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Differential Effects of Beta-Carbolines and Antidepressants on Rat Exploratory Activity in the Elevated Zero-Maze

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PÄHKLA, R., A. KASK AND L. RÄGO. *Differential effects of beta-carbolines and antidepressants on rat exploratory activity in the elevated zero-maze.* PHARMACOL BIOCHEM BEHAV **65**(4) 737–742, 2000.—Present experiments were designed to compare the effects of antidepressants desipramine (10 and 20 mg/kg IP) and fluoxetine (5 and 10 mg/kg IP) with anxiogenic β-carboline DMCM (0.5 and 1.0 mg/kg IP) in the elevated zero-maze test in rats. The second aim of this study was to assess the effects of pinoline (6-methoxy-1,2,3,4-tetrahydro-β-carboline) in the rat elevated zero-maze test in comparison with structurally unrelated β-carboline DMCM and antidepressants. The time spent in the open part of the elevated zero-maze was not significantly affected by antidepressants, but was decreased by β-carbolines pinoline and DMCM. The number of line crossings in the open parts and the number of head dips were also decreased more by β-carbolines in comparison with antidepressants. Latency to enter the open part was statistically significantly increased only by DMCM. Measurement of locomotor activity in a separate experiment indicated that activity of the rats' time moving, distance traveled, and number of rearings were reduced by all four drugs studied. These results demonstrate that the effects of antidepressants in the elevated zero-maze test differ from the effects of the reference anxiogenic compound DMCM. The effects of pinoline and DMCM in the zero-maze test were similar, which suggests the involvement of mechanisms other than serotoninergic in the action of pinoline. © 2000 Elsevier Science Inc.

Beta-carbolines Antidepressants Exploratory activity Elevated zero-maze

THE elevated zero-maze is a modification of the elevated plus-maze originally described by Shepherd and colleagues (21), and it has been suggested to increase the sensitivity of the test. The elevated zero-maze design incorporates both traditional and novel ethological measures in the analysis of drug effects. It differs from the traditional plus-maze in that it excludes the exploration of a central platform allowing continuous movement around the apparatus, and thus reduces ambiguities in the interpretation of time spent on the central square of the traditional design. The effects of benzodiazepines in the elevated zero-maze have been described before (3, 21), including data from our laboratory (13). There are several studies showing that antidepressants are inactive or anxiogenic-like in the elevated plus-maze test after acute administration (8,18). However, the effects of antidepressants in the zero-maze model are studied less extensively. The aim of the present experiments was to reveal possible differences in the effects of antidepressants in comparison with anxiogenic DMCM in the elevated zero-maze test. From antidepressants two different drugs were chosen for our experiment: fluoxetine, as an example of mainly a serotonergic compound, and desipramine as a noradrenergic compound.

The second aim of this study was to assess the effects of pinoline (6-methoxy-1,2,3,4-tetrahydro-β-carboline) in the rat elevated zero-maze test in comparison with anxiogenic β-carboline DMCM, and antidepressants fluoxetine and desipramine. Pinoline is a member of a large group of pharmacologically active compounds, beta-carbolines. Pinoline can be formed in the mammalian body under physiological conditions from serotonin (5-hydroxytryptamine, 5-HT) or melatonin (5,9,16). It inhibits the activity of monoamine oxidase-A (7), serotonin reuptake (11), and binds to the imipramine and citalopram recognition site (12,17). In a former experiment, pinoline exerted a dose-dependent antidepressant-like effect in the rat forced-swimming test, and decreased the exploratory activity in the elevated plus-maze (15).

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In addition, we assessed the effect of the same drugs on rat activity in apparatus enabling automated monitoring and recording of locomotor activity.

METHOD

Animals

Adult male Wistar rats weighing 240–320 g were used in all experiments. Animals obtained from the National Laboratory Animal Center, Kuopio, Finland, were housed five per cage under standard laboratory conditions for at least 2 weeks prior to testing. The animal room had a 12 L:12 D cycle, with lights on at 0800 h. Food and drinking water were available ad lib. One hour before the experiment the cages with animals were moved from the animal room to the behavioral testing room. All experiments were performed between 1200 and 1900 h. The study protocol was approved by the Ethics Committee for Animal Research of the University of Tartu.

Drugs and Treatment

Drugs used in the present experiments were pinoline (6-methoxy-1,2,3,4-tetrahydro-β-carboline), desipramine hydrochloride, fluoxetine hydrochloride (all from Sigma, St. Louis, MO), and DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate), donated by Schering AG, Germany. Desipramine and fluoxetine were dissolved in distilled water; pinoline was dispersed in an aqueous suspension with a few drops of Tween 85 [polyoxyethylene-(20)-sorbitan oleate]; DMCM was dissolved in 0.1 ml 0.1 N HCl and diluted with distilled water. Drugs were injected intraperitoneally in a volume of 1 ml/kg 30 min before testing. Control groups received injections of distilled water. After administration of a drug the animal was returned to the home cage.

Elevated Zero-Maze Test

The elevated zero-maze has been designed in accordance with the original description of Shepherd et al. (21), with a few modifications. The elevated zero-maze is an annular platform (width 10 cm) with a diameter of 105 cm, divided into two opposite open parts and two opposite closed parts (height of the side walls 40 cm). The open parts have borders (height 1 cm). All parts of the apparatus were made of black stained metal, and the apparatus was elevated 50 cm above the floor.

For a test, the animal was placed into one of the open parts facing the closed part of the apparatus, and observed for 240 s. Behavioral measures taken included: (a) the time of latency, i.e., the time of first entry with all four paws from the closed part into the open part; (b) number of open part entries; (c) time spent in the open part; (d) the number of line crossings in the open parts; (e) the number of head dips over the edge of the platform; (f) the number of stretch-attend postures.

Locomotor Activity Determination

Locomotor activity was measured using ActiMot system (TSE Technical and Scientific Equipment, Bad Homburg, Germany). In this test rats were placed at the center of rectangular box 40 × 40 cm, with 40-cm high side walls made of transparent plastic. The box was equipped with infrared sensors coupled to a personal computer through an interface. This system allowed all movements of the rat in the box to automatically register. Measures taken during 20 min (four periods of 5 min) were: (a) time resting; (b) distance traveled; and (c) number of rearings.

Statistics

One-way analysis of variance (ANOVA) was used to compare the data from zero-maze experiments and repeated measures ANOVA for locomotor activity data. A post hoc least-significant difference (LSD) test was applied to compare group means with the control. $p < 0.05$ was deemed significant.

RESULTS

Elevated Zero-Maze Test

Results of an elevated zero-maze test are presented in Figs. 1 and 2. Factorial ANOVA revealed that DMCM had a significant effect on latency to enter the open part, $F(2, 19) = 5.65$; $p < 0.05$. A post hoc LSD test revealed that both doses of DMCM (0.5 and 1.5 mg/kg) significantly increased the latency to enter the open part. All other drugs tested had no effect—both doses of pinoline and the highest dose of desipramine tested (20 mg/kg) only tended to increase this parameter. DMCM and pinoline-treated rats made significantly less open-part entries, $F(2, 19) = 27.5$ and $F(2, 18) = 30.0$, respectively, $p < 0.001$, and spent less time in the open part of the maze, $F(2, 19) = 9.3$ and $F(2, 18) = 11.6$, $p < 0.005$. Pinoline treatment had also a significant effect on the number of stretch-attend postures, $F(2, 18) = 3.7$, while the effect of DMCM did not reach significance, $F(2, 19) = 2.2$, $p = 0.13$, NS. Both doses of pinoline increased the number of stretch-attend postures, and so did, in fact, the lowest dose of DMCM (0.5 mg/kg). Both β-carbolines significantly reduced the number of head dips, $F(2, 18) = 21.3$, $p < 0.001$, for pinoline and $F(2, 19) = 23.71$, $p < 0.001$, for DMCM, respectively, and the number of line crossings in the open part, $F(2, 18) = 25.79$, $p < 0.001$, for pinoline and $F(2, 19) = 19.70$, $p < 0.001$, for DMCM, respectively.

Desipramine (10 and 20 mg/kg) and fluoxetine (10 mg/kg) had a significant effect on the number of open-part entries, $F(2, 20) = 3.6$ and $F(2, 20) = 3.5$, respectively, $p < 0.05$. Although both doses of desipramine and the highest dose of fluoxetine (10 mg/kg) decreased the number of open-part entries, these drugs did not decrease the time spent in the open part, $F(2, 20) = 0.82$ and $F(2, 20) = 0.3$, respectively, NS. Fluoxetine had significant effects on activity of the rats in the open part, $F(2, 20) = 5.1$, $p < 0.05$, while the effect of desipramine was just below a significance margin, $F(2, 20) = 3.1$, $p < 0.06$. When the LSD test was applied to these data it was found that rats treated with desipramine (10 and 20 mg/kg) and fluoxetine (10 mg/kg) made fewer number of line crossings in the open part. Stretch-attend postures remained unaltered with desipramine and fluoxetine treatment, $F(2, 20) = 0.84$ and $F(2, 20) = 1.3$, respectively, both NS. ANOVA revealed that from the antidepressants only fluoxetine had a significant effect on the number of head dips, $F(2, 20) = 4.9$, $p < 0.05$; a post hoc test indicated that the number of head dips was significantly reduced by a 20 mg/kg dose of fluoxetine only.

Locomotor Activity Determination

Results of the locomotor activity test are presented in Figs. 3 and 4. A one-way ANOVA revealed that pinoline and DMCM had a significant effect on time moving, $F(6, 54) = 4.94$ and $F(6, 57) = 2.77$, respectively, $p < 0.05$, distance traveled, $F(6, 54) = 5.82$ and $F(6, 57) = 10.53$, respectively, $p < 0.05$, and number of rearings, $F(6, 54) = 8.29$ and $F(6, 57) = 17.23$, respectively, $p < 0.05$. All the above cited results refer

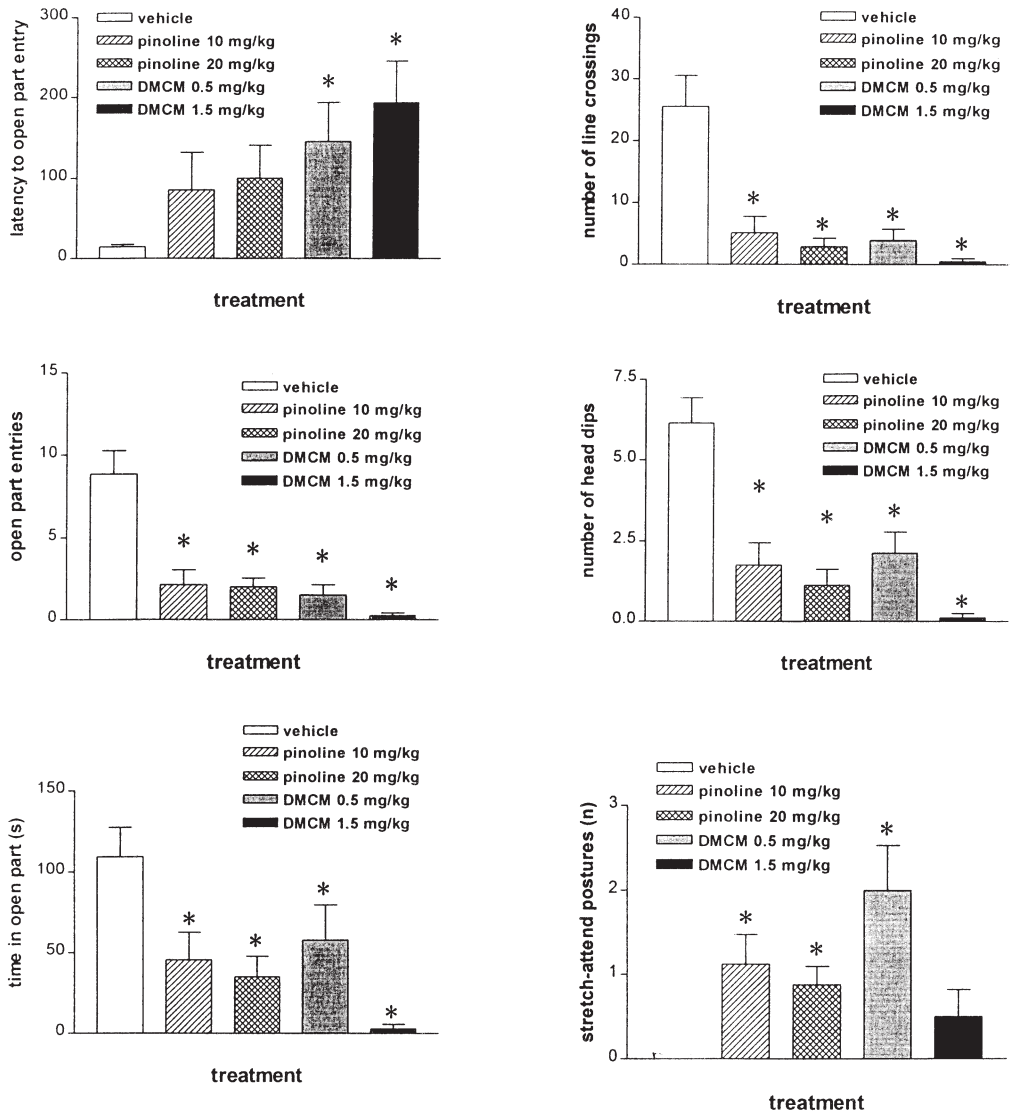

FIG. 1. The effects of DMCM (0.5 and 1.0 mg/kg IP) and pinoline (10 and 20 mg/kg IP) on elevated-zero maze behavior of the rats. Drugs were injected intraperitoneally 30 min before the test. *$p < 0.05$ LSD test. Rats of control group did not make any stretch-attend posture in this experiment.

to time and treatment interactions. Individual comparisons of measurements carried out at specific intervals indicated that the activity of pinoline- and DMCM- treated rats differed from the behavior of saline-treated animals during the first 15 min of the test. Thereafter, the exploratory activity of the control rats had decreased so much that their behavior become indistinguishable from the pinoline- and DMCM-treated rats.

In a second experiment on a separate group of the rats we studied the effects of desipramine and fluoxetine using the same experimental conditions. An ANOVA indicated that desipramine and fluoxetine had a significant effect on the time spent moving [treatment effect, $F(2, 20) = 8.68$ and $F(2, 20) = 5.50$, respectively; $p < 0.05$]. There was no significant interaction between time and treatment, $F(6, 60) = 1.538$, $p = 0.18$ for desipramine, and $F(6, 60) = 0.86$, $p = 0.51$ for fluoxetine. Desipramine and fluoxetine also reduced the distance traveled [treatment effect, $F(2, 20) = 9.21$ and $F(2, 20) = 4.70$, respectively, $p < 0.05$]. Interaction between time and treatment was just below the limits of statistical difference for desipramine, $F(6, 60) = 2.05$, $p = 0.07$, and nonsignificant for fluoxetine, $F(6, 60) = 1.043$, $p = 0.40$. Fluoxetine and desipramine reduced the number of rearings [time and treatment interaction, $F(6, 60) = 3.61$ for desipramine, and $F(6, 60) = 2.40$ for fluoxetine, respectively, $p < 0.05$.

DISCUSSION

The elevated plus-maze test has been well validated for testing the anxiolytic and anxiogenic effects of the drugs. In the plus-maze, the open/total arm entries ratio is used to assess the effect of a drug on the anxiety level, whereas the

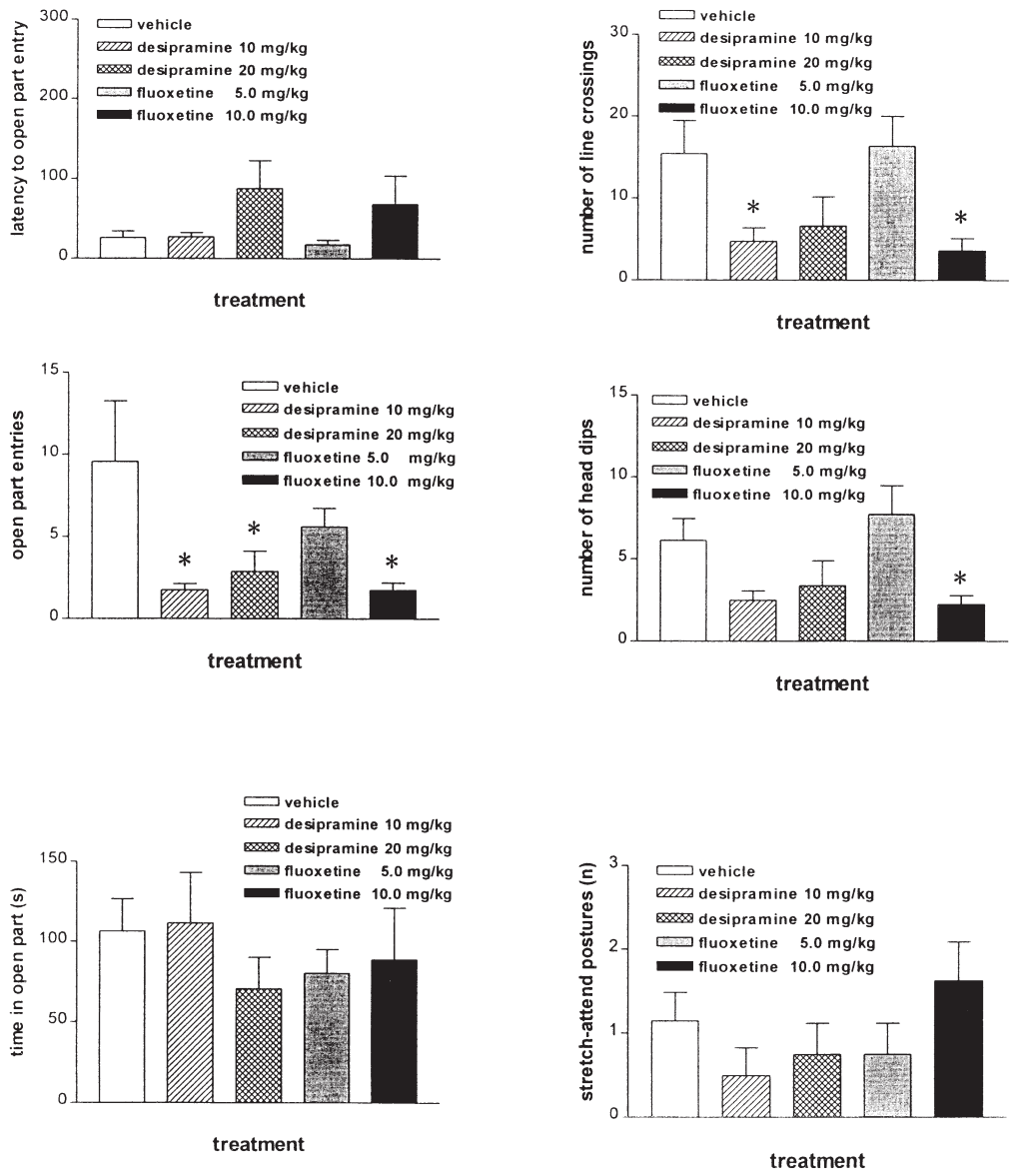

FIG. 2. The effects of desipramine (10 and 20 mg/kg IP) and fluoxetine (5 and 10 mg/kg IP) on elevated-zero maze behavior of the rats. Drugs were injected intraperitoneally 30 min before the test. *$p < 0.05$ LSD test.

number of total arm entries is taken to reflect a general locomotor activity (6,18). In the zero-maze test, due to the consecutive arrangement of the open and closed parts, the number of open and closed-part entries is essentially the same. The main criteria for the anxiolytic effect of the drug in the zero-maze are the increased number of open-part entries and the increased percentage of the time spent in the open parts. Several published studies are showing that diazepam dose dependently increased both of these criteria (3,13,21).

In the present experiment, all studied drugs decreased the number of open-part entries. However, this may reflect only the decreased motor activity of the animals, as in the locomotor activity box all drugs in both tested doses also decreased activity parameters—time spent moving, distance traveled, and the number of rearings. Also, some rats treated with fluoxetine and desipramine entered the open part and remained there immobile, showing no interest or fear towards the testing environment for a considerable amount of time. This observed behavioral profile is more likely related to general suppressant effects rather than an anxiogenic action of antidepressants. The time spent in the open part of the elevated zero-maze was not significantly affected by antidepressants, but was decreased by the β-carbolines pinoline and DMCM. The number of line crossings in the open parts and the number of head dips were also significantly more decreased by β-carbolines in comparison with antidepressants.

The number of stretch-attend postures is an ethological measure believed to assess the risk assessment behavior (6). In the present experiment, however, rats from all treatment groups made only a few stretch-attend postures. This favored

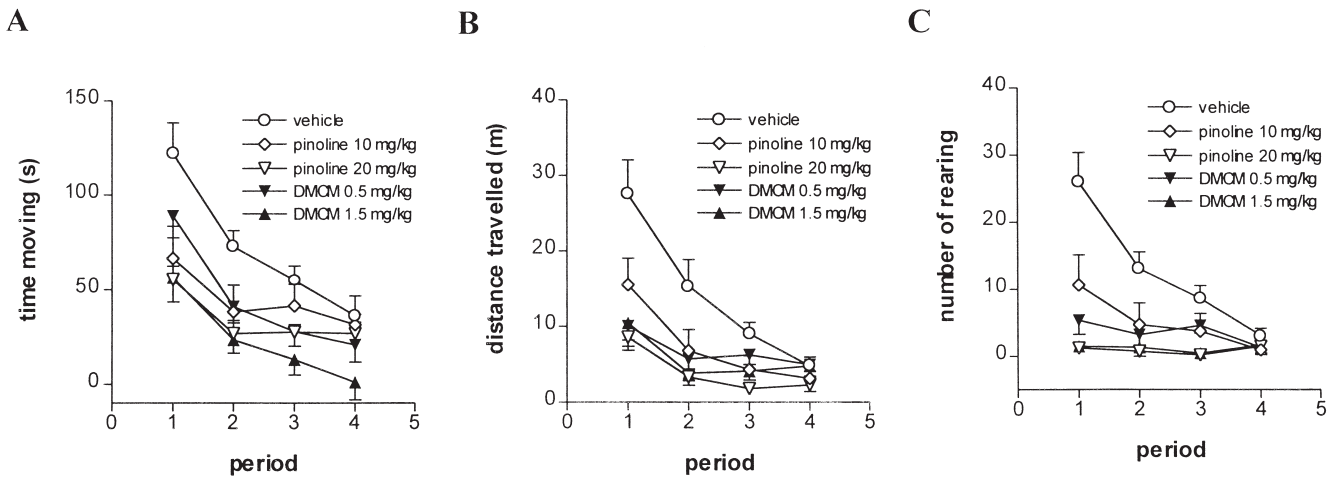

FIG. 3. The effects of DMCM (0.5 and 1.0 mg/kg IP) and pinoline (10 and 20 mg/kg IP) on locomotor activity of the rats. Drugs were injected 30 min before the test. Rats were then placed in transprent plexiglas boxes, and their behavior was monitored for 20 min. Graph A shows the effects of these treatments on time spent moving, graph B represents the distance traveled, and the number of rearing is shown on graph C. See the Results section for detailed statistical comparisons.

the random distribution in the number of stretch-attend postures, which is exemplified by the absence of this behavior in the rats of the one control group.

In our study, we also measured the time of latency before the first entry into the open part. This index was not measured in the previous studies by Bickerdike et al. (3) and Shepherd et al. (21). Matto et al. (13) found this parameter very variable, and not appropriate to characterize the anxiolytic or anxiogenic effects of the drug. In the present study, the latency was statistically significantly increased by DMCM, and also increased, though statistically nonsignificantly, by pinoline and higher doses of antidepressants.

In conclusion, the first part of our experiment revealed that the time spent in the open part of the zero-maze was a most reliable measure to differentiate between the effects of antidepressants and β-carbolines. The number of line crossings and the number of head dips were also parameters more affected by β-carbolines in comparison with antidepressants.

Concerning the effect of pinoline, our study revealed significant anxiogenic properties of this compound. The time spent in the open parts of the apparatus and the number of head dips were significantly reduced both by pinoline and DMCM but not by antidepressants. This finding is in line with former plus-maze experiments (15). In this study, the anxiogenic-like properties of pinoline were explained by the serotoninergic effects of the compound.

In the present study the effects of pinoline were compared with antidepressants with serotoninergic (fluoxetine) and noradrenergic (desipramine) properties (10). However, the effects of pinoline in the zero-maze test differed from those of fluoxetine and desipramine. This suggests the involvement of mechanisms other than serotoninergic.

In contrast to known anxiogenic β-carbolines, pinoline does not have any affinity for the benzodiazepine binding sites (2,19). The binding capacity of β-carbolines to the benzodiazepine receptor correlates with the convulsive potential of these compounds (19). In contrast, pinoline has no convulsive potential. Moreover, in some animal models anticonvul-

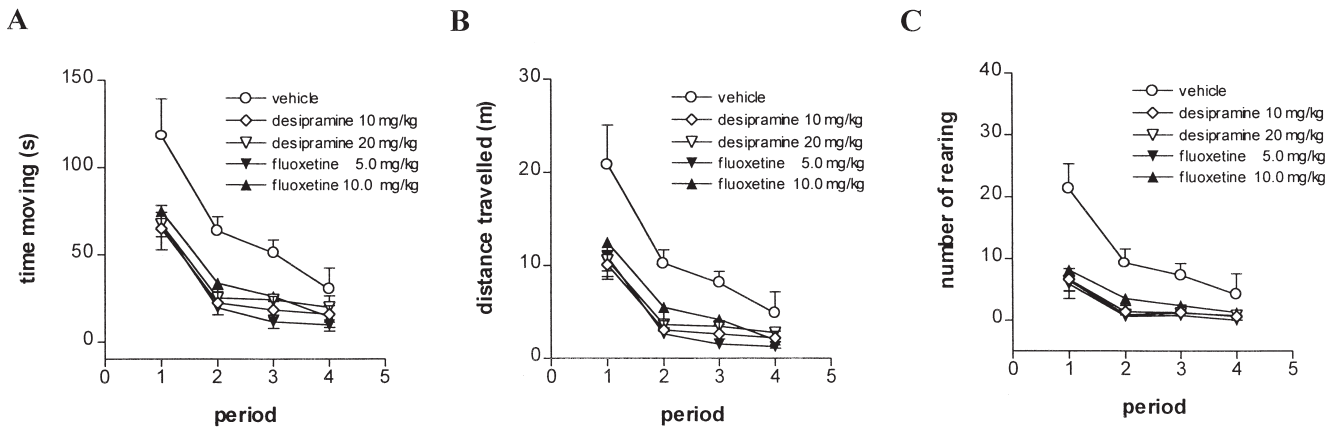

FIG. 4. The effects of desipramine (10 and 20 mg/kg IP) and fluoxetine (5 and 10 mg/kg IP) on locomotor activity of the rats. (A) Time spent moving; (B) distance traveled; (C) number of rearings. Detailed statistical comparisons are provided in the Results section.

sive properties of pinoline have been found (4). The acute toxicity of pinoline in mice (LD_{50}) is also several times lower than that of convulsive β-carbolines or even tricyclic antidepressants (1).

Initially, pinoline has been proposed to function as an endogenous ligand of a serotonin transporter (12,20). Later experiments confirmed the competitive interaction with the selective serotonin reuptake inhibitor citalopram in vitro (17). The present study indicates that there are also other mechanisms responsible for the complex effect of pinoline. In addition to the antidepressant action revealed in a former forced swimming experiment (15), pharmacological doses of pinoline also have a significant anxiogenic effect. Considering the endogenous occurrence of this compound, at least in certain conditions (e.g., after alcohol withdrawal) (14,22), the mechanism of action of pinoline on experimental anxiety and the possible role of endogenous pinoline in the modulation of anxiety levels needs further study.

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