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Possible antidepressant effects of some non-opioid analgesics

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Abstract

Several studies have suggested an association between depression and inflammation. Experimental and clinical trials on the effect of anti-inflammatory treatment on depression showed possible antidepressant properties of some nonsteroidal anti-inflammatory drugs in rodents and in humans. The aim of this study was to test in mice a possible influence of two chemically different non-opioid analgesics (ibuprofen and metamizole) on the antidepressant effect of amitriptyline. Swiss albino mice were used in a spontaneous locomotive activity test, a common measure of exploratory behavior and general activity, and the forced swimming test. Ibuprofen 30, 60, 120 and 240 mg kg-1 bw, metamizole 25, 50, 100 and 200 mg kg-1 bw, amitriptyline 5, 10 and 20 mg kg-1 bw were used alone or in combination. ANOVA and post-hoc tests were used for statistical analysis. Both non-opioid analgesics were not sedative in doses used in the forced swimming test. In this test amitriptyline had an antidepressant effect only at 10 and 20 mg kg-1 bw. Ibuprofen (60 mg kg-1 bw) and metamizole (100 mg kg-1 bw) did not have an antidepressant effect per se, but potentiated the effect of the non-antidepressant dose of amitriptyline (5 mg kg-1 bw), the results becoming statistically significant for the combinations ibuprofen-amitriptyline and metamizole-amitriptyline. The results suggest that non-sedative single doses of ibuprofen and metamizole could be used as adjuvants of classical antidepressant drugs to increase their antidepressant effect. The mechanism implicated may be interaction of these analgesics with serotonin and/or adrenergic central synapses, not with inflammatory and neuroproliferative processes involved in the pathogeny of depression.

Key words: ibuprofen, metamizole sodium, amitriptyline, sedation, antidepressant effect.

1. Introduction

Several studies have suggested an association between depression and inflammation (reviewed in DANTZER & al. [1], KRISHNADAS & CAVANAGH [2], ZUNSZAIN & al. [3], NAJJAR & al. [4]). Treatment with pro-inflammatory agents (Calmette-Guérin bacillus, endotoxins) causes depressive symptoms (SALEH & al. [5], DE PAIVA & al. [6], JAIN & al. [7]). A systematic review and meta-analysis of randomized clinical trials on the effect of anti-inflammatory treatment on depression, depressive symptoms, and their adverse effects showed possible antidepressant properties of some nonsteroidal anti-inflammatory drugs (NSAIDs), especially celecoxib (KÖHLER & al. [8]). Also, there is experimental evidence of antidepressant effects of NSAIDs (piroxicam, celecoxib, aspirin) in mice and rats (GUAN & al. [9], SANTIAGO & al. [10]). On the other side, there is evidence that NSAIDs decrease the effects of the antidepressant drugs. Certain NSAIDs showed depressant effects in humans in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) clinical trial. An analysis of this multicenter trial showed that concomitant administration of NSAIDs and antidepressants decreased the responder percentage to 45% in major depression, vs. 55% responder percentage when antidepressants were given without NSAIDs (WARNER-SCHMIDT & al. [11]). Other

experimental studies showed that certain NSAIDs (ketoprofen, naproxen, ibuprofen, aspirin, paracetamol) have depressant activity when given alone or decrease antidepressant activity of serotonin-specific reuptake inhibitors, SSRI when given in combination (WARNER-SCHMIDT & al. [11], RĂDUCANU & al. [12]). Experience of our laboratory showed a variable response – depressant, antidepressant and no effect for some agents of NSAIDs group (metamizole – possible depressant per se, parecoxib and ketorolac – antidepressant per se and meloxicam, lornoxicam – no effect) when administered in mice in single dose, 2 hours before the forced swimming test (FST) (PĂUNESCU [13], PĂUNESCU [14]). The aim of this work was to test in mice a possible influence of two chemically different non-opioid analgesics (ibuprofen and metamizole) on the antidepressant effect of amitriptyline.

2. Material and methods

Two behavioural tests were used in our approach: the spontaneous locomotive activity test and FST. The first test (spontaneous locomotive activity) was used to evaluate the possible sedative effect of various doses of metamizole and ibuprofen, which could interfere with movements of mice in the cylinder with water used in FST. The second test used (FST) evaluated the possible influence of the two NSAIDs on the antidepressant effects of amitriptyline, a tricyclic antidepressant (BORSINI & MELI [15]). The test was performed according the methodology of PORSOLT & al. [16].

Animals

Male NMRI mice, Swiss albino strain, bred in the biological hatchery of the "Carol Davila" University of Medicine and Pharmacy, Bucharest, were used. The animals were brought in from the hatchery 3 days before the experiments for adaptation to the new environment. They were kept under standard laboratory conditions, accommodated in acrylic plastic cages with the floor covered by wood shaving, 12 mice per cage, with ad libitum granulated food and water, at an environmental temperature of 21-22°C and relative humidity of 45-60%, under normal lighting conditions (between 07:00 – 19:00 hrs). All animal procedures were carried out with the approval of the local ethics committee for animal research of Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, in accordance with the European Communities Council Directive 86/609/EEC on the protection of animals used for scientific purposes.

Chemicals

The following substances were used: ibuprofen, metamizole sodium, amitriptyline. The substances were administered intraperitoneally (i.p.) in solutions. Pharmaceutical forms included the injectable form of metamizole sodium (Algocalmin – Zentiva, Romania) and powders of ibuprofen sodium and amitriptyline hydrochloride (both from Sigma Aldrich). The doses of ibuprofen and amitriptyline were calculated as free acid / free base not as salts.

Animal groups

Five groups were used in spontaneous locomotive activity test (n=10/group): in the first experiment a control group, that received normal saline and four test groups that received 25, 50, 100 and 200 mg kg-1body weight (bw) metamizole sodium, were used. In the second experiment (n=12/group), a control group, that received normal saline, and four test groups that received 30, 60, 120, 240 mg kg-1 bw ibuprofen were used. In FST experiments, a dose-effect relationship was performed for amitriptyline, using four groups treated with 5, 10, 20 mg kg-1 bw and normal saline, respectively (n=10/group). Afterwards, another FST experiment was performed using four groups (n=13/group): a control group, that received two injections of normal saline, one at 2 hours before the test and the second one 30 minutes before the test, a group that received metamizole sodium 100mg kg-1 bw and normal saline, a group that received amitriptyline 5 mg kg-1 bw and normal saline, a group that received amitriptyline 5 mg kg-1 bw and metamizole sodium 100 mg kg-1 bw. Metamizole sodium was given 2 hours before the test, while amitriptyline was given 30 minutes before the test. Normal saline was administered

2 hours before the test when given with amitriptyline, and 30 minutes before the test in the group that received metamizole alone.In the third FST experiment six groups of mice (n=11/group) were used as follows: a control group, that received two injections of normal saline, the first one 2 hours before the test and the second one 30 minutes before the test, a group that received amitriptyline 5 mg kg-1 bw and normal saline, a group that received ibuprofen 60 mg kg-1 bw and normal saline, a group that received ibuprofen 120 mg kg-1 bw and normal saline, a group that received amitriptyline 5 mg kg-1 bw and ibuprofen 60 mg kg-1 bw, a group that received amitriptyline 5 mg kg-1 bw and ibuprofen 120 mg kg-1 bw. Ibuprofen was given 2 hours before the test, while amitriptyline was given 30 minutes before the test. Normal saline was administered 2 hours before the test when given with amitriptyline, and 30 minutes before the test in groups that received ibuprofen alone, respectively. The vehicles of substances (normal saline) were administered in a volume of 10 mL kg-1 bw in spontaneous locomotive activity test in the left side of the abdomen and in a volume of 5 mL kg-1 bw, on each side of the abdomen (two doses that cumulate 10 mL kg-1) in FST. This experimental design was imposed by the administration of two different active substances intraperitoneally in the groups of mice in FST. Antidepressant drug amitriptyline was administered 30 minutes before test according to the method of SOCAŁA & al., 2012 [17]. Ibuprofen and metamizole were administered 120 minutes before test, according to our previous experience and to the method of DE PAIVA & al. [6]. This schedule of administration was chosen to give enough time for substances to penetrate the blood brain barrier and act centrally. After the peripheral administration of metamizole, some of its metabolites, namely 4-methylaminoantipyrine, 4-aminoantipyrine, 4-formylaminoantipyrin and 4-acetylaminoantipyrine were recovered from cerebrospinal fluid due to their lipophilicity (NIKOLOVA & al. [18]). 4-methylamino-antipyrine and 4-aminoantipyrine and other metabolites (arachidonic acid derivatives) were responsible for the pharmacodynamic effect of the parent drug in central nervous system (CNS) (ROGOSCH & al. [19]). Ibuprofen also crosses the blood-brain barrier as free acid, in a saturable mode (PAREPALLY & al. [20]). The pharmaceutical forms used were ibuprofen sodium salt and metamizole sodium salt which were highly soluble in saline, that allowed the administration of such high doses, of 240 mg kg-1 bw and 200 mg kg-1 bw, respectively.

Test equipment

In order to assess the exploratory behavior and general activity acrylic plastic cages illuminated with light from the ceiling of the room were used. The cage floor was divided into 20 equal 10 cm squares. The cage arena was cleaned following each trial. For the FST in mice, the test equipment consisted of Berzelius glasses 18 cm high, 10 cm diameter, water height 12 cm, water temperature of 28°C, and video recording systems.

Experimental protocol

In the spontaneous locomotive activity test the activity of mice was evaluated by calculating the number of squares crossed by mice (horizontal movements) in a period of 5 minutes. In the FST immobility was defined as lack of movements, except for respiratory movements and movements required for keeping the head above the water, with no significant active movements, either horizontally or vertically. Swimming was defined as active horizontal and vertical movements at the water surface. The time of swimming increases with the intensity of the antidepressant effect, and a low score seems to be associated with a possible depressant effect. The experiments were carried out in daylight conditions, between 08:30 and 16:30 hrs. The animals were left to swim for 6 minutes. Endpoint used was the swimming time calculated in seconds in the last 4 minutes of testing. The scoring was performed by staff trained for this purpose, 2 persons per experiment. The results were read in a blinded manner (the mice were assigned in three Berzelius glasses simultaneously, varying the immersion order into the glasses 1, 2, and 3 of each animal group), in accordance with the test protocol.

Statistical analysis

Microsoft Excel and SPSS version 15 were used for the purposes of statistical analysis. Homogeneity dispersion tests, ANOVA, and parametric post hoc tests – the Tukey test (based on homogenous dispersion) were used. $P<0.05$ was considered to indicate a statistically significant difference.

3. Results

In spontaneous locomotive activity test, in the first experiment, only the highest dose of metamizole sodium (200 mg kg-1 bw) diminished significantly the number of squares crossed by mice ($p<0.05$) compared with the control group, that received normal saline (Fig. 1).

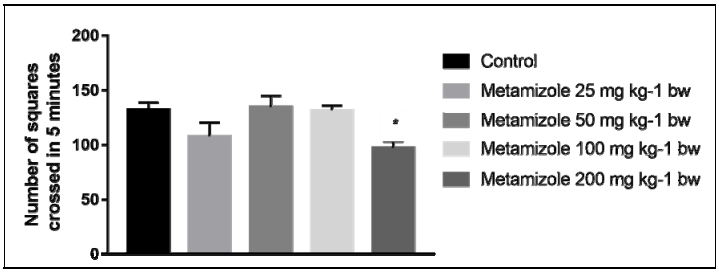

Fig. 1. Metamizole locomotive activity assessment. Each column represents the number of squares crossed by mice in 5 minutes ± standard error of mean (SEM). Control: 132.4±6.56; metamizole sodium 25 mg kg-1 bw: 107.8±12.74; metamizole sodium 50 mg kg-1 bw: 134.5±10.48; metamizole sodium 100 mg kg-1 bw: 131.6±4.47; metamizole sodium 200 mg kg-1 bw: 97.3±5.93. * $p<0.01$ vs control group.

In the same test, in the second experiment, only the highest dose of ibuprofen (240 mg kg-1 bw) diminished significantly the number of squares crossed by mice ($p<0.05$) compared with the control group, that received normal saline (Fig. 2).

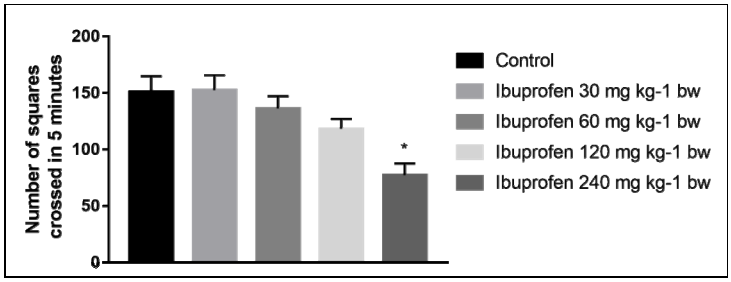

Fig. 2. Ibuprofen locomotive activity assessment. Each column represents the number of squares crossed by mice in 5 minutes ± SEM. Control: 151±13.68; ibuprofen 30 mg kg-1 bw: 152.33±13.46; ibuprofen 60 mg kg-1 bw: 136.5 ±11.01; ibuprofen 120 mg kg-1 bw: 118.25±9.14; ibuprofen 240 mg kg-1 bw: 77.58±10.57. * $p<0.01$ vs control group.

In order to establish the subeffective dose of amitriptyline, three geometric progressive doses were administred (ratio 2) in FST: 5, 10, 20 mg kg-1 bw. A dose-effect relationship was obtained, but only the doses of 10 and 20 mg kg-1 bw proved to be statistically significant, increasing the swimming periods of time. The dose of 5 mg kg-1 bw, which had a non-significant effect, was chosen to be used in the next experiments, in association with metamizole sodium and ibuprofen, respectively (Fig. 3).

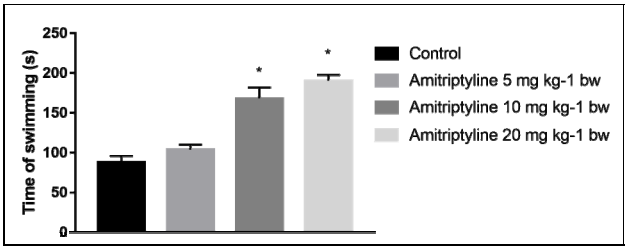

Fig. 3. Amitriptyline dose-effect relationship in FST. Each column represents the time of swimming (seconds) in the last 4 minutes of the trial ± SEM. Control: 87.5±8.34; amitriptyline 5 mg kg-1 bw: 103.5±6.59; amitriptyline 10 mg kg-1 bw: 168.75 ±14.01; amitriptyline 20 mg kg-1 bw: 190 ±7.61. * p<0.01 vs control group.

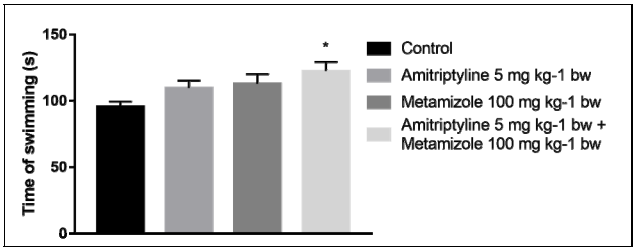

Fig. 4. Interaction between amitriptyline and metamizole sodium in FST. Each column represents the time of swimming (seconds) in the last 4 minutes of the trial ± SEM. Control: 95.39±.4.20; amitriptyline 5 mg kg-1 bw: 109.66±5.74; metamizole sodium 100 mg kg-1 bw: 112.69 ±7.52; amitriptyline 5 mg kg-1 bw and metamizole sodium 100 mg kg-1 bw: 122.31 ±7.06. * p<0.05 vs control group.

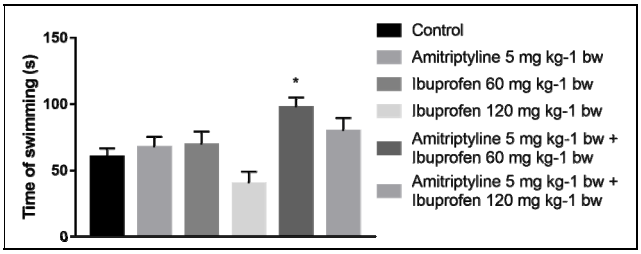

Fig. 5. Interaction between amitriptyline and ibuprofen in FST. Each column represents the time of swimming (seconds) in the last 4 minutes of the trial ± SEM. Control: 60±.6.68; amitriptyline 5 mg kg-1 bw: 67.27±8.13; ibuprofen 60 mg kg-1 bw: 69.45 ±9.94; ibuprofen 120 mg kg-1 bw: 40.18 ±9.18; amitriptyline 5 mg kg-1 bw and ibuprofen 60 mg kg-1 bw: 97.55 ±7.65; amitriptyline 5 mg kg-1 bw and ibuprofen 120 mg kg-1 bw: 79.64±10.09. * p<0.05 vs control group.

In the second FST, the subeffective doses of amitriptyline (5 mg kg-1 bw) and the non-sedative dose of metamizole sodium (100 mg kg-1 bw) did not modify the swimming periods of time, but their association induced a significant rise in this parameter. (Fig. 4). In the third FST, the subeffective doses of amitriptyline (5 mg kg-1 bw) and two non-sedative doses of ibuprofen (60 and 120 mg kg-1 bw) did not modify the swimming periods of time, but the association of ibuprofen 60 and amitriptyline 5 mg kg-1 bw induced a significant rise in this parameter. (Fig. 5)

4. Discussions

The spontaneous locomotive activity test, a common measure of exploratory behavior and general activity, and the forced swimming test, used to assess the possible antidepressant effect of a given substance, were used. In the spontaneous locomotive activity test were evaluated a wide range of doses including the ED50 for analgesic effect for metamizole sodium (90 mg

kg-1 bw), and ibuprofen (33 mg kg-1 bw), values according to literature – EMEA report [21], LILES & FLECKNELL [22]. The highest doses of metamizole sodium (200 mg kg-1 bw) and of ibuprofen (240 mg kg-1 bw) used had reduced significantly the number of squares crossed by mice, reflecting a possible sedative effect, which could interfere with the free movements of mice in FST. For this reason, in the following tests of forced swimming these doses were not tested. Only the highest non-sedative doses were used in these tests. In FST, a dose-effect relationship was obtained for amitriptyline, but only the doses of 10 and 20 mg kg-1 bw proved to be statistically significant, increasing the swimming periods of time. The dose of 5 mg kg-1 bw, which had a non-significant effect, was chosen to be used in the next experiments, in association with metamizole sodium and ibuprofen, respectively. In the second FST, the sub-effective doses of amitriptyline (5 mg kg-1 bw) and the non-sedative dose of metamizole sodium (100 mg kg-1 bw) did not modify the swimming periods of time, but their association induced a significant rise in this parameter, which signifies a possible antidepressant effect for this combination. In the third FST, the subeffective doses of amitriptyline (5 mg kg-1 bw) and two non-sedative doses of ibuprofen (60 and 120 mg kg-1 bw) did not modify the swimming periods of time, but the association of ibuprofen 60 and amitriptyline 5 mg kg-1 bw induced a significant rise in this parameter, which signifies a possible antidepressant effect for this combination. It may be mentioned that only the dose of 60 mg kg-1 bwof ibuprofen potentiated statistically significant the antidepressant effect of amitriptyline 5 mg kg-1 bw. The dose of 120 mg kg-1 bw proved a tendency to potentiate the effect of amitriptyline 5 mg kg-1 bw, but not statistically significant. Our results confirm data from literature that amitriptyline, in 10 and 20 mg kg-1 bw, had an antidepressant effect at 30 minutes after intraperitoneally injection (SOCAŁA [17], PARALE & KULKARNI [23]). In our experimental conditions, high doses of metamizole sodium and ibuprofen, two analgesic, antipyretic and anti-inflammatory substances which inhibit both cyclooxygenase isoenzymes COX1 and COX2, potentiated the antidepressant effect of amitriptyline administrated in subeffective doses. In this stage, it is difficult to state that this effect is definitory for the NSAIDs class, or is specific for singular substances, such as metamizole and ibuprofen, tested in our experiments. It may be mentioned that parecoxib, a COX2 selective inhibitor, tested before in our laboratory in the same experimental conditions, did not increase the effect of amitriptyline (5 mg kg-1 bw) (PĂUNESCU [14]). Although there is an inflammatory theory implicated in generation and progression of the depressive mood, the antiinflammatory action of these drugs cannot be involved in the potentiation of subeffective doses of amitriptyline, taking into account that these drugs were administered only 2 hours before evaluating their effect in FST. This period of time is not sufficient for the appearance of an antiinflammatory effect on cerebral tissue. Moreover, the animals studied were not under the influence of an inflammatory process in CNS. The forced swimming test statistically corelates with a clinical antidepressant effect, but this does not signify that animals tested are depressive. The mechanism implicated may be interaction of these analgesics with serotonin and/or adrenergic central synapses. The mechanism of action of amitriptyline is supposed to be the interaction with the norepinephrine transporter and serotonin transporter, key elements in the reuptake of the two neurotransmitters from synaptic cleft into presynaptic neurons (CHARNEY & al [24], STAHL [25]). This hypothesis might suggest that the antidepressant effect of the analgesics tested is not related to the inflammatory and/or neuroproliferative processes involved in the pathogeny of depression.

5. Conclusions

The results suggest that non-sedative single doses of ibuprofen and metamizole could be used as adjuvants of classical antidepressant drugs to increase their antidepressant effect. The mechanism implicated may be interaction of these analgesics with serotonin and/or adrenergic central synapses, not with inflammatory and neuroproliferative processes involved in the pathogeny of depression.

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