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Experimental Study on the Role of 5-HT3 Serotonin Receptors in the Mechanism of Anti-Inflammatory and Antihyperalgesic Action of Antidepressant Fluoxetine

KEYWORDS: fluoxetine, inflammation, 5-HT3 receptors

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ABSTRACT *The aim of the present study is to determine the role of 5-HT3 receptors in the mechanism of anti-inflammatory and antihyperalgesic action of antidepressant fluoxetine after single and repeated administration. Carrageenan-induced paw edema and test with mechanical pressure of inflamed paw were used. Fluoxetine showed significant anti-inflammatory and antihyperalgesic effect when compared with the control. After long-term treatment and in the first four hours, after single administration ondansetrone (5-HT3 antagonist) did not change significantly the anti-inflammatory effect of fluoxetine. At 24 hours in single dose treated animals the combination did not differ statistically when compared with the control. After acute and prolonged treatment the group that received fluoxetine + ondansetrone showed a statistically significant increase in paw pressure to withdraw the hind paw compared with that treated with fluoxetine alone. Fluoxetine has anti-inflammatory and antihyperalgesic effect, which is mediated through the action of serotonin on 5-HT3 receptors.*

Introduction

Antidepressants are class of drugs, which includes a heterogeneous in their structure and mechanism of action substances.Fluoxetine is an antidepressant of the group of selective serotonin reuptake inchibitors (SSRI). It blocks serotonin transporter protein by high-affinity mechanism and increases the concentration of this mediator in the central nervous system (CNS) and peripheral tissues [1].

Experimental data showed that along with its main pharmacological effect – antidepressive, fluoxetine has ant-inflammatory and analgesic activity [2]. Studies on the anti-inflammatory activity of antidepressants are of interest in several areas. According to the neuroinflammatory hypothesis, depressive disorders are related with inflammation in some brain structures [3].This raises the question about the possible involvementof the anti-inflammatory effect of antidepressants in their therapeutic efficacy in depression. On the other hand anti-inflammatory effect of antidepressants may be useful in the treatment of inflammatory diseases that accompany depression or antidepressants can be used alone for this purpose.For example, there is clinical evidence that some antidepressants, such as bupropion can induce remission in Crohn's disease, psoriasis and atopic dermatitis [4].

Fluoxetine has analgesic activity in different experimental models of pain and antinociceptive tests. Singh et al found that this antidepressant has pronounced analgesic effect in three classic pain tests – "tail-flick", hot plate and abdominal constrictor test. They observed this effect after systemic and intracerebroventricular administration of fluoxetine [5].The mechanism of this effect is not sufficiently understood. The role of serotonin is supposed.It is a key modulator of nociceptive transmission and has predominantly an inhibitory effect onpain. In genetically modified mice lacking central serotoninergic neurons (Lmx1b$^{f/f/p}$line)is observed persistent pain, whichis inhibited by intrathecal administration of 5-HT [6]. There are many evidence that the antinociceptive activity of many analgesics depends on the descending serotoninergic system5-HT3 receptors mediate antinociceptive effect in the spinaldorsal horn. There are experimental data for the involvement of this receptor subtype in the mechanism of the antinociceptive effect of SSRI antide pressants. 5-HT_3 antagonist ondansetrone inhibits analgesic effect of paroxetine in hot plate and abdominal constrictor test[7,8].The **aim** of the present study was to determine the role of 5-HT_3 receptors in the mechanism of anti-inflammatory and antihyperalgesic action of fluoxetine after single and repeated administration of the drug.

Material and methods

The experiment was approved by the Ethics Committee on Animal of the Bulgarian Agency for Food Safety permit No 56/19.03.2012 and decision of the Ethics Committee at the Medical University - Plovdiv, the protocol No 4/19.06.2013 year.

Animals

Male Wistar rats with average weight of 220 – 250 g were used. Animals were randomly divided in five groups (n = 8) treated for 14 days as follows:

1st group (control) – control group treated with saline intraperitoneal (i. p.);
2nd group (positive control group) - treated with a reference anti-inflammatory and analgesic drug diclofenac in a dose of 25 mg/kg bw (i. p.) ;
3rd group- treated with fluoxetine in a dose of 5 mg/kg bw (i. p.);
4th group - treated with 5-HT_3 receptor antagonist ondansetrone in a dose of 0,1 mg/kg bw (i. p.);
5th group treated with fluoxetine 20 mg/kg bw (i. p.) and ondansetrone 0,1 mg/kg bw (i. p.).

Groups were tested for analgesic and anti-inflammatory activity after a single and repeated (14 days) treatment. For the groups treated with the receptor antagonist the latter was applied only on the day of the experiments.

Experimental methods

1. Carrageenan-induced paw edema.

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Apparatus plethismometer (Ugo basile, Italy) was used. Prior to treatment the volume of the right hind paw of the animals from all groups was measured. Thereafter all animals were injected intraplantar with 0,1 ml of 1 % solution of carrageenan in 0,9 % sodium chloride to induce an inflammatory edema. Immediately after the injection of carrageenan animals from control group received 0,1ml/100 g bw saline; the positive control group was treated with diclofenac sodium 25 mg/kg bw and the animals in the experimental groups were injected i. p. the test substances. In the groups treated with more than one substance the second was introduced 30 minutes after the first one. Hind paw volume was measured immediately before carrageenan injection and on the 2nd, 3rd, 4th and 24th thereafter with a plethismometer. Percentage of paw edema was calculated using the following equation:

Paw edema (%) = $(V - V_0)/V_0 \times 100$,

where V is the paw volume at 2nd, 3rd, 4th, and 24th hour after carrageenan injection and V_0 is the paw volume prior to carrageenan injection.

Animals were tested for anti-inflammatory action on the 1st and 14th day of the treatment.

2. Nociceptive test with mechanical pressure of carrageenan-inflamed paw (analgesimeter).

The test was described by Randall & Selitto (1957). Apparatus analgesimeter (Ugo basile, Italy) is used. This method is used for rapid precise screening of analgesic drugs. Mechanical pain stimulus is applied on a normal or inflamed rat paw. Nociceptive threshold was measured by applying pressure on the inflamed rat hindpaw. The strength of the pressure at which the animal withdraws testing paw is recorded. The maximal possible pressure is 250 grams (cut off). The rats were tested one hour before the treatment and on the 1st, 2nd and 3rd hour after the treatment. For groups treated with two drugs rats were tested on the 1st, 2nd and 3rd hour after the treatment with the second drug. The pressure at which the animal withdraws the hindpaw was expressed as % of the maximum possible effect (MPE %), where MPE % = (post treatment threshold – pretreatment threshold) x 100/(250 – pretreatment threshold).

Statistical analysis

Data were analyzed using the method of analysis of variance - One Way ANOVA from the software product SPSS 21.0. Mean values ± SEM were calculated. Nonparametric test of Kolmogorov-Smirnov show a normal distribution. A comparison of the results between groups was performed using Independent – Samples T test. Results were considered significant at p<0,05.

Results and discussion

1. Effect of ondansetrone on the anti-inflammatory action of fluoxetine in carrageenan-induced inflammatory reaction.

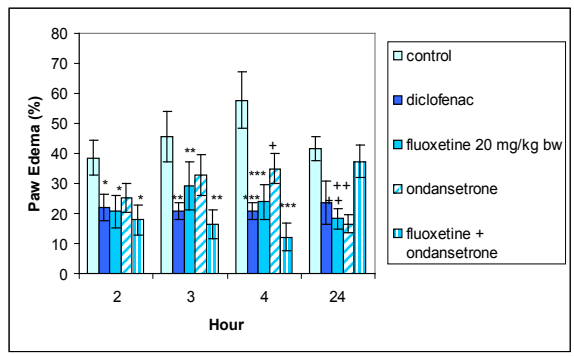

Fig. 1. Effect of 5-HT3 receptor antagonist ondansetrone on anti-inflammatory action of fluoxetine after a single dose.

* p < 0,05 compared with control on the 2nd hour; * * p < 0,05 compared with control on the 3rd hour; *** p < 0,05 compared with control on the 4th; + p < 0,05 compared with diclofenac on the 4th hour; ++ p < 0,05 compared with control on the 24th hour.

Anti-inflammatory effect of fluoxetine was detected in the early and late phase of inflammation after single and repeated administration (fig. 1 and fig. 2). Our results are in agreement with those of Abdel-Salam etal for anti-inflammatory activity of fluoxetin in this model of inflammation [2].

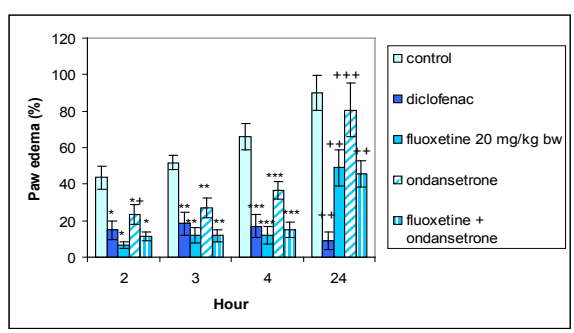

Fig. 2. Effect of 5-HT3 receptor antagonist ondansetrone on anti-inflammatory action of fluoxetine after repeated treatment.

* p < 0,05 compared with control on the 2nd hour; * * p < 0,05 compared with control on the 3rd hour; *** p < 0,05 compared with control on the 4th; + p < 0,05 compare with fluoxetine on the 2nd hour; ++ p < 0,05 compared with control on the 24th hour; +++ p < 0,05 compared with fluoxetine + ondansetrone on the 24th hour.

Ondansetrone significantly reduced the anti-inflammatory effect of fluoxetine only at the 24th hour after single administration (fig. 1). In the central nervous system, 5-HT_3 receptors are located primarily presynaptically and are associated with the axons and nerve terminals. Experimental data showed that 5-HT_3receptors play an important role in the self-regulation of serotonin release as they stimulate it, thus play the role of auto receptors[9]. Intracerebroventricular administration of serotonin in rats inhibits carrageenan-induced inflammation. Suppression of the immune function was observed by serotonin releasing substances such as amphetamine [10]. Data from experimental studies

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showed that on the level of the spinal cord 5-HT_3 receptors do not have modulating effect on carrageenan edema [11]. From our results we can speculate that supraspinal 5-HT_3 receptors are involved in the mechanism of the late anti-inflammatory effect of fluoxetine after single administration.

5-HT_3 receptor antagonist ondansetron did not change statistically significant the observed anti-inflammatory effect of fluoxetine in long term treated animals and in the first four hours in a single dose treated rats (fig. 1 and 2). T cells expressed on 5-HT_3 receptors have activation function [12]. The action of serotonin on peripheral 5-HT_3 receptors could lead to a decrease in the anti-inflammatory effect of fluoxetine in peripheral tissues. In this case the simultaneous administration of fluoxetine and ondansetron would potentiate their anti-inflammatory effect. This was not confirmed by our results. Probably agonistic action of increased by fluoxetine serotonin on 5-HT_3 receptors does not weaken the anti-inflammatory effect of the studied antidepressant.

2. Effect of ondansetrone on the antihyperalgesic action of fluoxetine in the test with mechanical pressure of inflamed rat hindpaw.

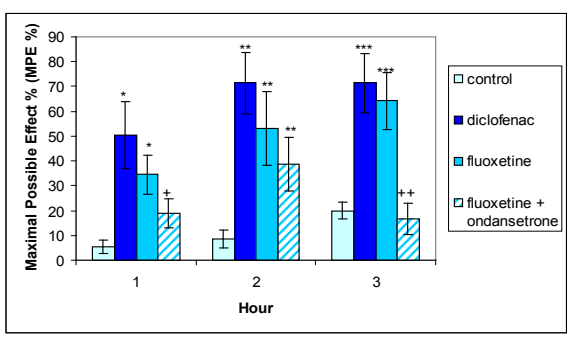

Fig. 3. Effect of 5-HT3 receptor antagonist ondansetrone on the antihyperalgesic effect of fluoxetine in carrageenan model of inflammation and single dose treatment.

* p < 0,05 compared with control on the 1st hour; ** p < 0,05 compared with control on the 2nd hour; *** p < 0,05 compared with control on the 3rd hour; + p < 0,05 compared with diclofenac on the 1st hour; ++ p < 0,05 compared with fluoxetine and diclofenac on the 3rd hour.

aconthe 1sthour; ++ p< 0,05 comparedwithfluoxetine and diclofenaconthe 3rdhour.

The 5-HT_3 receptor antagonist significantly reduced the strength of the pressure at which the animals withdraw the inflamed paw. This effect was observed in single dose (fig. 3) and long term treated animals (data not shown). Since behavioral responses in this test are mediated by centers in the spinal cord [13] we can assume that spinal 5-HT_3 receptors are essential in the mechanism of the antihyperalgesic effect of fluoxetine.5-HT_3 receptors with its localization in primary nociceptive afferents of spinal dorsal horn, monoaminergic descending inhibitory pathways, peripheral nerve endings and autonomous afferent fibers play a significant role in spinal pain transmission and endogenous suppression of pain [14].

In spinal dorsal horn, 5-HT3 receptors are expressed on postsynaptic and presynaptic terminals of GABAergic interneurons, where they enhance the inhibitory effect of GABA. Experimental data suggest that 5-HT_3 receptors are also expressed in a subpopulation of enkephalinergic neurons in spinal dorsal horn [15]. Ondansetrone as an antagonist of 5-HT_3 receptors interrupts the communication between the serotonin, GABA, and enkephalins, and significantly reduced the 5-HT-mediated antinociception. We can assume that in inflammatory conditions, the antinociceptive effect of fluoxetine is realized on spinal level and 5-HT_3 receptors play an important role.

Conclusion

Fluoxetine has anti-inflammatory and antihyperalgesic effects in carrageenan model of inflammation. Centrally localized 5-HT_3 receptor mediated its anti-inflammatory effect only in single dose treated animals in the late phase of carrageenan inflammation. Spinal 5-HT_3 receptors are involved in the antihyperalgesic effect of fluoxetine.

REFERENCE

1 Sghendo L, Mifsud J. Understanding the molecular pharmacology of the serotonergic system: using fluoxetine as a model. J Pharm Pharmacol 2012;64(3):317-25. | 2. Abdel-Salam OM, Baiuomy AR, Arbid MS. Studies on the anti-inflammatory effect of fluoxetine in the rat. Pharmacol Res. 2004;49(2):119-31. | 3. Maes, M., Yirmyia, R., Noraberg, J., Brene, S., et al. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab Brain Dis. 2009;24(1):27-53. | 4. Altschuler EL, Kast RE. Bupropion in psoriasis and atopic dermatitis: decreased tumor necrosis factor-alpha? Psychosom Med. 2003;65(4):719. | 5. Singh VP, Jain NK, Kulkarni SK. On the antinociceptive effect of fluoxetine, a selective serotonin reuptake inhibitor. Brain Res. 2000;915(2):218-26. | 6. Zhao, ZQ, Chiechio, S, Sun, YG, Zhang, KH, et al. Mice lacking central serotonergic neurons show enhanced inflammatory pain and an impaired analgesic response to antidepressant drugs. J Neurosci2007; 27: 6045–53. | 7. Duman, E.N.; Kesim, M.; Kadioglu, M.; Yaris, E.; Kalyoncu, N.I.; Erciyes, N. Possible involvement of opioidergic and serotonergic mechanisms in antinociceptive effect of paroxetine in acute pain. J. Pharmacol. Sci.2004, 94, 161-165. | 8. Kesim M, Duman EN, Kadioglu M, Yaris E, Kalyoncu NI, Erciyes N. The different roles of 5-HT(2) and 5-HT(3) receptors on antinociceptive effect of paroxetine in chemical stimuli in mice. J Pharmacol Sci. 2005 Jan;97(1):61-6. Epub 2005 Jan 15. | 9. Faerber L, Drechsler S, Ladenburger S, Gschaidmeier H, Fischer W.The neuronal 5-HT3 receptor network after 20 years of research--evolving concepts in management of pain and inflammation. Eur J Pharmacol. 2007 Mar 29;560(1):1-8. | 10. Conner ThJ, Kelly JP, Leonard BE. An assessment of the acute effects of the serotonin releasersmethylenedioxymethamphetamine, methylenedioxyamphetamine and fenfluramine on immunity in rats. Immunopharmacology 2000;46:223– 35. | 11. Daher JB, de Melo MD, Tonussi CR. Evidence for a spinal serotonergic control of the peripheralinflammation in the rat. Life Sci. 2005;76(20):2349-59. | 12. Khan NA, Poisson JP. 5-HT3 receptor-channels coupled with Na+ influx in human T cells: role in T cellactivation. J Neuroimmunol1999;99(1):53–60. | 13. LeBars D, Gozariu M, Cadden SW. Animalmodels of nociception. Pharmacol Rev. 2001;53(4):597-652. | 14. Bardin L, Lavarenne J, Eschalier A. Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. Pain. 2000 May;86(1-2):11-8. | 15. Huang J, Wang YY, Wang W, Li YQ, Tamamaki N, Wu SX. 5-HT(3A) receptor subunit is expressed in asubpopulation of GABAergic and enkephalinergic neurons in the mouse dorsal spinal cord. Neurosci Lett.2008;441(1):1-6.