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Increased antidepressant-like effect of desipramine combined with central stimulants (caffeine and amphetamine) in mice

Research Article

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Abstract: Desipramine is a widely used antidepressive agent that inhibits the reuptake of noradrenaline and serotonin, and central stimulants such as caffeine and amphetamine help to release noradrenaline and serotonin. This work aimed to evaluate whether the combination of these agents could produce a stronger antidepressant-like effect than either of the drugs alone. To this end, male mice were treated with different doses of desipramine, caffeine, amphetamine, desipramine-caffeine and desipramine-amphetamine. The results showed that all drugs produced decreased immobility time in the forced swimming model. The combined treatment of desipramine (0.31, 1.0 or 3.1 mg/kg i.p.) with caffeine or amphetamine (0.31 or 1 mg/kg i.p.) reduced immobility time greater than either of those drugs alone. The combined treatment of desipramine (0.31, 1 and 3.1 mg/kg i.p.) with amphetamine or caffeine (0.1 and 1 mg/kg i.p.) did not increase the motor activity significantly compared to the control. These results also suggested that drugs which promote the release of noradrenaline and serotonin could increase antidepressant-like effect of desipramine.

Keywords: *Forced swimming test • Depression • Desipramine • Caffeine • Amphetamine*

1. Introduction

Depression is an alteration of the state of mind which is characterized by the presence of sad or guilty feelings, having suicidal thoughts, and commonly comes with vegetative symptoms such as sleeping disorders, appetite disorders or decrease in locomotion [1]. Approximately 15 million people have suffered with depressive disorder and 90% of these cases which present suicidal thoughts suffer other mental disorders [2]. Within the systems involved in depression, the noradrenergic system was found altered, so agents which act through this system are used in the treatment of depression [3]. The existence of a decrease in noradrenergic activity in important cerebral regions has been described [4], based on the fact that the main metabolite of noradrenaline, 3-methoxy-4-hydroxyphenolglycol (MHPG), is low in depressive patients compared to healthy individuals [5,6].

On the other side, it has been reported that dysfunctions of the serotonergic system at different levels of the central nervous system (CNS) are related to the depressive disorder. Patients with higher depression levels and post- partum depression had lower levels of L-tryptophan, which is an amino acid required in the synthesis of serotonin (5-HT) [7]. Various studies have demonstrated low levels of 5-hydroxyindole-3-acetic acid (5-HIAA), the main metabolite of 5-HT, in individuals which tried or committed suicide through violent

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methods. These studies suggested that decreased levels of serotonin could be one of the causes related to depression [8,9].

Within the common drugs used for the treatment of this disorder, there are non-selective inhibitors of the recapture of serotonin and noradrenaline (tricyclic antidepressants). Desipramine belongs to the group of tricyclic antidepressants. Its chemical structure corresponds to a secondary amina, which blocks the recapture of biogenic amines such as noradrenaline (NA) and serotonin, therefore it helps postsynaptic stimulation [10]. The central stimulants are substances that increase the stimulation at different regions of the brain and spinal cord producing a series of effects such as an increase in motor functions. Central stimulants lead to the different subjective sensations of decrease in fatigue, higher concentration capacity, increase in motivation and some improvement in the state of mind [11].

Previous studies suggested that central stimulants could be used in combination with antidepressants such as desipramine [12]. The aim of this work is to study the effects of co-treatment of desipramine with central stimulants (caffeine or amphetamine) in the forced swimming model and open field motor activity test of mice.

2. Experimental Procedures

2.1 Animals

Male Balb-c mice weighing 30-35 g, maintained at 22-27°C with free access to water and food, under a 12 h light-dark cycle, were used. All experiments were performed in line with the allowed use of laboratory animals, which are protected by the international political laws and with the approval of the local ethics committee (Superior School of Medicine, National Polytechnic Institute).

2.2 Behavioral test and drugs

Forced swimming test (FST): Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1°C. The total duration of immobility during the 6 minute period was measured. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, and did only those movements necessary to keep its head above the water [13,14]. The agents including amphetamine, desipramine and caffeine (Sigma, USA) were dissolved in saline and administered i.p. to mice (10 ml/kg body weight) 30 minutes before the FST. Control mice received only saline.

Open field motor activity test: The apparatus consisted of an opaque Plexiglas box (40×30×20 cm) with the floor divided into 12 equal squares. The animals were placed in a corner of the apparatus and its behavior was videotaped during a 5 minute session in order to count the lines crossed when the animal entered each square (lines crossed per 5 minutes). The box was carefully cleaned between tests. A decreased number of counts are interpreted as a decrease in motor activity [15].

2.3 Data analysis

The results are expressed as means ± SEM in groups of six mice. Comparisons between the treated groups and control were performed by analysis of variance (ANOVA) followed by Duncan's multiple range test when appropriate. A value of $P<0.05$ was considered to be significant.

The interaction of two different drugs can be defined by the following conditions:

Additivity $d_A/DA + d_B/D_B = 1$

Antagonism $d_A/D_A + d_B/D_B > 1$

Synergism $d_A/D_A + d_B/D_B < 1$

Where DA and DB in the denominators are the doses for desipramine and caffeine or amphetamine alone that gives 77 seconds (the value, which all the combinations reached), whereas dA and dB are the doses of desipramine with caffeine or amphetamine in combination, which also produced 77 seconds (*i.e.* isoeffective) [16].

3. Results

The obtained results showed that desipramine, caffeine and amphetamine caused an antidepressant-like activity in a dose-dependent manner in the forced swimming test by shortening the immobility time of mice. The significant effects of desipramine, caffeine and amphetamine appeared at the doses of 10 mg/kg, 3.1 mg/kg and 3.1 mg/kg, respectively. There was no significant difference in the maximum effect induced by desipramine alone (31 mg/kg) and caffeine alone (31 mg/kg) (76.00±17.68 s *vs*. 46±11.58, $F(1,10)=2.015$, NS), however, amphetamine alone (31 mg/kg) exhibited a significantly high maximum effect on immobility time compared with desipramine alone (31 mg/kg) (5.33±3.48 s *vs*. 76.00±17.68, $F(1,10)=15.38$ $P<0.05$) (Figure 1).

Moreover, the present data showed that the co-treatment of desipramine (0.31, 1.0 or 3.1 mg/kg) with caffeine or amphetamine (0.31 or 1.0 mg/kg) reduced immobility time greater than that with either of those drugs alone (Figure 2). The synergism analysis showed that in the effect of 77 seconds of immobility time, caffeine (0.7026 mg/kg i.p.) and amphetamine

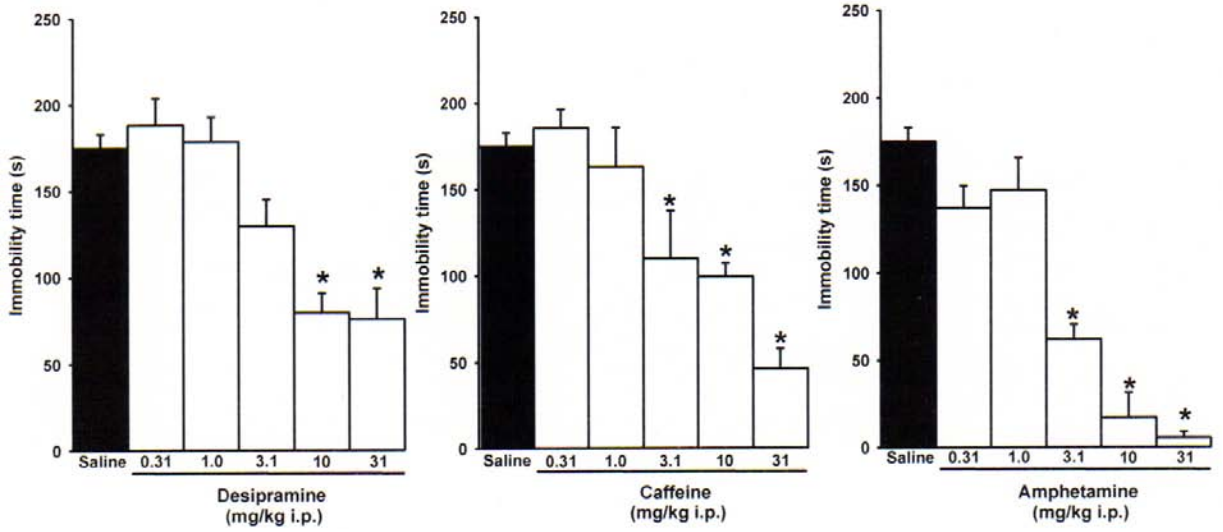

Figure 1. Effect of different desipramine, caffeine and amphetamine doses over immobility time in the forced swimming model. Black bars represent saline and the white ones desipramine, caffeine or amphetamine. The bars show the means ± SEM. * $P<0.05$ *vs*. saline.

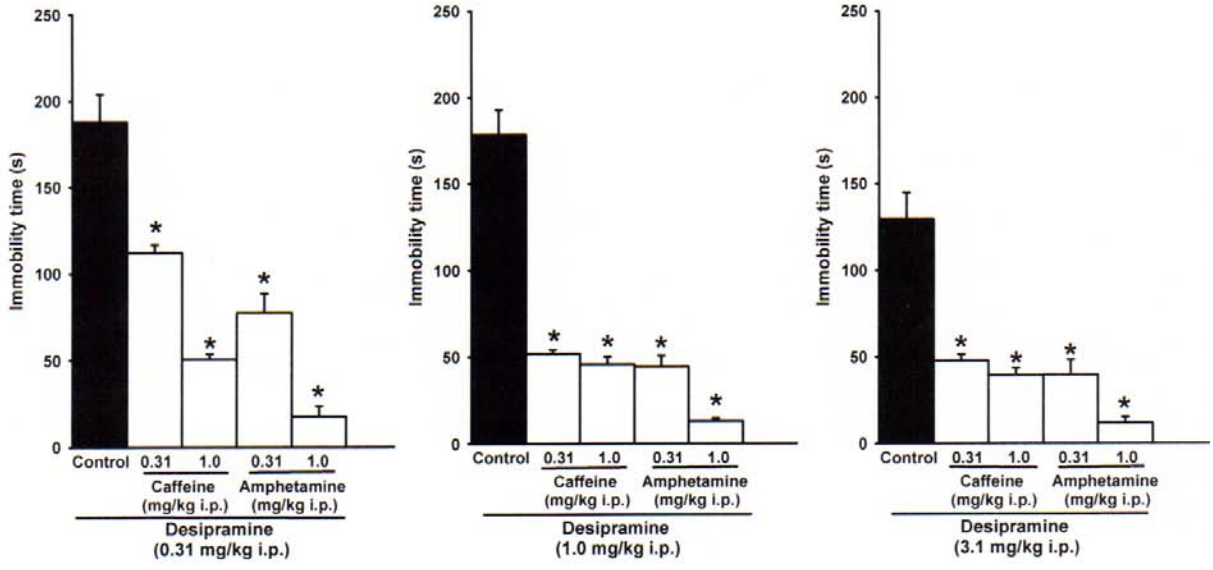

Figure 2. Effect of the combination of desipramine (0.3, 1 and 3.1 mg/kg i.p.) with caffeine (0.31 and 0.1 mg/kg i.p.) and amphetamine (0.31 and 0.1 mg/kg i.p.) over immobility time in the forced swimming model. Black bars represent desipramine alone and the whites ones all the combinations. The bars show the means ±SEM. * $P<0.05$ *vs*. desipramine alone.

(0.3138 mg/kg i.p.) produced a synergistic effect when they were combined with desipramine (0.31 mg/kg i.p.) (0.04956 and 0.1280, respectively). The analysis of motor activity showed that there was no significant difference in motor activity between the combined treatment of desipramine with caffeine and amphetamine to the control (Figure 3).

4. Discussion

The results showed that desipramine with central stimulants (amphetamine and caffeine) reduced the immobility time more than those drugs alone. Moreover, the combined treatment of desipramine with caffeine and amphetamine produced pronounced inhibition of immobility without modifying the motor activity.

Recent data suggested that the central stimulants could modify immobility time in model FST [17,18]. One study showed that a low dose of the non-selective adenosine receptor antagonist caffeine (1 mg/kg) inhibited the increase of immobility time in rats without modifying the spontaneous open field motor activity [18]. These data were in line with our present findings.

Binding studies have reported that serotonergic neurons of the dorsal raphe nucleus have adenosine

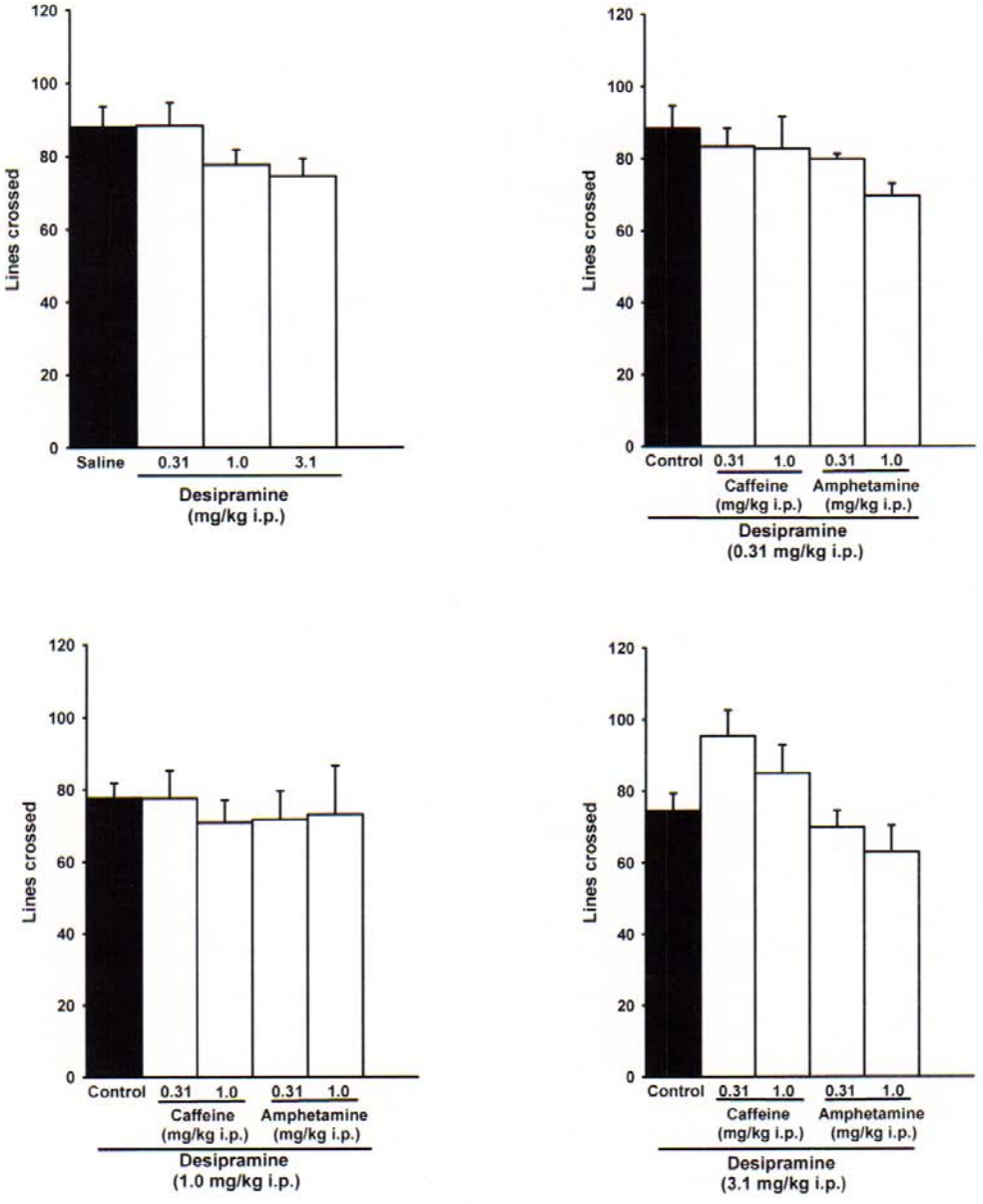

Figure 3. Effect of different desipramine, caffeine or amphetamine doses over locomotor activity in the open field test. Black bars represent control (saline or desipramine alone) and the white ones desipramine alone or combinations. The bars show the means ± SEM. * $P<0.05$ *vs.* saline.

receptors type 1 (A1R) [19]. Electrophysiology studies have also reported that the locus coeruleus contains receptors type A1R and the activation of these receptors by either adenosine or selective A1R agonists caused a decrease in the firing of these neurons [20], which leads to a decrease in adrenergic and serotonergic transmission [21].

On the other hand, the model of FST is related to the increase in the release of adenosine [22]. This increase leads to the activation of its receptors (A1R) to increase immobility time [23]. The possible mechanism involved in the FST model is partly due to the decrease of noradrenergic and serotoninergic activity [24,25]. Thus the administration of caffeine, which blocks the effects of endogenous adenosine, could prevent the decrease in nerve transmission and increase levels of serotonin and norepinephrine at a central level [23,26,27], and hence it produces a decrease of immobility time in FST model [28]. Caffeine promotes antidepressant effects of desipramine because this agent inhibits the reuptake of serotonin and norepinephrine in a non-selective way [10,29]. It helps activate postsynaptic receptors by increasing the

release and the inhibition recapture. Another important effect of the desipramine-caffeine combination is that desipramine can decrease the metabolism of caffeine, principally its demethylation and hydroxylation [30], this inhibition could contribute to the observed effect.

We observed that amphetamine exhibited its antidepressant characteristics in the FST model; this is consistent with the other results [23,31]. It has been suggested that the mechanism involved in its antidepressant activity is through the release of noradrenaline from sensitive and insensitive deposits to reserpine [32]. Thus amphetamine could increase the antidepressant activity of desipramine, on one hand it could increase neurotransmitter release, and on the other hand it could inhibit its recapture, which favors stimulation time for accumulation in synaptic space. The pharmacokinetic interactions between desipramine and amphetamine cannot be excluded because desipramine can inhibit the *in vivo* hydroxylation of amphetamine, and hence it increases the unmetabolized amphetamine in the brain. Although these effects are mainly observed in rats more than in mice [33,34].

Our synergism analysis showed that both combinations (desipramine-amphetamine and desipramine-caffeine) produced supra-additive synergism at low doses with which no antidepressant activity was observed in their treatment alone, indicating that these agents could be used to shorten the lag time for the effect and to increase its potency.

In conclusion, our results suggested that central stimulants such as caffeine and amphetamine could increase the antidepressant response of desipramine partly due to the facilitation of liberation of noradrenaline and serotonin and partly due to the inhibition of their recapture.

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