight into the nature of the relationship between adrenal medullary function and PVN KC obesity is provided by other findings from Experiment 3. Clearly, the obesity seen subsequent to disruption of the PVN cannot be attributed to a loss of adrenal medullary functions, because complete removal of the medullae did not potentiate the obesity of KC rats, nor did it cause obesity in brain intact rats. On the contrary, adrenal demedullated KC rats had lower cumulative weight gains than did adrenal intact KC rats.

Perhaps disruption of paraventricular connections alters the functional status of the adrenal medulla in some qualitative fashion so as to contributes to the obesity that results. One qualitative aspect of adrenal medullary function that is posited to be compromised by PVN disconnection is the activity of the medullary CA biosynthetic pathway. KCs alongside the PVN disrupt the

humoral and neural factors that modulate the enzymes governing the proportions of E and NE that are produced (see Introduction). The adrenal medullary CA response most compatible with the development of obesity is a reduction in the levels of E versus NE as this would encourage a tendency toward deposition of fuels over mobilization and utilization. PVN KC rats are reported to have lowered E levels along with reduced E/NE ratios (50). More work is needed to sufficiently determine their role in KC obesity.

Alternative explanations can be put forth for the reduced weight gain seen in demedullated KC rats. Demedullation may actually serve to increase sympathetic activity. For instance, a denervation supersensitivity response can follow the removal of sympathoadrenomedullary tissue and serve to increase the activity of the remaining SNS (60). Also distruction of one aspect of the SNS can be accompanied by reflex compensatory increases in CA synthesis in the complementary intact branch(es) of the SNS (60, 132, 133, 134, 127, 198, 196). Possibly the lower feed efficiency of demedullated KC rats reflects this enhanced sympathetic activity.

It is possible that the lower weight gain of demedullated KC rats is due to inaccuracies in PVN KC surgery or to insufficiencies in adrenocortical functions. Both the KC and demedullation surgeries were verified. The food intake of the demedullated KC rats was also

comparable to that of adrenal intact KC controls. This suggests that all of the KCs were equivalently placed. The adrenal demedullation surgery may have disturbed the function of the adrenal cortex to produce the attenuated weight gain of the demedullated KC rats. However, demedullation surgery did not eliminate the initial post-KC surgery weight gain or cause excessive postoperative consumption of a saline solution (data not shown), nor did it impair the weight gains of sham brain-operated rats. But ACTH did appear to potentiate the weight gain of the demedullated KC rats.

From the present work it appears that DEX's action at the adrenal medulla does not comprehensively explain its suppression of consummatory responses. DEX's negative feedback action at higher centers was therefore also examined. Supplementing DEX with ACTH in Phase 3b did not reverse the attenuation of body weight produced by DEX. Thus, it does not appear that DEX acts on food intake and body weight by inhibiting ACTH release.

Some of the responses that accompanied the administration of ACTH in experimental phase 3b seem to point instead to the adrenal medulla as capable of influencing ingestive behavior. For instance, an elevation in food intake accompanied the administration of ACTH in KC rats with intact adrenals but not in demedullated KC rats. Also, DEX-treated sham brain-operated rats with

intact adrenals normalized their weight and food intake when ACTH was administered, while DEX-treated demedullated rats did not.

The increases in circulating endogenous CORT stimulated by ACTH should be identical in rats with intact versus demedullated adrenals. However, exogenously-applied ACTH also increases the concentration of endogenous CORT perfusing the intra-portal adrenal system to the adrenal medulla. Adrenomedullary CA synthesis indirectly induced by ACTH would seem to account for the differential responses seen between rats possessing and those not possessing medullary tissue.

The exact physiological significance of the consummatory responses produced by ACTH is not clear. However, they imply that ACTH has the potential to alter the balance of adrenomedullary E and NE available to influence consummatory behavior.

The action of DEX at several sites may contribute to the responses seen. For instance, glucocorticoids maintain the level of adrenergic activity in the sympathetic nervous system ---particularly in the superior cervical ganglion (14, 22, 191, 195, 200). The effects of KCs alongside the PVN on the sympathetic nervous system are not exclusive to the adrenal medulla. Transection of the PVN is thought to alter the tonus of the entire sympathetic nervous system. Although indicated as an age-dependent effect by some (13, 74), many studies have

demonstrated that the administration of DEX can enhance CA levels and CA synthesizing enzymes including E and PNMT in sympathetic ganglia and extraadrenal chromaffin cells (12, 142, 191). This entertains the possibility that the actions of DEX encompass enhancement of sympathetic CA activity in general.

The influence of DEX at the liver, pancreas, adipose tissue, or muscle may also be involved. Glucocorticoids affect the metabolic processes or secretory states at all of these sites (19, 60, 100, 195). In addition, the effects of CA`s on these functions are potentiated by glucocorticoids (38, 100, 195). Hence, the suppressive effects of DEX in this study may relate to actions at several sites. For this to be the case DEX must exert itself in a fashion that differs qualitatively from CORT because the administration of CORT in a wide range of dosages does not produce the suppressive effects of DEX. Some have noted that CORT acts in the manner that differs from synthetic glucocorticoids, particularly prednisolone-based corticoids such as DEX (100, 140, 178).

Finally, the results from this experiment contrast findings from Experiment 2. In testing drug regimens in the first two experiments, it was demonstrated in Experiment 2 that a 0.1 mg/kg dose of DEX, when adjusted daily in a alcohol vehicle via drinking water, stabilized the weight gain of KC rats with intact adrenals and

reduced their food intake. This effect was produced in Experiment 2 with minimal glycosuria and without altering water intake. Thus, 0.1 mg/kg DEX via this administration procedure was considered suitable to use in the succeeding studies.

However, when this DEX regimen was extended to Experiment 3 and presented from the time of brain surgery, a significant glycosuric condition consistently appeared in adrenal intact KC rats treated with DEX. It was accompanied by a greatly elevated water intake, a compensatory response to renal fluid losses. Also contrasting the results of Experiment 2, no reduction in food intake was seen in DEX-treated KC rats with intact adrenals in Experiment 3. At least a component of the overeating seen under these latter circumstances may be in compensation for the glycosuria.

There is an intricate balance between the suppression of body weight and food intake by DEX, and the occurrence of glycosuria. DEX can influence body weight and food intake without glycosuria or polydipsia being a factor. On the other hand, the effects of DEX on KC animals can be obscured by secondary effects created by severe hyperglycemia and the excretion of calories. Thus, the use of the 0.1 mg/kg dose of DEX in Experiment 3 did not serve after all to capitalize on this experimental paradigm and maximize the production of coherent results. In other words, it produced confounded results. This dose given

every other day or a lower dose such as 0.05-0.075 mg/kg might have more clearly delineated the relationship between DEX's suppressive effects and its action at the medulla.

The time point of exposure following KC surgery may have been a factor. In Experiment 2 where glycosuria did not contribute significantly to responses produced, rats were not treated with 0.1 mg/kg DEX immediately after KC surgery as in Experiment 3, but were introduced to it approximately a week later following exposure first to a 0.05 mg/kg dose of DEX. Experiencing a gradual introduction to the higher 0.1 mg/kg DEX dose could play a role in the differential responses.

The age of the rats used in these studies also differed. The rats used in Experiment 2 were 2 - 2 1/2 months old at the beginning of the study, while the rats in Experiment 3 were 3 1/2 - 4 months old. Age differences in characteristics such as growth, metabolic rate, and thermogenic capacity may be a source of the incongruous responses produced by DEX in Experiment 2 versus 3.

C H A P T E R V

GENERAL DISCUSSION

KCs alongside the PVN sever CRF-positive paraventricular to infundibular neural projections, thus reducing the responsiveness of the pituitary-adrenocortical system. The present work indicates that deficits in the availability of circulating CORT do not produce the overeating and obesity that follow PVN KCs. CORT supplements did not block the hyperphagia or obesity, even when CORT was provided continuously (Experiment 1) or in resemblance to a normal diurnal cycle (Experiment 2). A deficit in ACTH also does not appear to be responsible for hypothalamic obesity, as KC hyperphagia and obesity were not blocked by ACTH and its enhancement of endogenous CORT (Experiment 3).

Deficits in CA biosynthesis within the adrenal medulla were also hypothesized as possibly contributing to the obesity seen after PVN KCs. DEX was employed to test this hypothesis because of its documented ability to restore CA deficits. KC rats treated with DEX did have attenuated feeding and weight gains. This response was seen in the absence of confounds associated with steroid diabetes (Experiment 2).

However, the reasoning that DEX produces this suppression of regulatory responses via the adrenal medulla was not fully supported. Suppressions in body

weight and sometimes food intake were still seen in adrenal demedullated animals treated with DEX (Experiment 3).

Yet, the effects of DEX are potentiated in the presence of the adrenal medulla as indicated by the dramatically raise peripheral glucose levels in KC rats. Incidence of a DEX-induced diabetic condition, consisting of severe hyperglycemia, glycosuria, polydipsia, and weight loss, were seen only in KC rats with intact adrenals and not in those with demedullated adrenals. Even when glycosuria was not present, significant elevations in blood glucose levels were found in adrenal intact KC rats administered DEX. Slight increases in serum glucose were also present in DEX-treated adrenal intact, but not demedullated, brain intact rats.

The extent to which the hyperglycemia mediated by the medulla relates to DEX's attenuation of appetitive responses is not determined from the present work. How this glucose response relates to the physiological changes underlying KC-induced obesity is also not clear. The sympathoadrenomedullary response to insulin-induced hypoglycemia is under central control (182). Also, activation of the extrahypophysial CRF system associated with the caudal hindbrain projection system of the PVN does result in increases in blood glucose (21, 23, 24, 25) presumably largely via peripheral CAs (21, 24).

Adrenomedullary E appears to be responsible for the altered carbohydrate metabolism produced by DEX in adrenal intact KC rats. E, acting primarily via α-adrenergic receptors, increases blood glucose and hepatic glycogenolysis (84). The dissociation of these E-mediated hyperglycemic effects from E's anorectic effects has been demonstrated (84). Similarly, the changes in glucose balance induced by DEX were shown to be exaggerated by the presence of adrenal medullary CAs, while this was not established for the changes DEX produced in consummatory responses.

However, the adrenal medulla was not found to be the only nor the major site at which DEX acts to suppress body weight. Hence, we cannot adhere to the premise that DEX reverses deficits in adrenomedullary CA activity produced by KCs to reduce the resultant obesity. In turn, we cannot draw definitive conclusions about the relationship between the obesity seen subsequent to KCs alongside the PVN and altered adrenomedullary CA activity. Other experimental approaches that utilize direct measures of adrenal medullary activity and function would more adequately examine this relationship.

For instance, it first should be established that this type of PVN KC procedure does indeed alter the balance of E to NE synthesized in the adrenal medulla. Direct measures of adrenal NE and E content, turnover, and release, along with the activity of CA biosynthetic

enyzmes in adrenal intact KC rats should be performed. If diminished CA production and secretion is found in these rats, then its role in the ingestion and weight gain seen after KC surgery should be explored. One approach might comprise the replacement of more than just one adrenal secretion following adrenalectomy in KC rats. In varying amounts and ratios, CORT, NE, E, (and mineralocoid) could be administered in an attempt to titrate the relative involvement of CAs in the obesity seen after KCs. To examine how alterations in the neural and hormonal inputs regulating CA production in medullary tissue relates to PVN obesity, another agent that activates (or inhibits) NE and/or E production, and that has less ubitiquitous effects on body tissues than does DEX, could be employed.

Another significant finding emerging from the present work is that DEX and CORT differ in their effects on body weight and food intake. As mentioned previously, these regulatory responses were attenuated by DEX, but were affected negligibly by CORT. Others have reported similar responses after administering DEX (7, 69, 104, 140, 178) or CORT (140, 178) to normal rats. One of several properties in which disparities between DEX and CORT exist may be responsible for the incongruous effects they have on consummatory responses. Agruments were made herewithin against the role of disparities in their potency, mineralocorticoid effects, and site(s) of negative

feedback. Neither did differences in their action at the adrenal medulla or their promotion of blood glucose fully account for it (see discussion sections, Experiments 1, 2, and 3). Other evidence suggests that alterations in the activity level of rats also do not underlie the deviations in food intake and body weight produced by DEX (7, 69). It still remains unclear what the basis of the contrasting consummatory responses produced by DEX and CORT is. Some other possible explanations exist:

Reduced growth and weight gain were not produced by chronic exposure to CORT in this work nor in that of others (140, 178). In contrast, some pieces of evidence suggest that the suppressive effects of DEX may relate to its interaction with the growth hormone (GH) system, but the exact nature of their interrelationship is difficult to elucidate. Chronic exposure to DEX reduces food intake, body weight, and linear growth, and also makes rats more responsive to the exogenous application of GHRF (203). Somatomedins, a family of peptides released from the liver under GH control, decrease food intake and body weight when centrally applied (194). This peptide also suppresses GH secretory bursts via its feedback stimulation of the inhibitory hypothalamic hormone, somatostatin (194). In turn, KCs alongside the PVN increase food intake, body weight, and linear growth (47, 48, 49). Somatostatin has been identified in the PVN (158, 163, 187). These data suggest that an interesting

interplay may exist between the action of DEX (at the brain or possibly the anterior pituitary), the regulation of the GH system, and consummatory responses. More work is needed to clarify and support this possibility.

The incongruous effects of DEX and CORT may be due to the differential action of these steroids at one or several peripheral sites such as the liver, pancreas, adipose tissue, or muscle. CORT and DEX may differ in their effects on metabolic processes that are mediated by glucocortiocoid action at these sites and that contribute to the homeostatic responses and the energy balance of an animal. An example is the discrepancy that exists in the glucose-induced insulin response seen in the presence of CORT and DEX (100) in an in vitro preparation. Insulin secretion is inhibited when CORT, but not DEX, is added. An another example can be derived from the differences in growth seen upon exposure to CORT versus DEX. This suggests that these glucocorticoids may differ in their promotion of the use of amino acids as a fuel and/or in their activation of gluconeogenesis.

CORT and DEX may also influence the nervous system in different ways leading to the elicitation of different regulatory responses. Some evidence does indicate that differences exist in modulatory effects of these steroids on CAc activity in the brain (127, 206) and superior cervical ganglion (191).

The differences in the effects of CORT versus DEX at various sites may entail differences in their intracellular binding properties. Dissimilarities in characteristics such as receptor type and location, and binding specificity and affinity have been revealed for DEX and CORT in some instances. Some sites at which dissimilar binding profiles have at times been noted between CORT and DEX include the superior cervical ganglion (14, 191, 200), liver (1), and brain (34, 147, 148, 180), anterior pituitary (34, 148), and in the blood (as stated in 1, 200).

Concluding, the present work indicates that marked differences exist between the effects of CORT and DEX. It revealed that the properties that characterize one glucocorticoid cannot necessarily be extended to another. Thus DEX (and probably other prenisolone-based steroids) should not be considered a glucocorticoid in the same sense that CORT is. These findings also bring into question whether all properties that are traditionally attributed to glucocorticoids as a class actually do represent the actions and effects of each compound considered a glucocorticoid. The recognition of this difference is particularly relevant with regard to the medical treatment of obese or diabetic-prone individuals with DEX or other prednisolone-based glucocorticoids.

REFERENCES

1. Agarwal, M. K. Evidence that natural versus synthetic steroid hormones bind to physicochemically distinct cellular receptors. Biochem. Biophys. Res. Com.73(3): 767-772, 1976.

2. Aravich, P. F., Sclafani, A. Paraventricular hypothalamic lesions and medial hypothalamic knife cuts produce similar hyperphagia syndromes. Behav. Neurosci. 97(6): 970-983,1983.

3. Aravich, P. F., Sclafani, A., Leibowitz, S.F. Effects of hypothalamic knife cuts on feeding induced by NE paraventricular norepinephrine injections. Pharmac. Biochem. Behav. 16: 101-111, 1982.

4. Axelrod, J., Reisine, T. D. Stress hormones: their interaction and regulation. Science 224: 452-459, 1984.

5. Azar, A. P., Shor-Posner, G., Filart, R., Leibowitz, S. F. Impact of medial hypothalamic 6-hydroxy-dopamine injections on daily food intake, diet selection, and body weight in freely-feeding and food-restricted rats. Proc. Ann. Meet. Eastern Psychol. Assoc. 55: 106,1984.

6. Barris, R. W., Ingram, W. R. The effect of experimental hypothalamic lesions upon blood sugar. Amer. J. Physiol. 114: 555-561, 1936.

7. Beatty, W. W., Scouten, C. W., Beatty, P. A. Differential effects of dexamethasone and body weight loss on two measures of activity. Physiol. Behav. 7: 869-871, 1971.

8. Becker, E. E., Kissileff, H. R. Inhibitory controls of feeding by the ventromedial hypothalamus. Am. J. Physiol. 226: 383-396, 1974.

9. Bellinger, L. L., Williams, F. E., Bernardis, L. L. Effect of ventromedial and dorsomedial hypothalamic lesions on circadian corticosterone rhythms. Neuroendocrinology 22: 216-225, 1976.

10. Bhakthavatsalam, P., Leibowitz, S. F. Alpha2-noradrenergic feeding rhythm in paraventricular nucleus: relation to corticosterone. Am. J. Physiol. 250(19): R83-R88, 1986.

11. Bloom, F. E., Battenberg. E. L. F., Rivier, J., Vale, W. Corticotropin releasing factor (CRF) immunoreactive neurons and fibers in rat hypothalamus. Regul. Pept. 4: 43-48, 1982.

12. Bohn, M. C., Bloom, E., Goldstein, M., Black, I. B. Glucocorticoid regulation of phenylethanolamine N-methyltransferase (PNMT) in organ culture of superior cervical ganglia. Dev. Biol. 105: 103-136, 1984.

13. Bohn, M. C., Goldstein, M., Black, I. B. Expression of phenylethanolamine N-methyltransferase in rat sympathetic ganglia and extra-adrenal chromaffin tissue. Dev. Biol. 89: 299-308, 1982.

14. Bohn, M. C., Mc Ewen, B., Luine, V. N., Black, I. B. Development and characterization of glucocorticoid receptors in rat superior cervical ganglion. Dev. Brain Res. 14: 211-218, 1984.

15. Bray, G. A., Inoue, S., Nishizawa, Y. Hypothalamic sity: the autonomic hypothesis and the lateral hypothalamus. Diabetologia 20: 366-377, 1981.

16. Bray, G. A., York, D. A. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol. Rev. 59: 719-809, 1979.

17. Britton, D. R., Hoffman, D. K., Lederis, K., Rivier, J. A comparison of the behavioral effects of CRF, Sauvagine, urotensin I. Brain Research 304: 201-205, 1984.

18. Britton, D. G., Koob, G, Rivier, J., Vale, W. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci. 31: 363-367, 1982.

19. Brown, J. H. U. Integration and coordination of metabolic processes- a systems approach to endocrinology. New York: Van Nostrand Reinhold Co., 1978, pp. 42-184.

20. Brown, M. R., Fisher, L. A. Brain peptide regulation of adrenal epinephrine secretion. Am. J. Physiol. 247: E41-E46, 1984.

21. Brown, M. R., Fisher, L. A. Corticotropin-releasing factor: effects on the autonomic nervous system and visceral systems. Federation Proc. 44: 243-248, 1985.

22. Brown, M. R., Fisher, L. A. Glucocorticoid suppression of the sympathetic nervous system and adrenal medulla. Life Sciences 39: 1003-1012, 1986.

23. Brown, M. R., Fisher, L. A., Rivier, J., Spiess, J., Rivier, C., Vale, W. Corticotropin-releasing factor: effects on the sympathetic nervous system and oxygen consumption. Life Sciences 30: 207-210, 1982.

24. Brown, M. R., Fisher, L. A., Spiess, J., Rivier, C., Rivier, J., Vale, W. Corticotropin-releasing factor: actions on the sympathetic nervous system and metabolism. Endocrinology 111(3): 928-931, 1982.

25. Brown, M. R., Fisher, L. A., Spiess, J., Rivier, J., Vale, W. Comparison of the biologic actions of corticotropin releasing factor and sauvagine. Reg. Peptides 4: 107-114, 1982.

26. Bruce, B. K., King, B. M., Phelps, G. R., Veitia, M. C. Effects of adrenalectomy and corticosterone administration on hypothalamic obesity in rats. Am. J. Physiol. 243(6): E152-E157, 1982.

27. Bruhn, T. O., Plotsky, P. M., Vale, W. W. Effect of paraventricular lesions on corticotropin-releasing factor (CRF)-like immunoreactivity in the adrenocorticotropin response to ether stress and exogenous CRF. Endocrinology 114(1): 57-62, 1984.

28. Camus, J., Gournay, J. J., Le Gran, A., Presse Med. 1: 249, 1925 (as stated in ref. #4a).

29. Carter, J. A., Sclafani, A. Pi-Sunyer, F. X. Spontaneous diabetes in VMH knife-cut rats fed a diet high in sugar and fat. Diabetes 32(suppl. 1): 423, 1983.

30. Ciaranello, R. D., Wooten, G. F., Axelrod, J. Regulation of dopamine B-hydroxylase in rat adrenal glands. J. Biol Chem. 250(8): 3204-3211, 1975.

31. Coscina, D. V., Chambers, J. W., Park, I., Hogan, S., Himms-Hagen, J. Impaired diet-induced thermogenesis in brown adipose tissue from rats made obese with parasagittal hypothalamic knife-cuts. Brain Res. Bull. 14(6): 585-593, 1985.

32. Dallman, M. F. Viewing the ventromedial hypothalamus from the adrenal gland. Am. J. Physiol. 246(15): R1-R12, 1984.

33. DeKloet, E. R., Van der Vies, J., De Wied, D. The site of the suppressive action of dexamethasone on pituitary-adrenal activity. Endocrinology 94: 61-73, 1974.

34. DeKloet, E. R., Wallach, G., McEwen, B. S. Differences in corticosterone and dexamethasone binding to rat brain and pituitary. Endocrinology 96: 598-609, 1975.

35. Edmonds, S. C., Adler, N. T. Food and light as entrainers of circadian runnng activity in the rat. Physiol. Behav. 18(5): 915-919, 1977.

36. Eng, R., Gold, R. M., Nunez, A. Elemental hypothalamic obesity after discrete lesions of the paraventricular nucleus. Soc. Neurosci. Abstr. 5: 216, 1979.

37. Eng, R., Gold, R. M., Sawchenko, P. E. Hypothalamic hypoactivity prevented but not reversed by subdiaphragmatic vagotomy. Physiol. Behav. 20(5): 637-641, 1978.

38. Exton, J. H., Friedmann, N., Wong, E. A-H., Brineaux, J. P., Corbin, J. D., Park, C. R. Interaction of glucocorticoids with glucagon and epinephrine in the control of gluconeogenesis and glycogenolysis in liver and of lipolysis in adipose tissue. J. Biol. Chem. 247(11): 3570-3588, 1972.

39. Gewirtz, G. P., Kvetnansky, R., Weise, V. K., Kopin, I. J. Effect of hypophysectomy on adrenal dopamine B-hydroxylase activity in the rat. Mol. Pharmacol. 7: 163-168, 1971.

40. Gibbs, D. M. Measurement of hypothalamic corticotropin-releasing factors in hypophyseal portal blood. Federation Proc. 44: 203-206, 1985.

41. Gladfelter, W. E., Brobeck, J. R. Decreased spontaneous locomotor activity in the rat induced by hypothalamic lesions. Am. J. Physiol. 203: 811-817, 1962.

42. Goadsby, P. J. Brainstem activation of the adrenal medulla in the cat. Brain Res. 327: 241-248, 1985,

43. Gold, R. M. Hypothalamic hyperphagia produced by parasagittal knife cuts. Physiol. Behav. 5: 23-25, 1970.

44. Gold, R. M. Hypothalamic hyperphagia: males get just as fat as females. J. Comp. Physiol. Psychol. 71: 347-356, 1970.

45. Gold, R. M. Hypothalamic obesity: the myth of the ventromedial nucleus. Science 182: 488-490, 1973.

46. Gold, R. M. Mouse chow: diet of choice for rat hypothalamic obesity. Paper presented at the 53rd Annual Meeting of the Eastern Psychological Association, Baltimore, MD, 1982.

47. Gold, R. M., Ieni, J. R., Simson, E. L. Delayed or precocious hyperphagia after symmetrical or assymmetrical hypothalamic knife cuts in male and female weanling rats. Physiol. Behav. 18(2): 275-281, 1977.

48. Gold, R. M., Jones, A. P., Sawchenko, P. E., Kapatos, G. Paraventricular area: critical focus of a longitudinal neurocircuitry mediating food intake. Physiol. Behav. 18(6): 1111-1119, 1977.

49. Gold, R. M., Kapatos, G. Delayed hyperphagia and increased body length after hypothalamic knife cuts in weanling rats. J. Comp. Physiol. Psychol. 88(1): 202-209, 1975.

50. Gold, R. M., Simson, E. L. Perturbations of serum insulin, glucagon, somatostatin, epinephrine, norepinephrine, and glucose after obesifying hypothalamic knife-cuts. In: The Neural Basis of Feeding and Reward, edited by B. G. Hoebel and D. Novin. Brunswick, ME: Haer Institute, 1982, pp 187-201.

51. Gold, R. M., Sumprer, G., Ueberacher, H. M., Kapatos, G. Hypothalamic hyperphagia despite imposed diurnal or nocturnal feeding and drinking rhythms. Physiol. Behav. 14(6): 861-865, 1975.

52. Goldman, J. K., Bernardis, L. L. Glucocorticoid and estrogen administration in weanling rats with hypothalamic obesity. Horm. Metab. Res. 7: 65-70, 1975.

53. Goldman, J. K., Frohman, L. A. Effects of glucocorticoid administrayion to rats on in vitro intermediary metabolism. J. Pharmacol. Exp. Ther. 188: 310- 317, 1973.

54. Gosnell, B. A., Morley, J. E., Levine, A. S. Adrenal modulation of the inhibitory effect of corticotropin releasing factor on feeding. Peptides 4(6): 807-812, 1983.

55. Goth, A. Adrenal steroids. In: Medical Pharmacology: Principles and Concepts. St. Louis: C. V. Mosby Co., 1974, pp 477-489.

56. Grijalva, C. V., Lindholm, E. The role of the autonomic nervous system in hypothalamic feeding syndromes. Appetite 3: 111-124, 1982.

57. Grossie, J., Turner, C. W. Effect of thyroxine, hydrocortisone, and growth hormone on food intake in rats. Proc. Soc. Exp. Biol. Med. 118: 28-30, 1965.

58. Guillemin, R. T., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W., Bloom, F. B-endorphin and adrenocorticotropin are secreted concomitantly by pituary gland. Science 197: 1367-1369, 1977.

59. Gunion, M. W., Tache, Y. Intrahypothalamic corticotropin-releasing factor decreases gastric output but increases secretion volume. Gastroenterology 86(5): 1102, 1984.

60. Guyton, A. C. The adrenocortical hormones. In: Textbook of Medical Physiology. Philadelphia, PA: W. B. Saunders Co., 1976, pp 1019-1035.

61. Hamilton, J. M. Brown adipose tissue of hypothalamic knife-cut rats: effects of high-carbohydrate and high-fat diets. Master's thesis, University of Massachusetts, Amherst, MA.

62. Hashimoto, K., Murakami, K., Hattori, T., Niimi, M., Fujino, K., Ota, Z. Corticotropin-releasing factor (CRF)-like immunoreactivity in the adrenal medulla. Peptides 5(4): 707-711, 1984.

63. Haynes, R. C., Murad, F. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of adrenocortical steroid biosynthesis. In: Goodman and Gilman's The Phamacological Basis of Therapeutics, edited by A. G. Gilman, L. S. Goodman, T. W. Rall, F. Murad. New York: MacMillan Publishing Co., 1985, pp 1459-1489.

64. Hethering, A. W., Ranson, S. W. The spontaneous activity and food intake of rats with hypothalamic lesions. Am. J. Physiol. 136: 609-617.

65. Ingram, W. R., Barris, R. W. Evidence of altered carbohydrate metabolism in cats with hypothalamic lesions. Amer. J. Physiol. 114: 562-571, 1936.

66. Inoue, S. -I. Ventromedial hypothalamic lesions eliminate anticipatory activities of restricted daily feeding schedules in the rat. Brain Res. 250: 183-187, 1982.

67. Ixart, G., Alonso, G., Szafarczyk, A., Malaval, F., Nouguler-Soule, J., Assenmacher, I. Adrenocorticotropic regulations after bilateral lesions of the paraventricular or supraoptic nuclei and in Brattleboro rats. Neuroendocrinology 35: 270-276, 1982.

68. Kakolewski, J. W., Deaux, E., Christensen, J., Case, B. Diurnal patterns in water and food intake and body weight changes in rats with hypothalamic lesions. Am. J. Physiol. 221: 711-718, 1971.

69. Katz, R. J., Carroll, B. J. Endocrine control of psychomotor activity in the rat: effect of chronic dexamethasone upon general activity. Physiol. Behav. 20: 25-30, 1978.

70. King, B. M., Banta, A. R., Tharel, G. N., Bruce, B. K., Frohman, L. A. Hypothalamic Hyperinsulinemia and obesity: role of adrenal glucocorticoids. Am. J. Physiol. 245(8): E194-199, 1983.

71. Kiss, J. Z., Mezey, E., Skirboll, L. Corticotropin-releasing factor-immunoreactive neurons of the paraventricular nucleus become vasopressin positive after adrenalectomy. Proc. Natl. Acad. Sci. USA 81: 1854-1858, 1984.

72. Kobayashi, K., Takahashi, K. Prolonged food deprivation abolishes the circadian adrenocortical rhythm but not endogenous rhythm in rats. Neuroendocrinology 29: 207-214, 1979.

73. Koob, G. F., Bloom, F. E. Corticotropin-releasing factor and behavior. Federation Proc. 44: 259-263, 1985.

74. Koslow, S. H., Bjegovic, M., Costa, E. Catecholamines in sympathetic ganglia of rat: effects of dexamethasone and reserpine. J. Neurochem. 24: 277-281, 1975.

75. Krahn, D. D., Gosnell, B. A., Levine, A. S., Morley, J. E. Localization of the effects of corticotropin releasing factor on feeding. Soc. Neurosci. Abstr. 10: 302, 1984.

76. Kramer, T. H., Gold, R. M. Facilitation of hypothalamic obesity by greasy diets: palatability vs. lipid content. Physiol. Behav. 24(1): 151-156, 1980.

77. Krieger, D. Effect of ocular enucleation and altered lighting regimens at various ages on the circadian periodicity of plasma corticosteriod levels in the rat. Endocrinology 93: 1077-1091, 1973.

78. Krieger, D. T. Food and water restriction shifts corticosterone, temperature, activity, and brain amine periodicity. Endocrinology 95: 1195-1201, 1974.

79. Krieger, D. T. Rhythms in CRF, ACTH, and corticosteriods. In: Endocrine Rhythms, edited by D. T. Krieger. New York: Raven Press, 1979, pp 123-142.

80. Krieger, D. T. Ventromedial hypothalamic lesions abolish food-shifted circadian adrenal and temperature rhythmicity. Endocrinology 106: 649-654, 1980.

81. Krieger, D. T., Hauser, H. Comparison of synchronization of circadian corticosteroid rhythms by photoperiod and food. Proc. Natl. Acad. Sci. USA 75: 1577-1581, 1978.

82. Krieger, D. T., Liotta, A., Brownstein, M. J. Corticotropin releasing factor distribution in normal and Brattleboro rat brain, and effect of deafferentation, hypophysectomy and steriod treatment in normal animals. Endocrinology 100: 227-237, 1977.

83. Kvetnansky, R., Gewirtz, G. P., Weise, U. K., Kopin, I. J. Effect of hypophysectomy on immobilization-induced elevation of tyrosine hydroxylase and phenylethanolamine-N-methyl transferase in the rat adrenal. Endocrinology 87: 1323-1329, 1970.

84. Langhans, W., Pantel, K., Scharrer, E. Dissociation of epinephrine's hyperglycemic and anorectic effect. Physiol. Behav. 34(3): 457-463, 1985.

85. Leach, C. S., Lipscomb, H. S. Adrenal cortical control of adrenal medullary function. Proc. Soc. Exp. Biol. Med. 130: 448-451, 1969.

86. Lee, M. O. Determination of the surface area of the white rat with its application to expression of metabolic results. Am. J. Physiol. 89: 24-33, 1929.

87. Leibowitz, S. F. Adrenergic stimulation of the paraventricular nucleus and its effects on ingestive behavior as a function of the drug dose and time of injection in the light-dark cycle. Brain Res. Bull. 3: 357-363, 1978.

88. Leibowitz, S. F. Brain cathecolaminergic mechanisms for control of hunger. In: Hunger: Basic Mechanisms and Clinical Implications, edited by D. Novin, W. Wyrwicka, and G. Bray. New York: Raven Press, 1976, pp 1-18.

89. Leibowitz, S. F. Functional and anatomical studies of noradrenergic system of the paraventricular hypothalamus that controls feeding behavior. Soc. Neurosci. Abstr. 5: 220, 1979.

90. Leibowitz, S. F. Hypothalamic catecholamine systems in relation to control of eating behavior and to mechanisms of reward. In: The Neural Basis of Feeding and Reward, edited by B. G. Hoebel and D. Novin. Brunswick, ME: Haer Institute, 1982, pp 241-258.

91. Leibowitz, S. F. Neurochemical systems of the hypothalamus: control of feeding and drinking behavior and water-electrolyte excretion. In: Handbook of the Hypothalamus, vol 1, Part A, Behavioral Studies of the Hypothalamus, edited by P. J. Morgane and J. Panksepp. New York: Marcel Dekker, 1980, pp 299-437.

92. Leibowitz, S. F. Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. Pharmac. Biochem. Behav. 8: 163-175, 1978.

93. Leibowitz, S. F. Reciprocal hunger-regulating circuits involving alpha- and beta-adrenergic receptors located, respectively, in the ventromedial and lateral hypothalamus. Proc. Natl. Acad. Sci. USA 67: 1063-1070, 1970.

94. Leibowitz, S. F., Brown, L. L. Histochemical and pharmacological analysis of noradrenergic projections to the paraventricular hypothalamus in relation to feeding stimulation. Brain Res. 201: 289-314,1980.

95. Leibowitz, S. F., Hammer, N.J., Brown, L. L. Analysis of behavioral deficits produced by lesions in the dorsal and ventral midbrain tegmentum Physiol. Behav. 25: 819-834, 1980.

96. Leibowitz, S. F., Hammer, N. J., Chang, K. Feeding behavior induced by central norepinephrine injection is attenuated by discrete lesions in the hypothalamic paraventricular nucleus. Pharmac. Biochem. Behav. 19(6): 945-950, 1983.

97. Leibowitz, S. F., Hammer, N. J., Chang, K. Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat. Physiol. Behav. 27: 1031-1040, 1981.

98. Leibowitz, S. F., Roland, C. R., Hor, L., Squillari, V. Noradrenergic feeding elicited via the paraventricular nucleus is dependent upon circulating corticosterone. Physiol. Behav. 32: 857-864, 1984.

99. Leibowitz, S. F., Roossin, P., Rosenn, M. Chronic norepinephrine injection into the hypothalamic paraventriculr nucleus produces hyperphagia and increased body weight in the rat. Pharmac. Biochem. Behav. 21(5): 801-808, 1984.

100. Lenzen, S., Bailey, C. J. Thyroid hormones, gonadal and adrenocortical steroids and the function of the islets of Langerhans. Endocrine Reviews 5(3): 411-434, 1984.

101. Levine, A. S., Rogers, B., Kneip, J., Grace, M., Morley, J. E. Effect of centrally administered corticotropin releasing factor (CRF) on multiple feeding paradigms. Neuropharmacology 22: 337-339, 1983.

102. Lichenstein, S. S., Marinescu, C., Leibowitz, S. F. Chronic infusion of norepinephrine and clonidine into the hypothalamic paraventricular nucleus. Brain Res. Bull. 13(4): 591-595, 1984.

103. Louis-Sylvestre, J. Neuroendocrinology of hyperphagias and obesities. Reprod. Nutr. Develop. 20(5B): 1545-1562, 1980.

104. Lowy, M. T. and G. K. W. Yim. Selective reduction by dexamethasone of stress-related hyperphagias. Life Sci. 27: 2553-2558, 1980.

105. Luiten, P. G. M., ter Horst, G. J., Karst, H., Steffens, A. B. The course of paraventricular hypothalamic efferents to autonomic structures in medulla and spinal cord. Brain Res. 329: 374-378, 1985.

106. Lytle, L. D. Control of eating behavior. In: Nutrition and the Brain, vol 2, edited by R. J. Wurtman and J. J. Wurtman. New York: Raven Press, 1977, pp. 1-145.

107. Makara, G. B. Mechanisms by which stressful stimuli activate the pituitary-adrenal system. Federation Proc. 44: 149-153, 1985.

108. Makara, G. B., Stark, E., Kapocs, G., Antoni, F. A. Long-term effects of hypothalamic paraventricular lesion on CRF content and stimulated ACTH secretion. Am. J. Physiol. 250(13): E319-E324, 1986.

109. Makara, G. B., Stark, E., Karteszi, M., Palkovits, M., Rappay, G. Effects of paraventricular lesions on stimulated ACTH release and CRF in stalk-median eminence of the rat. Am. J. Physiol. 240(3): E441-E446, 1981.

110. Marubayashi, U., Antunes-Rodrigues, J., McCann, S. M. Blockade of adrenal compensatory hypertropy by unilateral hypothalamic lesions. Brain Res. Bull. 14(4): 297-300, 1985.

111. McCabe, J. T., DeBellis, M. D., Leibowitz, S. F. Clonidine-induced feeding: analysis of central sites of action and fiber projections mediating this response. Brain Res. 309: 85-104, 1984.

112. McCarty, R., Kopin, I. J. Stress-induced alterations in plasma catecholamines and behavior of rats: effects of chlorisondamine and bretylium. Behav. Neural Biol. 27: 249-265, 1979.

113. McLean, S., Hoebel, B. G. Feeding induced by opiates injected into the paraventricular hypothalamus. Peptides 4(3); 287-292, 1983.

114. McLean, S., Hoebel, B. Opiate and norepinephrine-induced feeding from the paraventricular nucleus of the hypothalamus are dissociable. Life Sciences 31: 2379-2382, 1982.

115. Menguy, R. Effects of restraint stress on gastric secretion in the rat. Am. J. Dig. Dis. 5: 911-916, 1960.

116. Merchenthaler, I. Corticotropin releasing factor (CRF)-like immunoreactivity in the rat central nervous system. Extrahypothalamic distribution. Peptides 5: Suppl. 1, 53-69, 1984.

117. Merchenthaler, I., Hynes, M. A., Vigh, S., Schally, A. V., Petrusz, P. Corticotropin releasing factor (CRF): origin and course of afferent pathways to the median eminence (ME) of the rat hypothalamus. Neuroendocrinology 39: 296-306, 1984.

118. Merchenthaler, I., Hynes, M. A., Vigh, S., Schally, A. V., Petrusz, P. Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat spinal cord. Brain Res. 275: 373-377, 1983.

119. Merchenthaler, I., Vigh, S. Petrusz, P., Schally, A. V. Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. Am. J. Anat. 165: 385-396, 1982.

120. Merchenthaler, I., Vigh, S., Petrusz, P., Schally, A. V. The paraventriculo-infundibular corticotropin releasing factor (CRF) pathway as revealed by immunocytochemistry in long-term hypophysectomized or adrenalectomized rats. Regul. Pept. 5: 295-305, 1983.

121. Meyer, J. S., Micco, D. J., Stephenson, B. S., Krey, L. C., McEwen, B. S. Subcutaneous implantation method for chronic glucocorticoid replacement therapy. Physiol. Behav. 22(5): 867-870, 1979.

122. Meyers, F. H., Jawetz, E., Goldfien, A. The adrenocortical steroids. In: Review of Medical Pharmacology. Los Altos, CA: Lange Medical Publications, 1980, pp 353-367.

123. Mezey, E., Kiss, J. Z., Skirboll, L. R., Goldstein, M., Axelrod, J. Increase of corticotropin-releasing factor staining in rat paraventricular nucleus neurones by depletion of hypothalamic adrenaline. Nature 310(5973): 140-141, 1984.

124. Moberg, G. P., Bellinger, L. L., Mendel, Y. E. Effect of meal feeding on daily rhythms of plasma corticosterone and growth hormone in the rat. Neuroendocrinology 19: 160-169, 1975.

125. Moldrow, R. L., Fischman, A. J. Physiological changes in rat hypothalamic CRF: circadian, stress and steroid suppression. Peptides 3(5): 837-840, 1982.

126. Molinoff, P. B., Brimijoin, S., Weinshilboum, R., Axelrod, J. Neurally mediated increase in dopamine-B-hydroxylase activity. Proc. Nat. Acad. Sci. 66: 453-458, 1970.

127. Moore, K. E., Phillipson, O. T. Effects of dexamethasone on phenylethanolamine N-methyltransferase and adrenaline in the brain and superior cervical ganglia of adult and neonatal rats. J. Neurochem 25: 289-294, 1975.

128. Morimoto, Y., Oishi, T., Arisue, K. Yamamura, Y. Effect of food restriction and its withdrawal on the circadian adrenocortical rhythm in rats under constant dark or constant lighting conditions. Neuroendocrinology 29: 77-83, 1979.

129. Morley, J. E., Levine, A. S. Corticotropin releasing factor, grooming, and ingestive behavior. Life Sciences 31: 1459-1464, 1982.

130. Morley, J. E., Levine, A. S., Gosnell, B. A., Billington, C. J. Neuropeptides and appetite: contribution of neuropharmacological modeling. Federation Proc. 43: 2903-2907, 1984.

131. Morley, J. E., Levine, A. S., Murray, S., Kneip, J. Pepidergic regulation of norepinephrine induced feeding. Pharmac. Biocem. Behav. 16(2): 225-228, 1982.

132. Mueller, R. A., Thoenen, H., Axelrod, J. Adrenal tyrosine hydroxylase: compensatory increase in activity after chemical sympathectomy. Science 163: 468-469, 1969.

133. Mueller, R. A., Thoenen, H., Axelrod, J. Increase in tyrosine hydroxylase activity after reserpine administration. J. Pharmacol. Exp. Ther. 169: 74-79, 1969.

134. Mueller, R. A., Thoenen, H., Axelrod, J. Inhibition of neuronally induced tyrosine hydroxylase by nicotinic receptor blockade. Eur. J. Pharmac. 10: 51-56, 1970.

135. O'Donohue, T. L., Browley, W. R., Jacobowitz, D. M. Changes in ingestive behavior following interruption of a noradrenergic projection to the paraventricular nucleus: histochemical and neurochemical analyses. Pharmac. Biochem. Behav. 9: 99-105, 1978.

136. Olschowka, J. A., O'Donohue, T. L., Mueller, G. P., Jacobowitz, D. M. Hypothalamic and extra-hypothalamic distribution of CRF-like immunoreactive neurons in the rat brain. Neuroendocrinology 35: 305-308, 1982.

137. Olschowka, J. A., O'Donohue, T. L., Mueller, G. P., Jacobowitz, D. M. The distribution of corticotropin-releasing factor-like immunoreactive neurons in rat brain. Peptides 3(6): 995-1015, 1982.

138. Palkovitz, M., Brownstein, M. J, Vale, W. Distribution of corticotropin-releasing factor in rat brain. Federation Proc. 44: 215-219, 1985.

139. Paull, W. K., Gibbs, F. P. The corticotropin releasing factor (CRF) neurosecretory system in intact, adrenalectomized, and adrenalectomized-dexamethasone treated rats. Histochemistry 78: 303-316, 1983.

140. Pericas, I., Jolin, T. Plasma insulin levels, and insulin sensitivity in adrenalectomized rats: effects of corticosterone and dexamethasone. Steriods Lipids Res. 5: 286-296, 1974.

141. Petrusz, P., Merchenthaler, I., Maderdrut, J. L., Heitz, P. U. Central and peripheral distribution of corticotropin-releasing factor. Federation Proc. 44: 229-235, 1985.

142. Phillipson, O. T., Moore, K. E. Effects of dexamethasone and nerve growth factor on phenylethanolamine N-methyltransferase and adrenaline in organ cultures of newborn rat superior cervical ganglion.J. Neurochem. 25: 295-298, 1975.

143. Pohorecky, L. A., Piezzi, R. S., Wurtman, R. J. Steroid induction of phenylethanolamine-N-methyl transferase in adrenomedullary explants: independence of adrenal innervation. Endocrinology 86: 1466-1468, 1970.

144. Powley, T. L. The ventromedial hypothalamic syndrome, satiety, and a cephalic phase hypothesis. Psychol. Rev. 84: 89-126, 1977.

145. Powley, T. L., Laughton, W. Neural pathways involved in the hypothalamic integration of autonomic responses. Diabetologia 20: 378-387, 1981.

146. Powley, T. L., Walgren, M. C., Laughton, W. B. Effects of guanethidine sympathectomy on ventomedial hypothalamic obesity. Am. J. Physiol. 245(14): R408-R420, 1983.

147. Rhees, R. W., Grosser, B. I., Stevens, W. Effect of steroid competition and time on the uptake of [3H]corticosterone in the rat brain: an autoradiographic study. Brain Research 83: 293-300, 1975.

148. Rhees, R. W., Grosser, B. I., Stevens, W. The autoradiographic localization of [3H]dexamethasone in the brain and pituitary of the rat. Brain Research 100:151-156, 1975.

149. Richardson, J. S., Jacobowitz, D. M. Depletion of brain norepinephrine by intraventricular injection of 6-hydroxydopa: a biochemical, histochemical and behavioral study in rats. Brain Research 58: 117-133, 1973.

150. Rietvald, W. J., Ten Hoor, F., Kooij, M., Flory, W. Changes in 24-hour fluctuations of feeding behavior during hypothalamic hyperphagia in rats. Physiol. Behav. 21: 615-622, 1978.

151. Rivier, C., Rivier, J., Vale, W. Inhibition of adrenocorticotropic hormone secretion in the rat by immunoneutralization of corticotropin-releasing factor. Science 218: 377-379, 1982.

152. Rivier, C., Vale, W. Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature 305: 325-327, 1983.

153. Roland, C. R., Bhakthavatsalam, P., Leibowitz, S. F. Interaction between corticosterone and alpha2-noradrenergic system of the paraventricular nucleus: relation to eating behavior. Neuroendocrinology 42: 296-305, 1986.

154. Roland, C. R., Oppenheimer, R. L., Chang, K., Leibowitz, S. F. Hypophysectomy disturbs the noradrenergic feeding system of the paraventricular nucleus. Psychoneuroendocrinology 10(1): 109-120, 1985.

155. Roth, K. A., Weber, E., Barchas, J. D. Immunoreactive corticotropin releasing factor (CRF) and vasopressin are colocalized in a subpopulation of the immunoreactive vasopressin cells in the paraventricular nucleus of the hypothalamus. Life Sci. 31: 1857-1860, 1982.

156. Sakakura, M., Yoshioka, M., Kobayashi, M., Takebe, K. Degree of inhibition of ACTH release by glucocorticoids in adrenalectomized rats. Neuroendocrinology 32: 38-41, 1981.

157. Sakakura, M., Yoshioka, M., Kobayashi, M., Takabe, K. The site of inhibitory action of a natural (corticosterone) and synthetic steroid (dexamethasone) in the hypothalamus-pituitary-adrenal axis. Neuroendocrinology 32: 174-178, 1981.

158. Sawchenko, P. E. Anatomic relationships between the paraventricular nucleus of the hypothalamus and visceral regulatory mechanisms: implications for the control of feeding behavior. In: The Neural Basis of Feeding and Reward, edited by B. G. Hoebel and D. Novin. Brunswick, ME: Haer Institute, 1982, pp 259-274.

159. Sawchenko, P. E., Gold, R. M. Effects of gastric vs. complete subdiaphragmatic vagotomy on hypothalamic-hyperphagia and obesity. Physiol. Behav. 26(2): 281-292, 1981.

160. Sawchenko, P. E., Gold, R. M., Alexander, J. Effects of selective vagotomies on knife cut-induced hypothalamic obesity: differential results on lab chow vs. high-fat diets. Physiol. Behav. 26(2): 293-300, 1981.

161. Sawchenko, P. E., Gold, R. M., Leibowitz, S. F. Evidence for vagal involvement in the eating elicited by adrenergic stimulation of the paraventricular nucleus. Brain Research 225: 249-269, 1981.

162. Sawchenko, P. E., Swanson, L. W. Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. Science 214: 685-687, 1981.

163. Sawchenko, P. E., Swanson, L. W. Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. J. Comp. Neurol. 205: 260-272, 1982.

164. Sawchenko, P. E., Swanson, L. W. Localization, colocalization, and plasticity of corticotropin-releasing factor immunoreactivity in rat brain. Federation Proc. 44: 221-228, 1985.

165. Sawchenko, P. E., Swanson, L. W., Vale, W. W. Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in paravocellular neuroscretory neurons of the adrenalectomized rat. Proc. Natl. Acad. Sci. USA 81: 1883-1887, 1984.

166. Sawchenko, P. E., Swanson, L. W., Vale, W. W. Corticotropin-releasing factor: co-expression within distinct subsets of oxytocin-, vasopressin-, and neurotensin-immunoreactive neurons in the hypothalamus of the male rat. The Journal of Neuroscience 4(4): 1118-1129, 1984.

167. Schipper, J., Steinbusch, H. W. M., Vermes, I., Tilders, F. J. H. Mapping of CRF-immunoreactive nerve fibers in the medulla oblongata and spinal cord of the rat. Brain Res. 267: 145-150, 1983.

168. Schipper, J., Werkman, T. R., Tilders, F. J. H. Quantitative immunocytochemistry of corticotropin-releasing factor (CRF). Studies on nonbiological models and on hypothalamic tissues of rats after hypophysectomy, adrenalectomy and dexamethasone treatment. Brain Res. 293: 111-118, 1984.

169. Schwille, P. O., Engelhardt, W., Wolf, U. Haniisch, E. Infusion of somatostatin antiserum in the rat--failure to raise stress induced low gastric acid secretion. Horm. Metab. Res. 13: 710-711, 1981.

170. Sclafani, A. The role of hyperinsulinema and the vagus nerve in hypothalamic hyperphagia reexamined. Diabetologia 20: 402-410, 1981.

171. Scalfani, A. Aravich, P. F. Macronutrient self-selection in three forms of hypothalamic obesity. Am. J. Physiol. 244(13): R686-R694, 1983.

172. Sclafani, A., Berner, C. N. Hyperphagia and obesity produced by parasagittal and coronal hypothalamic knife cuts: further evidence for a longitudinal feeding inhibitory pathway. J. Comp. Physiol. Psychol. 91: 1000-1018, 1977.

173. Sclafani, A., Kirchgessner, A. The role of the medial hypothalamus in the control of feeding: an update. In: Feeding Behavior: Neural and Hormonal Controls, edited by S. Ritter and C. D. Barnes. New York: Academic Press, 1986, pp 27-66.

174. Shor-Posner, G., Azar, A. P., Insinga, S., Leibowitz, S. F. Deficits in the control of food intake after hypothalamic paraventricular nucleus lesions. Physiol. Behav. 35(6): 883-890, 1985.

175. Siegel, P. S. Food intake in the rat in relation to the dark-light cycle. J. Comp. Physiol. Psychol. 54: 294-301, 1961.

176. Sims, S., Lorden, J. F. Effect of paraventricular nucleus lesions on body weight, food intake, and insulin levels. Behav. Brain Res. 22: 265-281, 1986.

177. Simonyi, A., Fekete, M. I. K., Kenessey, A., Paldi-Haris, P., Graf, L. Prolonged ACTH treatment increases trypsin-like and phenylethanolamine-n-methytransferase (PNMT) activity in the adrenals European Journal of Pharmacology 106: 465-466, 1984.

178. Simpson, C. W., Dicara, L. V., Wolf, G. Glucocorticoid anorexia in rats. Pharmac. Biochem. Behav. 2(1): 19-25, 1974.

179. Sofroniew, M. V., Schrell, U. Evidence for a direct projection from oxytocin and vasopressin neurons in the hypothalamic paraventricular nucleus to the medulla oblongata: immunohistochemical visualization of both the horseradish peroxidase transported and the peptide produced by the same neurons. Neurosci. Lett. 19: 257-263, 1980.

180. Stevens, W., Reed, D. J., Grosser, B. I. Binding of natural and synthetic glucocorticoids in rat brain. J. Steroid Biochem. 6: 521-527, 1975.

181. Storlien, L. H., Albert, D. J. The effect of VMH lesions, lateral cuts, and anterior cuts on food intake, activity level, food motivation, and reactivity to task. Physiol. Behav. 9: 191-197, 1972.

182. Stricker, E. M., Rowland, N., Saller, C. F., Friedman, M. I. Homeostasis during hypoglycemia: central control of adrenal secretion and peripheral control of feeding. Science 196: 79-81, 1977.

183. Sutton, R. E, Koob, G. F., LeMoal, M., Rivier, J., Vale, W. Corticotropin releasing factor produces behavioural activation in rats. Nature 297: 331-333, 1982.

184. Swanson, L. A. Immunohistochemical evidence for a neurophysin-containing autonomic pathway arising in the paraventricular nucleus of the hypothalamus. Brain Res. 128: 346-353, 1977.

185. Swanson, L. W., Hartman, B. K. Biochemical specificity in central pathways related to peripheral and intracerebral homeostatic functions.Neuroscience Letters 16: 55-60, 1980.

186. Swanson, L. W., Kuypers, H. G. J. M. The paraventricular nucleus of the hypothalamus: Cytoarchitectonic subdivisions and organizatoin of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J. Comp. Neurol. 194: 555-570, 1980.

187. Swanson, L. W., Sawchenko, P. E. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Annu. Rev. Neurosci. 6: 275-325, 1983.

188. Swanson, L. W., Sawchenko, P. E. Paraventricular nucleus: A site for the interaction of autonomic and neuroendocrine mechanisms. Neuroendocrinology 31: 410-417, 1980.

189. Swanson, L. W., Sawchenko, P. E., Berod, A., Hartman, B. K., Helle, K., VanOrden, L. S. An immunohistochemical study of the organization of catecholaminergic cells and terminal fields in the paraventricular and supraoptic nuclei of the hypothalamus. J. Comp. Neurol. 196: 271-285, 1981.

190. Swanson, L. W., Sawchenko, P. E., Rivier, J., Vale, W. W. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: a immunohistochemical study. Neuroendocrinology 36: 165-186, 1983.

191. Sze, P. Y., Hedrick, B. J. Effects of dexamethasone and other glucocorticoid steroids on tyrosine hydroxylase activity in the superior ganglion. Brain Res 265: 81-86, 1983.

192. Tache, T., Goto, Y., Gunion, M. W., Vale, W., Rivier, J., Brown, M. Inhibition of gastric acid secretion in rats by intracerebral injection of corticotropin-releasing factor. Science 222: 935-937, 1983.

193. Tache, Y., Gunion, M. Corticotropin-releasing factor: central action to influence gastric secretion. Federation Proc. 44: 255-258, 1985.

194. Tannenbaum, G. S., Guyda, H. J., Posner, B. I. Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. Science 220: 77-79, 1983.

195. Tepperman, J. Metabolic and endocrine physiology. Chicago: Year Book Medical Publishers, Inc., 1980, pp. 167-293.

196. Thoenen, H., Mueller, R. A., Axelrod, J. Increased tyrosine hydroxylase activity after drug-induced alteration of sympathetic transmission. Nature 221:1264, 1969.

197. Thoenen, H., Mueller, R. A., Axelrod, J. Neuronally dependent induction of adrenal phenylethanolamine-N-methyltransferase by 6-hydroxydopamine. Biochem. Pharmacol. 19: 669-673, 1970.

198. Thoenen, H., Mueller, R. A., Axelrod, J. Trans-synaptic induction of adrenal tyrosine hydroxylase. J. Pharmac. Exp. Ther. 169: 249-254, 1969.

199. Tilders, F. J. H., Schipper, J., Lowry, P. J., Vermes, I. Effects of hypothalamus lesions on the presence of CRF immunoreactive nerve terminals in the median eminece and on the pituitary-adrenal response to stress. Regulat. Peptides 5: 77-94, 1982.

200. Towle, A. C., Sze, P. Y. [3H] Corticosterone binding in rat superior cervical ganglion. Brain Res. 253: 221-229, 1982.

201. Valentino, R. J., Foote, S. L., Aston-Jones, G. Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. Brain Res. 270: 363-367, 1983.

202. Vernikos-Danelles, J. Effect of stress, adrenalectomy, hypophysectomy and hydrocortisone on the corticotropin-releasing activity of rat median eminence. Endocrinology 76: 122-126, 1965.

203. Wehrenberg, W. B., Baird, A. Ling, N. Potent interaction between glucocorticoids and growth hormone-releasing factor in vivo. Science 221: 556-558, 1983.

204. Weiss, G. F., Leibowitz, S. F. Efferent projections from the paraventricular nucleus mediating - noradrenergic feeding. Brain Res. 347:225-238, 1985.

205. Wilkinson, C. W., Shinsako, J., Dallman, M. F. Daily rhythms in adrenal responsiveness to adrenocorticotropin are determined primarily by the time of feeding in the rats. Endocrinology 104: 350-359, 1979.

206. Wolkowitz, O., Sutton, M., Koulu, M., Labarca, R., Wilkinson, L., Doran, A., Hauger, R., Pickar, D., Crawley, J. Chronic corticosterone administration in rats: behavioral and biochemical evidence of increased central dopaminergic activity. European J. Pharmacol. 122: 329-338, 1986.

207. Wurtman, R. J., Axelrod, J. Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. J. Biol. Chem. 241(10): 2301-2305, 1966.

208. Wurtman, R. J. Control of epinephrine synthesis in the adrenal medulla by the adrenal cortex: hormonal specificity and dose-response characteristics. Endocrinology 79: 608-614, 1966.

209. Zimmerman, E. A. The organization of oxytocin and vasopressin pathways. In: Neurosecretion and Brain Peptides, edited by J. B. Martin, S. Reichlin, and K. L. Bick. New York: Raven Press, 1981, pp. 63-75.