Original paper

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Comparative Effect between Antidepressants and D-phenylalanine, a Phenethylamine Precursor, in an Animal Model of Depression

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Abstract - A relevant role has been attributed to phenethylamine in depressive disorders. It has been measured in human urine and rat brain in pathological conditions and after drug administration. Furthermore, a clinical correlation has been proposed between urinary elimination and depressive symptoms. Furthermore, its metabolic predecessor, D-phenylalanine, has been used as an antidepressant drug in the treatment of depressive disorders. The use of this amino acid has been realized alone, or in combination with classical antidepressants. In the present study, we tried to characterize its behavioural profile comparing it with imipramine and fluoxetine. Antidepressant drugs have been studied using diverse animal models. We used here the Porsolt test, or Forced Swimming Test (FST), measuring times of climbing, swimming and resting. When a comparison was performed between groups in climbing behaviour, significant differences were observed between imipramine treated group and saline controls ($p < 0.05$), and imipramine versus fluoxetine and D-phenylalanine ($p < 0.01$). When swimming was evaluated, clear differences between D-phenylalanine and the other groups were observed ($p < 0.001$). Additionally, a significant difference was also observed between imipramine and fluoxetine ($p < 0.01$). When resting was evaluated, high differences between D-phenylalanine versus all other groups were shown ($p < 0.001$). Observed behavioural profile was according to serotonergic antidepressant drugs effects. It is supported by the fact that swimming behaviours were increased, and a correlative decrease in resting was also present. We conclude that D-phenylalanine showed higher antidepressant potency than other classical antidepressants, at least at the doses used.

Key words: Phenylalanine; imipramine; fluoxetine; antidepressive agents; behaviour

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Introduction

A relevant role has been attributed to phenethylamine in depressive disorder [1-5]. It has been measured it in human urine and rat brain in pathological conditions and after drug administration [1,3-5]. Furthermore, a clinical correlation has been proposed between uri-

nary elimination and depressive symptoms. Furthermore, its metabolic predecessor, D-phenylalanine (DPA), has been used as an antidepressant drug in the treatment of depressive disorders [6]. In Argentina, early studies of Fisher and co-workers in 23 patients gave relevant evidences regarding the efficacy of D-phenylalanine association in patients who were not responders to classical tricyclic antidepressants or mono amino oxidase inhibitors [7]. This study was extended to 472 cases, also in Argentina, by Mesones and Cia [6]. They found important findings regarding urinary phenylethylamine concentration and depressive disorders, and a relevant response to D-phenylalanine in these patients. Similar findings were obtained by Beckmann and Ludolph in Germany [8,9]. The use of this amino acid has been realized alone [8,10], or in combination with classical antidepressants [6]. In the present study, we tried to characterize its translational behavioural profile comparing it with imipramine and fluoxetine. Antidepressant drugs have been studied using diverse animal models. We use here the Porsolt test, or Forced Swimming Test (FST), measuring time of climbing, swimming and resting.

In the last years, the FST has been largely used in the study of antidepressant actions [11-14]. The neurotransmitter systems involved in the action of antidepressants have also been identified using this method [15]. The chosen antidepressants have different mechanisms of action. Imipramine is one of the most classical tricyclic antidepressants, and its main mechanism of action has been linked to noradrenaline reuptake inhibition [16]. Fluoxetine has been characterized as a selective serotonin reuptake inhibitor [17]. The mechanism of action of D-phenylalanine has been matter of several possibilities. Its role as amphetamine homolog, and as antidepressant has been reported, as a precursor of phenylethylamine [18,19]. Its efficacy has been compared to imipramine [19]. Consecutively, its use as antidepressant has been widely proposed [8,9,19]. Additionally, action of trace amines on a specific receptor (trace-amine associated receptor, TAAR1), has been considered matter of future studies in this research area [20]. Taking into account all mentioned evidences, we considered interesting to study a comparative effect of the precursor D-phenylalanine with so called classical antidepressants in a translational approach.

The aim and rationale of this preliminary study was to compare the action of DPA with the above mentioned two classical antidepressants, aiming to experimentally validate its antidepressant behavioural action. Additionally, results may allow knowing if DPA action is mainly mediated by noradrenergic or serotonergic systems.

Materials and Methods

Subjects

A group of 38 male rats of a Holtzman derived colony, weighing between 280-310 g and aged 90 days old was used. Animals were maintained under controlled temperature conditions (22 – 24 °C) and lighting (lights on 0500 – 1900 h). They were fed with Standard rat pellets. Pellets and water were freely available.

Drugs

Three drugs were used: imipramine (20 mg/kg), fluoxetine (10 mg/kg) and D-phenylalanine (30 mg/kg). They were all for use in humans. Drugs were kindly provided by Mrs. Sarita Roitman (Sevilla Pharmacy, Mendoza, imipramine and fluoxetine) and Mr. Henry Wittaker (Magister Pharmacy, Buenos Aires, D-phenylalanine).

Apparatus

The apparatus is a plexiglass cylinder (height 60 cm, diameter 30 cm) containing water (25 °C) up to 20 cm of the cylinder's height.

Test Procedure

Forced swimming test consists of two different expositions to the apparatus. The first one, or pretest treatment, was done placing the rats 15 min in the test apparatus, without drugs, 24 h prior to the experiments. After 24 h of the pretest, the second one or test phase was initiated. During it, the group of 38 rats was divided in four groups: saline (n = 9), imipramine

20 mg/kg (n = 9), fluoxetine 10 mg/kg (n = 10) and D-phenylalanine 30 mg/kg (n = 10). After 5 min of injection, the behavioural test was displayed according classical criteria, measuring time spent in climbing, swimming and resting. Test phase durations was 5 min [11-15].

Statistics

Kolmogorov Smirnov test was used to determine the parametric distribution of data. Data were processed with ANOVA 1 followed by Student Newman-Kewls. In all cases, a level of $p < 0.05$ was considered significant.

Bioethics

Experiments were realized according to bioethical rules. Bioethical and legal dispositions were considered in animal's care. All housing and all experimental procedures were carried taking into account the rules of the project approval criteria of the National University of Cuyo. We followed also the guidelines set by European Community Council (Directive 86/609/EEC).

Results

When a comparison was performed between groups in climbing behaviour, a significant difference was observed between them (ANOVA, F 3.34 = 6.547; $p < 0.01$). When groups were compared using the Student Newmann Kewls post-test, significant differences were observed between imipramine treated group and saline controls ($p < 0.05$), and imipramine versus fluoxetine and DPA ($p < 0.01$, Figure 1).

An intergroup difference was observed in swimming (ANOVA, F 3.34 = 28.77, $p < 0.0001$). The Student Newmann Keuls post-test showed differences between DPA and the other groups ($p < 0.001$, Figure 2). A significant

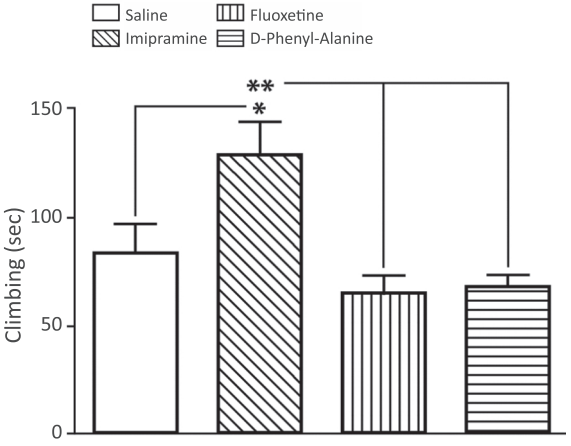

Figure 1. Effects of imipramine (20 mg/kg), fluoxetine (10 mg/kg) and D-phenylalanine (30 mg/kg) on climbing behaviour time in the Forced Swimming Test. A significant statistical difference was observed between imipramine and control rats ($p < 0.05$). A high statistical difference was observed between imipramine and other antidepressant drugs ($p < 0.01$). Data are presented as means ± SEM. A value of $p < 0.05$ was considered significant. * = $p < 0.05$; ** = $p < 0.01$.

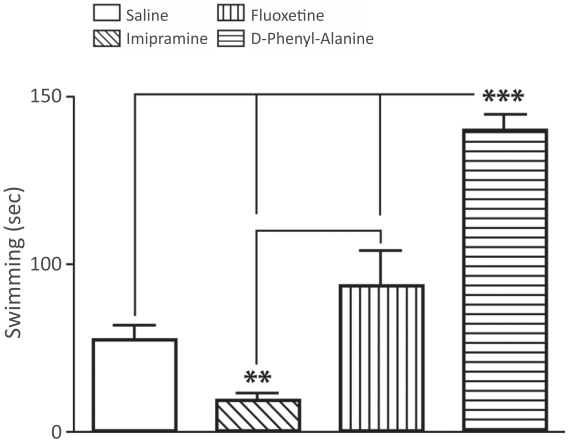

Figure 2. Effects of imipramine (20 mg/kg), fluoxetine (10 mg/kg) and D-phenylalanine (30 mg/kg) on swimming behaviour time in the Forced Swimming Test. A high statistical difference was observed when D-phenylalanine effect on swimming was compared to controls, imipramine and fluoxetine treated rats ($p < 0.001$). A very significant statistical difference was also observed between imipramine and fluoxetine groups ($p < 0.01$). Data are presented as means ± SEM. A value of $p < 0.05$ was considered significant. ** = $p < 0.01$; *** = $p < 0.001$.

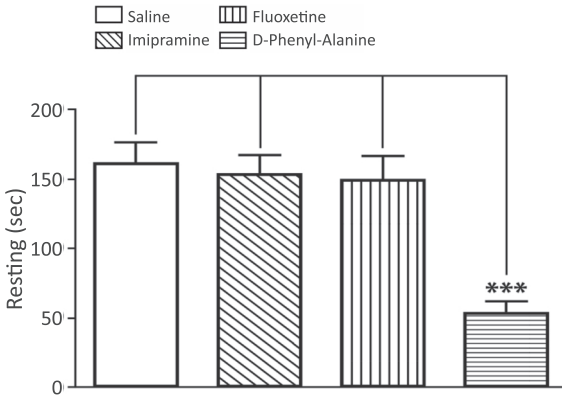

Figure 3. Effects of imipramine (20 mg/kg), fluoxetine (10 mg/kg) and D-phenylalanine (30 mg/kg) on resting behaviour time in the Forced Swimming Test. A very high statistical difference was observed when D-phenylalanine effect on resting was compared to controls, imipramine and fluoxetine treated rats ($p < 0.001$). Data are presented as means ± SEM. A value of $p < 0.05$ was considered significant. *** = $p < 0.001$.

difference was also observed between imipramine and fluoxetine ($p < 0.01$, Figure 2).

When resting was evaluated, intergroup differences were found (ANOVA, $F\ 3.34 = 12.15$, $p < 0.0001$). The Student Newmann Kewls post-test showed differences between DPA versus all other groups ($p < 0.001$, Figure 3).

Discussion

Considering different behavioural parameters, a clear effect was observed using these drugs. Imipramine induced an important increase in climbing behaviour when compared to saline controls ($p < 0.05$), or fluoxetine and D-phenylalanine treated groups ($p < 0.01$, Figure 1). When swimming behaviour was studied, relevant differences between D-phenylalanine and the other groups were observed ($p < 0.001$, Figure 2). A significant difference was also observed between imipramine and fluoxetine ($p < 0.01$, Figure 2). Resting was also considered, and relevant differences between D-phenylalanine versus all other groups were observed ($p < 0.001$, Figure 3). Synthesizing, imipramine increased climbing, showing a prevalent noradrenergic profile. Swimming was decreased as showing a typical behavioural displacement. An increase in climbing reduced significantly swimming time. Fluoxetine did not increased swimming behaviour when compared to control group. However, this difference was observed when compared to imipramine that reduced swimming increasing climbing. It may be interpreted as a behavioural displacement. In this experiment, D-phenylalanine was surprisingly active, increasing some serotonergic parameters. In this way, climbing behaviour was not modified by this drug, but swimming and decreases in resting were observed. This last parameter, resting as an immobility state, has been over lighted as representative of a mood lowered state, and it has been considered the best sign of antidepressant drug actions [11]. In this experiment, this was the most affected by D-phenylalanine administration. It strongly suggests a clinical predictive value for this drug. The relevance of the FST to assess antidepressant-like activity has been remarked in several reviews. An important number of evidences have signalled that different antidepressants, acting on different neurotransmitter systems, may modify different responses in FST [15,21-28]. In this way, it has been reported that an elevation in serotonergic neurotransmission increases predominantly swimming behaviour [25-28]. By the opposite way, noradrenergic neurotransmission increase induces higher climbing behaviours [25-28]. In our experimental schedule, imipramine produced a noradrenergic like effect, and D-phenylalanine induced a clear serotonergic like behavioural effect. The effect of fluoxetine at the dose used was not significant when compared to controls, but an increase in swimming behaviour was insinuated, and it was different of imipramine when both groups were compared. Negative results have been reported using DPA as antidepressant in a small group of patients [29]. It was attributed to a low absorption of D-ami-

no acids in the digestive apparatus. However, other studies performed in important group of patients and delimitating subgroups with low urinary phenylethylamine excretion led to clear therapeutic results [6,8]. These evidences clearly point DPA as a therapeutic resource, in combination with other classical antidepressants or even alone [6, 8], as it has been extensively reviewed [10]. Present findings give additional support to the evidences observed previously in our country [6] and corroborated in Germany [7]. Relation with urinary levels of Phenylethylamine [1,4] was also observed in Argentina. Recently, new interest has been paid to trace amine associated receptor [30]. Treatment based in the stimulation of these receptors has been proposed for several psychiatric pathologies [31]. Administration of D-phenylalanine has been used in our country since a lot of years ago in the treatment of depressive disorder [6].

This study is preliminary in nature and, strictly speaking, would constitute a pilot study. Successive additional studies should be carried out to see the scope of these findings. We may conclude that DPA was very active in this predictive translational model. It was also interesting that this drug induced a serotonergic-like behavioural pattern in the FST.

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Conflict of Interest

None to declare.

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