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Comparative Studies With Somatostatin and Cysteamine in Different Behavioral Tests on Rats

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VÉCSEI, L., C. KIRÁLY, I. BOLLÓK, A. NAGY, J. VARGA, B. PENKE AND G. TELEGDY. *Comparative studies with somatostatin and cysteamine in different behavioral tests on rats.* PHARMACOL BIOCHEM BEHAV 21(6) 833–837, 1984.—In the present study the effects of somatostatin and cysteamine (a selective decreaser of the somatostatin level in the body) were compared in different behavioral tests on rats. Somatostatin inhibited the extinction of active avoidance behavior 8 hr and 24 hr after intracerebroventricular (ICV) treatment, while cysteamine facilitated it 4 hr and 8 hr after subcutaneous (SC) treatment. Somatostatin did not significantly influence the cysteamine-induced facilitation of the extinction. Somatostatin did not have a significant effect on T-maze spatial discrimination learning and reverse learning, whereas cysteamine markedly attenuated the performance 4 hr (1st day) after treatment. Somatostatin in a dose of 4 μg (ICV) increased the locomotor activity 10 min after treatment, while cysteamine markedly decreased all parameters of the open-field test. These effects of the drug had disappeared 24 hr after treatment. If different doses of somatostatin (4 μg or 10 μg ICV) were administered to cysteamine-pretreated rats, the peptide did not modify the drug-induced changes in the open-field test. The data suggest that the brain somatostatin might have a physiological role in the organization of certain types of behavior.

Somatostatin Open field Cysteamine Locomotor activity

THE first evidence of a hypothalamic factor capable of inhibiting the release of growth hormone was obtained sixteen years ago by Krulich *et al.* [7]. Guillemin *et al.* [3,16] isolated this factor from ovine hypothalami and named it somatostatin. Later it was isolated from porcine and human hypothalami too [1, 13, 14]. The first indication of a behavioral effect of somatostatin was the observation of a transient tranquilizing effect of a large dose administered intravenously to monkeys [15]. Further, the peptide influenced the self-stimulation behavior [20, 21, 22], increased the LD50 of strychnine [5,6], prolonged the pentobarbital anesthesia time [5,6], potentiated the behavioral effect of L-dopa [10], had an antiamnesic effect [17] and inhibited the extinction of active avoidance behavior [18,19].

Cysteamine decreased the brain somatostatin-like immunoreactivity (SLI), consistent with the lowering of both somatostatin-14 and somatostatin-28 [12]. The specificity of cysteamine in decreasing the brain SLI has been demonstrated by the lack of effect of this treatment in specific brain areas on the concentrations of vasopressin enkephalin, vasoactive intestinal peptide and cholecystokinin, as determined by radioimmunoassay [9]. Thus, the existing evidence suggests that cysteamine specifically lowers tissue concentrations of SLI by increasing the intracellular degradation of somatostatin-14, possibly by disrupting the "vesicular" storage of the neuropeptide. The somatostatin-14 thereby released would be inactivated by peptidases located within synaptic terminals [2].

On the basis of these results, in the present study we have compared the effects of increased brain somatostatin (exogenous administration of somatostatin) and a decreased level of somatostatin (treatment with cysteamine) on the active avoidance behavior, T-maze discrimination and open-field activity.

METHOD

Animals

Experiments were performed on male CFY rats weighing 160–180 g. The animals were housed 5 per cage and were kept on a standard diet with food and water ad lib, under an artificial light schedule (12 hr light, 12 hr dark); the light period started at 6.00 a.m. The experiment was begun at 8.00 a.m.

Behavioral Procedure

Active avoidance behavior. Active avoidance behavior was studied in a platform jumping conditioning apparatus [18,19]. The conditional stimulus (CS) was the light of a 40 W

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electric bulb, while the unconditional stimulus (US) was an electric shock of 0.2 mA delivered through the grid floor of the apparatus to the paws of the rat. Each day for three consecutive days, 10 trials were performed, with a mean intertrial interval of 60 sec. On the fourth day extinction trials were run and the US was no longer applied. The CS was presented for a maximum of 10 sec, or it was terminated as soon as the animal had made the response. Animals which made at least 8 conditional responses out of 10 trials in the first extinction session were used for further experimentation. These animals were divided into different groups (9–22 rats/group) and immediately after the first extinction session were treated with saline (4 μl ICV and 2 ml/kg SC), somatostatin (4 μg/4 μl ICV), cystamine (300 mg/kg/2 ml SC), or cysteamine + somatostatin. The second and third extinction sessions were performed 4 and 8 hr after the treatment on day 4. The fourth extinction session was run on day 5, 24 hr after the treatment. Data are presented as percentage of correct responses.

Spatial discrimination learning: T-box. On the first day the animals were trained to avoid foot shocks by going to the right arm of the T-appartus. Training started by allowing the animals to explore the apparatus for 5 min. The animals were then placed on the start (far end of the stem), and after 10 sec, electric shocks (0.5 sec, 50 Hz, 0.2 mA) were applied a maximum of five times. The correction allowed the animal to continue the search until the goal in the right arm was reached. The rat was left there for the rest of the intertrial interval (20 sec) and was then placed on the start. The training continued until the criterion of 9 correct choices. The total number of incorrect choices was recorded. On the next day the safe goal area was shifted to the other arm of the T-maze and the discrimination was reversed until animal made 9 correct choices one after another. On the third day the task was reversed again. Somatostatin (4 μg/4 μl ICV) or saline (4 μl ICV) was administered only on the first day, immediately before the training session, while cysteamine (300 mg/kg/2 ml SC) or saline (2 ml/kg SC) was given 4 hr before the learning session on the first day. Data were obtained on days 1, 2 and 3 and are expressed as the total number of incorrect choices.

Open-field behavior. The animals were placed in an open-field box, which consisted of 36 squares measuring 10×10 cm each. Activity was characterized by the total numbers of squares explored, of rearings and groomings and the defecation boluses produced during the 3 min session. In this test somatostatin (4 μg/4 μl or 10 μg/4 μl ICV) was administered 10 min or 24 hr before the test session, and cysteamine (300 mg/kg 2 ml SC) 4 hr or 24 hr before the session, while the controls received saline. In the combined treatment group the cysteamine pretreatment was followed 4 hr later by the administration of somatostatin (4 μg/4 μl or 10 μg/4 μl ICV).

Surgical Procedure

For ICV administration, the animals were anesthetized with pentobarbital-Na (Nembutal, 40 mg/kg IP) and a cannula was placed into the lateral cerebroventricle and fixed to the skull with dental cement. The correct positioning of the cannula was checked by dissection of the brain.

Drugs

Cysteamine (Cysteaminiumchlorid, Laborpraparate Merck) was used. Somatostatin (cyclic) was synthetized by Botond Penke.

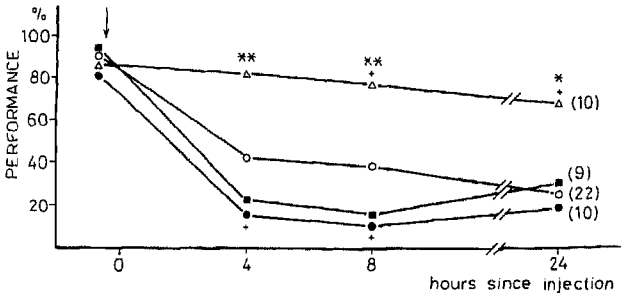

FIG. 1. Effect of somatostatin and cysteamine on the active avoidance behavior. Number of animals used appear in parentheses. Asterisks represent significant difference. Arrow indicates the time of the administration of the substances. ○—○—Control; △—△—somatostatin (4 μg/4 μl ICV); ●—●—cysteamine (300 mg/kg SC); ■—■—cysteamine + somatostatin. †$p<0.05$ (M.W. test), *$p<0.02$ (K.W. test), **$p<0.01$ (K.W. test).

Statistical Analysis

The Mann-Whitney U-test (M.W. test) was used for comparisons between two treatment groups as opposed to the overall H-test of Kruskal-Wallis (K.W. test).

RESULTS

As concerns the active avoidance behavior, the ICV and SC saline-treated control groups did not differ significantly from each other, and therefore their results were expressed in one control group. Somatostatin (4 μg/4 μl ICV) significantly inhibited the extinction of the active avoidance behavior 8 hr ($U=50$, $p<0.05$ M.W. test; $p<0.01$ K.W. test) and 24 hr ($U=53$, $p<0.05$ M.W. test; $p<0.02$ K.W. test) after treatment. Cysteamine (300 mg/kg 2 ml SC) facilitated the extinction 4 hr ($U=46$, $p<0.05$ M.W. test; $p<0.01$ K.W. test) and 8 hr ($U=43$, $p<0.05$ M.W. test) after treatment, but after 24 hr the performance of the cysteamine-treated animals did not differ from that of the control group. If the cysteamine-treated animals received somatostatin (ICV), the peptide did not significantly influence the drug-induced facilitation of the extinction (Fig. 1).

In the T-maze spatial discrimination test, saline-treated groups (ICV and SC) did not differ significantly from each other, and they were therefore combined into one control group. Somatostatin (4 μg/4 μl ICV) did not significantly influence the T-maze discrimination learning. The cysteamine-treated animals learned the test paradigm significantly more slowly ($U=44$, $p<0.02$ M.W. test; $p<0.01$ K.W. test) than their controls. On the second and third days (reverse learning), the performances of the cysteamine and saline-treated animals did not differ from each other (Fig. 2).

With respect to the open-field behavior, somatostatin (4 μg/4 μl ICV) treatment increased the ambulation ($U=30$, $p<0.05$ M.W. test; $p<0.01$ K.W. test) of the rats 10 min after treatment, but after 24 hr this effect had disappeared. The peptide in a dose of 10 μg/4 μl did not modify the activity of the rats, though in several cases uncoordinated movements were observed. Cysteamine markedly decreased the ambulation ($U=18$, $p<0.01$ M.W. test), rearing ($U=16$, $p<0.001$

TABLE 1

THE INTERACTION BETWEEN CYSTEAMINE AND SOMATOSTATIN ON THE OPEN-FIELD BEHAVIOR OF RATS

Treatment	Total number of squares	Total number of rearings	Total number of groomings	Defecation boluses
	Open-field behavior 10 min after the ICV somatostatin or saline			
sal+sal	72.5 ± 7.6* (12)†	20.5 ± 2.8 (12)	4.6 ± 0.6 (12)	3.0 ± 0.4 (12)
sal+som (4 μg)	116.6 ± 7.8‡ (11)	24.3 ± 2.9 (11)	6.0 ± 0.8 (11)	2.1 ± 0.6 (11)
sal+som (10 μg)	58.3 ± 7.6 (11)	14.2 ± 3.5 (11)	2.8 ± 0.9 (11)	1.7 ± 0.6 (11)
cyst+sal	32.1 ± 3.7§ (10)	3.5 ± 0.4¶ (10)	2.1 ± 0.3‡ (10)	1.2 ± 0.4‡ (10)
cyst+som (4 μg)	45.6 ± 6.1 (10)	7.5 ± 2.1 (10)	3.5 ± 0.6 (10)	1.8 ± 0.7 (10)
cyst+som (10 μg)	44.2 ± 6.9 (10)	7.1 ± 2.0 (10)	3.4 ± 0.7 (10)	1.9 ± 0.6 (10)
	Open-field behavior 24 hr after the ICV somatostatin or saline			
sal+sal	63.2 ± 5.4 (12)	18.9 ± 1.9 (12)	7.1 ± 0.9 (12)	1.7 ± 0.6 (12)
sal+som (4 μg)	75.3 ± 6.2 (11)	20.6 ± 2.6 (11)	7.5 ± 0.7 (11)	1.5 ± 0.5 (11)
sal+som (10 μg)	78.4 ± 5.2 (11)	23.1 ± 1.8 (11)	8.2 ± 0.9 (11)	1.1 ± 0.6 (11)
cyst+sal	70.6 ± 6.7 (10)	22.0 ± 2.9 (10)	7.9 ± 0.6 (10)	2.0 ± 0.6 (10)
cyst+som (4 μg)	72.4 ± 7.1 (10)	21.1 ± 2.2 (10)	6.6 ± 0.8 (10)	2.3 ± 0.3 (10)
cyst+som (10 μg)	67.5 ± 6.9 (11)	19.2 ± 2.4 (11)	7.3 ± 0.9 (11)	2.5 ± 0.5 (11)

Rats were tested 10 min and 24 hr after administration of somatostatin (4 μg or 10 μg/4 μl, ICV) or saline (4 μl, ICV). Animals were pretreated with cysteamine (300 mg/kg) or saline subcutaneously 4 hr prior to ICV injection.
*Mean ± SEM (standard error of the mean), †Numbers in parenthesis are the numbers of rats in the treatment group; ‡$p<0.05$ (M.W. test); $p<0.01$ (K.W. test); §$p<0.01$ (M.W. test); $p<0.01$ (K.W. test); ¶$p<0.001$ (M.W. test); $p<0.01$ (K.W. test).

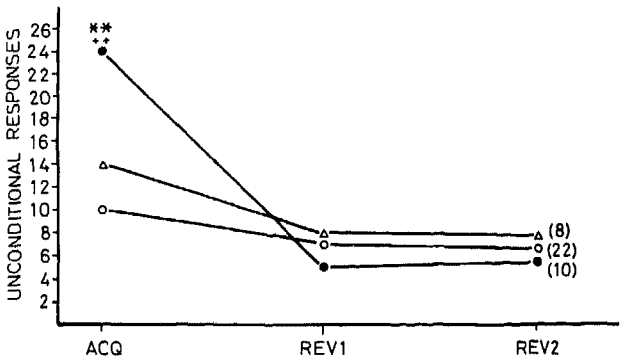

FIG. 2. Effect of somatostatin and cysteamine on the T-discrimination learning. Number of animals used appear in parentheses. Asterisks represent significant difference. ACQ=acquisition, REV 1=first reversal, REV 2=second reversal. ○—○—control; △—△—somatostatin (4 μg/4 μl ICV); ●—●—cysteamine (300 mg/kg SC). ††$p<0.02$ (M.W. test) **$p<0.01$ (K.W. test).

M.W. test; $p<0.01$ K.W. test), grooming ($U=25$, $p<0.05$ M.W. test, $p<0.01$ K.W. test) and defection ($U=25$, $p<0.05$ M.W. test, $p<0.01$ K.W. test) 4 hr after treatment. Twenty-four hr after administration of the drug, this effect had disappeared. Somatostatin (4 μg/4 μl; 10 μg/4 μl ICV) did not significantly modify the open-field parameters of cysteamine-pretreated animals (Table 1).

DISCUSSION

In the present study the effects of exogenously administered somatostatin and of endogenous somatostatin depletion by cysteamine were investigated in different behavioral tests. Somatostatin was administered ICV, for in previous preliminary studies peripheral administration of the peptide did not modify the aversively motivated behavior of rats (unpublished observation). In contrast, cysteamine was administered peripherally, as a dose of 300 mg/kg (SC) decreased the somatostatin level of the brain [9].

Somatostatin inhibited the extinction of active avoidance behavior. Delay in the extinction of an active avoidance response may be due, to the increased selective attention to the conditioned stimulus of the light signal, a more general state of arousal, enhanced motivation, and an improved memory function [8]. Furthermore, the peptide had an antiamnesic effect [17]. This might mean that somatostatin is able to influence the memory processes, so this antiamnesic effect might participate in the extinction inhibition of active avoidance behavior induced by the peptide. Somatostatin did not influence the T-maze discrimination learning or reverse learning, indicating that selective attention may not be influenced by peptide treatment. In the open-field behavior the peptide (4 μg/4 μl ICV) increased the locomotor activity, but this effect had disappeared 24 hr after treatment. Somatostatin administered in a higher dose produced coordination difficulties and stereotypy, as observed earlier [11]. The locomotor stimulation induced by somatostatin (lower dose) might also be an important factor in the extinction inhibition of active avoidance behavior. Indeed, in earlier experiments we found that the intertrial activity was also increased during the extinction session [19].

Cysteamine, which selectively decreases the somatostatin level, displayed characteristic inhibitory action in all three behavioral tests. Four hr after administration of the drug, a time when the somatostatin level is extremely de-

pleted [4,9], the performance was decreased in all behavioral tests, but this effect had disappeared 24 hr later, when the somatostatin depression had nearly been eliminated [4].

As concerns the active avoidance behavior, 4 hr and 8 hr after treatment with cysteamine the performance had decreased significantly, but 24 hr later it had returned nearly to the control level. Similarly, in the T-discrimination learning test, 4 hr after cysteamine treatment the performance of the animals was markedly decreased, but on the next day it had returned to the control level. Many other effects of cysteamine (such as depression of the rectal temperature, increase of the plasma glucose level [4]) had disappeared 24 hr after administration of the drug. In the open-field behavior all parameters of this behavioral test were markedly decreased 4 hr after treatment, but 24 hr later the activity of the treated animals was the same as that of their controls. It is possible that the inhibitory effects of cysteamine in the different tests were mediated mainly by the depression of the locomotor activity, but 24 hr after treatment the effects of the drug had disappeared and the performance of the rats in the behavioral tests was at the level of the controls. The time-related parallel changes of the endogenous somatostatin with these behavioral changes are well documented [4].

On the basis of these findings, the effects of ICV administered somatostatin on the open-field and active avoidance behavior of cysteamine-pretreated rats were investigated. These rats were hyperactive immediately after treatment (approximately 5 min), while later grooming and scratching were seen. Four hr after treatment with the drug the animals were calm. The activity (ambulation and rearing) of cysteamine-pretreated animals increased in the open-field test after somatostatin treatment, but this was not significant statistically. Similarly, in the active avoidance behavior the facilitated extinction induced by cysteamine was not influenced significantly by the administration of somatostatin. Brown *et al.* [4] have found that ICV administration of a potent somatostatin agonist to cysteamine-pretreated animals (treated with the same dose as used in our experiment) inhibited the rises in plasma epinephrine and glucose, but had no effect on the elevated norepinephrine level. This means that the potent analog only partially antagonized the effects of cysteamine on the metabolic pathways. As regards our experiments, it is possible that the ICV administered somatostatin did not reach the receptor sites in a sufficient concentration. It is also possible that some peripheral mechanisms too are involved in the behavioral action of cysteamine.

The data obtained following somatostatin and cysteamine administration show opposite effects in most of the behavioral studies. In other words, exogenously administered somatostatin (which increased the brain somatostatin level) and cysteamine administration (which decreased the somatostatin level in the body) exert opposite action. This might suggest that the endogenous brain somatostatin is of physiological significance in the organization of certain types of behavior.

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