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## THE EFFECTS OF CHOLECYSTOKININ A AND B RECEPTOR ANTAGONISTS, DEVAZEPIDE AND L 365260, ON CITALOPRAM-INDUCED DECREASE OF EXPLORATORY BEHAVIOUR IN RAT

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The present study has been divided into two sets. In the first set, the aim of the experiments was to investigate the dose-response effect of selective serotonin re-uptake inhibitor (SSRI) citalopram on rat exploratory behaviour in the elevated plus-maze. In the second set of experiments, the effect of cholecystokinin (CCK) CCK<sub>A</sub> and CCK<sub>B</sub> receptor antagonists, devazepide and L 365260, on citalopram-induced decrease of exploratory behaviour in the elevated plus-maze was studied. Citalopram (5 and 10 mg/kg) decreased the number of open and total arm entries, line crossings on open arms, and percentage of time spent exploring in open arm. Dose 15 mg/kg was without any effect on rat exploratory behaviour. Devazepide (0.01 and 1.0 mg/kg) failed to modify any of the citalopram-induced changes observed. L 365260 (1.0 mg/kg) reversed most of the effects of citalopram: the numbers of open and total arm entries, the number of line crossings, and the percentage of time spent exploring in open arms. L 365260 at dose level 0.01 mg/kg was ineffective. These results support the involvement of the CCK<sub>B</sub> receptor subtype in SSRI-induced anxiogenic-like effects in rodents.

**Key words:** *cholecystokinin, CCK receptors, citalopram, anxiety, behaviour, elevated plus-maze.*

### INTRODUCTION

Cholecystokinin (CCK), originally discovered in the gastrointestinal tract, is also present in different regions in the mammalian central nervous system (CNS). It has been found that CCK-ergic neurotransmission is involved in many different neuronal systems, being a comodulator of dopaminergic (1), gamma-aminobutyric acid- (GABA-) (2), opioid-, 5-HT-, and glutamatergic (3) neurotransmission. CCK is involved in the control of nociceptive messages (4), anxiety (5—7), satiety (8), and in the control of mediation of seizure activity (9).

It has been suggested that CCK plays an important role in the neurobiology of anxiety both in animals (5, 7, 10—12) and humans (13, 14). In

some experiments it has been found that CCK-peptides produce a significant decrease of exploratory behaviour in the elevated plus-maze in rats. The effects of CCK in the CNS are mediated through distinct receptor subtypes, CCK<sub>A</sub> and CCK<sub>B</sub> (7, 10). It has been reported that CCK-peptides may induce decrease of exploratory behaviour and this effect can be blocked by CCK<sub>B</sub> receptor antagonists (6, 15). These results suggest that CCK<sub>B</sub> receptor antagonists might be effective in anxiety and panic states.

There are many clinical reports that the new group of antidepressants, the selective serotonin re-uptake inhibitors (SSRI-s), may initiate or enhance the negative emotional symptoms such as nervousness (16, 17), jitteriness (18), or even anxiety (19) in depressed patients during the first days of administration. On the other hand, only in a few number of animal studies of anxiety it has been found that SSRI-s may produce acute anxiogenic effect in rodents. For example, it has been found that a single injection of cianopramine or citalopram (both SSRI-s) increase neophobic reactions in the free exploration tests (20). In the elevated plus-maze test, paroxetine and indalpine have been reported to produce an anxiogenic-like effect (21).

The link between CCK-ergic neurotransmission and 5-HT re-uptake blockade is unclear; there are only a few number of reports concerning this field. Thus, for example, Bickerdike and colleagues (22) have found that the anxiolytic-like effect of devazepide, a selective CCK<sub>A</sub> receptor antagonist, in the elevated zero-maze, was blocked by Wy 27587 and zimelidine (SSRI-s).

In the present study, we investigated the role of CCK-ergic neurotransmission on the effects of the SSRI citalopram in the elevated plus-maze. The first set of our experiments aims to investigate the dose-response effect of citalopram on rat exploratory behaviour in the elevated plus-maze. In the second set of experiments we investigated the effect of co-administration of citalopram and either CCK<sub>A</sub> or CCK<sub>B</sub> receptor antagonist, devazepide or L 365260, respectively, on rat exploratory behaviour. In our previous studies using the exploration box we have found that the administration of devazepide or L 365260 always failed to reveal a significant anxiolytic effect (23). Therefore, the effect of devazepide and L 365260 was not investigated independently and all experiments were performed as the "one-factor" studies.

## MATERIALS AND METHODS

### *Animals*

Male Wistar rats (from Grindex Breeding Center, Riga, Latvia) weighing 300—400 g were used in all experiments. The animals were housed four per cage under standard laboratory conditions; water and food were available *ad libitum*. The animal room had a controlled temperature (20°C, ±2°C) and light/dark cycle (light on from 8.00 a.m. to 8.00 p.m.).

One hour before an experiment the animals were moved from animal room into behavioural testing room in their home cages.

Each test group consisted of 8 to 12 animals. Each animal was used only once.

### *Experimental apparatus*

The elevated plus-maze test was carried out according to the description of Pellow et al. (23) with few modifications (24). The elevated plus-maze apparatus consists of a maze with two open arms 50 × 10 cm and two enclosed arms 50 × 10 × 30 cm with an open roof. The arms were placed such that the two open and two enclosed arms were opposite each other. All parts of the apparatus were made from wood and the apparatus was elevated 50 cm above floor. The surface of an open arm was divided with dark lines into three parts of equal size.

### *Procedure*

All experiments were carried out between 1.00 p.m. and 7.00 p.m. For a test, the animal was placed facing an enclosed arm and observed during 240 seconds for following criterions: (1) the time of latency (time of first entry with all four paws from an enclosed arm into an open arm); (2) the number of entries into open arms; (3) the number of total entries; (4) the number of line crossings in open arms; and (5) time spent exploring in open arms. The criterions (1) to (4) were scored only when the animal has moved with all four paws across a line. The criterion "time spent exploring in open arm" was measured when the animal explored with two paws across a line.

### *Drugs and drug administration*

The following drugs were used: citalopram, donated by Lundbeck, Denmark; devazepide donated by Merck, Sharp & Dohme, UK, and L 365260, [3R-(+)-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3yl)-N'-(methyl-phenyl)urea], donated by Merck, Sharp & Dohme, UK.

Citalopram was dissolved in distilled water and adjusted up to volume 1 ml/kg. Devazepide and L 365260 were suspended with few drops of TWEEN-85® (polyoxyethylene-(20)-sorbitan oleate) and adjusted with distilled water up to volume 1 ml/kg body weight.

Vehicle or citalopram were administrated 30 min before test. In the case of co-administration of two drugs, the CCK receptor antagonist was always administrated five minutes before citalopram. After the administration of a drug the animal was returned to its home cage.

All drugs were injected intraperitoneally.

### *Statistics*

The results were analyzed by one-factor analysis of variance (ANOVA). For post hoc comparison the data were subjected to Fisher's Least Significant Difference (LSD) test. The probability levels  $p < 0.05$  were considered statistically significant.

## RESULTS

### *The dose-response effect of citalopram*

One-factor ANOVA revealed significant main effect after acute citalopram treatment on most criterions measured [the number of line crossings

( $F(3.36) = 5.49$ ;  $p < 0.01$ ), the number of open arm entries ( $F(3.36) = 5.00$ ;  $p < 0.01$ ), the number of total arm entries ( $F(3.36) = 5.5$ ;  $p < 0.01$ ), and the percentage of time spent exploring in open arms ( $F(3.36) = 3.9$ ;  $p < 0.05$ ); only in the case of time of latency ANOVA failed to show a significant effect. Fisher's LSD test demonstrated a significant effect in doses 5 mg/kg and 10 mg/kg. Citalopram in dose range 0–15 mg/kg elicited a clear U-shaped dose-response curve (Figures 1A, B, C, D).

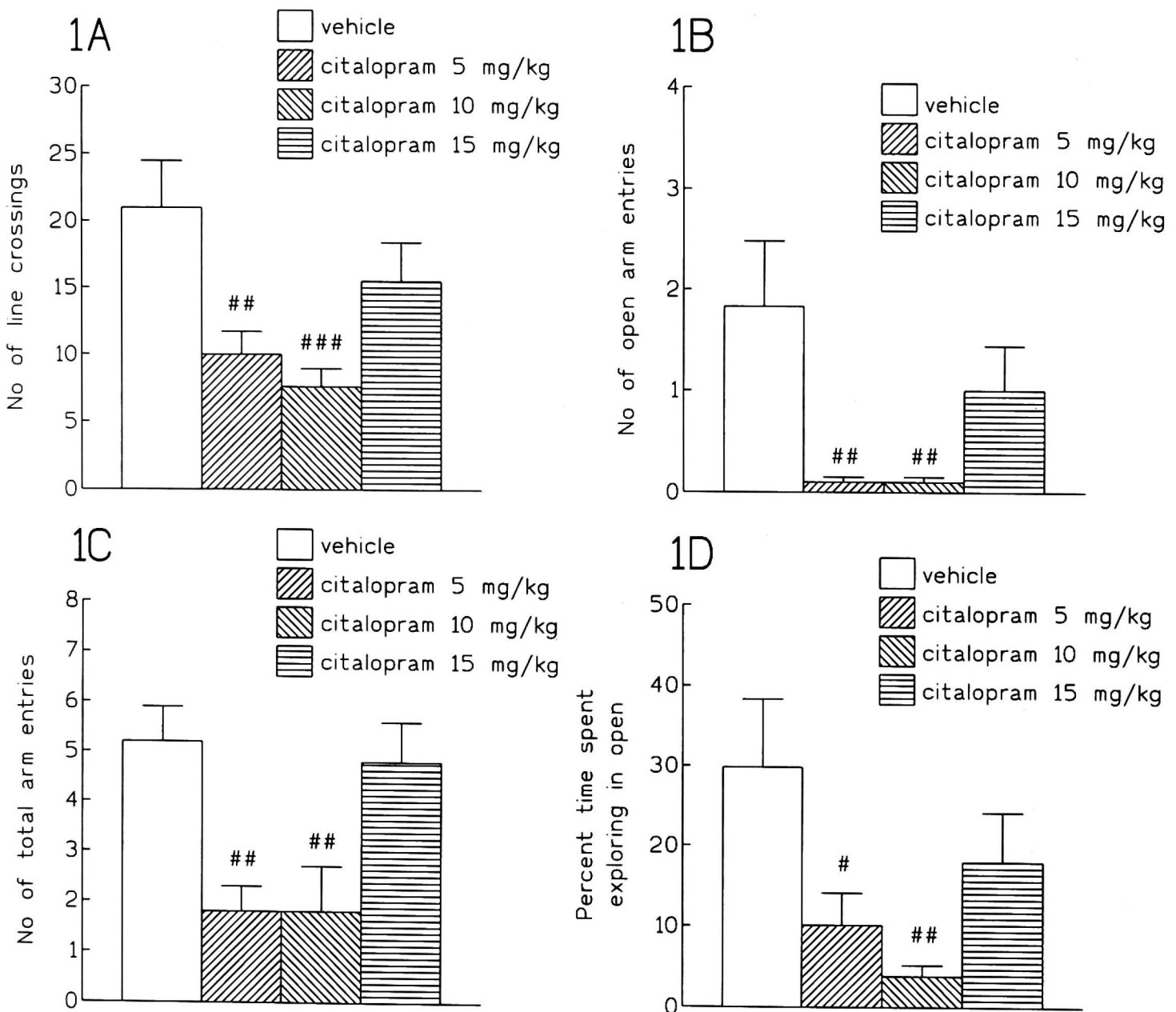


Fig. 1A, 1B, 1C, and 1D. The effect of citalopram on exploratory behaviour in the elevated plus-maze in rats.

Number of exploratory events and percentage time spent exploring on open arms in the elevated plus-maze. All data presented are obtained values  $\pm$  SEM. The data are subjected to Fisher's LSD test. \* $p < 0.05$ ; # $p < 0.01$ , ### $p < 0.001$ .

### The effect of devazepide and L 365260

Rats were administrated either vehicle, citalopram 5 mg/kg, citalopram 5 mg/kg and devazepide 0.01 mg/kg or citalopram 5 mg/kg and devazepide

1.0 mg/kg. One-factor ANOVA revealed a significant main effect [the number of line crossing ( $F(3.30) = 3.93$ ;  $p < 0.05$ ), the number of open arm entries ( $F(3.30) = 2.84$ ;  $p < 0.05$ ), the number of total arm entries ( $F(3.30) = 3.41$ ;  $p < 0.01$ ), and the percentage time spent exploring in open arms ( $F(3.30) = 5.57$ ;  $p < 0.01$ )]. Post hoc data analysis demonstrated a significant anxiogenic-like effect both of citalopram alone and of citalopram and devazepide co-administration in comparison to corresponding vehicle group (*Table 1*) on all criteria measured except time of latency. Moreover, Fisher's LSD test did not reveal a significant difference between the citalopram alone and the simultaneous devazepide plus citalopram administration in all three drug-treated groups investigated.

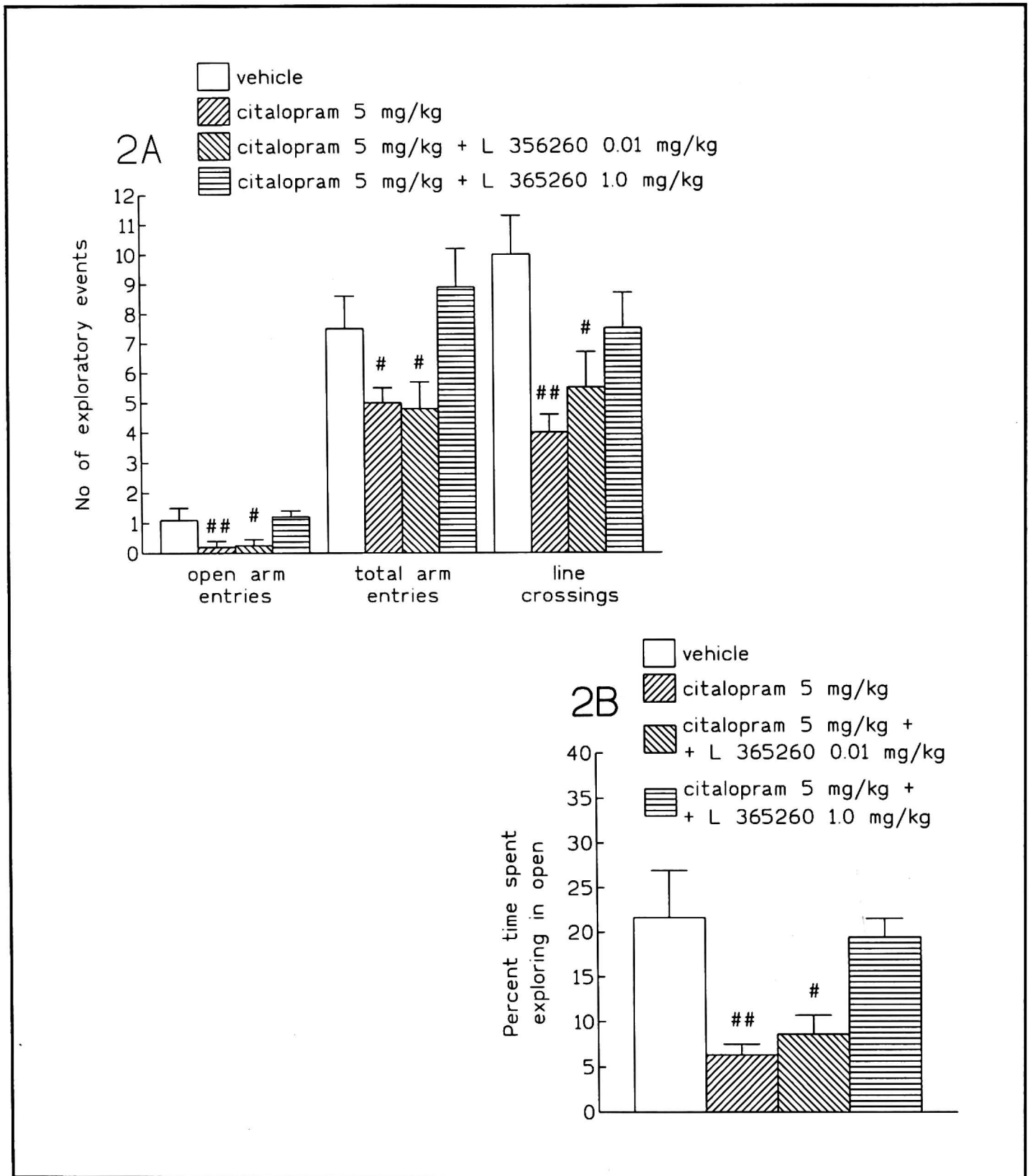
*Table 1.* The effect of citalopram and devazepide co-administration on exploratory behaviour in the elevated plus-maze in rats.

	Latency (s)	Open arm entries	Total arm entries	Line crossings	Percent time spent exploring in open
1. Vehicle	10.4 ± 1.0	1.1 ± 0.4	9.2 ± 1.2	11.0 ± 2.5	24.8 ± 6.0
2. Citalopram 5 mg/kg i.p.	8.5 ± 0.9	0.25 ± 0.2 <sup>##</sup>	5.6 ± 0.9 <sup>#</sup>	4.5 ± 2.0 <sup>#</sup>	11.5 ± 2.6 <sup>#</sup>
3. Citalopram 5 mg/kg i.p. + devazepide 0.01 mg/kg i.p.	11.7 ± 0.6	0.5 ± 0.4 <sup>#</sup>	4.3 ± 1.0 <sup>#</sup>	6.5 ± 2.1 <sup>#</sup>	14.2 ± 2.7 <sup>#</sup>
4. Citalopram 5 mg/kg i.p. + devazepide 1.0 mg/kg i.p.	7.2 ± 1.3	0.4 ± 0.3 <sup>#</sup>	5.9 ± 1.3 <sup>#</sup>	5.5 ± 2.7 <sup>#</sup>	13.4 ± 2.1 <sup>#</sup>

Number of exploratory events and percentage of time spent exploring on open arms in the elevated plus-maze. All data presented are obtained values ± SEM. The data are subjected to Fisher's LSD test. <sup>#</sup> $p < 0.05$ ; <sup>##</sup> $p < 0.01$ .

In the following experiment, rats were administered either vehicle, citalopram 5 mg/kg, citalopram 5 mg/kg and L 365260 0.01 mg/kg or citalopram 5 mg/kg and L 365260 1.0 mg/kg. A significant effect of L 365260 treatment on four behavioural measures was revealed, by one-factor ANOVA: the numbers of open ( $F(3.31) = 2.98$ ;  $p < 0.05$ ) and total ( $F(3.31) = 2.54$ ;  $p < 0.05$ ) entries, the number of line crossings ( $F(3.31) = 3.02$ ;  $p < 0.05$ ), and the percentage time spent exploring in open arms ( $F(3.31) = 3.97$ ;  $p < 0.05$ ). As in previous studies, citalopram 5 mg/kg decreased significantly the rat exploratory behaviour in the elevated plus-maze (except time of latency).

Fisher's LSD test demonstrated that L 365260 (1.0 mg/kg) reversed significantly the citalopram-induced decrease of exploratory behaviour. In lower dosage (0.01 mg/kg), L 365260 was unable to modify any of the observed citalopram-suppressed criteria of exploratory behaviour (*Figures 2A and 2B*).



*Fig. 2A and 2B.* The effect of citalopram and L 365260 co-administration on exploratory behaviour in the elevated plus-maze in rats.

Number of exploratory events and percentage time spent exploring on open arms in the elevated plus-maze. All data presented are obtained values  $\pm$  SEM. The data are subjected to Fisher's LSD test. \* $p < 0.05$ ; \*\* $p < 0.01$ .

## DISCUSSION

The first set of the present experiments demonstrates that citalopram elicits anxiogenic-like effect in the elevated plus-maze in rat. This experiment manifests the decrease of exploratory behaviour after a single injection of selective serotonin re-uptake inhibitor citalopram in lower dose range (5 and 10 mg/kg, respectively). Loss of this effect at the dose level of 15 mg/kg excludes the interpretation of the decrease of exploratory activity after citalopram treatment as a sedative effect. In our previous study with classic tricyclic antidepressants (26) using elevated plus-maze test no effect on exploratory behaviour after acute administration of desipramine or imipramine was found. Thus, the moderate serotonin-positive effect of a drug after acute administration might be one of the neurochemical factors which augments the neophobia in the elevated plus-maze (20). It must be emphasized, that such a decrease of exploratory activity after acute administration of a SSRI is not exhibited in all animal models of exploratory behaviour (27, 28). We have demonstrated previously that in a free exploration paradigm (the exploration box) citalopram did not impair exploratory behaviour (29). This suggests that citalopram augments neophobia in forced exploration tests such as the elevated plus-maze.

There is strong evidence that both  $CCK_A$  and  $CCK_B$  receptors are present in the CNS, although  $CCK_B$  receptor subtype predominates (30). The physiological role of these subtypes is still unclear. In the animal models of anxiety, the anxiety-related suppression of behaviour is supposed to be mediated through the  $CCK_B$  receptor subtype (5, 10, 30).

In our second set of experiments, the  $CCK_A$  receptor antagonist devazepide failed to modify the anxiogenic-like effect of citalopram. Contrary, the  $CCK_B$  receptor antagonist L 365260 at the dose level of 1.0 mg/kg reversed most of the effects of citalopram. These results suggest the predominant role of  $CCK_B$  receptors in anxiolytic-like effects in rodents.

On the other hand, as a neuropeptide co-transmitter, CCK requires high frequency neuronal activity or bursting (31), and CCK receptor antagonists should not have any effect under normal neuronal activity. Thus, our data demonstrate that a neurobiological link between serotonergic and CCK-ergic neurotransmission could exist and the  $CCK_B$  receptor antagonists might be useful as a novel pharmacological tool to avoid the anxiogenic-like effects of SSRI-s during the first days of treatment.

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