Experimental

ANTINOCICEPTIVE EFFECT OF CLOMIPRAMINE THROUGH INTERACTION WITH SEROTONIN 5-HT2 AND 5-HT3 RECEPTOR SUBTYPES

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ABSTRACT

INTRODUCTION: Tricyclic antidepressants are used in the treatment of various pain syndromes. The antidepressant clomipramine inhibits predominantly the reuptake of serotonin in the central nervous system. The mechanism of its analgesic effect is not fully understood.

The aim of the present study was to find experimentally any dose-effect dependence in the analgesic effect of clomipramine and the involvement of the 5-HT2 and 5-HT3 receptors in the mechanism of this effect. Material and methods: Fifty male Wistar rats were used in the study allocated to five groups (10 animals each): a saline treated control group, one positive control group treated with metamizole and three experimental groups treated with intraperitoneally administered clomipramine in doses of 5, 10 and 20 mg/kg bw, respectively. To study the role of 5-HT2 and 5-HT3 receptors in this effect we used another five groups (10 animals each): control, positive control and three experimental groups treated with clomipramine only, clomipramine and granisetron and clomipramine and cyproheptadine, respectively. Three nociceptive tests were used: the hot plate test, analgesimeter and the acetic acid-induced writhing test. To gauge the antinociceptive action we used the increased latency in the hot plate test expressed as maximum possible effect % (%MPE), the increase in paw pressure to withdraw the hind paw in analgesimeter and decrease in the number of spinal cord writhes in the acetic acid test.

RESULTS: Clomipramine in a dose of 20 mg/kg bw significantly increased the %MPE in hot plate test and the pressure to withdraw the hind paw in the analgesimeter when compared with the control. In the acetic acid test clomipramine decreased non-significantly the number of writhes compared with the controls. Granisetron reduced non-significantly the antinociceptive effect of clomipramine in all tests. Cyproheptadine potentiated the analgesic effect of clomipramine in acetic acid test and decreased it significantly in the hot plate test.

In analgesimeter cyproheptadine decreased significantly the paw pressure to withdraw the tested hind paw at 1 hour and non-significantly at 2 hours.

CONCLUSION: Clomipramine in the dose of 20 mg/kg bw has a pronounced antinociceptive affect towards thermal and mechanical pain stimulation. The 5-HT2 and 5-HT3 receptor subtypes are very likely involved in the mechanism of this effect.

Key words: clomipramine, antinociception, serotonin, 5-HT2 receptor, 5-HT3 receptor

INTRODUCTION

Use of antidepressants alone or as adjuvant therapy for pain control has been increasingly practiced lately. Tricyclic antidepressants are effectively used in the management of chronic pain syndromes such as painful diabetic neuropathy, postherpetic neuralgia, tension-type headache, migraine and oral-facial pain. They are also effective in pain control of central etiology and in post-stroke conditions. There is evidence of an effect in controlling pain caused by arthrosis and arthritis which can be associated with a likely anti-inflammatory effect. Unlike the
antidepressive effect which has drug specific latency, the analgesic effect of an antidepressant has a very short latency. It usually occurs independently of the antidepressive effect and the doses needed to produce antinociception lower than those inducing an antidepressive effect.4

Clomipramine is tricyclic antidepressant. It inhibits the serotonin (5-hydroxytryptamine) and noradrenaline reuptake in the synapses of the central nervous system. The fact that the basic action of clomipramine is based on this mechanism suggests that we should explore the serotonergic and adrenergic pain inhibiting systems in the central nervous system. Clomipramine exhibits an antinociceptive effect in experiments with male rats after acute and chronic administration.5 The mechanism of this effect is not fully understood. The antidepressive effect of clomipramine is closely related to the inhibition of serotonin and noradrenaline reuptake. The major mechanism of action suggests that serotonergic and adrenergic pain-inhibiting systems may be implicated in its antinociceptive action. There are reports in the literature about the role of serotonin in the modulation of nociceptive transmission in the spinal cord.6,7 Autoradiographic studies showed high densities of 5-HT3 in superficial laminae of the spinal dorsal horn in rats where nociceptive messages are transmitted from primary afferents to spinal neurons.8 5-HT2 receptors are located in the periphery mediating there the serotonin-induced hyperalgesia. At peripheral level serotonin is involved in the pathogenesis of pain and inflammation potentiating the effects of bradykinins, prostaglandins and the like. There is evidence that this receptor subtype, and especially 5-HT2a subtype, is also located in the central nervous system.9 These somatodendrite-located receptors release upon stimulation the substance P which activates the nociceptive systems. The mechanism of the nociceptive effect of antidepressants and the participation of various mediators, the types of receptors, their agonists and antagononists are still obscure.

The aim of the present study was to investigate experimentally the dose-effect dependence of the analgesic action of clomipramine and find what role the 5-HT2 and 5-HT3 receptors play in the mechanism of this effect.

MATERIAL AND METHODS

ANIMALS

To study the dose-effect dependence of the analgesic action of clomipramine 50 male Wistar rats were randomly divided in five groups, ten animals each as follows:

- Group I – a control group comprising animals treated with saline (i.p.);
- Group II – a positive control group treated with the analgesic drug metamizole in a dose of 150 mg/kg bw (i.p.);
- Group III – clomipramine-treated animals in a dose of 5 mg/kg bw (i.p.);
- Group IV – clomipramine treated animals in a dose of 10 mg/kg bw (i.p.);
- Group V – animals treated with 20 mg/kg bw (i.p.) of clomipramine (Cl).

To study the role of serotonergic mediation in the antinociceptive effect of clomipramine male Wistar rats were randomly divided in five groups, ten animals each as follows:

- Group I – a control group with animals treated with saline (i.p.);
- Group II – a positive control group treated with the analgesic drug Metamizole in a dose of 150 mg/kg bw (i.p.);
- Group III – clomipramine-treated animals in a dose of 20 mg/kg bw (i.p.);
- Group IV – animals treated with clomipramine in a dose of 20 mg/kg bw (i.p.) and granisetron (5-HT3 antagonist) in a dose of 0.1 mg/kg bw (i.p.);
- Group V – animals treated with 20 mg/kg bw (i.p.) of clomipramine (Cl) and cyproheptadine (5-HT2 antagonist) in a dose of 5 mg/kg bw (i.p.).

Animals were housed under standard laboratory conditions (light-dark cycle: 12 : 12 h, 45% air humidity, room temperature - 26.5 ± 1 °C and water and food ad libitum.

DRUGS

Metamizole sodium (Sopharma), clomipramine hydrochloride (Novartis), granisetron (Roche) and cyproheptadine (Egis) were dissolved in distilled water. Emulsions with Tween emulsifier were prepared for lipid-soluble drugs. The antagonists granisetron and cyproheptadine were injected 30 minutes after the administration of clomipramine.

NOCICEPTIVE TESTS

HOT PLATE TEST

The test was described by Eddy and Leinbach10 and modified by Scheiber and Sawynok (1990). In this test rats were individually placed on a hot surface (Ugo Basile, Italy) with the temperature adjusted to 55 ± 1 °C. The latency to the first sign of paw licking or response to avoid the heat was taken as an indicator of the pain threshold; the cut-off time was 30 s to avoid tissue damage. The rats were
tested one hour before treatment and at 1 and 2 hours after treatment. For groups treated with two drugs, the rats were tested at 1 and 2 hours after the treatment with the second drug.

Latency was expressed as % of the maximum possible effect (%MPE), where MPE = (post-treatment threshold – pre-treatment threshold) x 100/(cut-off time – pre-treatment threshold).

**ANALGESIMETER**

The test was described by Randall & Selitto. In this test mechanical nociceptive stimulus was used. The nociceptive threshold was measured in centimeters with an analgesimeter (Ugo Basile) by applying an increasing pressure to the right hind paw of unrestrained rats. Paw withdrawal was used. The rats were tested one hour before the treatment and at 1 and 2 hours after the treatment. For groups treated with two drugs, the animals were tested at 1 and 2 hours after the treatment with the second drug.

**ACETIC ACID TEST (ABDOMINAL CONSTRICTOR TEST)**

The abdominal constrictor test was performed using 3% acetic acid (0.1 ml/100 g bw) administered intraperitoneally. The reaction of the animals to the chemical irritation of the peritoneum is spinal cord writhes. The number of writhes were counted for 20 minutes after the injection of the acetic acid. The rats were tested one hour after treatment.

**STATISTICAL ANALYSIS**

The data of the results were analyzed using the independent-samples t-test of SPSS version 11.0. Mean values ± SEM were calculated. Results were considered significant at p < 0.05.

**RESULTS**

**EFFECT OF CLOMIPRAMINE IN THE HOT PLATE TEST**

In the hot plate test the latency expressed as percent maximum possible effect (%MPE) in the controls was 21.18 ± 7.28 and 24.35 ± 6.52 at 1 and 2 hours, respectively. Metamizole decreases non-significantly this index compared to controls in both tests (%MPE = 35.87 ± 11.05% and %MPE = 42.5 ± 7.95%, respectively). Systemically administered clomipramine in a dose of 20 mg/kg bw significantly increases the latency at 2 hours compared with that caused by saline. There is no statistical difference in this index between groups treated with metamizole and clomipramine in a dose of 20 mg/kg bw. At 2 hours the latency in the animals treated with clomipramine in doses of 5 mg/kg bw and 10 mg/kg bw was statistically significantly shorter than that in the group treated with clomipramine in a dose of 20 mg/kg bw (Fig. 1). Fig. 2 shows the dose-effect dependence of the antinociceptive effect of clomipramine in the hot plate test.

**EFFECT OF CLOMIPRAMINE IN NOCICEPTIVE MECHANICAL PAW TEST**

In Randall & Selitto test (analgesimeter) the pressure at which the tested animal withdraws its hind paw (expressed in cm) in the control was 3.33 ± 1.19 cm and 5.33 ± 1.19 cm at 1 and 2 hours, respectively. In both tests metamizole treated group increases this index significantly compared with the control (p = 0.001 and p = 0.003, at 1 and 2 hours, respectively). There were no statistical differences in this effect between groups treated with clomipramine in the three tested doses and the group that received metamizole. In the three tested doses of clomipramine the paw pressure at which the animal withdraws its paw at 1 and 2 hours increases statistically significantly in comparison with that obtained in the controls (Fig. 3). Fig. 4 shows the dose-effect dependence of the antinociceptive effect of clomipramine in Randall & Selitto test.

**EFFECT OF CLOMIPRAMINE IN THE ACETIC ACID TEST**

In the acetic acid test metamizole decreases significantly the number of spinal cord writhes when compared with the control. Groups treated with clomipramine in the three tested doses show a non-significant decrease in the number of writhes compared to the saline treated group. There was no significant difference between groups treated with metamizole and clomipramine in a dose of 20 mg/kg bw (Fig. 5).

**EFFECT OF 5-HT2 AND 5-HT3 RECEPTOR ANTAGONISTS ON THE ANTINOCICEPTIVE EFFECT OF CLOMIPRAMINE IN THE HOT PLATE TEST**

In the clomipramine + granisetron treated group we had a non-significant decrease of %MPE when compared with the clomipramine treated group at 1 hour in the hot plate test. Animals treated with clomipramine and granisetron did not show any significant difference in this index compared with the saline treated group at 1 hour. At 2 hours the co-administration of clomipramine and granisetron caused a statistically significant increase in %MPE in comparison with the control. There was no significant difference in this index between the clomipramine + granisetron treated group and clomipramine only treated group. Cyproheptadine + clomipramine treated group showed significant increase in %MPE in comparison with the control at 1 and 2 hours. There was no significant difference
in %MPE between the clomipramine treated group and the clomipramine + cyproheptadine group at 1 and 2 hours (Table 1).

**Effect of 5-HT2 and 5-HT3 receptor antagonists on the antinociceptive effect of clomipramine in the nociceptive mechanical paw test**

In Randall & Selitto test (analgesimeter) there was no significant difference in the pressure to withdraw the hind paw at 1 and 2 hours between the groups treated with clomipramine + granisetron and clomipramine + cyproheptadine and the controls. We found a non-significant decrease of the pain threshold in the clomipramine + granisetron treated group in comparison with the clomipramine only treated animals. There was a significant decrease of the pressure at which the animal withdraws its paw in the group with co-administration of clomipramine and cyproheptadine at 1 hour and non-significant decrease at 2 hours in comparison with the pressure measured in the clomipramine only treated group (Fig. 6).

**Effect of 5-HT2 and 5-HT3 receptor antagonists on the antinociceptive effect of clomipramine in the acetic acid nociceptive test**

In this test all groups decreased significantly the number of writhes in comparison with those caused

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* p = 0.046 in comparison with the controls; ** p = 0.006 in comparison with clomipramine in a dose of 20 mg/kg bw; *** p = 0.046 in comparison with clomipramine in a dose of 20 mg/kg bw.

**Figure 1.** Effect of clomipramine in doses of 5, 10 and 20 mg/kg bw on the nociceptive threshold in thermal pain stimulation (hot plate test).

**Figure 2.** Dose-effect dependence of clomipramine antinociceptive effect in the hot plate test.
in the saline treated group. When compared with the clomipramine only treated group there was a non-significant increase in the writhing number in the clomipramine + granisetron treated group while in clomipramine + cyproheptadine group they decreased (Fig. 7).

**DISCUSSION**

The antinociceptive effect of different classes of antidepressants, including those with tricyclic structure, has been experimentally documented. As a representative of this class of drugs, clomipramine exerts an analgesic effect in experimental models of pain in intact rats and in rats in a neuropathic pain experimental model. There are single reports about the absence of such an effect and even about the inhibition of morphine induced analgesia. The effectiveness of clomipramine in the treatment of pain syndromes of various genesis is also confirmed by randomized clinical studies. A. Eschaller, et al. found that the intensity of the analgesia induced by antidepressants varies according to the pain stimulation in the following order: chemical > mechanical > electric > thermal. In the current study clomipramine demonstrated a pronounced dose dependent antinociceptive effect at the test with
thermal pain stimulation. In this test the effect is significant at 120 minutes with a dose of 20 mg/kg. The analgesic effect caused by the drug in smaller doses failed to reach significance, the intermediate dose causing the weakest effect. Rosland JH et al. demonstrated such an effect even at a dose of 10 mg/kg, in the hot plate test with acute and chronic administration of the drug.5 The same authors also found an effect of potentiation of morphine-induced analgesia by clomipramine. Using a model of mechanical nociception at 1 and 2 hours we found that pain threshold was lower for all tested doses. The intensity of the observed antinociception is the lowest at the intermediate dose tested. As an

Figure 5. Effect of clomipramine in doses of 5, 10 and 20 mg/kg bw on the nociceptive threshold in chemical pain stimulation (writhing test).

Table 1. Comparison between the clomipramine treated groups and the 5-HT2 and 5-HT3 receptor antagonists in thermal pain stimulation (hot plate test)

<table>
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<tr>
<th>Groups</th>
<th>Number</th>
<th>Min ± SEM</th>
<th>Minutes</th>
<th>t</th>
<th>P</th>
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<td>Controls</td>
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<td>Cyproheptadine</td>
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<td>81.05 ± 11.37</td>
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<td>31.13 ± 7.54</td>
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<td>Granisetrone</td>
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exception from the above rule about the influence of the nature of the stimulation on the observed effect, in our study, the test with acetic acid was not significant at all the three doses applied. Nevertheless, the high dose failed to reach statistically significant difference from metamizole, a standard analgetic, while the number of writhings was the highest when the intermediate dose was used. The intensity of the observed analgetic effect is not in direct ratio to the dose. It is due to the influence over a number of mediator systems, and receptor subtypes. According to the data we obtained we used 20 mg/kg dose for testing the possible mechanism of the antinociceptive action of clomipramine.

Figure 6. Response of clomipramine induced analgesia of 5-HT2 and 5-HT3 receptor antagonists in mechanical pain stimulation (Randall & Selitto test).

Figure 7. Response of clomipramine induced analgesia of 5-HT2 and 5-HT3 receptor antagonists in chemical pain stimulation (writhing test).
mechanical and chemical pain stimulation ("hot plate" test, tail flick test, Randall and Selitto test and formalin test).17

On the contrary the agonists of these receptors, such as 1-(m-chlorophenyl)-bi guanide induce antinociception at thermal (hot plate) and mechanical (colorectal distension test) pain stimulation.18,19 In the current study the 5-HT3 receptor antagonist granisetron inhibits the antinociceptive effect of clomipramine at mechanical pressure on the hind paw, at the acetic acid test and at 1 hour at the hot plate test. These results confirm the role of the of the spinal 5-HT receptors in the antinociception at three types of pain stimulation. The absence of significant decrease of analgetic effect of clomipramine at the thermal test at 2 hours can be explained with the fact that the behavioural responses to this test (licking of the hind paw and jump) are integrated by the supraspinal structures and are realized with the participation of C and Aδ type I and II fibres.20 Most probably the 5-HT3 receptors are not the leading ones in the realization of antinociception at supraspinal level.

The 5-HT2 receptors are heterogeneous by localization and functions. In the peripheral tissues they participate in the transmission of the nociceptive effect of serotonin as a mediator of pain and inflammation. In the peripheral pain model of peritoneal stimulation with acetic acid, cyproheptadine as an antagonist of these receptors facilitates the analgetic effect of the clomipramine.

In the central nervous system the 5-HT2 receptors are present in the superficial and the deep lamina of the dorsal horn of the spinal cord.21 But here their concentration is lower than that of 5-HT3 receptors. Their stimulation facilitates the nociceptive transmission through release of substance P by the presynapses. Experimental data show that the treatment with antagonists of substance P inhibits the nociception mediated by this receptor type.22 These data could explain the lowering of the pain threshold in clomipramine and cyproheptadine treated rats at the thermal nociceptive test. In a model of mechanical nociception the 5-HT2 receptor antagonist antagonizes the antinociceptive effect of clomipramine, which suggests that these receptors take place in the pain inhibiting descendent paths as well.23,24 Presumably, the receptor-mediated release of inhibiting neurotransmitters at supraspinal level (GABA in the prefrontal cortex of rats) or by the spinal interneurons is in the base of the antinociception observed.25

CONCLUSION

Clomipramine in the dose of 20 mg/kg has a pronounced antinociceptive affect on thermal and mechanical pain stimulation. Most probably the 5-HT3 and 5-HT3 receptor subtypes are involved in the mechanism of this effect at spinal level.

REFERENCES

Антиоцицептивный эффект кломипрамина посредством влияния на серотониновые 5-HT2 и 5-HT3 рецепторные субтипы

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Резюме

Введение. Трициклические антидепрессанты находят применение при лечении болевых синдромов различного происхождения. Кломипрамин ингибирует преимущественно обратный возврат серотонина в центральную нервную систему. Механизм его анальгетического эффекта еще не вполне выяснен.

Цель: Установить экспериментально доза-эффект» зависимость анальгетического эффекта кломипрамина и роль серотониновых 5-HT2 и 5-HT3 рецепторов в механизме этого эффекта.

Материал и методы: С целью установления доза-эффект зависимости анальгетического действия кломипрамина мускульные крьсы породы Wistar разделены на 5 групп по 10 (контрольная группа: подвергнутая воздействию физиологического раствора; позитивная группа: подвергнутая воздействию метамизола и 3 опытные группы, подвергнутые воздействию кломипрамина в дозах 5, 10 и 20 мг/кг м.т. интраперитонеально). В целях анализа различных ролей 5-HT2 и 5-HT3 рецепторов использовались и другие 5 групп животных по 10 в группе (контрольная группа, позитивная группа и 3 опытные группы, подвергнутые воздействию кломипрамина, кломипрамина + гранисетрона и кломипрамина + ципрогептадина). Животные подвергали воздействию трех нецицептивных тестах (горячая плита, анальгезиметр, индуцированное уксусной кислотой перидонечальное раздражение). В качестве критерия антиоцицептивного эффекта использованы уменьшение латентного времени при тесте „горячая плита”, представлено как процент максимального возможного эффекта (MPE %), увеличение силы давления при аналогезиметрии и уменьшение числа повоночных изгибов при тесте с уксусной кислотой.

Результаты: Кломипрамин в дозе 20 мг/кг м.т. синьицифицирует MPE MÆ и увеличивает силу давления соответственно при тестах „горячая плита” и анальгезиметр при сравнении контрольной групп. При техе с уксусной кислотой кломипрамин синьицифицирует числа повоночных изгибов при сравнении контрольной групп.

Гранисетрон несиньицифицирует рецидивирует антиоцицептивный эффект кломипрамина при трех примененных тестах. Ципрогептадин потенцирует эффект кломипрамина при тесте с уксусной кислотой и не снижает его синьицифицировано при тесте „горячая плита”. При аналогезиметрии ципрогептадин синьицифицирует силу давления при отодвигании на первом и втором час.

Заключение: Кломипрамин в дозе 20 мг/кг м.т. обладает выраженным антиоцицептивным эффектом относительно тепловых и механических болевых стимулов как 5-HT2 и 5-HT3 рецепторных субтипов и по всей вероятности играет роль в механизме этого эффекта.