ANTINOCICEPTIVE ACTIVITY OF TADALAFIL AND ADRENERGIC AGENTS IN THE WRITHING TEST IN MICE

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Summary

The authors investigated the antinociceptive activity of tadalafil 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) tadalafil (2.5 or 5 mg/kg), Metoprol (2 or 5 mg/kg) or methyl dopa (62.5 or 125 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 metoprolol and methyl dopa. After 30 min, mice also received the lower ineffective dose of tadalafil followed by acid injection. The combination of ineffective doses of metoprolol and methyldopa with tadalafil significantly inhibited the nociceptive response induced by acetic acid injection. Data obtained from these experiments showed that ineffective doses of tadalafil associated with ineffective doses of adrenergic agents provided analgesic effects in the writhing test.

Key words: Cyclic Adenosine Monophosphate, Cyclic Guanosine Monophosphate, Phosphodiesterase

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Introduction

The mechanism of hyperalgesia results from excitatory mediators that sensitize the nerve terminals that are the nociceptors. Inflammatory mediators evokes pain by either direct such as histamine or indirect mechanism such as norepinephrine, cytokines, or prostaglandins (1, 2). The sympathetic nervous system can play an important role in modulation of nociceptors by increase in cyclic adenosine monophosphate (cAMP) (3). Inflammatory pain depend on cAMP mediated protein kinase A pathway and cyclic guanosine monophosphate (cGMP) mediated protein kinase G pathway has opposite function. The cytosolic levels of cAMP and cGMP are regulated by phosphodiesterase (PDE). Two major families of PDE have been implicated in nociception, including cAMP specific PDE-4 and cGMP specific PDE-5 (4). Considering that inflammatory pain can result from a complex coordination involving sympathomimetic amines, the cAMP and cGMP levels, and the present study was designed to investigate the effect of adrenergic agents on the tadalafil-induced antinocicecption.

Materials and Methods

Animals

Sixty Swiss mice (25–30 g) 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 Institutional Animal Ethics Committee of Animal in accordance with the CPCSEA.

Measurement of antinociceptive activity

The nociception was assessed by the writhing test (5). Briefly, acetic acid, (0.1 ml of a 1% 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.

Study design

To assess the effect of the test drugs, animals received either tadalafil (2.5 or 5 mg/kg, p.o), a selective β_1-adrenoceptor antagonist metoprolol (2 or 5 mg/kg, p.o.), α_2-adrenoceptor agonist methyl dopa (62.5 or 125 mg/kg, p.o.) 30 min before acetic acid injection. Tadalafil was administered 15 min prior to stimulus injection. To validate the data, a positive control was pretreated with diclofenac (1 mg/kg, p.o.). Nontreated groups (NT) consisted of mice that received just acetic acid (ip) followed by 0.9% sterile saline (ip). The doses of tadalafil and adrenergic agents were selected by trial and error. To analyze the effect of adrenergic agents on tadalafil- induced antinociception, another series of experiments was performed. At this time, animals were pretreated with the lower doses of adrenergic agents: metoprolol (2 mg/kg, p.o.), methyl dopa (62.5 mg/kg,

p.o.). After 30 min, the animals received the lower dose of tadalafil (2.5 mg/kg, p.o) followed 15 min later by acetic acid injection (*ip*). The total number of writhes was counted for the next 30 min.

Statistical Analysis

Data are expressed as mean ± SEM. One way ANOVA and tuckey's multiple comparison test were used for the statistical analysis.

Results

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

The injection (ip) of 1% (v/v) solution of acetic acid (1 ml/100 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 diclofenac sodium (1 mg/kg). Tadalafil (5 mg/kg) administered orally 15 min prior to the stimulus injection showed a significant inhibition of the nociceptive response ($p < 0.05$) as compared to NT group (Fig. 1). Although the lower dose of tadalafil (2.5 mg/kg) tended to reduce the number of writhes, it failed to exhibit significant ($p > 0.05$) antinociceptive effect.

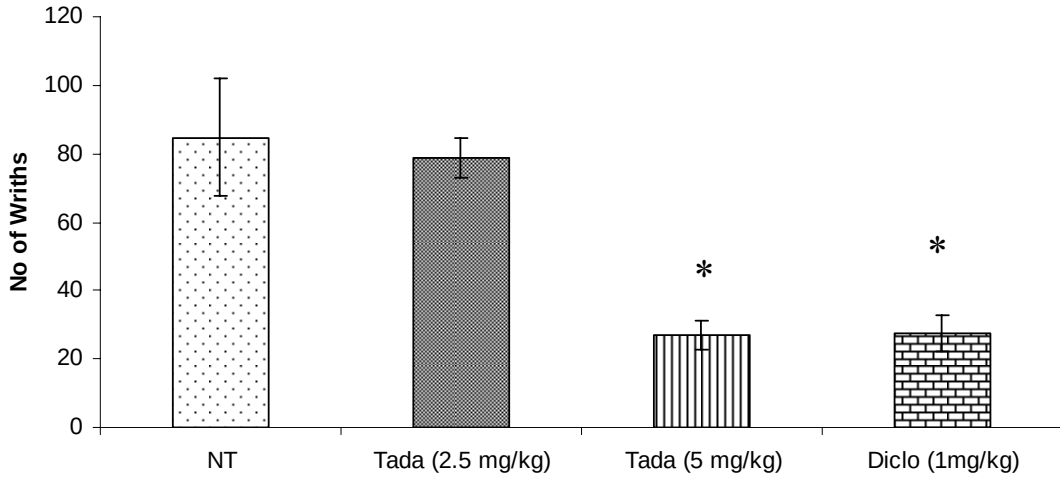

Fig.1: Effect of systemic administration of tadalafil on writhing response induced by acetic acid in mice. The number of wriths was determined between 0 and 30 min after injection (ip) of acetic acid, 1% v/v of solution, 0.1 ml/ 10 gm of body weight. A positive control was pretreated with diclofenac sodium (Diclo) (1 mg/kg, p.o.). Tadalafil (Tada) (2.5 or 5 mg/kg, p.o.) was given 15 min before acetic acid injection. Data are expressed as mean ± SEM of 6 mice of each group. *$p<0.05$ indicates significant difference from nontreated group (NT) group (One way ANOVA, tuckey's test).

Effect of pretreatment with adrenergic agents (metoprolol and methyldopa) on the writhing response to acetic acid

Although the lower dose of metoprolol (2 mg/kg) or methyl dopa (62.5 mg/kg) tended to reduce the number of writhes, both failed to exhibit significant ($p > 0.05$) effect. However, metoprolol (5 mg/kg) and methyl dopa (125 mg/kg) significantly inhibited ($p < 0.05$) the nociceptive response as compared to NT group (Fig. 2).

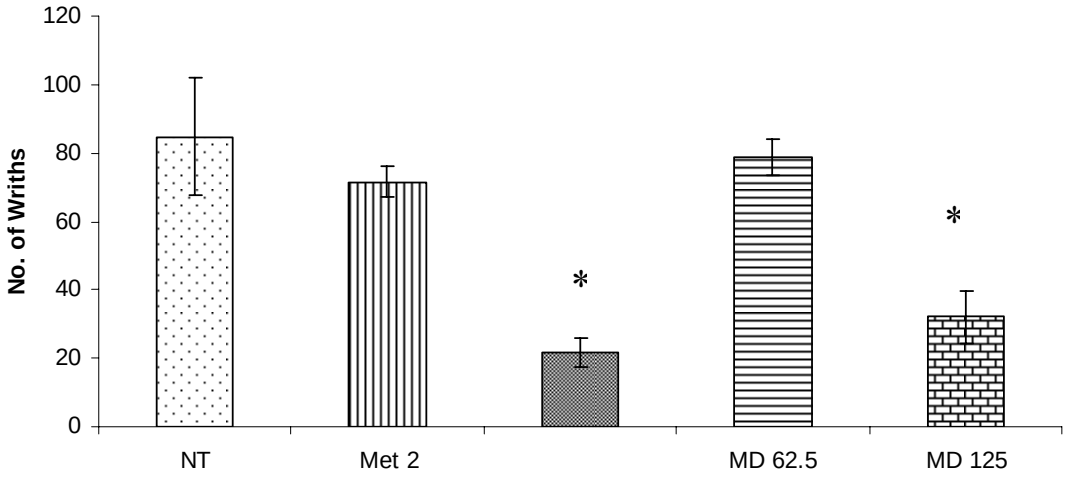

Fig.2: Effect of systemic administration of adnergic agents on writhing response induced by acetic acid in mice. The number of writhes was determined between 0 and 30 min after injection (ip) of acetic acid, 1% v/v of solution, 0.1 ml/ 10 gm of body weight. Metoprolol (Met) (2 or 5 mg/kg, p.o.) or Methyl dopa (MD) (62.5 or 125 mg/kg, p.o.) was given 15 min before acetic acid injection. Data are expressed as mean ± SEM of 6 mice of each group. *$p<0.05$ indicates significant difference from nontreated group (NT) group (One way ANOVA, tuckey's test).

Effect of metoprolol and methyl dopa on the tadalafil-induced antinociception on the writhing response to acetic acid

The combination of ineffective doses of tadalafil (2.5 mg/kg) with metoprolol (2 mg/kg), or methyl dopa (62.5 mg/kg) significantly ($p < 0.05$) inhibited the nociceptive response by as compared to NT group (Fig. 3).

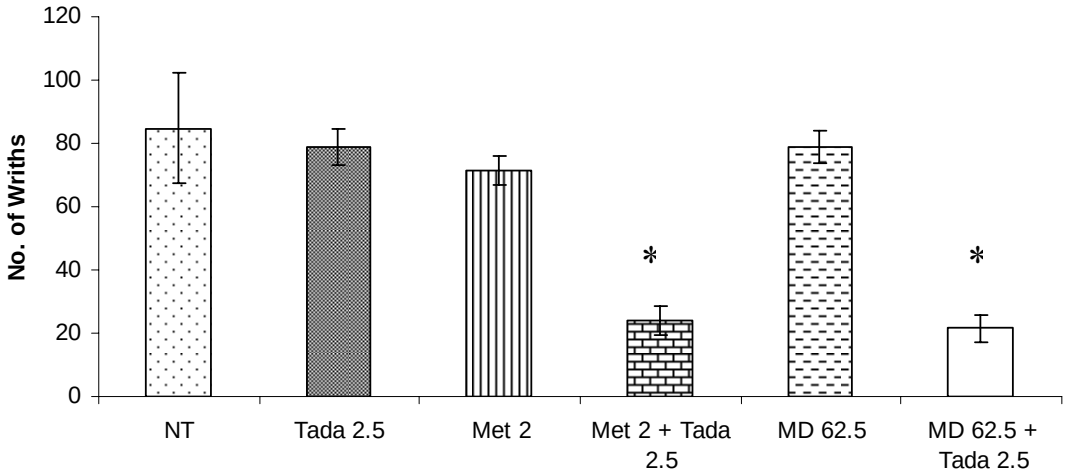

Fig.3: Effect of systemic administration of adnergic agents on tadalafil induced antinociception on writhing response induced by acetic acid in mice. The number of wriths was determined between 0 and 30 min after injection (ip) of acetic acid, 1% v/v of solution, 0.1 ml/ 10 gm of body weight. Metoprolol (Met) (2 mg/kg, p.o.) or Methyl dopa (MD) (62.5 mg/kg, p.o.) was given. After 15 min tadalafil (2.5 mg/kg, p.o.) was given; 15 min after acetic acid was injected. Data are expressed as mean ± SEM of 6 mice of each group. *$p<0.05$ indicates significant difference from nontreated group (NT) group (One way ANOVA, tuckey's test).

Discucssion

In inflammatory pain modulation there is an interaction of the noradrenergic system and the cGMP pathway. Intracellular cGMP concentrations are regulated by the action of guanylyl cyclases and by the rate of degradation by GMP-specific PDE (6). Tadalafil works by inhibiting cGMP, specifically effect on PDE-5 (7). Tadalafil's pharmacologic distinction is its longer half-life (17.50 hours). According to Mixcoatl-Zecuatl T, (4), sildanafil produces peripheral antinociception, and increase the cGMP level would account for the sildanafil -induced antinociceptive effect. Whenever there is activation of cGMP pathway, as there is inhibition of hyperalgesia, cAMP is produced during inflammatory reactions, and its enhancement is associated with worsening of inflammatory hyperalgesia (8).

Here in the present experimental study, ineffective dose of tadalafil with ineffective doses of adrenergic agents (metoprolol, Methyldopa) and diclofenac sodium that resulted in significant inhibition of the nociceptive response in the writhing test. The effect of tadalafil with α adrenoceptor agonist was larger than with β adrenoceptor antagonists.

This increased effect of the tadalafil is not due to drug metabolic interaction because tadalafil is metabolized primarily by cytochrome P450 3A4 and adrenergic agents are not considered to be inhibitors of this enzyme (9). There is an increased antinociceptive response produced by the combination of tadalafil and adrenergic agents.

Metoprolol (selective β_1-adrenoceptor antagonist) inhibited the nociceptive response. When ineffective doses of tadalnafil and metoprolol were administered concomitantly, the writhing response was significantly inhibited. Although the antinociceptive mechanisms of β -adrenoceptor antagonists are not known, metoprolol effects might be a result of changes in cAMP levels, because *in vitro* β_1-adrenoceptor stimulation leads to cAMP accumulation (10). Some authors suggested the role of β adrenoceptors in nociception. Although, both types of β receptors are involved in pain perception but β_1 receptor activation is involved in chemically induced nociception. Thus, small doses of tadalafil and metoprolol when administered concomitantly could provide increased antinociception displaying less adverse effects.

Besides, α_2-adrenoceptor agonists are implicated for the treatment of acute pain events and prevention of postoperative pain (11). In the present study, methyl dopa (an α_2-adrenoceptor agonist) significantly substantiated the writhing counts. This result was consistent with several observations indicating that the activation of α_2-adrenoceptors inhibits substance P release (12), decreases tumor necrosis factor-α (TNF-α) and interleukin (IL)-1 plasma concentration in postoperative patients (13) and produces analgesic effects in different types of pain models (14, 15). In this regard, it has been demonstrated that analgesic effects are probably mediated by changes in K^+ current as α_2 agonist administration results in cell hyperpolarization by increasing K^+ conductance (16).

Conclusion

It is extrapolated from the results that tadalafil and adrenergic agents must be promoted for designing new analgesics. However, the pharmacological profile of these associations must be subject to further investigations.

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