9-2005

THE TEMPORAL HORMESIS OF DRUG THERAPIES

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□   Recent publications in the field of asthma therapeutics and studies performed over the last decade in the treatment of chronic heart failure suggest a phenomenon called 'temporal hormesis'. This phenomenon can be defined as the beneficial action of drug after chronic administration as opposed to its detrimental acute effects. Temporal hormesis may be related to the classification of the drug molecule as an agonist, antagonist or an inverse agonist. This phenomenon may be a more general principal applicable in the treatment of other diseases apart from asthma and chronic heart failure.

Keywords: Temporal hormesis, Beta-adrenoceptor antagonist, Chronic therapy, Asthma, Heart failure

SUMMARY

In the past decade a major paradigm shift has occurred in the treatment of chronic heart failure, wherein some contraindicated drugs have been shown to be beneficial in the disease treatment (Bond, 2002). Furthermore, a recent paper from our laboratory suggests the possibility of using a currently contraindicated drug for chronic asthma therapy (Callaerts-Vegh *et al.*, 2004). These drugs (β-adrenoceptor blockers) are or have been contraindicated for asthma and CHF because when given acutely they worsen the symptoms of the disease. Possibly because it was assumed that the chronic effect would be similar to their acute effect, the chronic effects of these drugs were never investigated until recently. However, in both CHF and asthma chronic administration of the drugs resulted in improvement in the hallmark symptoms of the disease. In CHF the beneficial effect has been demonstrated both in animal models (Asanuma *et al.*, 2004) and human patients (Hall et al, 1995) whereas in asthma it has been studied only in animal models (Callaerts-Vegh *et al.*, 2004). Also, both disease treatments involve a G protein-coupled receptor (GPCR) termed the β2-adrenoceptor (β2AR). Evidence suggests that in CHF and in our murine model of asthma only drugs classified as β2AR inverse agonists are capable of producing the beneficial chronic effect. The data from both heart failure and the asthma model studies suggests that the duration of drug administration plays a major role in determin-

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414

ing its response (Bond, 2002 & Ellis, 2004). The beneficial action of the drug after chronic administration as opposed to its detrimental acute effects can be defined as temporal hormesis. This is different from classical hormesis, which is defined as the 'dose-response phenomenon characterized by low dose stimulation, high dose inhibition, resulting in either a J-shaped or an inverted U-shaped dose response' (Calabrese, 2004). Hence, classical hormesis is defined by the dose of the drug as the main criterion, while temporal hormesis can be defined relative to the duration of therapy. Examples and possible explanations of the phenomenon of temporal hormesis are discussed in more detail in the following sections.

BRIEF HISTORY OF INVERSE AGONISM AT G PROTEIN-COUPLED RECEPTORS

GPCRs are the largest superfamily of receptors and also the target molecules for almost two-thirds of the drugs on the market. In classical receptor theory, only two classes of ligands were considered to interact with receptors: agonist and antagonist. Receptors were believed to exist in a single quiescent, non-signaling state. Only after agonist binding would the activated receptor induce signaling. In this model, binding by antagonists produced no cellular signaling but simply prevented receptors from being bound and activated by agonists. Then, Costa and Herz demonstrated that receptors could be manipulated into a constitutive or spontaneously active state that produced cellular signaling in the absence of agonist occupation (Costa and Hertz, 1989). They also provided evidence that certain compounds could 'turn-off' or inactivate these spontaneously active receptors. The authors termed these compounds 'negative antagonists'. The term 'negative antagonist' has now been largely replaced by the term 'inverse agonist'. In the ensuing years, many studies have provided further evidence that GPCRs exist in constitutively or spontaneously active states that are inactivated by inverse agonists [for reviews see, (de Ligt *et al.*, 2000 & Milligan *et al.*, 1995)]. The β_2AR is a prototype GPCR for studying inverse agonism, the first *in vivo* report of constitutive activity and inverse agonism was shown in β_2AR (Samama *et al.*, 1993; Chidiac *et al.*, 1994; Samama *et al.*, 1994 & Bond *et al.*, 1995).

ANTAGONISTS VERSUS INVERSE AGONISTS

Inverse agonists are often viewed as a subset of antagonists because under conditions of low numbers of spontaneously active receptors, inverse agonists produce effects qualitatively similar to those of antagonists. However, it is important to differentiate the properties of antagonists from those of inverse agonists. In the two-state model, receptors exist in equilibrium between an inactive state (R), and a spontaneously active state (R*) (Milligan *et al.*, 1995; Bond *et al.*, 1995 & Leff, 1995). Agonists preferentially bind to and enrich the number of receptors in the R* state

415

N. Dudekula et al.

and decrease the receptors in the R state. Inverse agonists preferentially bind to and enrich the number of receptors in the inactive R state thereby decreasing the numbers in the R* state. Antagonists bind with equal affinity to both R and R* and do not change their numbers, but do prevent both agonists and inverse agonists from producing their effects.

Agonists and inverse agonists modulate cellular activity in a reciprocal manner (Table 1). For example, inverse agonists move 'baseline' receptor activity in a direction opposite to that produced by agonist. This 'reciprocity' of agonists and inverse agonists seems to hold true for all parameters investigated so far. Furthermore, this reciprocity implies that inverse agonists alter signaling on their own. Their effects are not solely attributable to the prevention of agonist activity as would be true for antagonists. For example, ligands now classified as inverse agonists can produce upregulation of β_2ARs after the depletion of agonists (Elfellah & Reid, 1989). It is possible that the reciprocity of agonists and inverse agonists extends to their ability to modulate cellular signaling. Agonists given acutely increase signaling by receptor activation and chronic agonist treatment desensitizes and decreases signaling, this cellular signaling is manifested in the organism as the physiological effects (hence, increased signaling = increased effect/response and decreased signaling = decreased effect/response). In contrast, inverse agonists acutely decrease cellular signaling yet may be able to chronically increase signaling (highlighted box of Table 1). We believe it is this reciprocity that accounts for the temporal hormesis observed with drug treatment of certain diseases (Figure 3).

TABLE 1 Comparison of agonist and inverse agonist properties

Agonist	Inverse agonist
Promotes formation of more **active** receptor (**R***)	Promotes formation of more inactive receptor (R)
Promotes receptor-G protein coupling	Decreases receptor-G protein coupling
Promotes phosphorylation by GRK	Prevents phosphorylation by GRK
Promotes endocytosis and downregulation of receptor	Promotes upregulation of cell surface receptors
Promotes conformational changes (decreases fluorescence emission)	Promotes conformational changes (increases fluorescence emission)
Homologous desensitization	Homologous sensitization
Heterologous desensitization	Heterologous sensitization
ACUTELY PROMOTES EFFECT	**ACUTELY INHIBITS EFFECT**
CHRONICALLY INHIBITS EFFECT	**CHRONICALLY PROMOTES EFFECT**

Both agonists and inverse agonists effects are blocked by antagonist (modified from Bond, *et al.*, 2001)
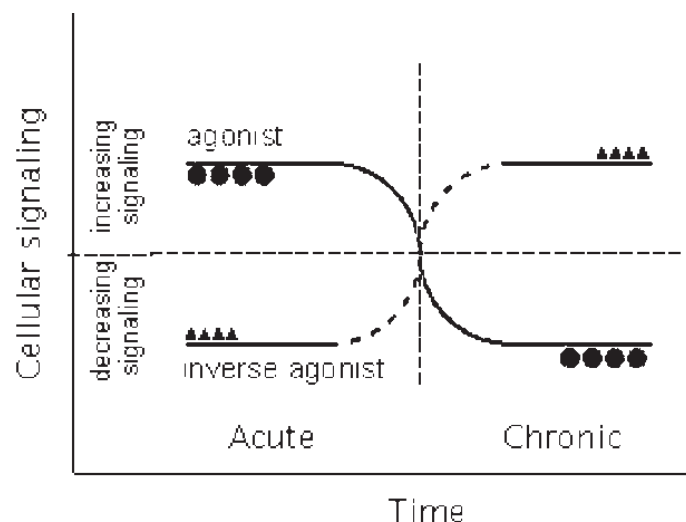
416

The Temporal Hormesis of Drug Therapies

FIGURE 1 A schematic representing the fundamental hypothesis of temporal Hormesis, that agonists and inverse agonists have temporally reciprocal effect on cellular signaling.

BENEFICIAL EFFECTS OF CHRONIC TREATMENT WITH 'β-BLOCKERS'

CHF is a major health concern associated with a high degree of mortality. Recently, a major paradigm shift in the chronic treatment of CHF has lead to the widespread clinical use of a previously contraindicated class of drugs, 'β-blockers'. In CHF there is a decrease in cardiac contractility and the heart is unable to meet the blood and oxygen demands of the body. The body responds to the diminished output by elevating the levels of norepinephrine and epinephrine delivered to the heart. This results in the chronic activation of the βAR system, eventually leading to desensitization of the receptors and a decrease in the βAR density in the heart (Bristow *et al.*, 1982).

Since the failing heart shows a decreased contractility, positive inotropic agents are used to increases cyclic AMP levels to improve cardiac output. The βAR agonists dobutamine and xamoterol increase myocardial contractility and short-term administration of dobutamine results in an improved cardiac performance (Weber *et al.*, 1982; Stoner *et al.*, 1977 & Leier *et al.*, 1977). However, placebo-controlled clinical trials reported an increase in mortality among patients treated long term with intravenous dobutamine (Weber *et al.*, 1982 & Dies *et al.*, 1986) and xamoterol (1990, The Xamoterol in Severe Heart Failure Study Group). Therefore the use of βAR agonists produces initial cardiac improvements, but long-term administration results in loss of βAR function and a drop of cardiac function in failing hearts, and ultimately in an increased risk of mortality.
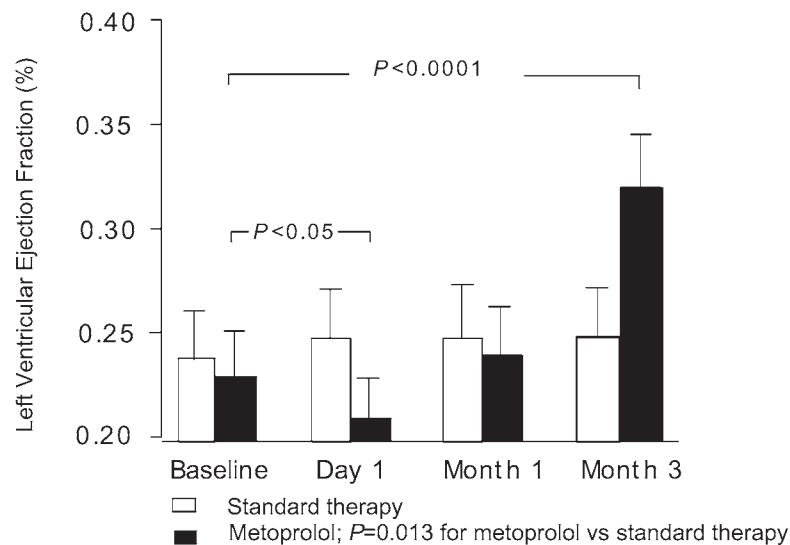
417

N. Dudekula et al.

FIGURE 2 The left ventricular ejection fraction was measured using Echocardiograph. Changes in the left ventricular ejection fraction from baseline to day 1, month 1 and month 3 in the metoprolol and standard therapy groups were recorded. The standard therapy was a long term angiotensin converting enzyme inhibitor except in two patients who received isosorbide dinitrate/hydralazine. Ejection fraction decreased about 25% on day 1 with acute metoprolol administration and increased only after 1 month of metoprolol therapy and by the 3rd month the metoprolol treated patients showed a significant increase in the left ventricular ejection fraction. *(Reprinted with permission from JACC)*
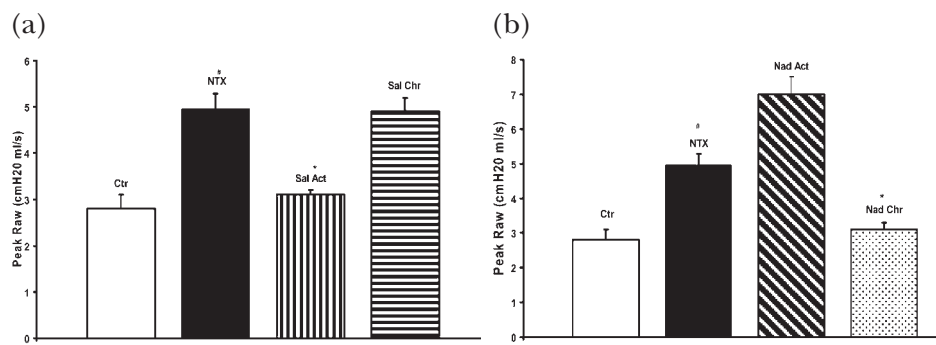
(a)   (b)

FIGURE 3 Effects of administration of β-AR ligands on the peak airway responsiveness to cholinergic stimulation. Peak Raw was determined for each mouse by examining the individual methacholine dose-response curves and choosing the highest Raw value produced by any of the methacholine doses (most often next to the last dose, 408 µg.kg^{-1}.min^{-1}). Shown are the mean peak Raw $\pm$ SEM after treatments with the β-AR agonist salbutamol (A) and after treatments with β-AR inverse agonist nadolol (B), after both acute and chronic administration, in comparison with non treated asthmatic mice (NTX; black bars, n = 7–25) and control mice (Ctr; white bars, n = 6–21). Note the change in the scale of y axis for B. *, $P \leq 0.05$ compared to NTX; #, $P \leq 0.05$ compared to Ctr (ANOVA). *(Reprinted with permission from PNAS)*

The class of drugs termed 'β-blockers', is comprised of βAR antagonists and inverse agonists, and has been shown to have negative inotropic activity and produce decreases in cardiac output (Kukin *et al.*, 1999). Therefore treating a system with decreased contractility, such as heart fail-

418

The Temporal Hormesis of Drug Therapies

ure, with β-blockers was contraindicated. However, studies have shown that chronic treatment with certain 'β-blockers' can improve cardiac function and decrease mortality (Lechat *et al.*, 1998). Though initially this treatment may be associated with a further decrease in cardiac output and detrimental effects, chronic treatment over several months leads to a significant improvement of cardiac function (Hall *et al.*, 1995 & Packer *et al.*, 1996). Several long-term clinical trials have been conducted using the 'β-blockers' carvedilol (Packer, 1996) and metoprolol (Hjalmarson *et al.*, 2000 & MERIT-HF Study Group, 2000) each resulting in improved cardiac function and decreased mortality (Krum, 1997). In contrast, a recent large-scale trial with the 'β-blocker' bucindolol failed to show a significant beneficial effect (β-blocker Evaluation of Survival trial Investigators, 2001). In summary, chronic treatment with certain 'β-blockers', has been shown to decrease mortality and improve cardiac function, while bucindolol produced no effect.

IS THE INVERSE AGONISM OF 'β-BLOCKERS' THE REASON FOR THERAPEUTIC BENEFIT?

The differential therapeutic effects of carvedilol, metoprolol and bucindolol raise the question as to what distinguishes these compounds at the mechanistic level. A recent study examined the antagonist or inverse agonist properties of these three compounds. In cardiac myocytes from failing human hearts pretreated with forskolin to amplify any constitutive signaling, metoprolol and carvedilol both functioned as inverse agonists, while bucindolol behaved as an antagonist (Maack *et al.*, 2000). Thus, both compounds that are beneficial in the chronic treatment of heart failure and have acute adverse effects are inverse agonists. In contrast, the compound with no chronic benefits, bucindolol behaved as an antagonist. Though this study did not examine which βAR subtype was involved in mediating the inverse agonist effects of the compounds, it is likely to be the β_2-subtype for the following reasons: (1) Although metoprolol is classified as a preferential or cardio selective β-blocker, its selectivity for β_1AR over β_2AR is very poor and can be as low as 2 fold (Baker, 2005 & Flesch *et al.*, 2001). Thus at the concentrations normally used in heart failure, this ligand would also be occupying β_2ARs. (2) Reduction in mortality was greater in patients treated with non-selective as compared to selective β_1AR blocking agents (Lechat *et al.*, 1998). (3) β_1ARs are downregulated in heart failure, while β_2ARs are not (Brodde *et al.*, 1992) resulting in an increase in the β_2/β_1 ratio. Several studies have shown that the β_2AR exhibits more spontaneous activity than the β_1AR. For example, mice with cardiac overexpression of the β_2AR exhibit greater increases in cardiac contractility than mice overexpressing the β_1AR (Engelhardt *et al.*, 1999 & Milano *et al.*, 1994). (4) In a murine coronary artery occlusion model of heart failure, we have observed beneficial effects of an inverse agonist (carvedilol) but not an

419

N. Dudekula et al.

antagonist (alprenolol) suggesting the murine model behaves similarly (Callaerts-Vegh *et al.*, 2004). In summary, currently available data all indicate that chronic treatment with inverse agonists is beneficial in a disease state characterized by impaired βAR signaling.

ASTHMA, SIMILAR DRUGS, OUTCOMES AND RECEPTORS: PARADIGM SHIFT?

Asthma affects an estimated 20 million Americans and its incidence is increasing. Although diagnosis and medications have improved the management of asthma, the age adjusted mortality rate increased 55.6% between 1979 and 1998 (American Lung Association: Epidemiology and Statistics Unit, 2004). The increased incidence and mortality clearly asks for a better understanding of the physiology and pharmacology underlying the asthma disease state. Interestingly, asthma and CHF display significant similarities in their pharmacology.

Acute treatment with βAR agonists is beneficial in both diseases yet chronic treatment can be detrimental. For example, β_2AR agonists induce bronchodilation, and are the most commonly used drugs for treatment of asthma. A clinical trial testing the chronic use by asthmatics of a long lasting β_2AR agonist, salmeterol, was recently terminated due to increased deaths in some patient groups (FDA, 2003), similarly chronic use of βAR agonists has been reported to increase mortality in heart failure patients (Packer, 1995). The detrimental effect of chronic βAR agonist treatment has also been demonstrated in animal models of asthma (Hoshiko & Morley, 1993 & Mazzoni *et al.*, 1994). It was shown recently that modest overexpression of the human β_2AR in transgenic mice increased the airway response to spasmogens via an upregulation of phospholipase C-β1 (McGraw *et al.*, 2003). Conversely, knocking out β_1 and β_2-ARs led to a *decrease* in airway response to spasmogens. The authors termed the phenomena 'antithetical regulation' and suggested their results warrant testing of 'β-blockers' in asthma. While the mortality issue due to chronic agonist use in asthmatics is multifactorial, one of the risk factors for death from asthma is the excess use of inhaled β_2AR agonists (Spitzer *et al.*, 1992 & National Asthma Education and Prevention Program, 1997).

Conversely, acute treatment with βAR inverse agonists is detrimental for both CHF and asthma patients. βAR inverse agonists can acutely result in bronchoconstriction and are currently contraindicated in asthmatics (Hua *et al.*, 1978). Asthma patients may experience severe airway narrowing after an acute dose of β-blocker, similar to the initial reduction in left ventricular ejection fraction (an index of cardiac contractility), in CHF patients. Thus, there are remarkable similarities between the pharmacological modulation of the β_2AR in both CHF and asthma. Nevertheless, aside from our recent study (Callaerts-Vegh *et al.*, 2004) the

420

The Temporal Hormesis of Drug Therapies

effect of chronic treatment with βAR antagonists or inverse agonists in asthma has not been tested. Although asthma and heart failure are different diseases, very similar ligands are being used in their treatment; the ligands act at the same receptor systems, and produce similar outcomes, i.e. acutely detrimental and chronically beneficial.

β-ADRENOCEPTOR LIGANDS AND ASTHMA

We administered the β2AR agonist salbutamol and the inverse agonist nadolol acutely and chronically to study their effects on the airway responsiveness in a murine model of asthma. When the drugs were administered acutely, we observed the classical response of the β-adrenoceptor agonists in asthma therapy. Salbutamol effectively reduced airway resistance by causing bronchodilation, while nadolol worsened the symptom triggering exaggerated bronchoconstrictor response (Figure 3a & 3b), which is in accordance with previously published data (Boskabady & Snashall, 2000). This exaggerated bronchial response of airways on acute β-blocker administration is responsible for this drug class to be contraindicated in asthma therapy.

However, some of the chronic results were not as expected. As has been reported in earlier studies, we also observed a loss in response to the agonist salbutamol with chronic therapy, wherein the airway response to methacholine was similar to that of an untreated asthmatic mouse (Figure 3a) (Tamaoki *et al.*, 2004). However, chronic treatment with nadolol had an opposite response compared to nadolol administered acutely. Chronic treatment with nadolol produced significantly reduced airway responsiveness (Figure 3b). This supports our hypothesis that given chronically, some β-adrenoceptor antagonists/inverse agonists may effectively alleviate the symptoms of asthma. The mechanism by which this beneficial effect occurs is still not clear but may involve an increase in β-adrenoceptor number in the airways of the animals treated chronically with nadolol (Callaerts-Vegh *et al.*, 2004).

These studies suggests that treatment with some β-blockers may be useful in asthma therapy, but a major concern of using these drugs in patients would be the bronchoconstrictor response observed when the drug is administered initially. Ways to avoid this unwanted effect of the drug could be by starting with a very low dose of the antagonist, as is done in heart failure treatment, and then increasing the dose over a period of time, or the blocker can initially be given in combination with an agonist to reduce the unwanted response in patients. While there is always risk in extrapolating from mice to men, this study may provide another example of the temporal hormesis observed with some drug therapies.

421

N. Dudekula et al.

IS TEMPORAL HORMESIS AN ISOLATED INCIDENT OR A GENERAL PHENOMENON?

Both the above examples clearly show opposing effects of drugs depending on the duration of therapy. The agonist and the antagonist/inverse agonist produce opposite responses dependent upon whether given acutely or chronically. To assume that this phenomenon of temporal hormesis is an isolated incident seen only in heart failure or asthma (since both involve β-adrenoceptors) may be presumptuous. A good example of the temporal hormesis is in pain management. Upon chronic administration of morphine (an opioid agonist), tolerance develops to its analgesic effect. *Crain and Shen*, in 1998, reviewed the literature showing that chronic administration of morphine with an opioid antagonist/inverse agonist (such as naltrexone) was more effective in pain management then morphine alone (Crain & Shen, 1998). They speculated this enhancement in morphine activity in conjunction with naltrexone was due to the antagonist preventing the morphine-induced tolerance. These authors helped to form a company, *Pain Therapeutics*, which now has a combination product of opioid agonist and antagonist/inverse agonist in Phase III trials. There are also other diseases in which patients become refractory to chronic agonist treatment. For example, many studies have shown a loss of response in Parkinson's patients treated chronically with dopamine agonists (Gerlach & Riederer, 2003). All of the above diseases involve receptors that belong to the superfamily of G protein-coupled receptors (GPCRs). These receptors are often regulated by related kinases involved in desensitization; therefore, it is possible that these diseases could also be treated with either blockers or the combination of agonist and blocker. Temporal hormesis may turn out to be a general phenomenon of GPCRs, at least in cases where the agonist loses effectiveness when administered over a long period of time.

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422

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423

N. Dudekula et al.

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424