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ORIGINAL ARTICLE

Fluoxetine action on acute pain in rats submitted to sciatic nerve constriction*

Ação da fluoxetina sobre a dor aguda em ratos submetidos à constrição do nervo ciático

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SUMMARY

BACKGROUND AND OBJECTIVES: Selective serotonin reuptake inhibitors, such as fluoxetine, have been suggested as alternative to tricyclic antidepressants to treat chronic pain, due to the lower incidence of side effects. This study aimed at observing the effects of serotonin on acute pain modulation, by the administration of fluoxetine through the formalin test in rats previously submitted to sciatic nerve constriction.

METHOD: We used 24 male Wistar rats, with mean weight of 300 g and distributed in 5 groups: 1. Control untreated; 2. Sciatic nerve constriction; 3. Sciatic nerve constriction and treated with 5 $mg.kg^{-1}$. day oral fluoxetine for 15 days; 4. Sciatic nerve constriction treated with 5 $mg.kg^{-1}$ oral reserpine every 72 hours and with 5 $mg.kg^{-1}$.day oral fluoxetine for 15 days; 5. Sciatic nerve constriction treated with 5 $mg.kg^{-1}$ oral reserpine every 72 hours for 15 days. All animals were submitted to modified formalin test after treatment.

RESULTS: Response in phase I, intermediate phase and phase II formalin test was not changed by sciatic nerve constriction. Treatment with reserpine or fluoxetine has not interfered with first and intermediate formalin test phases in the groups submitted to sciatic nerve constriction. The number of flinches in the second formalin test phase has increased in animals treated with fluoxetine and has decreased in animals treated with reserpine. There has been decrease in the number of flinches in animals treated with the association reserpine and fluoxetine as compared to animals treated with fluoxetine alone.

CONCLUSION: Fluoxetine has increased painful sensation after acute stimulation in rats submitted to sciatic nerve constriction, showing the algogenic action of the drug in this experimental model.

Keywords: Fluoxetine, Formalin test, Neuropathy, Pain, Reserpine.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Os inibidores seletivos da recaptação de serotonina como a fluoxetina, têm sido apontados como alternativa ao uso dos antidepressivos tricíclicos para o tratamento da dor crônica, pela menor incidência de efeitos colaterais. O objetivo deste estudo foi estudar o efeito da serotonina na modulação da dor aguda, pela administração de fluoxetina, por meio do teste da formalina, em ratos, anteriormente submetidos à constricção do nervo ciático.

MÉTODO: Foram estudados 24 ratos Wistar, machos, com peso médio de 300 g, distribuídos em 5 grupos: 1. Controle sem tratamento; 2. Constrição do nervo ciático; 3. Constricção do nervo ciático tratados com 5 $mg.kg^{-1}$.dia de fluoxetina, por via oral durante 15 dias; 4. Constrição do nervo ciático tratados

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com 5 mg.kg^{-1} de reserpina, por via oral a cada 72h e com 5 mg.kg^{-1}.dia de fluoxetina por via oral, durante 15 dias; 5. Constrição do nervo ciático tratados com 5 mg.kg^{-1} de reserpina por via oral em intervalos de 72h, durante 15 dias. Todos os animais foram submetidos ao teste da formalina modificado após os tratamentos especificados.

RESULTADOS: A resposta na fase I, na fase intermediária e na fase II do teste da formalina não foi alterada pela constrição do ciático. O tratamento com reserpina ou fluoxetina não interferiu com as fases I e intermediária do teste da formalina nos grupos submetidos à constrição do ciático. O número de elevações da pata na fase II do teste da formalina aumentou nos animais tratados com fluoxetina e diminuiu nos animais tratados com reserpina. Nos animais tratados com a associação reserpina e fluoxetina houve redução do numero de elevações da pata em comparação com os animais tratados apenas com a fluoxetina.

CONCLUSÃO: O tratamento com fluoxetina aumentou a sensação dolorosa após estímulo agudo em ratos submetidos à constrição do ciático, evidenciando ação algogênica do fármaco neste modelo experimental.

Descritores: Dor, Fluoxetina, Neuropatia, Reserpina, Teste da formalina.

INTRODUCTION

Drugs inhibiting 5-hydroxytryptamine uptake (5-HT), or selective serotonin uptake inhibitors (SSUI), do not interfere with neurotransmitters other than serotonin[1]. Fluoxetine, which is the prototype of the group, is a selective serotonin uptake inhibitor in cerebral cortex, serotoninergic neurons and platelets. It does not inhibit other neurotransmitters uptake and has no affinity for adrenergic, muscarinic, cholinergic H_1-histamines, serotonin or dopamine receptors[2]. Fluoxetine hydrochloride has become one of the most widely used antidepressants to treat some neurological disorders due to its pharmacological and therapeutic importance, in addition to the relative absence of severe adverse reactions and low abuse potential. Most common adverse reactions related to fluoxetine, even in therapeutic doses, are: dry mouth, sweating, headache, diarrhea, sleepiness and insomnia[3].

Several animal studies with SSUI in experimental pain models showed a possible interaction of such drugs with the endogen opioid system or analgesic effect potentiation mediated by serotoninergic and/or noradrenergic pathways[4]. In addition, SSUI may indirectly act on pain, because they also promote a mild endorphin levels increase[5].

This study aimed at studying the effects of serotonin in acute pain modulation, by the administration of fluoxetine through the formalin test in rats previously submitted to sciatic nerve constriction.

METHOD

After the approval of the Animal Experiment Ethics Committee, University of Taubaté (UNITAU), approval 003/2009, 24 male Wistar rats were used, weighing between 300 and 350 g, supplied by UNITAU's vivarium and maintained in groups of 5 animals per compartment in UNITAU's Pharmacology Laboratory, where they remained for at least 15 days before the experiment, for adequate adaptation, with free access to commercial balanced feed and water. Light was controlled with 12-h light-dark cycles and room temperature of 22 ± 3º C (19 to 25º C). Ethical standards of the international Association for the Study of Pain (IASP) regulating animal experiments were followed (Committee for Research and Ethical Issues of the IASP, 1983). Only healthy animals were used in the research.

Animals were randomly divided in 5 groups, according to the pharmacological treatment: 1. Control group (C) – without surgical intervention (n = 4); 2. Sciatic constriction group (SC) – animals submitted to sciatic nerve constriction (n = 6); 3. Reserpine group (SC + R) – animals were submitted to sciatic constriction and treated with oral reserpine (5 mg.kg^{-1}) in 72-h intervals, for 15 days (n = 4); 5. Fluoxetine group (SC + F) – animals were submitted to sciatic nerve constriction and daily treated with oral fluoxetine (5 mg.kg^{-1}) for 15 days (n = 4); 5. Fluoxetine + reserpine group (SC + FR) – animals were submitted to sciatic nerve constriction and treated with oral reserpine (5 mg.kg^{-1}) every 72 hours during 15 days and with oral amitriptyline (5 mg.kg^{-1}) daily for 15 days (n = 4).

All animals, except for C group, were submitted to sciatic nerve constriction in the right paw under anesthesia with halothane (3 vol%). Right sciatic nerve was exposed and a 5 to 7 mm segment was dissected. Four ligatures with chrome catgut suture 4-0 type C, with 1 mm intervals were performed around the nerve[6].

After sciatic nerve constriction and pharmacological treatment, both treated and control animals were submitted to sensitivity perception test. The test consisted

in touching right hind paw plantar region (treated) and left (control) with filaments of standardized thickness using Von Frey filaments[7].

Flinching at touch indicates sensitivity. Filament thickness is recorded and results are statistically analyzed. Animals were submitted to Von Frey test 15 days after sciatic constriction. Two measurements were taken at 3-day intervals. After Von Frey test, all animals were submitted to the modified formalin test injected in the sciatic nerve constriction paw.

Modified formalin test – before the procedure, animals were placed in a glass chamber measuring 30 x 30 x 30 cm for 15 minutes to adapt to the study environment. A mirror was placed behind the chamber in 45º angle, to help visualization of flinches in all directions.

Test consisted of 50 µL injection of 2% formalin solution in hind paw dorsum producing a two-phase nociceptive response. Number of flinches was counted during 60 minutes. Phase I corresponded to the number of flinches during 5 min after injection. Phase II corresponded to the number of flinches as from 21 until 60 min after injection; and the intermediate phase consisted of the number of flinches from 6 to 20 min after injection. Phase I was interpreted as being due to the acute activation of peripheral nociceptors, while phase II as resulting from acute inflammatory response or from central sensitization. The period between both nociceptive response phases is considered inactive and attributed to the involvement of a central antinociceptive mechanism[8].

All flinches unrelated to gait were considered, regardless of the time the paw remained elevated. Counting was continuous during 60 min and partial number of flinches was recorded every 5 min.

Analysis of variance for independent samples was used for statistical analysis of results, followed by Bonferroni test. Significance level was less than 5% ($p < 0.05$).

RESULTS

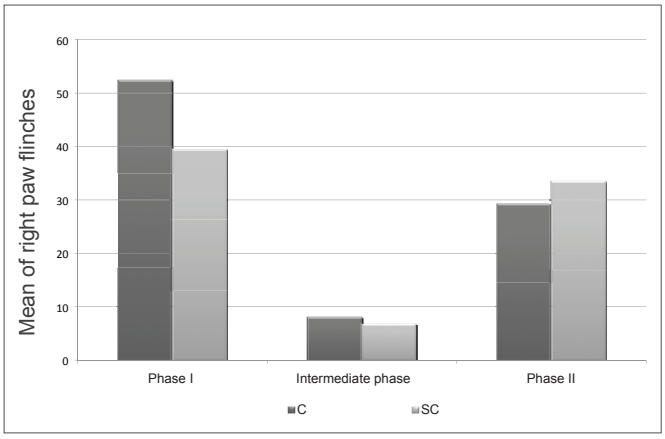
Sciatic nerve constriction has not changed phase I, phase II and intermediate phase responses to formalin test (Graph 1).

Treatment with reserpine, fluoxetine or with the association reserpine-fluoxetine has not interfered with phase I and intermediate phase of formalin test in groups submitted to sciatic nerve constriction (Graph 2).

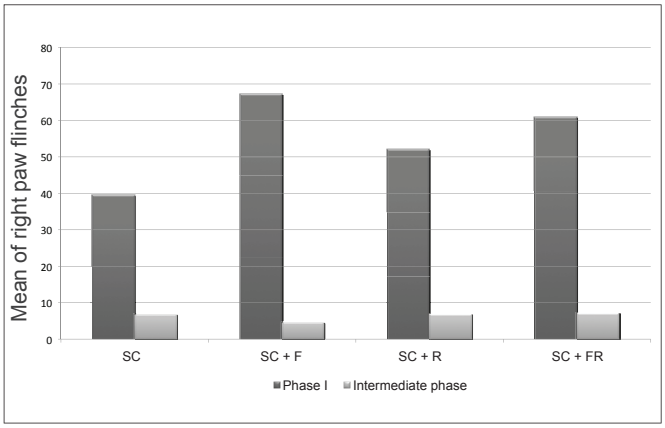
During formalin test phase II, treatment with fluoxetine has increased the number of flinches as compared to untreated group. Treatment with reserpine has decreased the number of flinches as compared to untreated group. There has been decreased number of flinches in the group treated with the association reserpine-fluoxetine as compared to animals treated with fluoxetine alone (Graph 3).

DISCUSSION

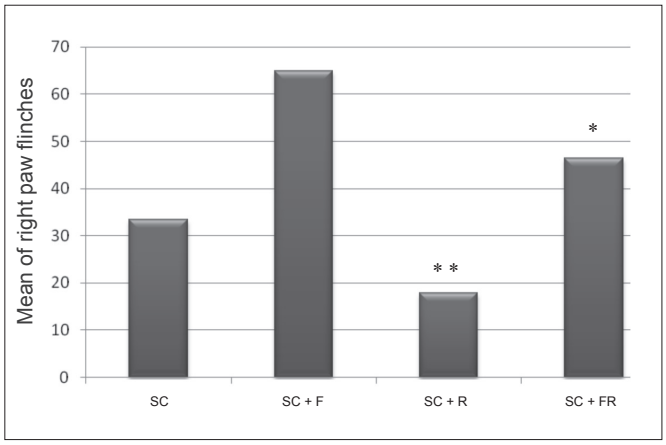
The formalin test also causes tissue injury and inflammatory response activation, but sciatic ligature seems to involve neuronal plasticity mechanisms which may increase nociceptors sensitivity to acute inflammatory response mediators[9]. This was not observed in



Graph 1 – Mean of right paw flinches in control and sciatic nerve constriction groups.
C = Control group; SC = sciatic constriction group.



Graph 2 – Mean of right paw flinches in rats submitted to sciatic nerve constriction and untreated or treated with reserpine, fluoxetine or reserpine + fluoxetine in formalin test first and intermediate phases.
SC = sciatic constriction group; SC + F = fluoxetine group; SC + R = fluoxetine + reserpine group; SC + FR = fluoxetine + reserpine group.



Graph 3 – Mean of right paw flinches in rats submitted to sciatic nerve constriction and untreated or treated with reserpine, fluoxetine or reserpine + fluoxetine in formalin test second phase.
SC = sciatic constriction group; SC + F = fluoxetine group; SC + R = fluoxetine + reserpine group; SC + FR = fluoxetine + reserpine group.
*p < 0.05 as compared to SC or SC + FR.
** p <0.05 as compared to SC + F.

our study since phase I, intermediate phase and phase II responses were not changed by sciatic nerve constriction because there were no differences between responses of C an and SC groups.

Treatment with reserpine or fluoxetine has not interfered with formalin test phase I and intermediate phase in groups submitted to sciatic nerve constriction.

The first formalin test phase is related to nociception[8]. Treatment with reserpine has maintained such response. These results are in line with a previous study where intact animals, without surgical procedures, treated with reserpine, showed unchanged response during the first test phase, allowing inferring that the drug does not interfere with pain signal transduction[10]. Reserpine does not seem to interfere with pain signal transduction and with pain descending inhibitory pathway in the sciatic nerve constriction model.

Fluoxetine has not changed the number of flinches during intermediate formalin test phase, with or without reserpine, indicating that this drug does not interfere with pain descending inhibitory pathway in this experimental model. These results indicate that norepinephrine, serotonin and dopamine depletion may not interfere with pain signal transduction or with central nociception, characteristics of the described phases in this experimental model.

There has been increased number of right paw flinches during phase II, after treatment with fluoxetine. These data point to an algogenic effect of fluoxetine in acute conditions with pre-existing hyperalgesia.

Descriptions of fluoxetine action on pain are conflicting. Fluoxetine has shown peripheral antinociceptive action in inflammatory formalin test and in neuropathic pain model, differently from what was found in our study[11]; studies with knockout mice on serotoninergic neurons of the brainstem have shown that the involvement of serotonin with the action mechanisms of antidepressant drugs varies with the type of pain. SSUI analgesic effects during thermal pain test were very mild or absent in such mice, suggesting that serotonin reuptake blockade is involved with the acute analgesic effect of antidepressants[12]. In a different study, duloxetine analgesic effects on persistent pain models in rodents have not been affected by the loss of serotoninergic neurons, suggesting a critical norepinephrine role in antidepressant-induced analgesia in chronic pain conditions[13].

A review including 59 controlled randomized studies has compared the analgesic effect of antidepressants to placebo in patients with diabetic neuropathy and no evidences were found that fluoxetine was more effective than placebo for pain relief[14]. This statement is in line with our phase I and intermediate phase results, however different from phase II since in the latter fluoxetine has increased pain sensation in the experimental models, which presented higher number of flinches.

Since fluoxetine has increased the number of flinches during phase II of the formalin test and this number was decreased with reserpine, one may infer that changes in the synthesis / release of neurotransmitters, such as serotonin, may induce central modulation adaptations manifested as sensitivity changes. Increased response to formalin of animals treated with reserpine and fluoxetine favors this hypothesis. Fluoxetine had no effect on Wistar rats pain during the electric and thermal stimulation test[1], however it increased motor response to induced pain. Experimental data suggest that the acute administration of SSUI may exacerbate an acute type of pain. In addition, the fluoxetine group had higher number of flinches in response to pain during formalin test phase II, which is the inflammatory phase, thus with a pro-inflammatory behavior[10]. These conclusions are in line with our results.

Serotonin action in its different subtypes of receptors

is able to modulate different signals, which primarily depend on the type of coupled receptor, on the number of receptors in the cell, on protein interaction, and on the number and type of G protein expressed in cell membranes[10].

The activation of 5-HT_{1A} receptors may show paradoxical effects, triggering both analgesic and hyperalgesic effects. This pre-synaptic receptor is broadly distributed in the central nervous system as well as in peripheral tissues, primarily acting through the activation of Gi/o protein, which is inhibitory, with inhibition of adenylcyclase, even in the hippocampus and cortical neurons. The hyperalgesic action would be consequence of partial opioid release inhibition in spinal cord dorsal horn, decreasing pain modulation by the pain descending inhibitory system. This is caused by the blockade of just encephalins release which, in association with dynorphins, would activate the opioid system. By activating Gi/o protein one may also inactivate voltage-dependent calcium channels and inhibit opioid release. On the other hand, it has also been shown that medullar 5-Ht_{1A} receptors mediate antinociceptive effects by interacting with NMR descending pathways[10].

One may also associate the hyperalgesia found in this study during the second formalin test phase with a possible interaction of serotonin with 5-HT_{1A}-type receptors present in large quantities in the somatosensory and prefrontal cortex. This receptor is coupled to G protein which, through the activation of phospholipase C, produces two second messengers which are diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG is a cofactor of protein kinase C, with facilitation of glutamatergic transmission in spinal cord dorsal horn neurons, and IP_3 promotes mobilization of calcium reserves, thus leading to hyperexcitability of motor neurons and medullar reflexes[10]. These arguments are a possible explanation for the findings of this study[10].

So, fluoxetine does not influence formalin test phase I and intermediate phase. However, in phase II, increased serotonin levels obtained by the administration of fluoxetine to rats has worsened pain, fact that was confirmed by the increased number of flinches. It is worth highlighting that when norepinephrine and dopamine were depleted by administering reserpine before fluoxetine, there has been acute pain improvement, result which should be further investigated by future studies.

CONCLUSION

Fluoxetine has increased pain sensation after acute stimulation of rats submitted to sciatic nerve constriction, showing the algogenic action of the drug in this experimental model.

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