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SCIENTIFIC ARTICLE

Hyperalgesic effect of subarachnoid administration of phentolamine in mice[☆]



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KEYWORDS

Mice;
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Abstract

Background and objectives: Painful phenomenon is one of the most important and complex experiences. Phentolamine is a non-selective alpha-adrenergic antagonist. The objective of this study was to compare the effect of increasing doses of phentolamine into subarachnoid space in rats in the modulation of painful phenomenon.

Methods: 84 male Wistar rats were divided into formalin and plantar incision groups, subdivided into six subgroups ($n = 7$). Control group received only saline ($10 \mu\text{L}$); active subgroups received phentolamine $10 \mu\text{mg}$ (GF10), 20 mg (GF20), 30 mg (GF30), 40 mg (GF40), and 50 g (GF50). In formalin group, pain was induced by injection of $50 \mu\text{L}$ of 2% formalin in dorsal region of right posterior paw. In plantar incision group, pain was induced by plantar incision and evaluated using von Frey filaments. Induction and maintenance of anesthesia were performed with 3% halothane for catheter placement into subarachnoid space and plantar incision. Statistical analysis was performed using the JMP program from SAS with 5% significance level.

Results: Phentolamine at doses of 20 and 30 g increased the algescic response in the intermediate phase of the formalin test. In plantar incision test, it had hyperalgesic effect on first, third, fifth, and seventh days at a dose of 10 g and on first, third, and fifth days at a dose of 20 g and on fifth day at a dose of 30 g.

[☆] Study conducted at Faculdade de Medicina do ABC, Santo André, SP, Brazil.

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PALAVRAS-CHAVE

Ratos;
Fentolamina;
Dor;
Teste da formalina;
von Frey

Conclusion: Subarachnoid administration of phentolamine showed hyperalgesic effect, possibly due to the involvement of different subclasses of alpha-adrenergic receptors in modulating pain pathways.

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Efeito hiperálgico da fentolamina, por via subaracnoidea, em ratos

Resumo

Justificativa e objetivos: O fenômeno doloroso é uma das mais importantes e complexas experiências. A fentolamina é antagonista alfa-adrenérgico não seletivo. O objetivo foi comparar os efeitos de doses crescentes da fentolamina, por via subaracnoidea, em ratos na modulação do fenômeno doloroso.

Método: Foram usados 84 ratos Wistar machos, divididos nos grupos formalina e incisão plantar, subdivididos em seis subgrupos ($n=7$). No subgrupo controle (GC) apenas salina ($10\ \mu\text{L}$), nos subgrupos ativos, $10\ \mu\text{g}$ de fentolamina (GF10), $20\ \mu\text{g}$ (GF20), $30\ \mu\text{g}$ (GF30), $40\ \mu\text{g}$ (GF40) e $50\ \mu\text{g}$ (GF50). No grupo formalina, a dor foi induzida com injeção de $50\ \mu\text{L}$ de formalina a 2%, na região dorsal da pata posterior direita. No grupo incisão plantar, a dor foi induzida por incisão plantar e avaliação pelos filamentos de von Frey. Indução e manutenção anestésica com halotano a 3% para introdução de cateter no espaço subaracnoideo e feitura da incisão plantar. Análise estatística dos resultados pelo programa JMP do SAS com nível de significância 5%.

Resultados: A fentolamina nas doses de 20 e $30\ \mu\text{g}$ produziu aumento da resposta álgica na fase intermediária do teste da formalina. No teste da incisão plantar, promoveu efeito hiperálgico no primeiro, terceiro, quinto e sétimo dias na dose de $10\ \mu\text{g}$, no primeiro, terceiro e quinto dias na dose de $20\ \mu\text{g}$ e no quinto dia na dose de $30\ \mu\text{g}$.

Conclusão: A fentolamina por via subaracnoidea promoveu efeito hiperálgico, possivelmente pela participação de diferentes subclasses de receptores alfa-adrenérgicos nas vias modulatórias da dor.

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Introduction

The passage of information through the posterior horn of the spinal cord (PHSC) toward the rostral levels of the central nervous system (CNS) undergoes profound excitatory and inhibitory influences. The pharmacology elucidation of these modulatory systems has guided the ability assessment of specific receptors.¹

Neurotransmitters, amino acids, and neuropeptides are released by primary afferent terminals into PHSC, where they act in nociceptive transmission modulation. Among others, the excitatory amino acids (glutamate and aspartate); neurotransmitters and neuropeptides, including the tachykinins (substance P [SP], neurokinin A [NKA], and neurokinin B [NKB]); calcitonin related gene peptide (CGRP); cholecystokinin (CCK); somatostatin; nitric oxide (NO); prostaglandin (PG); galanin; enkephalins; and endorphins are highlighted.²

Phentolamine, an alpha-adrenergic competitive antagonist, which belongs to the non-selective imidazoline group and has similar efficacy on alpha 1 (α_1) and alpha 2 (α_2) can also block 5-HT receptors and potassium channels (K^+) and cause the release of histamine by mast cells.³

This study evaluated the effects of escalating doses of phentolamine, administered in the subarachnoid space, on pain-induced in rats using the modified formalin test and the incision plantar test to verify if the involvement of adrenergic pathways in descending pain inhibitory system is phentolamine dose-dependent.

Method

Experimental procedures followed the Ethical Standards of the International Association for the Study of Pain (IASP), which regulates animal experiments (Committee for Research and Ethical Issues of the IASP, 1983), and the project was approved by the Comitê de Ética no Uso de Animais Ceua/Unitau, protocol # 019/11. In total, we used 84 male Wistar rats, weighing 220–300 g, which were individually placed in transparent glass chamber measuring $15\ \text{cm} \times 25\ \text{cm} \times 15\ \text{cm}$, with a hole in front and back to allow entrance and exit of oxygen, anesthetic gases, and carbon dioxide. The rats were anesthetized with 3% halothane in 100% oxygen. When the rats showed inability to get around the chamber, he was removed and placed in the supine

position with the abdomen over a plastic cylinder, with his muzzle in a mask through which he continued to receive the same halothane concentration.

Subsequently, a polyethylene catheter (PE 10) was caudally introduced into the subarachnoid space, by puncture with a 22G Tuohy needle, at the midline of the intervertebral space above the penultimate lumbar vertebra to the subarachnoid space, which was identified by the reflex movement of the tail or one of the hind paws. The catheter was fixed in the subcutaneous tissue, the skin sutured with needled nylon 4-0, and at the end of the experiment, after the animal sacrifice with sodium thiopental, lumbar spine was sectioned to confirm the catheter presence in subarachnoid space.⁴

The animals were randomly allocated into two groups: formalin and plantar incision. Rats in formalin group were allocated into six subgroups ($n=7$ each); the control group (CG) received 10 μL subarachnoid saline immediately after catheter placement; active groups PG10, PG20, PG30, PG40, and PG50 received via the same route 10 μL of phentolamine solution with the respective doses 10 μg , 20 μg , 30 μg , 40 μg , and 50 μg , which, respectively, corresponded to 31.5 nmol, 63 nmol, 94 nmol, 126 nmol and 157 nmol of phentolamine in sterile saline.

The formalin induced pain (formalin test modified) was carried out by administering 50 μL 2% formalin solution into the dorsal region of the right hind paw, 25 min after saline or phentolamine administration.⁵

All paw withdrawals, which were not related to gait, were recorded regardless of the time that it remained suspended. Counting was done continuously for 60 min. The partial number of withdrawals was recorded every 5 min. The test was divided into three phases: I, Intermediate, and II. Phase I included the number of flinches during the first 5 min, the Intermediate Phase from the 6th to the 20th minute, and Phase II from the 21st to the 60th minute.

Rats in plantar incision group were allocated into six subgroups of seven animals each. Control group (CG) received 10 μL subarachnoid saline immediately after catheter placement; active groups PG10, PG20, PG30, PG40, and PG50 received via the same route 10 μL of phentolamine solution with the respective doses 10 μg , 20 μg , 30 μg , 40 μg , and 50 μg , which, respectively, corresponded to 31.5 nmol, 63 nmol, 94 nmol, 126 nmol and 157 nmol of phentolamine in sterile saline.

Surgical, longitudinal incision of 1 cm extension was made in the posterior limb of the anesthetized animal, with a scalpel blade number 11. Skin and fascia of the plantar region were incised, starting 0.5 cm from the calcaneus edge and extending toward the toes. The plantaris muscle was elevated and incised longitudinally and the incision remained intact. After hemostasis with slight pressure on the surgical site, all plans were approximated and sutured with two separate points with needled mononylon 4-0. Hyperalgesia assessment was done by applying von Frey's filaments in the 2nd hour and the 1st, 3rd, 5th, 7th, 14th, and 21st day after saline or phentolamine administration.⁶

The solution volume (10 μL) injected into the subarachnoid space was defined from previous studies.^{4,7,8}

For statistical analysis, we used the JMP® Statistical software by SAS Institute (Statistical Analysis System) and the

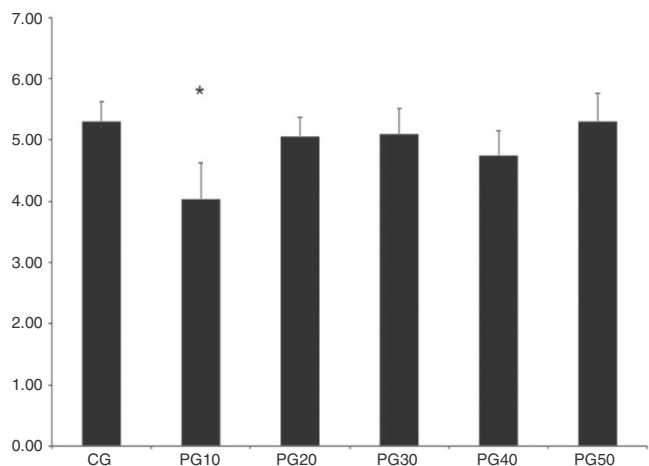


Figure 1 Mean number of animal flinches during the Intermediate phase of the formalin test in study groups. *Statistically significant difference ($p < 0.05$).

analysis of variance followed by Dunnett's test, considering a significance level lower than 5% ($p < 0.05$).

Results

Pain intensity assessed by modified phentolamine test

Phentolamine, administered in the subarachnoid space at doses of 20 μg and 30 μg produced increased algescic response in the intermediate phase of the modified formalin test, as the average number of flinches during the formalin test Intermediate Phase ranged from 16.28 in PG10 to 27.95 in PG30. The highest values were found in PG20 and PG30 groups, which showed statistically significant differences compared with the control group (Figs. 1 and 2).

The mean number of flinches during the formalin test Phase I and Phase II showed no statistically significant differences between groups.

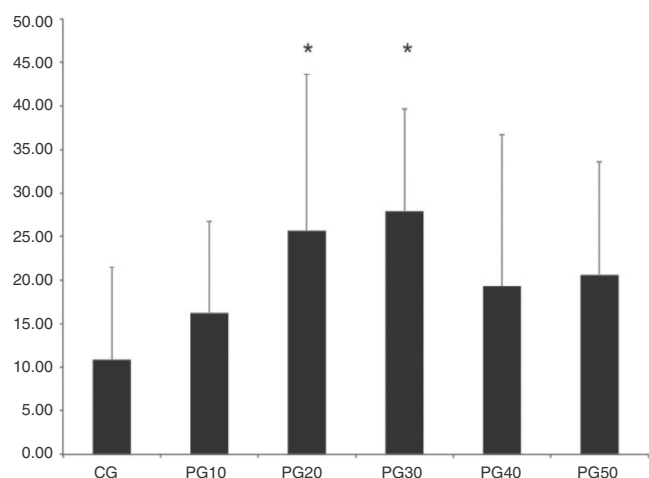


Figure 2 Mean number of animal flinches during all phases of the formalin test in study groups. *Statistically significant difference ($p < 0.05$).

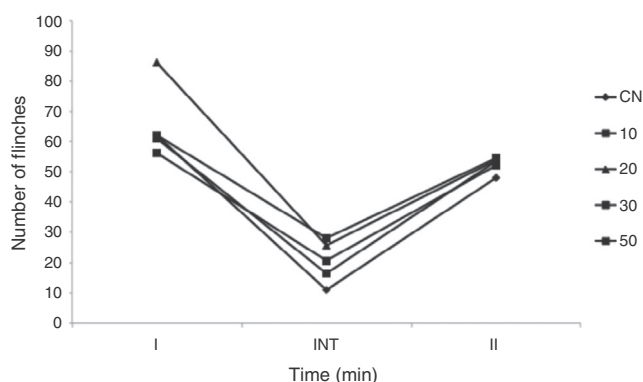


Figure 3 Response to von Frey's filaments at the 2nd hour after subarachnoid phentolamine.

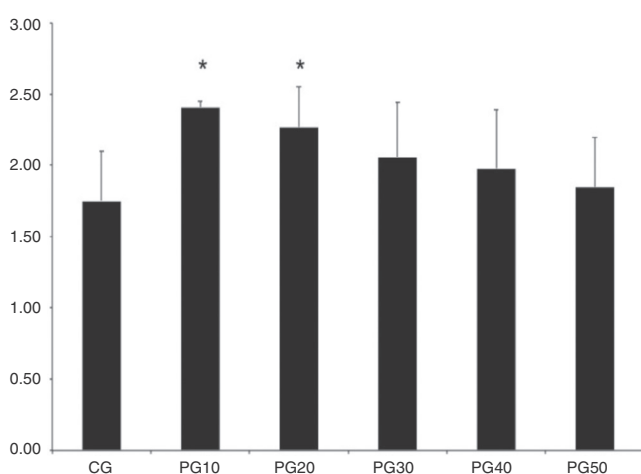


Figure 4 Response to von Frey's filaments on the first day after subarachnoid phentolamine. * Statistically significant difference ($p < 0.05$).

Pain intensity measured by von Frey's filaments, in surgical incision plantar test

Subarachnoid phentolamine at a dose of 10 µg provided hyperalgetic effect on plantar incision-induced pain in the first, third, fifth, and seventh days; at a dose of 20 µg, it provided hyperalgetic effect on the first, third and fifth days of plantar incision-induced pain; and at a dose of 30 µg, it provided hyperalgetic effect on the fifth day of plantar incision-induced pain.

At the 2nd hour and the first, third, and fifth days after subarachnoid phentolamine application there was less sensitivity to von Frey's filaments in PG10 and PG20, with statistically significant differences when compared to CG (Figs. 3–6).

On the seventh day of assessment, increased sensitivity to von Frey's filaments was seen in PG10, with a statistically significant difference compared to CG (Fig. 7).

Assessment in the 14th and 21st days showed no statistically significant differences between groups in sensitivity to von Frey's filaments.

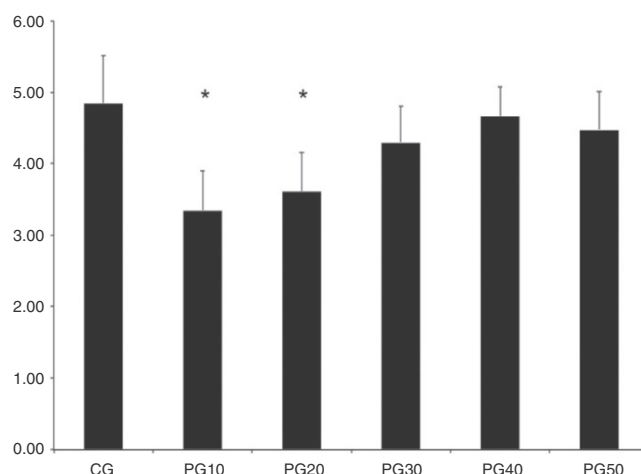


Figure 5 Response to von Frey's filaments on the third day after subarachnoid phentolamine. *Statistically significant difference ($p < 0.05$).

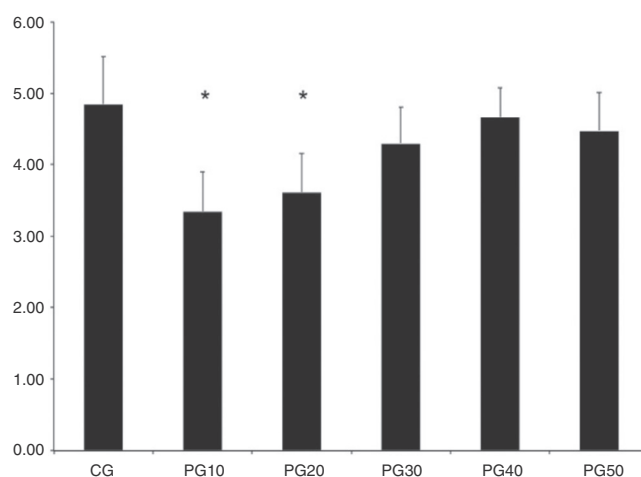


Figure 6 Response to von Frey's filaments on the fifth day after subarachnoid phentolamine. *Statistically significant difference ($p < 0.05$).

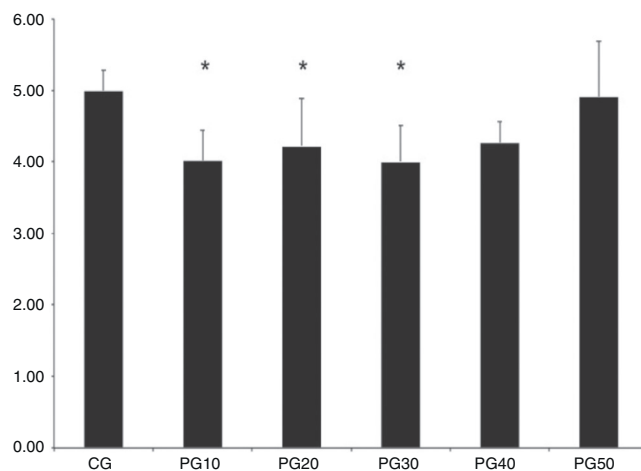


Figure 7 Response to von Frey's filaments on the seventh day after subarachnoid phentolamine. *Statistically significant difference ($p < 0.05$).

Discussion

The descending pain inhibitory system mainly consists of four interconnected parts of the CNS: (a) cortical and diencephalic systems; (b) periaqueductal gray (PAG) and periventricular matter rich in enkephalins and opioid receptors; (c) rostroventral portion of the bulb, especially the nucleus raphe magnus (NRM) and (d) adjacent nuclei that receive excitatory impulses from PAG and, in turn, send noradrenergic and serotonergic fibers, via the dorsolateral funiculus, which project to the dorsal horn of the spinal cord and bulb. The fibers of the descending inhibitory system mainly terminate in laminae I, II, and V where they inhibit nociceptive neurons, including interneurons and ascending tracts that project rostrally, among them the spinothalamic, spinoreticular, and spinomesencephalic tracts. Another important fiber group that has been included in the endogenous pain control system formation is locus coeruleus noradrenergic neurons and central cholinergic system.^{2,9–11}

To assess the effects of phentolamine on pain pathways, multiple doses are administered into subarachnoid space in rats. In this study, we used escalating doses, from previously reported doses usually administered by other authors who have shown conflicting results and highlighted the need for studies with varying doses, which motivated this dose-escalation study.^{4,8,12}

Modified formalin test is widely used as animal model of nociception because it produces responses similar to those occurring in humans. The number of flinches as a device for quantifying pain behavior induced by formalin is closely related with the classical formalin test and cardiovascular changes in response to pain caused by formalin in the paw, showing close correlation with pain behavior in conscious animals.^{13–16}

During Phases I and II of the modified formalin test, there was no difference between groups, suggesting no interference from the noradrenergic system. However, during the Intermediate Phase, proposed as related to central pain inhibition, we found a significant increase in the number of flinches, compared with control group, when doses of 20 and 30 μ g were administered via subarachnoid, without statistically significant difference compared with the other groups.

Studies reporting analgesic effect in the presence of spinal alpha-2 receptor stimulation suggest that 10 μ g were insufficient to have agonist effects on these receptors and that doses higher than 30 μ g may also have agonist effects on alpha-1 receptors, an effect that antagonizes the effects of alpha-2 receptor stimulation.¹⁷

The mouse model of plantar incision pain proposed by Brennan et al.⁶ is very useful for understanding the pathophysiological mechanisms of pain. In clinical practice, it is very similar to the pain experienced by patients in postoperative period, involuntary and less intense at rest.

Subarachnoid administration of phentolamine in rats at doses of 10 and 20 μ g showed analgesic characteristics during the second hour after administration. This can be explained by the predominance of the central modulatory effect on low doses of the drug. These low doses are unable to antagonize the effect of descending noradrenergic release, as with the increased dose, this antagonism

prevailed, showing no reduction in sensitivity (analgesia). However, the dose of 10 μ g administered via subarachnoid presented hyperalgesic effect on the first, third, fifth, and seventh days, as well as the dose of 20 μ g on the first, third, and fifth days. The dose of 30 μ g provided hyperalgesic effect only on the fifth day after administration, which suggests that in this period without modulatory action, the blockade of adrenergic receptors with greater selectivity, possibly on alpha-2 receptors, promoted greater sensitivity and hyperalgesia.

Other studies have reported analgesic effect with alpha-2 receptor stimulation, located in the spinal cord, by neuronal discharge inhibition^{17,18}. This could explain the results of this experiment, as higher doses of phentolamine (alpha-adrenergic antagonist) could also stimulate alpha-1 receptor, produce antagonist effect to the effects of alpha-2 receptor stimulation, and potentiate the adrenergic effect on alpha-1 receptors.

The hypothesis that larger doses of the antagonist phentolamine could stimulate the alpha-1 adrenergic receptors and produce an antagonist effect to the effect of alpha-2 adrenergic receptors stimulation and therefore enhance the adrenergic effect on alpha-1 adrenergic receptors should be elucidated in further studies comparing blockers with greater selectivity for alpha-1 adrenergic receptors with high doses of phentolamine.

Conclusion

Different doses of subarachnoid phentolamine provide different effects on pain sensitivity, possibly by the participation of different subclasses of alpha-adrenergic receptors in modulating pain pathways.

Conflicts of interest

The authors declare no conflicts of interest.

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