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Antinociceptive activity of sildenafil and adrenergic agents in the writhing test in mice

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Abstract:
The authors investigated the antinociceptive activity of sildenafil and adrenergic agents co-administered in the writhing test in mice. The intensity of nociception was quantified by the number of writhes occurring between 0 and 30 min after stimulus injection. Nontreated groups (NT) received acid intraperitoneally (*ip*) followed by sterile saline (*ip*). Animals received (*ip*) sildenafil (2.5 or 5 mg/kg), propranolol (0.5 or 2 mg/kg), atenolol (0.05 or 2 mg/kg), prazosin (0.05 or 0.25 mg/kg) or clonidine (0.01 or 0.1 mg/kg) 30 min before acid injection. It was observed that only the largest doses of every drug inhibited the number of writhes in mice. In another series of experiments, animals were pretreated with the lower ineffective doses of propranolol, atenolol, prazosin or clonidine. After 30 min, mice also received the lower ineffective dose of sildenafil followed by acid injection. The combination of ineffective doses of propranolol, atenolol, prazosin or clonidine with sildenafil significantly inhibited the nociceptive response induced by acetic acid injection. Data obtained from these experiments showed that ineffective doses of sildenafil associated with ineffective doses of adrenergic agents provided analgesic effects in the writhing test.

Key words:
sildenafil, antinociception, pain, acetic acid, mice

Introduction

The mechanism of inflammatory hyperalgesia results from excitatory actions of endogenous mediators that sensitize or stimulate the free nervous terminals, that is, the nociceptors. Therefore, it has been proposed that inflammatory pain mediators should be classified into two types: direct activators of nociceptors, such as histamine; and nociceptor up-regulators that directly sensitize the nociceptors, such as prostaglandins, cytokines or sympathomimetic amines [16, 29].

The sympathetic nervous system can modulate the functions of nociceptors by a neuronal increase of Ca^{2+}/3'-5'-cyclic adenosine monophosphate (cAMP) [14]. Inflammatory pain depends on the stimulation of the cAMP-protein kinase A pathway, whereas the 3'-5'-cyclic guanosine monophosphate (cGMP)-protein

kinase G pathway has the opposite action [7, 24]. The cytosolic levels of cAMP and cGMP are controlled by peripheral phosphodiesterases (PDE). Two major families of PDE have been implicated in nociception, including cAMP-specific PDE-4 and cGMP-specific PDE-5 [19].

Sildenafil is a selective PDE-5 inhibitor that is widely known because of its therapeutic efficacy in erectile dysfunction by increasing intracellular cGMP levels [9, 22]. While maintaining an excellent safety and tolerability profile in the management of erectile dysfunction, sildenafil also provides a prolonged benefit in various other diseases [30]. It has been suggested that increasing intracellular cGMP levels were also associated with antinociceptive properties of the sildenafil [12].

Considering that inflammatory pain can result from a complex coordination involving sympathomimetic amines, the cAMP and cGMP levels, the present study was designed to investigate the effect of adrenergic agents on the sildenafil-induced antinocicecption.

Materials and Methods

Animals

Hundred and eight male Swiss mice (25–30 g) from the authors' own animal facilities were used throughout the experiments. They were maintained at controlled ambient temperature (20–22°C) to avoid environmental disturbances that might influence the response of animals. All efforts were made to minimize animal suffering and the number of animals used. The study protocol was approved by the local Committee of Animal Use and Care, in accordance with the "Guide for the Care and Use of Laboratory Animals" from the Brazilian College of Animal Experimentation (COBEA).

Measurement of antinoceptive activity

The nociception was assessed by the writhing test [2]. Briefly, acetic acid, (0.1 ml of a 0.6% v/v solution per 10 g of body weight) was injected *ip* in mice. These animals were placed in a large glass cylinder and the intensity of nociception was quantified by counting the total number of writhings occurring between 0 and 30 min after stimulus injection. The writhing response is characterized by a wave of contractions of the abdominal musculature followed by extension of the hind limbs.

Drugs

All agents (indomethacin, propranolol, atenolol, prazosin, clonidine) were purchased from either Sigma Chemical Company (St. Louis, MO, USA) or Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, UK). Sildenafil (Viagra®) was kindly provided by Pfizer (Sandwich, Kent, UK).

Study design

To assess the effect of the test drugs *per se*, animals received (*ip*) either sildenafil (2.5 or 5 mg/kg), a non-selective β-adrenoceptor antagonist propranolol (0.5 or 2 mg/kg), selective β_1-adrenoceptor antagonist atenolol (0.05 or 2 mg/kg), selective α_1-adrenoceptor antagonist prazosin (0.05 or 0.25 mg/kg) or α_2-adrenoceptor agonist clonidine (0.01 or 0.1 mg/kg) 30 min before acetic acid injection. Sildenafil was administered 5 min prior to stimulus injection [5]. To validate the data, a positive control was pretreated (*ip*) with indomethacin (5 mg/kg). Nontreated groups (NT) consisted of mice that received just acetic acid (*ip*) followed by 0.9% sterile saline (*ip*). Sildenafil dose selection was based on a previous study [12]. The largest doses of adrenergic agents were selected in accordance with literature [10, 11, 26, 27, 31].

To analyze the effect of adrenergic agents on sildenafil-induced antinociception, another series of experiments was performed. At this time, animals were pretreated (*ip*) with the lower doses of adrenergic agents: propranolol (0.5 mg/kg), atenolol (0.05 mg/kg), prazosin (0.05 mg/kg), or clonidine (0.01 mg/kg). After 30 min, the animals received (*ip*) the lower dose of sildenafil (2.5 mg/kg) followed 5 min later by acetic acid injection (*ip*). The total number of writhes was counted for the next 30 min.

Statistical analysis

Results are presented as the mean ± SEM of measurements made on at least five animals in each group. Differences between means were compared using one-way ANOVA followed by Tukey's test. In the test, the criterion for statistical significance was $p < 0.05$.

Results

Effect of pretreatment with sildenafil (2.5 or 5 mg/kg) on the writhing response to acetic acid

The injection (*ip*) of 0.6% (v/v) solution of acetic acid (0.1 ml/10 g) in mice induced a significant writhing response between 0 and 30 min later, which was significantly ($p < 0.05$) inhibited by the pretreatment with indomethacin (5 mg/kg). Sildenafil (5 mg/kg) injected (*ip*) 5 min prior to the stimulus injection showed a significant 57.1% inhibition of the nociceptive response ($p < 0.05$) as compared to NT group (Fig. 1). Although the lower dose of sildenafil (2.5 mg/kg) tended to reduce the number of writhes, it failed to exhibit significant ($p > 0.05$) antinociceptive effect.

Effect of pretreatment with adrenergic agents (propranolol, atenolol, prazosin, clonidine) on the writhing response to acetic acid

Although the lower dose of propranolol (0.5 mg/kg) or atenolol (0.05 mg/kg) tended to reduce the number of writhes, both failed to exhibit significant ($p > 0.05$) effect. However, propranolol (2 mg/kg) and atenolol (2 mg/kg) significantly inhibited ($p < 0.05$) the nociceptive response by 53.6% and 57.4%, respectively, as compared to NT group (Fig. 2A). Similarly, the lower doses of prazosin (0.05 mg/kg) or clonidine (0.01 mg/kg) tended to reduce the writhing response, although without reaching statistical significance ($p > 0.05$). Curiously, the largest inhibition (almost 100%) was observed when α-adrenergic agents were given. In fact, prazosin (0.25 mg/kg) or clonidine

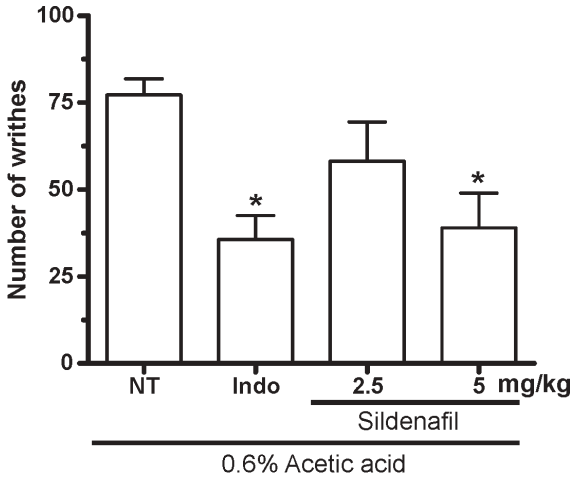

Fig. 1. Effect of systemic administration of sildenafil on the writhing response induced by acetic acid in mice. The number of writhes was determined between 0 and 30 min after injection (*ip*) of acetic acid at 0.6% (v/v), 0.1 ml/10 g of body weight. A positive control was pretreated (*ip*) with indomethacin (Indo) (5 mg/kg). Sildenafil (2.5 or 5.0 mg/kg, *ip*) was given 5 min before acetic acid. Data are expressed as the mean ± SEM of 6 mice for each group. * $p < 0.05$ indicates significant difference from the nontreated (NT) group (ANOVA, Tukey's test)

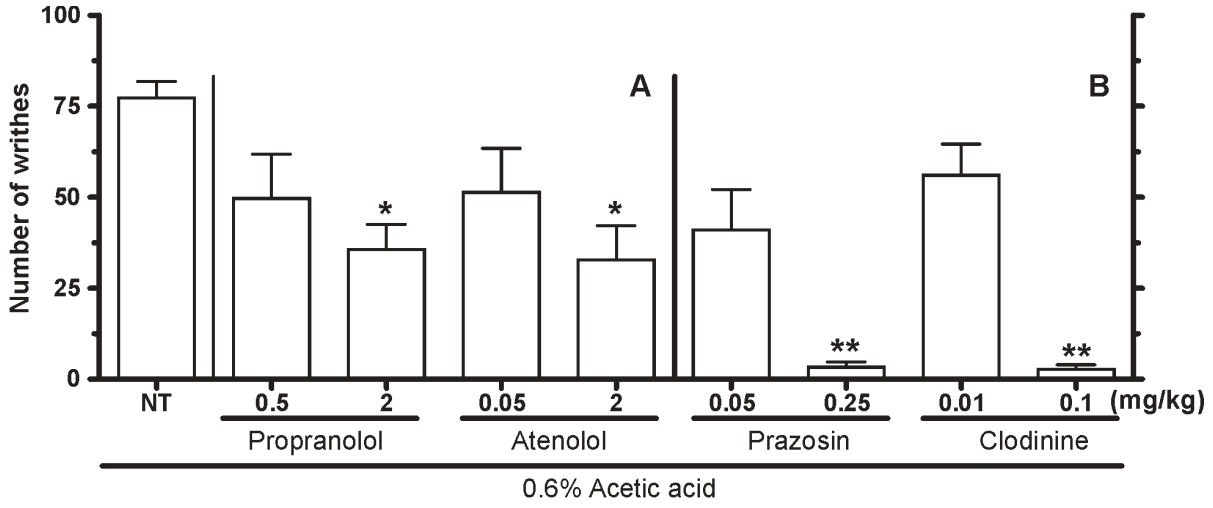

Fig. 2. Effect of systemic administration of adrenergic agents on the writhing response induced by acetic acid in mice. The number of writhes was determined between 0 and 30 min after *ip* injection of acetic acid at 0.6% (v/v), 0.1 ml/10 g of body weight. Propranolol (0.5 or 2.0 mg/kg), atenolol (0.05 or 2.0 mg/kg), prazosin (0.05 or 0.25 mg/kg) or clonidine (0.01 or 0.1 mg/kg) was given *ip* 30 min before acetic acid. Data are expressed as the mean ± SEM of 6 mice for each group. * $p < 0.05$ or ** $p < 0.01$ indicates significant difference from the nontreated (NT) group (ANOVA, Tukey's test)

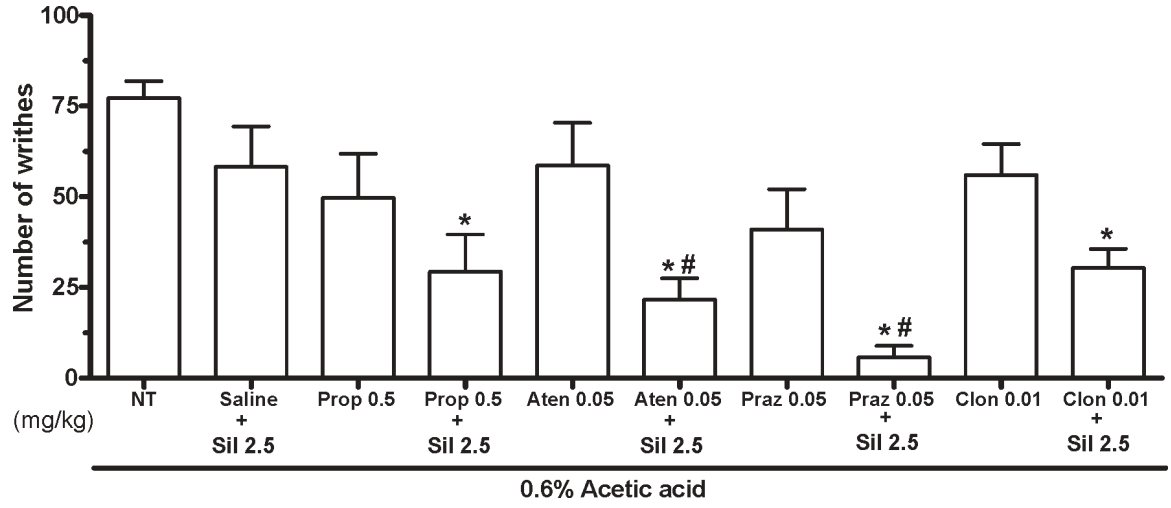

Fig. 3. Effect of systemic administration of adrenergic agents on sildenafil-antinociception on the writhing response induced by acetic acid in mice. The number of writhes was determined between 0 and 30 min after *ip* injection of acetic acid at 0.6% (v/v), 0.1 ml/10 g of body weight. Propranolol (Prop 0.5 mg/kg), atenolol (Aten 0.05 mg/kg), prazosin (Praz 0.05 mg/kg) or clonidine (Clon 0.01 mg/kg) was given *ip*. After 30 min, sildenafil (2.5 mg/kg, *ip*) was given; 5 min after that acetic acid was injected. Data are expressed as the mean ± SEM of 6 mice for each group. * $p < 0.05$ indicates significant difference from the nontreated (NT) group and # $p < 0.05$ indicates significant difference from the respective adrenergic agent (ANOVA, Tukey's test)

(0.1 mg/kg) significantly ($p < 0.01$) inhibited the nociceptive response by 95.7% and 96.5%, respectively, as compared to NT group (Fig. 2B).

Effect of propranolol, atenolol, prazosin or clonidine on the sildenafil-induced antinociception on the writhing response to acetic acid

The combination of ineffective doses of sildenafil (2.5 mg/kg) with propranolol (0.5 mg/kg), atenolol (0.05 mg/kg), prazosin (0.05 mg/kg) or clonidine (0.01 mg/kg) significantly ($p < 0.05$) inhibited the nociceptive response by 62%, 72%, 92.7% and 60.6%, respectively, as compared to NT group (Fig. 3).

Discussion

This paper presents a limited amount of data concerning the interaction of the noradrenergic system and the cGMP pathway in inflammatory pain modulation. Intracellular cGMP concentrations are regulated by the action of guanylyl cyclases and by the rate of degradation by GMP-specific PDE [23]. Sildenafil is a novel inhibitor of the cGMP-specific PDE-5 [9]. Experimental evidence suggests that sildenafil produces peripheral antinociception, and the cGMP level increase would account for the sildenafil-induced antinociceptive effect [19]. Whereas the activation of cGMP pathway is associated with inhibition of hyperalgesia, cAMP is produced during inflammatory reactions, and its enhancement is associated with worsening of inflammatory hyperalgesia [4]. In the present study, the authors used an ineffective dose of sildenafil with ineffective doses of adrenergic agents (propranolol, atenolol, prazosin or clonidine) that resulted in significant inhibition of the nociceptive response in the writhing test. Furthermore, the effect of sildenafil with α-adrenoceptor agonist was larger than with β-adrenoceptor antagonists. This increased effect of the sildenafil is not due to drug metabolic interaction because sildenafil is metabolized primarily by cytochrome P450 3A4 and adrenergic agents are not considered to be inhibitors of this enzyme [13]. To the best of the authors' knowledge, this is the first report to explore this increased antinociceptive response produced by the combination of sildenafil and adrenergic agents.

Some authors have focused on the role of α_1-adrenoceptors in nociception as their blockade attenuates pain [11]. In the present study, the authors observed that prazosin (selective α_1-adrenoceptor antagonist) inhibited (by almost 100%) the nociceptive response. Furthermore, when ineffective doses of sildenafil and

prazosin were administered concomitantly, the writhing response was significantly inhibited. Although the antinociceptive mechanisms of α-adrenoceptor antagonists are not clear, prazosin effects might be a result of changes in cAMP levels, because *in vitro* α_1-adrenoceptor stimulation leads to cAMP accumulation [8]. In humans, the α_1-adrenoceptor blockade can be associated with the occurrence of cardiovascular intolerance, orthostatic hypotension, headaches, dizziness, and tiredness [17]. Thus, small doses of sildenafil and prazosin when administered concomitantly could provide increased antinociception displaying less adverse effects. Besides, α_2-adrenoceptor agonists are used in clinical practice for the treatment of acute pain events and prevention of postoperative pain [6]. In the present study, clonidine (an α_2-adrenoceptor agonist) significantly reduced the writhing counts. This result was consistent with several observations indicating that the activation of α_2-adrenoceptors by clonidine inhibits substance P release [15], decreases tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β plasma concentration in postoperative patients [20], and produces analgesic effects in different types of pain models [21, 25]. In this regard, it has been demonstrated that clonidine analgesic effects are probably mediated by changes in K^+ current as clonidine administration results in cell hyperpolarization by increasing K^+ conductance [1].

Besides the role of α-adrenoceptors in analgesia, several authors also suggest that β-adrenoceptors play a role in inflammatory pain [3]. These receptors have been located in areas directly related to pain pathways and possess proinflammatory properties leading to IL-1β and IL-6 production [18]. The present report showed that although atenolol (a selective β_1-adrenoceptor antagonist) was effective at dose of 0.5 mg/kg (data not shown) at this same dose, propranolol (a nonselective β-adrenoceptor antagonist) was ineffective. Probably, this effect was because of the stimulus used in the present study, since it has been suggested that the class of β-adrenoceptors involved in pain control depends on the type of nociceptive stimulus. Although β_1- and β_2-adrenoceptors are both implicated when the stimulus is physical, only β_1 subtype is involved when the stimulus is chemical [17]. Furthermore, the authors also observed that ineffective doses of propranolol and sildenafil administered concomitantly significantly reduced the writhing response. It has been described that β-adrenoceptor stimulation is associated with the accumulation of cAMP [28]. Those associations may contribute to the therapeutic effectiveness and might further reduce side effects mediated by the blockade of β-adrenoceptors.

Taking into account these results, they are very encouraging for designing new analgesics. However, the pharmacological profile of these associations must be subject to further investigations.

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