INVOLVEMENT OF 5-HT_2 AND 5-HT_3 RECEPTORS IN THE ANALGESIC EFFECT OF CLOMIPRAMINE ACCORDING TO THE FORMALIN PAIN TEST IN RATS

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ABSTRACT

Introduction: In recent years antidepressant drugs are widely used in the treatment of chronic pain, alone or more frequently as adjuvant of analgesics. Experimental and clinical studies indicate that the tricyclic antidepressant clomipramine has an analgesic effect. The possible mechanism of this action remains unclear. The **aim** of the present study was to investigate the possible involvement of 5-HT_2 and 5-HT_3 receptors in the analgesic effect of clomipramine after single and repeated administration**. Material and methods**: Male Wistar rats were divided in five groups (n=8), treated for 14 days with: saline (control), metamizole 150 mg/kg bw (positive control), clomipramine 20 mg/kg bw, clomipramine + 5-HT_2 receptor antagonist cyproheptadine (5mg/kg bw) and clomipramine + 5-HT_3 receptor antagonist ondansetrone (0,1 mg/kg bw) intraperitoneally. Antinociceptive test which used chemical (formalin test) stimuli was used. To evaluate the analgesic effect was used reduction the licking time of hind paw with intraplantar formalin injection. **Results**: In single dose treated animals clomipramine non significantly reduced licking time of inflamed paw when compared with control in both registered phases. After repeated administration this antidepressant reliably decreased licking time only in the late phase of formalin test. In single dose treated rats with clomipramine + ondansetron an increase of licking time in the early phase and a decrease in the late phase of formalin test was observed when compared with the group that received only clomipramine.After repeated administration ondansetron significantly increased licking time in the early phase as well as in the late phase of formalin test when compared with clomipramine treated group. In rats repeatedly treated with both clomipramine and ondansetrone the observed effect did not differ from the controls. In the group with acute and repeated co-administration of cyproheptadine and clomipramine statistically significant reduction of licking time was observed in both phases of formalin test when compared with the control and clomipramie group. **Conclusion:** Clomipramine has analgesic effect in formalin model of pain after repeated administration and this effect is mediated through spinal 5-HT_3 receptors.

Key words: *clomipramine, 5-HT_2 receptors, 5-HT_3 receptors, analgesia*

Introduction

Clomipramine is a tricyclic antidepressant that inhibits the reuptake of serotonin and norepinephrine in synapses of the CNS. Rojas-Corrales MO et al. found that both clomipramine and its active metabolite desmethylclomipramine have significant analgesic effect in experimental pain models (1). In a clinical trial has been established efficacy of treatment with clomipramine on musculoskeletal pain, as better therapeutic results were obtained in patients without depression. Fouquet B et al found that hypochondria and conversion disorders are predictors of better clinical response (2). The mechanism of the analgesic effect of clomipramine is not fully understood.

Serotonin is a key modulator of nociceptive transmission. It has mainly an inhibitory effect on pain. In genetically modified mice lacking central serotonergic neurons ($Lmx1b^{f/f/p}$ line) was observed persistent pain, which is inhibited by the intrathecal administration of 5-HT (3). Evidence suggest that the antinociceptive activity of many analgesics depends on the descending serotonergic system

In the rat spinal cord 5 -HT_2 receptors are present in the superficial and deep lamina of the dorsal horn. Experimental data indicate that the analgesic effect of antidepressants is dependent on

these receptors (4). The intraperitoneal injection of 5-HT_2 receptor antagonist ketanserin antagonized the analgesic effect of antidepressants of various groups in formalin test (5).

5-HT_3 receptors mediate antinociceptive effect in the spinal dorsal horn. There is evidence of involvement of 5-HT_3 receptors in the antinociception induced by antidepressants. 5-HT_3 receptor antagonist ondansetron inhibits the antinociceptive effect of imipramine after intraperitoneal administration , but not after intracerebroventricular injection in formalin test in rats (6).

The **aim** of this study was to determine the role of the 5 -HT2 and 5- HT3 receptors in the mechanism of the analgesic action of clomipramine after single and repeated administration in the formalin pain model in rats.

Material and methods

The experiment was approved by the Ethics Committee on Animal of the Bulgarian Agency for Food Safety permit N 56/19.03.2012 and decision of the Ethics Committee at the Medical University - Plovdiv, the protocol N 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 intraperitoneally (i. p.);

2nd group (positive control group) - treated with a reference analgesic drug metamizole in a dose of 150 mg/kg bw (i. p.) ;

3rd group - treated with clomipramine in a dose of 20 mg/kg bw (i. p.);

4th group - treated with clomipramine + 5-HT_2 receptor antagonist cyproheptadine 5mg/kg bw(i. p.);

5th group - treated with clomipramine + 5-HT_3 receptor antagonist ondansetrone 0,1 mg/kg bw (i. p.).

Formalin test

In one of the rat hind paw was injected intraplantar 200 μl 0.2% formalin. Licking time of inflamed paw was recorded for the first 10 minutes and thereafter between 20 and 30 minutes. As a sign of the analgesic action was reported reduction of licking time of the experimental animals compared with the saline control. Treated with metamizole control group was used as a standard for an analgesic effect.

Statistical analysis

Data were analyzed using the method of analysis of variance - One Way Anova from the software product SPSS 11.0. Mean values ± SEM were calculated. Nonparametric test of Kolmogorov-Smirnov show a normal distribution. A comparison of the results between groups was done using Independent – Samples T test. Results were considered significant at $p<0,05$.

Results

In single dose treated animals clomipramine non significantly reduced licking time of inflamed paw when compared with control in both registered phases. Metamizole as reference analgesic drug showed reliable increase in this index in the early and late phase of formalin test when compared with saline. In single dose treated rats with clomipramine + ondansetron an increase of licking time in the early phase and a decrease in the late phase of formalin test was observed when compared with the group that received only clomipramine. The group treated with both clomipramine and ondansetrone showed a significant increase in the licking time in the second phase of formalin test when compared with saline. Cyproheptadine significantly increased the effect of clomipramine on licking time in the early and late phase of the used pain model. In both phases the group treated with clomipramine and cyproheptadine showed significant difference when compared with saline and clomipramine treated group (Fig. 1).

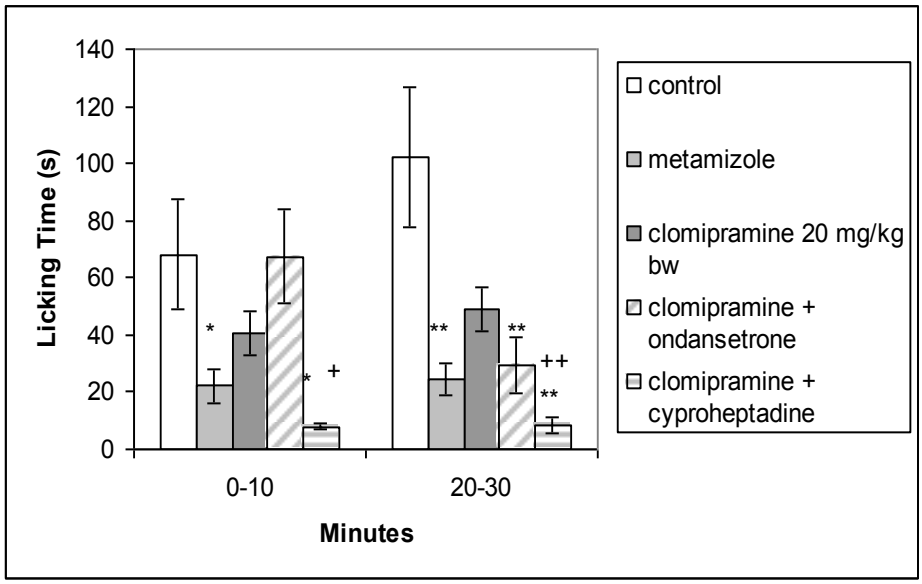

Fig. 1. Effect of the 5-HT_2 receptor antagonist cyproheptadine and 5-HT_3 receptor antagonist ondansetron on the analgesic effect of clomipramine in the formalin test after single treatment.

* p <0,05 compared with control on 0-10 min; + p <0.05 compared with clomipramine on 0-10 min; ** p <0,05 compared with control on 20-30 min; + + p <0,05 compared with clomipramine on 20-30 minute.

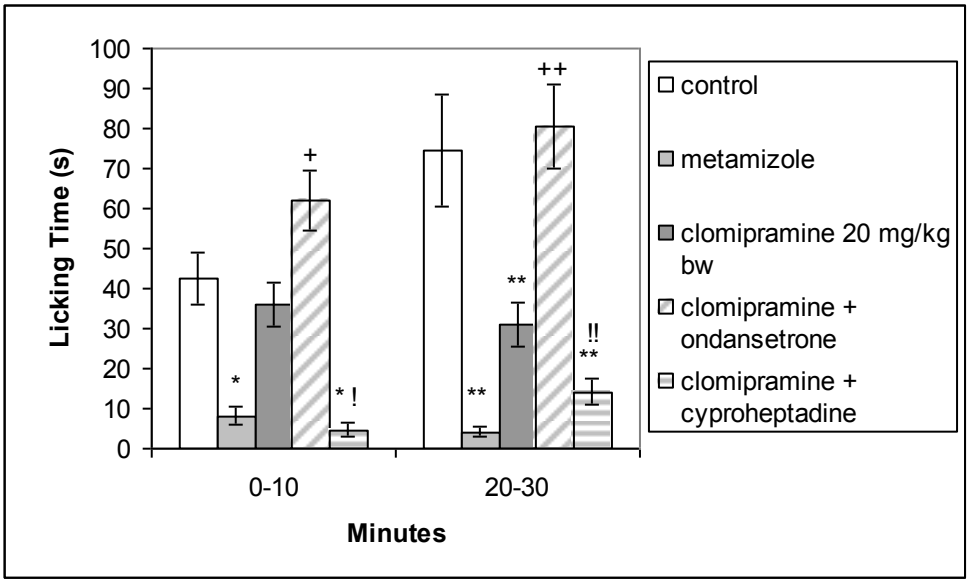

Fig. 2. Effect of 5-HT_2 receptor antagonist cyproheptadine and 5-HT_3 receptor antagonist ondansetron on the analgesic effect of clomipramine in the formalin test and repeated treatment.

* p <0,05 compared with control on 0-10 min; ! p <0.05 compared with clomipramine on 0-10 min; + p = 0.016 compared with clomipramine on 0-10 min; ** p <0,05 compared with control on 20-30 min; ++ p = 0.002 compared with clomipramine on 20-30 min; !! p <0,05 compared with clomipramine on 20-30 minute.

After repeated administration clomipramine reliably decreased licking time only in the late phase of formalin test. In contrast, metamizole showed significant effect in both phases in comparison with the control. Cyproheptadine significantly potentiated the effect of clomipramine in both phases of the test. The group treated with clomipramine + cyproheptadine have reliable antinociceptive effect in both phases of the used pain model, when compared with saline.

Ondansetron significantly increased licking time in the early phase as well as in the late phase of formalin test when compared with clomipramine treated group. In rats repeatedly treated with both clomipramine and ondansetrone the observed effect did not differ from the controls (Fig. 2).

Discussion

The results in our study indicate that the antidepressant clomipramine used in a dose of 20 mg/bw has analgesic effect in the formalin pain model, which is registered in the late phase of the test after repeated treatment. Nociceptive response to intraplantar formalin injection is biphasic. There is an initial acute period of 7 ± 10 min (phase 1). After a short period of remission from about 10 minutes, phase 2 begins, consisting of a longer period of sustained activity. Phase 1 is thought to be produced by the acute activation of nociceptors and primary afferent fibers by formalin injection, whereas phase 2 has been associated with the release of local endogenous mediators responsible for sensitization of primary and spinal sensory neurons and subsequent activation of the nociceptors (7). Our results show that clomipramine block nociceptive stimuli, due to the sensitization of spinal sensory neurons. In formalin test of normal and genetically modified mice lacking central serotonergic neurons (line $Lmx1b^{f/f/p}$) was established no significant difference in the first phase of the test and enhancement of behavioral responses in $Lmx1b^{f/f/p}$ line in the second phase (3). Probably central serotonergic pathways modulate the effects of clomipramine on spinal nociceptive mechanisms.

Our results show that cyproheptadine potentiate the analgesic effect of clomipramine in both phases of the formalin test . This effect in the first phase is intended, as the 5-HT_2 receptors in the periphery potentiate the pronociceptive effect of serotonin. Several studies have emphasized their role in the activation of nociceptors (8). These receptors interact with the receptors of prostaglandin E_2 and noradrenaline, leading to nociceptive reaction (9). The latter can be blocked by 5-HT_2 antagonists, as in the case of cyproheptadine. Abbott V et al reported the influence of 5-HT_2 antagonists on phase 2 of the formalin test similar to our results (9). Although in the second phase is observed sensitization of neurons in dorsal spinal horn, where these receptors mediate antinociceptive action of serotonin, the effect of peripheral inflammation can not completely be ignored.

Sasaki M et al. established the role of 5-HT_3 receptors in the antinociceptive effect of serotonin in both phases of the formalin test. This effect is antagonized by intrathecal administration of a 5-HT_3 receptor antagonist MDL-72222 (10). Our results showed that after single and repeated administration of ondansetrone antagonizes the non- significant analgesic effect of clomipramine in the first phase of formalin. Probably these receptors are responsible for the peripheral antinociceptive effect of clomipramine.

Our results showed significant involvement of these receptors in the analgesic effects of clomipramine in the late phase of formalin test only in continuously treated animals. Since in this phase the behavioral reactions are mediated by sensitization of the spinal dorsal horn this result indicates that the 5-HT_3 receptors play a role primarily in the antinociceptive effect of clomipramine at the level of the spinal cord. There is some evidence that serotonin -induced antinociception at spinal cord level is mediated by these receptors (11). Several studies indicate that these receptors are located on spinal GABAergic and enkephalinergic neurons (12). Their activation is followed by the release of GABA and enkephalins in the superficial dorsal horn,leading to antinociceptive effect (13).

Conclusion: Clomipramine has analgesic effect in formalin model of pain after repeated administration and this effect is mediated through spinal 5-HT_3 receptors.

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