JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1997, 48, 2, 239—251

V. MATTO [1], J. HARRO [2], L. ALLIKMETS [1]

THE EFFECT OF DRUGS ACTING ON CCK RECEPTORS AND RAT FREE EXPLORATION IN THE EXPLORATION BOX

[1] Department of Pharmacology, University of Tartu, Tartu;
[2] Department of Public Health, University of Tartu, Tartu, Estonia

The effects of cholecystokinin (CCK) CCK_A receptor antagonist devazepide (10 μg/kg and 1.0 mg/kg), CCK_B receptor antagonist L 365260 (1.0 mg/kg), and CCK_B receptor agonist CCK tetrapeptide (CCK-4, 75 μg/kg), and their concomitant administration with antidepressants desipramine (10 mg/kg) and citalopram (10 mg/kg) on rat exploratory behaviour were studied in the recently developed exploration box test. In addition, the effects of repeated administration of desipramine (10 mg/kg) and citalopram (10 mg/kg) were studied. After acute administration, CCK-4 decreased significantly the number of line crossings, rears, investigative approaches, and the time spent exploring. The time of latency and the number of entries into large arena were unchanged. Desipramine reduced all observed criterions of rat behaviour, but citalopram was ineffective. Devazepide (1.0 mg/kg) and L 365260 (1.0 mg/kg) had no effect on rat behaviour after acute or repeated administration. L 365260 (1.0 mg/kg) blocked the antiexploratory effect of CCK-4, whereas devazepide (10 μg/kg) did not. No interaction of CCK-4, devazepide, or L 365260 treatment with antidepressant treatment was found. Our results suggest that the administration of a CCK_B agonist diminishes rat exploratory behaviour, but neither CCK_A nor CCK_B receptor blockade induces changes on rat exploratory behaviour in the free exploration paradigm.

Key words: *cholecystokinin, desipramine, citalopram, anxiety, exploratory behaviour, exploration box, rat*

INTRODUCTION

Cholecystokinin- (CCK-) ergic neurotransmission in the central nervous system (for a review, see (1)) is one of the neurobiological systems which appears to regulate the neurobiology of anxiety and panic disorders both in humans and animals (2—4). The effects of CCK in the CNS are mediated

through distinct receptor subtypes, CCK_A and CCK_B (5, 6). In the majority of studies it has been found that the anxiogenic effects are mediated through the CCK_B receptor subtype (7—10). Thus, it has been found that the cholecystokinin tetrapeptide (CCK-4) which is a selective CCK_B receptor agonist, may produce a significant decrease of exploratory behaviour in rodents (11, 12) and may dose-dependently induce signs of anxiety in healthy volunteers (13). CCK receptor antagonists have been found to elicit anxiolytic-like effects (7, 14, 15) and it has been proposed that CCK_B receptor antagonists could be used as novel anxiolytic drugs. During the last years, several new compounds with high CCK_B receptor affinity and good lipid solubility have been synthesized (16).

Antidepressive drugs, widely in clinical use (17, 18), may in some cases cause anxiety after acute administration (19, 20). There are also data that antidepressants may produce acute anxiogenic effect in rodents in behavioural tests which are based on exploratory behaviour (21—23).

The exploratory behaviour in rodents is an evolutionary shaped drive since it is essential to adapt in a new environment. Two main domains of exploratory behaviour are the motivation to collect new information from the environment (curiosity drive) and fear (neophobia), (for a review, see (24)). A wide variety of animal models of exploratory behaviour has been developed to investigate the anxiolytic and anxiogenic profiles of drugs (24—27). For example, the widely used elevated plus-maze test, the elevated zero-maze test (15, 28—31) and the classic open field test (32) are tools for the identification of anxiolytic and anxiogenic effects. On the other hand, neither the elevated plus-maze, the open field test nor the other recently used animal screens are very suitable to distinguish the emotional and motivational components of the exploratory behaviour since these techniques use forced exploration and very limited time period (33). The exploration box, initially designed to distinguish the emotional and motivational components of exploratory behaviour after noradrenergic denervation (34), has been found to be also an appropriate tool to differentiate true anxiogenic effects from false positives. Furthermore, in the exploration box (for description, see Materials and Methods), several behavioural criterions can be observed simultaneously and the neophobia drive (emotional part) could be distinguished from the curiosity drive (motivational part). Thus, the number of line crossings, the number of rears, the number of investigative approaches, and the time spent exploring in open arena reflect neophobia. On the other hand, the number of entries into open arena, and the time of latency reflect rather curiosity. When the animal is repeatedly exposured to the exploration box, the habituational component of exploratory behaviour could be studied.

The link between CCK-ergic neurotransmission and anxiogenic effects of antipressants is not known; and our study attempts to clarify whether such a link exists when the animal is exposed to the exploration box. Therefore, we

investigated the effects of a CCK_B receptor agonist CCK-4 (75 μg/kg), a CCK_A receptor antagonist devazepide (10 μg/kg or 1.0 mg/kg), a CCK_B receptor antagonist L 365260 (1.0 mg/kg), and their interaction with desipramine (10 mg/kg) or citalopram (10 mg/kg) coadministration on rat exploratory behaviour in the exploration box. In addition, the effects of repeated devazepide (10 μg/kg and 1.0 mg/kg), L 365260 (1.0 mg/kg), desipramine (10 mg/kg), and citalopram (10 mg/kg) administration were studied.

MATERIAL AND METHODS

Animals

Female Wistar rats (from Grindex Breeding Center, Riga, Latvia) weighing 200—250 g were used in all experiments. The animals were housed five per cage under standard laboratory conditions; water and food were available *ad libitum*. The animal room had 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 in their home cages from the animal room into the behavioural testing room. All experiments were carried out between 1.00 p.m. and 7.00 p.m. Each test group consisted of four to ten animals. Each animal was used only once. In the experiments with repeated exposure to the exploration box, the same animal was used in five consecutive days.

Experimental apparatus

The exploration box (34) consists of a stainless steel rectangular arena with dimensions 50 × 100 cm and 40 cm side walls; on the shorter side of apparatus, a small chamber 20 × 20 × 20 cm similar to home cage (with sawdust floor) was situated. The surface of the floor of the arena was divided into eight squares of equal size. Onto large arena, few objects such as a cardboard box, a glass jar, a food pellet, and a wooden handle were placed.

Procedure

For the test, the animal has been placed into the small chamber and has been observed during 15 min for following criterions: (1) time of latency (time spent before first entry into the large arena); (2) horizontal (number of line crossing on the floor) and (3) vertical (number of rears) activity; (4) total number of entries into the large arena; (5) time spent exploring on the arena, and (6) number of investigative approaches to the novel objects. On the basis of the number of line crossings, the number of rears, and the number of the investigative approaches to the novel objects, (7) sum of exploratory events has been calculated.

In some experiments with antidepressant treatment, the time of latency was not measured.

In the experiments with repeated exposure to the exploration box, the animals were tested in five consecutive days.

Drugs and drug administration

The following drugs were used: CCK-4, from Bachem AG, Switzerland; citalopram, donated by Lundbeck, Denmark; desipramine, from Sigma, USA; 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′-(3-methyl-phenyl)urea], donated by Merck, Sharp & Dohme, UK.

Desipramine and citalopram were dissolved in distilled water. CCK-4 was dissolved in distilled water by addition of minimal amount of 0.1 N $NaHCO_3$ and stored as stock solution at −28°C. Devazepide and L 365260 were suspended with few drops of TWEEN-85® (polyoxyethylene-(20)-sorbitan oleate). All drugs were adjusted with distilled water up to volume 1 ml/kg body weight.

Desipramine (10 mg/kg), citalopram (10 mg/kg), devazepide (10 μg/kg, 1.0 mg/kg), and L 365260 (1.0 mg/kg) were injected intraperitoneally 30 min before test session, CCK-4 (75 μg/kg) subcutanously 15 min before test session.

Statistics

The data obtained from one day experiments were analyzed by one-factor analysis of variance (ANOVA) and those of five day experiments by repeated measures ANOVA. When appropriate, for post hoc data comparison, the data were subjected to Scheffé test. The probability levels $p<0.05$ were considered statistically significant.

RESULTS

The acute experiments

After acute CCK-4 administration ANOVA revealed a significant main effect on rat exploratory behaviour in the exploration box ($F(3.20) = 18.20$; $p<0.001$ for the time spent exploring, $F(3.20) = 17.31$; $p<0.001$ for the number of investigative approaches, $F(3.20) = 15.87$; $p<0.001$ for the sum of exploratory events, $F(3.20) = 14.24$; $p<0.001$ for the number of line crossing, $F(3.20) = 13.80$; $p<0.001$ for the number of rears). Post hoc tests revealed significant differences in all three CCK-4 treated groups in comparison to the corresponding vehicle group. Thus, CCK-4 treatment decreased the time spent exploring, the number of investigative approaches, the number of line crossings, and the number of rears (*Table 1*).

Table 1. The effect of CCK-4 treatment on rat exploratory behaviour.

	Latency	Number of line crossings	Number of rears	Number of entries into large arena	Time spent exploring on large arena (s)	Number of investigative approaches	Sum of investigational events
1. vehicle	13±3	53±9	20±5	4.6±0.9	323±47	29±5	102±18
2. CCK-475 μg/kg s.c.	386±144	14±6$^{\#\#}$	3±2$^{\#\#}$	3.6±1.4	103±42$^{\#\#}$	7±5$^{\#\#}$	25±10$^{\#\#}$
3. desipramine 10 mg/kg+CCK-475 μg/kg s.c.	355±146	10±3$^{\#\#\#}$	6±2$^{\#\#\#}$	2.2±0.6	15±5$^{\#\#\#}$	1.0±±0.6$^{\#\#\#}$	8±3$^{\#\#\#}$
4. citalopram 10 mg/kg+CCK-4 75 μg/kg s.c.	315±147	8±6$^{\#\#\#}$	0.6±±0.6$^{\#\#\#}$	3.0±1.0	37±±17$^{\#\#\#}$	5±2$^{\#\#\#}$	13±6$^{\#\#\#}$

$^{\#\#}$ $p<0.01$; $^{\#\#\#}$ $p<0.001$ treatment vs vehicle group (Scheffé test)

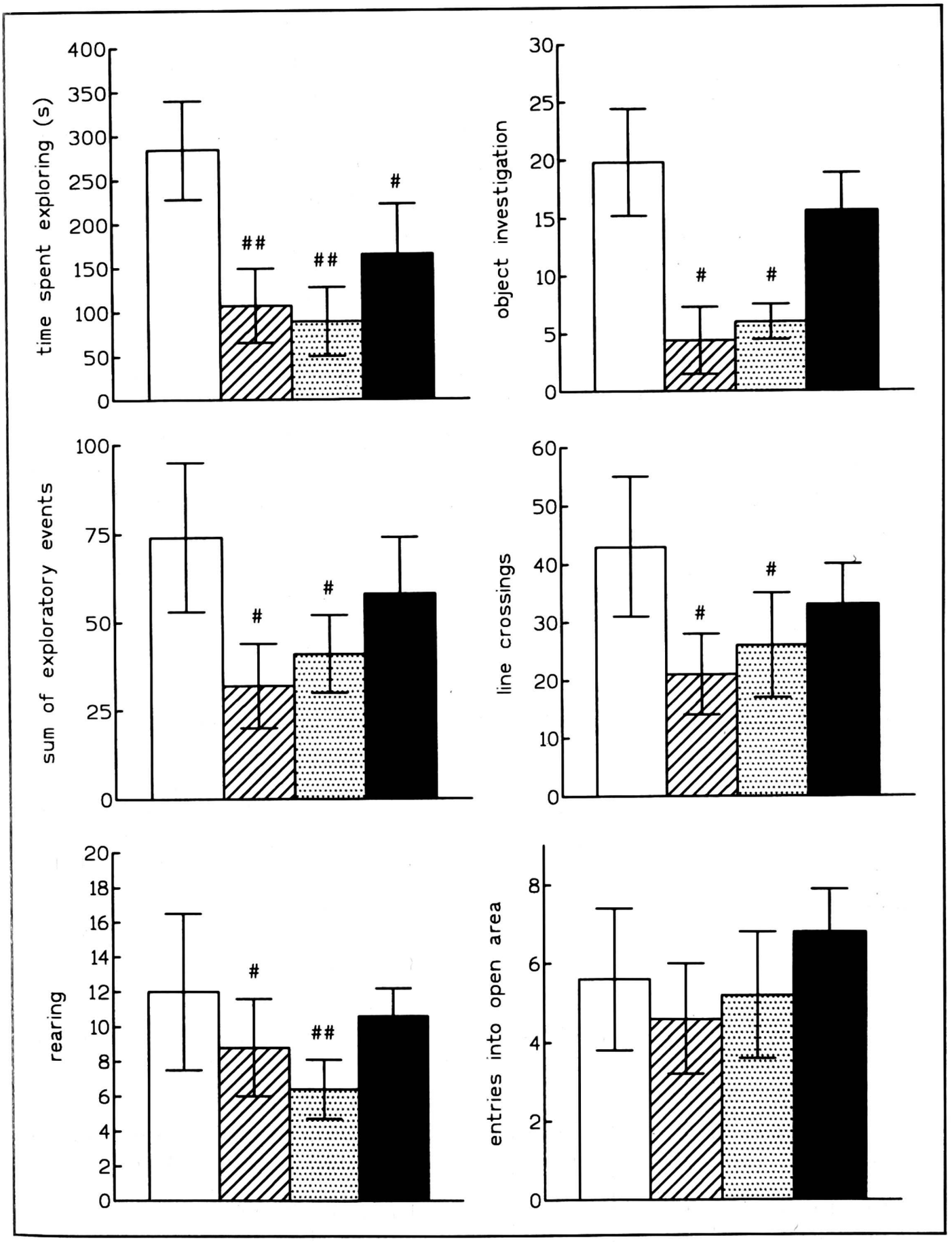

Fig. 1. The effect of CCK-4, CCK-4 and devazepide, and CCK-4 and L 365260 on rat exploratory behaviour in the exploration box.

Number of exploratory events in the exploration box. All data presented are obtained values ± SEM. The data are subjected to Scheffé test. (1) open columns, vehicle group; (2) hatched columns, CCK-4 group; (3) speckled columns, CCK-4 + devazepide group, (4) dark columns, CCK-4 + L 365260 gruop. $^{\#}p<0.05$, $^{\#\#}p<0.01$ treatment vs vehicle group.

In the experiment of the coadministration of CCK-4 and CCK receptor antagonists, ANOVA demonstrated significant differences ($F(3.16) = 3.25$; $p < 0.05$ for the number of line crossings, $F(3.16) = 3.87$; $p < 0.05$ for the number of rears, $F(3.16) = 4.06$; $p < 0.05$ for the sum of exploratory events, $F(3.16) = 3.61$; $p < 0.05$ for the number of investigative approaches, and $F(3.16) = 3.64$; $p < 0.05$ for the time spent exploring in open arena). The results of post hoc (Scheffé) tests are presented on *Fig. 1.* Thus, L 365260 1.0 mg/kg blocked the antiexploratory effects of CCK-4.

After acute devazepide, devazepide plus desipramine, or devazepide plus citalopram treatment ANOVA revealed a significant effect on rat behaviour ($F(3.20) = 3.51$; $p < 0.05$ for the tim spent exploring, $F(3.20) = 3.95$; $p < 0.05$ for the number of investigative approaches, $F(3.20) = 3.11$; $p < 0.05$ for the sum of exploratory events, $F(3.20) = 3.31$; $p < 0.05$ for the number of line crossings, and $F(3.20) = 4.95$; $p < 0.01$ for the number of rears). Post hoc tests revealed significant differences only in the devazepide plus desipramine treated group in comparison to the corresponding vehicle group. Thus, devazepide plus desipramine treatment decreased the time spent exploring, the number of investigative approaches, the sum of exploratory events, the number of line crossings, and the number of rears (*Table 2*).

Table 2. The effect of devazepide and L 365260 treatment on rat exploratory behaviour.

	Latency	Number of line crossings	Number of rears	Number of entries into large arena	Time spent exploring on large arena (s)	Number of investigative approaches	Sum of investigational events
1. vehicle	5±0	62±12	24±8	7.6±1.4	408±62	30±6	118±18
2. devazepide 1.0 mg/kg i.p.	7±2	58±8	31±7	6.6±0.9	365±49	15±6	115±13
3. desipramine 10 mg/kg + devazepide 1.0 mg/kg i.p.	455± ±126#	10±3#	2±1##	2.0±0.7	32±7#	3±1#	15±4#
4. citalopram 10 mg/kg + devazepide 1.0 mg/kg i.p.	20±3	70±6	12±4	6.8±2.1	385±80	26±9	116±23
1. vehicle	18±6	42±7	16±2	5.7±0.9	280±81	20±4	78±14
2. L 365260 1.0 mg/kg i.p.	20±5	50±9	22±5	4.6±1.4	308±43	15±6	87±14
3. desipramine 10 mg/kg + L 365260 1.0 mg/kg i.p.	375±96#	5±1##	1.3±0.6#	1.8±0.7	46±17#	8±1#	13±3#
4. citalopram 10 mg/kg + L 365260 1.0 mg/kg i.p.	32±14	51±14	10±3	5.0±2.0	365±40	21±6	71±12

# $p < 0.05$; ## $p < 0.01$ treatment vs corresponding vehicle group (Scheffé test)

After acute L 35260, L 365260 plus desipramine, or L 365260 plus citalopram treatment ANOVA revealed a significant main effect on rat exploratory behaviour in the exploration box ($F(3.20) = 3.21$; $p<0.05$ for the time spent exploring, $F(3.20) = 3.35$; $p<0.05$ for the number of investigative approaches, $F(3.20) = 3.59$; $p<0.05$ for the sum of exploratory events, $F(3.20) = 3.26$; $p<0.05$ for the number of rears, and $F(3.20) = 4.72$; $p<0.01$ for the number of line crossings). Post hoc tests revealed significant differences only in the L 365260 plus desipramine treated group in comparison to the corresponding vehicle group. Thus, L 365260 plus desipramine treatment decreased the time spent exploring, the number of investigative approaches, the sum of exploratory events, the number of rears, and the number of line crossings (*Table 2*).

The experiments with repeated exposure to the exploration box

In the 1 mg/kg devazepide experiment repeated measures ANOVA revealed significant day effects. Thus, repeated exposure to the exploration box decreased the number of line crossings ($F(4,24) = 5.37$; $p<0.01$), the number of entries into large arena ($F(4,24) = 8,81$; $p<0.001$), and the sum of exploratory events ($F(4,24) = 4.45$; $p<0.01$). Similar tendencies were found also for the number of rears, the time spent exploring, and the number of investigative approaches, although these effects were statistically not significant. The time of latency was unchanged in all five consecutive days. Neither treatment effect nor treatment × day interaction was found (data not shown).

In the 10 μg/kg devazepide experiments neither treatment effect nor day × treatment interaction was found whereas the day effect was similar to the 1.0 mg/kg experiment (data not shown).

In the L 365260 experiment repeated measures ANOVA revealed significant day effects. Thus, repeated exposure to the exploration box decreased the number of investigative approaches ($F(4,24) = 2.82$; $p<0.05$), the number of entries into large arena ($F(4,24) = 2.83$; $p<0.05$), and the sum of exploratory events ($F(4,24) = 2.92$; $p<0.05$). Similar tendencies were found also for the number of line crossings, the number of rears, and the time spent exploring, although these effects were statistically not significant. The time of latency was unchanged in all five consecutive days. Similar to the devazepide treatments, neither treatment effect nor treatment × day interaction was found (data not shown).

After repeated 10 mg/kg desipramine treatment repeated measures ANOVA revealed a significant treatment effect on rat behaviour ($F(1.6) = 11.37$; $p<0.05$ for the time spent exploring, $F(1.6) = 15.80$; $p<0.01$ for the number of investigative approaches, $F(1.6) = 21.10$; $p<0.01$ for the sum of exploratory events, $F(1.6) = 20.73$; $p<0.01$ for the number of line crossings, $F(1.6) = 22.04$; $p<0.01$ for the number of entries into large arena, and $F(1.6) = 11.22$; $p<0.05$ for the number of rears). In this experiment, the day

effect just did not reach significance level ($p<0.05$). This result could be explaned with small number of animals per group ($n = 4$). No treatment × day interaction was found. Thus, desipramine treatment decreased the time spent exploring, the number of investigative approaches, the sum of exploratory events, the number of line crossings, the number of entries into large arena, and the number of rears (*Tabl. 3*, statistics not shown).

Table 3. The effect of repeated desipramine and citalopram treatment on rat exploratory behaviour.

	Day	Vehicle treatment	Desipramine 10 mg/kg treatment	Vehicle treatment	Citalopram 10 mg/kg treatment
Number of line crossings	1 st	91±5	26±10	92±8	81±8
	2 nd	104±17	12±6	73±8	61±13
	3 rd	79±22	22±9	64±9	58±14
	4 th	80±17	20±10	65±7	51±12
	5 th	96±19	14±11	54±8	61±12
Number of rears	1 st	23±5	8±5	24±2	16±2
	2 nd	25±5	1±1	17±2	9±2
	3 rd	14±2	8±3	11±1	8±2
	4 th	20±4	7±4	12±1	7±1
	5 th	26±8	4±3	12±3	6±2
Entries into large arena	1 st	7.5±1.0	3.2±1.0	6.2±0.5	6.2±0.8
	2 nd	5.7±1.1	1.7±0.8	4.8±0.5	4.5±1.0
	3 rd	5.5±1.7	1.5±0.2	3.8±0.7	4.5±1.1
	4 th	5.7±0.2	1.5±0.8	4.2±0.6	3.5±0.7
	5 th	6.7±1.1	1.2±0.9	4.5±0.7	5.5±1.0
Time spent exploring (s)	1 st	458±68	173±96	450±36	435±19
	2 nd	373±61	120±65	362±41	266±48
	3 rd	312±65	216±85	311±33	252±54
	4 th	298±24	198±111	301±34	295±86
	5 th	385±93	93±79	225±39	255±53
Number of object investigations	1 st	44±6	13±6	42±3	38±4
	2 nd	46±11	7±4	35±3	24±5
	3 rd	37±9	15±6	29±3	24±5
	4 th	35±6	14±8	29±3	23±5
	5 th	45±12	7±6	23±4	24±5
Sum of investigational events	1 st	159±22	47±22	159±14	136±12
	2 nd	176±29	22±11	125±13	95±20
	3 rd	132±31	46±18	105±13	90±22
	4 th	135±25	42±22	107±12	82±19
	5 th	167±37	25±21	90±16	92±18

After repeated 10 mg/kg citalopram treatment repeated measures ANOVA failed to reveal any significant treatment effect on rat behaviour. However, in this experiment an obvious day effect was found ($F(4.48) = 8.18$; $p<0.001$ for

the time spent exploring, $F(4.52) = 6.34$; $p < 0.01$ for the number of investigative approaches, $F(4.52) = 7.82$; $p < 0.001$ for the sum of exploratory events, $F(4.52) = 6.48$; $p < 0.001$ for the number of line crossings, $F(4.52) = 3.49$; $p < 0.01$ for the number of entries into large arena, and $F(4.52) = 8.67$; $p < 0.001$ for the number of rears). No treatment × day interaction was found, (*Table 3*, statistics not shown).

DISCUSSION

In previous experiments it has been demonstrated that the exploration box might be used to study the neurobiological basis of the emotional and motivational responses in rodents. Thus, using acute and repeated administration of the standard anxiogenic β-carbolines DMCM and FG 7142 Otter *et al* (35, 36) demonstrated that the attenuation of exploratory behaviour in the exploration box could be considered as an anxiogenic effect.

In our earlier experiments we have also found that acute or repeated imipramine and desipramine elicited antiexploratory effect but citalopram did not modify rat behaviour in the exploration box (37). In the present study, these findings were confirmed. It should be emphasized that the antiexploratory effects of desipramine were different from those of β-carbolines, and these effects should not be considered as anxiogenic effects. Thus, the desipramine-induced decrease of exploratory behaviour was not blocked by diazepam, whereas diazepam blocked the antiexploratory effects of β-carbolines and there was no fear conditioning with antidepressant treatment. Furthermore, the antiexploratory effect of antidepressants did not habituate (unpublished) and no day × treatment interaction was found whereas using standard anxiogenic drugs, this kind of interaction was demonstrated in the experiments of Otter *et al* (35, 36). Finally, it has been found that the ambivalent behavioural element stretched-attend posture (SAP) is a sensitive parameter to characterisize the „true" anxiogenic/anxiolytic properties of a drug in rodents (38—40). Thus, the increased number of SAP-s reflects anxiogenic properties of a drug. Although SAP-s were not specially scored in our study, after desipramine treatment the animals exhibited l e s s SAP-s. Thus, the antidepressive drugs have no major influence on rat emotional behaviour when the animal is exposured to the exploration box; the desipramine-induced decrease of exploratory behaviour should be considered rather as diminished motivation to explore the large arena of the experimental apparatus.

Our present experiments confirm the ability of CCK-4 to produce antiexploratory effects (11, 41). Thus, the decrease of line crossings, rearings,

investigative approaches, sum of exploratory events, and time spent exploring demonstrates that the CCK-4 treatment induces anxiogenic-like changes in rat exploratory behaviour. This effect might be explained rather due the enhanced neophobia than attenuated motivation (number of entries into large arena and time of latency were unchanged). The antiexploratory effect of CCK-4 was blocked with CCK_B receptor antagonist L 365260 (1.0 mg/kg) but not with devazepide (10 μg/kg) and this finding supports the predominant role of CCK_B receptor subtype in anxiety. The devazepide dose 1.0 mg/kg was not tested in the acute CCK-4 experiment, since at this dose level devazepide may block also CCK_B receptors (11).

It has been demonstrated, that CCK_B receptor stimulation and blockade induce changes in central extracellular serotonin levels associated with "anxious" and "anxiolytic" behaviour (12). In contrast, our experiments demonstrate that neither desipramine nor citalopram were able to modify the anxiogenic-like effects of CCK-4 in the exploration box. It could be explained due the different method and different animals used (the elevated plus-maze and guinea-pigs in the experiment of Rex *et al* (12) vs exploration box and rats in our experiments). Moreover, it has been demonstrated to be crucial differences in exploratory behaviour in different rodent gender and lines. Johnston and File (42) reported gender differences in exploratory behaviour in the elevated plus-maze. Rex *et al* (43) found robust behavioural differences in anxiety or exploration between different rat lines. Despite the fact that females are in general less sensitive to the anxiolytic or anxiogenic effect of a drug, we used females, because of in accordance to the previous experiments females are relative stabile in their exploratory behaviour in the exploration box (34). Therefore, the effective CCK-4 dose used (75 μg/kg) is higher than in many reference experiments (the dose 50 μg/kg was ineffective in preliminary experiments; unpublished data).

In the present study, both CCK_A and CCK_B receptor antagonists failed to show any effect on exploratory behaviour or to modify the desipramine-induced decrease of exploratory behaviour in the one day experiment. Since in the previous experiments of Harro *et al* (34) it has been found that the CCK_B receptor antagonist LY 288513 treatment increased the number of line crossings and rearings on 3rd day, and sum of exploratory events on 5th day, the effect of repeated CCK receptor antagonist treatment was studied. We were unable to demonstrate neither drug effect nor drug × day interaction. This contradiction could be explained due the different baseline activity curve in all five days. Thus, in our experiments the habituation (decrease of the baseline activity curve) was significantly more pronounced then in those of Harro *et al*, (34). On the other hand, during the last few years it has been also reported on the lack of anxiolytic-like effects of both CCK_A and CCK_B receptor antagonists (44—47). It has been found that the efficacy of

anxiolytic agents in animal models of anxiety is likely to vary not only according to the animal gender or line used but also to the level of fear or anxiety induced by the aversive stimulus used. (44). The exploration box as a "pure" free exploration paradigm has evidently low capability to induce such aversive stimuli. Moreover, a neuropeptide cotransmitter requires high frequency neuronal activity or bursting and therefore the CCK receptor antagonists should not have any effect under normal neuronal activity (48).

In conclusion, our results suggest that the enhancement of CCK-ergic neurotransmission is involved in the neurobiological mechanisms of neophobia in rat, but any robust link between monoaminergic and CCK-ergic neurotransmission could not be found when the animal was exposured to the exploration box.

Acknowledgements: This study was supported by grants No 2469 (to J. H.) and No 07 (to L. A.) from the Estonian Science Foundation. We thank Lundbeck and Merck, Sharp & Dohme for generous gift of their drugs.

REFERENCES

1. Lindefors N, Lindén A, Brené S, Sedvall G, Persson H. CCK peptides and mRNA in the human brain. *Prog Neurobiology* 1993; 40: 671—690.
2. Bradwejn J, Koszycki D, Shriqui Ch. Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorders. Clinical and behavioral findings. *Arch Gen Psychiatry* 1991; 48: 603—610.
3. Harro J, Vasar E. Cholecystokinin-induced anxiety: how is it reflected in studies on exploratory behaviour? *Neurosci Biobehav Rev* 1991; 15: 473—477.
4. Harro J, Vasar E, Bradwejn J. CCK in animal and human research on anxiety. *Trends Pharmacol Sci* 1993; 14: 244—249.
5. Moran TH, Robinson PH, Goldrich MS, McHugh PH. Two brain cholecystokinin receptors: implications for behavioral actions. *Brain Res* 1986; 362: 175—179.
6. Beinfeld MC. Cholecystokinin/gastrin. In Psychopharmacology. The Fourth Generation of Progress FE Bloom, DJ Kupfer (eds). N. Y. Raven Press, Ltd., 1995; pp. 585—594.
7. Singh L, Lewis AS, Field MJ, Hughes J, Woodruff GN. Evidence for an involvement of the brain cholecystokinin B receptor in anxiety. *Proc Natl Acad Sci* USA 1991; 88: 1130—1133.
8. Koszycki D, Cox BJ, Bradwejn J. Anxiety sensitivity and response to cholecystokinin tetrapeptide in healthy volunteers. *Am J Psychiatry* 1993: 150: 1881—1883.
9. Pavlasevic S, Bednar I, Quereshi GA, Södersten P. Brain cholecystkinin tetrapeptide levels are increased in a rat model of anxiety. *Neuroreport* 1993; 5: 225—228.
10. Männistö PT, Lang A, Harro J, Peuranen E, Bradwejn J, Vasar E. Opposite effects mediated by CCK_A and CCK_B receptors in behavioural and hormonal studied in rats. *Naunyn-Schmiedebergs Arch Pharmacol* 1994; 349: 478—484.
11. Harro J, Vasar E. Evidence that CCK_B receptors mediate the regulation of exploratory behaviour in the rat. *Eur J Pharmacol* 1991; 193: 379—381.
12. Rex A, Fink H, Marsden CA. Effects of BOC-CCK-4 and L 365260 on cortical 5-HT release in guinea-pigs on exposure to the elevated plus-maze. *Neuropharmacology* 1994; 33: 559—565.
13. Bradwejn J, Koszycki D, Burin M. Dose ranging study of the effects of cholecystokinin in healthy volunteers. *J Psychiatr Neurosci* 1991; 16: 91—95.

14. Hughes J, Boden P, Costall B *et al.* Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc Natl Acad Sci* USA 1990; 87: 6728—6732.
15. Bickerdike MJ, Marsden CA, Dourish CT, Fletcher A. The influence of 5-hydroxytryptamine re-uptake blockade on CCK receptor antagonist effects in the rat elevated zero-maze. *Eur J Pharmacol* 1994; 271: 403—411.
16. Bock MG, DiPardo RM, Evans BE *et al.* Development of 1,4-benzodiazepine cholecystokinin type B antagonists. *J Med Chem* 1993; 36: 4276—4292.
17. Tyrer P, Tyrer J. Antidepressive drugs for treatment of anxiety disorders — and vice versa. In Handbook of depression and anxiety. A biological approach. JA den Boer, JM Ad Sitsen (eds). N. Y. Marcel Decker, Inc., 1994; pp. 497—514.
18. Schweizer E, Rickels K, Uhlenhuth EH. Issues in the long term treatment of anxiety disorders. In Psychopharmacology. The Fourth Generation of Progress. FE Bloom, DJ Kupfer (eds). N. Y. Raven Press, Ltd., 1995; pp. 1349—1360.
19. McElroy SL, Keck PE, Friedman LM. Minimizing and managing antidepressant side effects. *J Clin Psychiatry* 1995; 56 (Suppl 2): 49—55.
20. Preskorn SH. Comparison of the tolerability of bupropion, fluoxetine, imipramine, nefazodone, paroxetine, sertraline, and venlafaxine. *J Clin Psychiatry* 1995; 52 (Suppl 6): 12—21.
21. Chopin P, Briley M. Animal models of anxiety: the effect of compounds that modify 5-HT neurotransmission. *Trends Pharmacol Sci* 1987; 8: 383—388.
22. Griebel G, Moreau J-L, Misslin R, Martin JR. Acute and chronic treatment with 5-HT reuptake inhibitors differentially modulate emotional responses in anxiety models in rodents. *Psychopharmacology* 1994; 113: 463—470.
23. Griebel G, Blanchard DC, Agnes RS, Blanchard RJ. Different modulation of antipredator defensive behaviour in Swiss-Webster mice following acute or chronic administration of imipramine and fluoxetine. *Psychopharmacology* 1995; 120: 57—66.
24. Harro J. Measurement of exploratory behaviour in rodents. In Methods in Neurosciences, RA Conn (ed). Volume 14. San Diego Academic Press, Inc., 1993; pp. 359—377.
25. Lister RG. Ethologically-based animal models of anxiety disorders. *Pharmac Ther* 1990; 46: 321—340.
26. Blanchard JR, Yudko EB, Rodgers RJ, Blanchard DC. Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. *Behav Brain Res* 1993; 58: 155—165.
27. Treit D. Animal models of anxiety and anxiolytic drug action. In Handbook of depression and anxiety. A biological approach. JA den Boer, JM N. Y. Marcel Dekker, Inc., 1994; pp. 201—224.
28. Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav* 1986; 24: 525—529.
29. Handley SL, McBlane JW. An assessment of the elevated X-maze for studing anxiety and anxiety-modulating drugs. *J Pharmac Toxic Meth* 1993; 29: 129—138.
30. Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology, and ethology. In Ethology and Psychopharmacology. SJ Cooper, CA Hendrie (eds). N. Y. Wiley Press, Inc., 1994; pp. 9—44.
31. Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT. Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology* 1994; 116: 56—64.
32. Walsh RN, Cummins RA. The open field test: a critical review. *Psychol Bull* 1976; 83: 482—504.
33. Kelley AE, Cador M, Stinus L. Exploration and its measurement. In Neuromethods 13: Psychopharmacology. AA Boulton, GL Baker, AJ Greenshow (eds). Clifton, N. J. The Humana Press, Inc., 1989; pp. 95—144.

34. Harro J, Oreland L, Vasar E, Bradwejn J. Impaired exploratory behaviour after DSP-4 treatment in rats: implications for the increased anxiety after noradrenergic denervation. *Eur Neuropsychopharmacol* 1995; 5: 447—455.
35. Otter MH, Sõukand R, Oreland L, Harro J. Pharmacological validation of the exploration box: preliminary studies. *Pharmacol Toxicol* 1995; 76 (Suppl 3): 60.
36. Otter M, Skrebuhhova T, Matto V, Harro J. Studies on validation of a method to measure exploratory behaviour in rat. In Welfare and science. Proceedings of the sixth symposium of the Federation of European Laboratory Animal Science Association. PN O'Donoghue (ed). London, U. K. Royal Society of Medicine Press, 1997, in press.
37. Matto V, Allikmets L, Harro J. The mechanism of anxiogenic-like effect of antidepressants on exploratory behaviour in rats. *Pharmacol Toxicol* 1995; 76 (Suppl 3): 53.
38. Kaesermann HP. Stretched attend posture, a non-social form of ambivalence, is sensitive to a conflict-reducing drug action. *Psychopharmacology* 1986; 89: 31—37.
39. Kaesermann HP, Dixon AK. Further validation of the SAP test, a simple behavioural conflict test in mice. *Psychopharmacology* 1986; 89: S19.
40. Matto V, Harro J, Allikmets L. The effects of cholecystokinin A and B receptor antagonists on exploratory behaviour in the elevated zero-maze in rat. *Neuropharmacology* 1997; in press.
41. Rex A, Barth T, Voigt J-P, Domeney AM, Fink H. Effects of cholecystokinin tetrapeptide and sulfated cholecystokinin octapeptide in rat models of anxiety. *Neurosci Lett* 1994; 172: 139—142.
42. Johnston AL, File SE. Sex differences in animal tests of anxiety. *Physiol Behav* 1990; 49: 245—250.
43. Rex A, Sondern U, Voigt JP, Franck S, Fink H. Strain differences in fear-motivated behavior of rats. *Pharmacol Biochem Behav* 1996; 54: 107—111.
44. Dawson GR, Rupniak NMJ, Iversen SD *et al.* Lack of effect of CCK_B receptor antagonists in ethological and conditioned animal screens for anxiolytic drugs. *Psychopharmacology* 1995; 121: 109—117.
45. Kramer MS, Cutler NR, Balander JC *et al.* A placebo-controlled trial of L-365260, a CCK_B antagonist, in panic disorder. *Biol Psychiatry* 1995; 37: 462—466.
46. Charrier D, Dangoumau L, Puech AJ, Hamon M, Thiébot M-H. Failure of CCK receptor ligands to modify anxiety-related behavioural suppression in an operant conflict paradigm in rats. *Psychopharmacology* 1995; 121: 127—134.
47. Johnston NJT, Rodgers RJ. Ethological analysis of cholecystokinin (CCK_A and CCK_B) receptor ligands in the elevated plus-maze test of anxiety in mice. *Psychopharmacology* 1996; 124: 355—364.
48. Hökfelt T. Neuropeptides in perspective: the last ten years. *Neuron* 1991; 7: 867—879.

Received: December 2, 1996
Accepted: April 4, 1997

Author's address: V. Matto, Department of Pharmacology, University of Tartu, Ülikooli 18, EE 2400 Tartu, Estonia.