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RAPID COMMUNICATION

Effects of Calcitonin Gene-Related Peptide on Extrapyramidal Motor System

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CLEMENTI, G., M. GRASSI, C. VALERIO, A. PRATO, C. E. FIORE AND F. DRAGO. *Effects of calcitonin gene-related peptide on extrapyramidal motor system.* PHARMACOL BIOCHEM BEHAV 42(3) 545–548, 1992.—The effects of central administration of calcitonin gene-related peptide (CGRP, 1 or 100 ng/rat) on behavioral and biochemical parameters related to the extrapyramidal motor system were investigated in male rats. The peptide-induced catalepsy occurred only at the dose of 100 ng/rat and hypomotility at both doses used. Calcitonin gene-related peptide increased haloperidol-induced catalepsy and decreased apomorphine-induced hypermotility at the doses of 1 and 100 ng/rat. Although these behaviors are related to dopamine, no significant change of striatal DA or DOPAC concentration were observed after central administration of the peptide. Other neurotransmitters may be directly or indirectly involved in these behavioral effects of CGRP.

CGRP Catalepsy Hypomotility Dopaminergic system

CALCITONIN gene-related peptide (CGRP) is a neuropeptide abundantly found and heterogenously distributed within the central nervous system (CNS). It is formed in nervous tissue by alternative splicing of primary RNA-transcript of the calcitonin (CT) gene (1). In fact, whereas the m-RNA encoding calcitonin predominates in the thyroid, CGRP m-RNA is the major transcription product in the CNS (15). Binding sites for CGRP are present in the brain and spinal cord (9,19), and to a minor extent in peripheral tissues of man (17) and rat (21). Also CT has been shown to bind to specific receptor sites within the CNS (14). Interaction of CGRP with CT receptor sites has been observed in presence of a large excess of the peptide (9) raising the possibility of a common action. Both CGRP and CT exert a number of biological actions after central administration (8), including inhibition of food intake (10), inhibition of gastric acid secretion (11), and inhibition of prolactin release (5).

Since CT induces behavioral changes after central administration, possibly via an involvement of the nigrostriatal sistem (12), we have investigated the effects of intracerebroventricular (ICV) injection of rat CGRP (r-CGRP) on behavioral and biochemical parameters related to the extrapyramidal motor system, and specifically to the nigrostriatal dopaminergic system in rats. As a direct biochemical index of the nigrostriatal dopaminergic function, we have measured the striatal concentration of dopamine (DA) and of its main catabolyte, dehydroxyphenylacetic acid (DOPAC).

METHOD

Animals

Male Sprague-Dawley rats weighing 200–220 g (purchased from Charles River, Italy) were used. The animals were housed 5 per cage and kept at room temperature (20°C). All animals had free access to commercial food and water, under a constant light-dark cycle (lights on between 8:00 and 20:00). Seven days prior to the experimental session, 70 animals were implanted with a permanent plastic cannula into their right lateral ventricle (foramen interventriculare, Koning and Klippel, A6360). The operation was performed according to the method described by Brakkee et al. (4), under ether anesthesia. All animals showed good physical conditions at the beginning of experimental procedures. The rats were used only once in the behavioral experiments.

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Treatment

r-CGRP (Peninsula, U.K.) was dissolved in saline. Peptide solution was freshly prepared and injected ICV to the rats, 30 min prior to the behavioral test, at the doses of 1 or 100 ng/rat. Control animals received ICV injection of saline. Haloperidol (0.5 mg/kg, Sigma, U.S.A.) or apomorphine (1 mg/kg, Sigma, U.S.A.) were dissolved in saline and injected intraperitoneally (IP) and subcutaneously (SC), respectively, 30 min prior to behavioral tests.

Biochemical Assays

Three groups of seven animals were sacrificed by decapitation 30 min after the injection of r-CGRP or saline, and the brains were rapidly removed, the regions dissected and immediately frozen. The rostral pole of corpus striatum was taken as +5 mm anterior to bregma, as described by Pellegrino and Cushmann (13). Ten serial sections of 600-μm thickness were made from this plane using a cryostat kept at −10°C.

For DA and DOPAC assay, striatal tissue pellets were homogenized on ice-bath in 0.1 N $HClO_4$ centrifuged for 15 min, 15000 *g* at 4°C. Dopamine and DOPAC were assayed in 50-μl samples of the supernatant using the radioenzymatic method described by Argiolas and Fadda (2), based on the conversion of DA and DOPAC, respectively, into [^{3}H]methoxytyramine and [^{3}H]homovanillic acid after incubation with S-adenosyl-1-[methyl-^{3}H]methionine (The Radiochemical Centre, Amersham; 5–10 Ci/mmol) in the presence of catechol-*O*-methyl transferase (COMT).

Behavioral Tests

Catalepsy was measured using four tests, according to the method of Dunstan et al. (6), slightly modified: a) the animals were placed on the workbench. A score of 1 was given for each 15 s of forepaw immobility; b) the rats were positioned on a grid inclined with the nose pointing downward. A score of 1 was given for each 15 s of forepaw immobility; c) the forepaws of the animals were put on a platform 8 cm above the bench. A score of 1 was given if the animal rested for 15 s without descending into the bench or mounting onto the platform; d) the animals were put at the corner of the cage in the "buddha position." A score of 1 was given for each 15 s of maintainance of the position. Each test was repeated three times for 24 min (1 min for test) starting 8 min after the treatment.

Spontaneous motor activity was studied with the open field test. Animals were put inside a circular lit-up arena, with the bottom divided into 27 sections of equal size. The behavior of each animal was observed for a period of 3 min and ambulation was recorded as the number of sections explored with at least the forelegs.

Experimental Design

In the first experiment, cataleptic score was measured in animals receiving ICV injection of saline or r-CGRP together with IP administration of saline or haloperidol. Since the dose of 1 ng/rat of r-CGRP was not found to be effective in increasing cataleptic score, this dose was used to study the possible potentiation of catalepsy induced by IP injection of

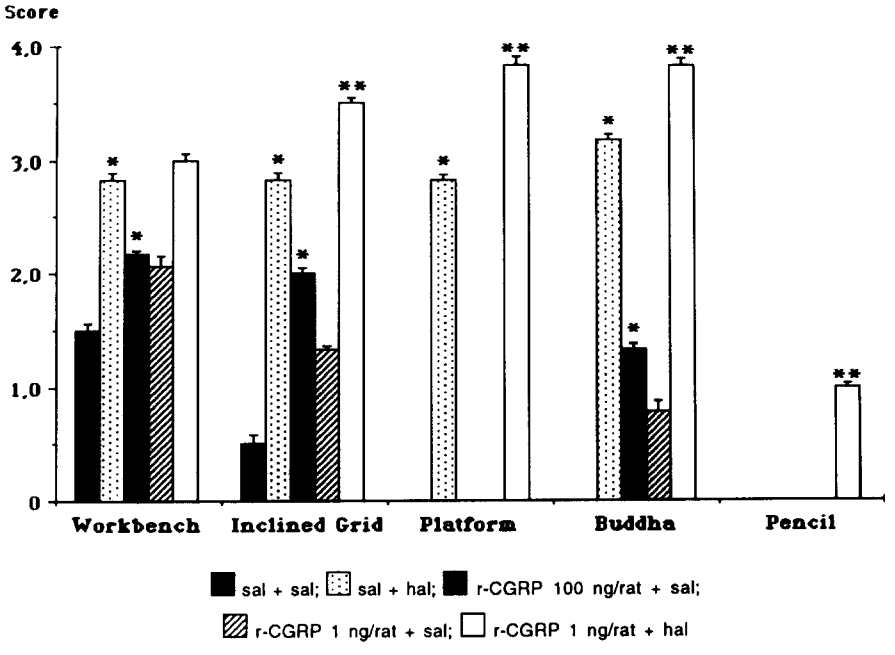

FIG. 1. Catalepsy induced by ICV injection of r-CGRP at different doses and the interaction with IP administration of haloperidol in rats. Values are means S.E. The number of animals was 7 per each group.
*Significant difference vs. saline + saline-injected controls ($p < 0.05$, Dunnett's test for multiple comparison).
**Significant difference vs. saline + haloperidol-injected rats ($p < 0.05$, Dunnett's test for multiple comparison).

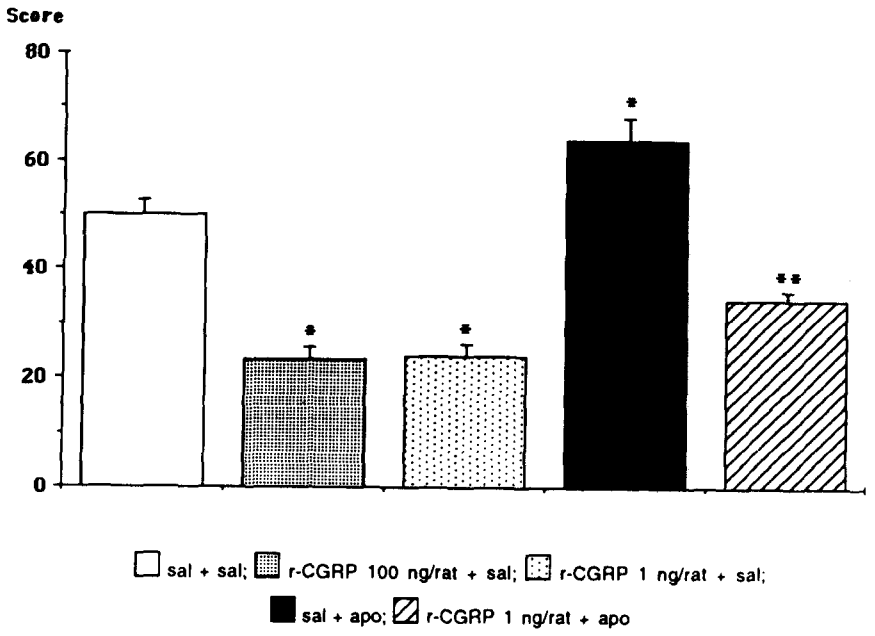

FIG. 2. Changes of motor activity induced by ICV acute injection of r-CGRP at different doses and the interaction with SC administration of apomorphine in rats. Values are means ± SE. The number of animals was 7 per each group.
*Significant difference vs. saline + saline-injected controls ($p < 0.05$, Dunnett's test for multiple comparison).
**Significant difference vs. saline + apomorphine-injected rats. ($p < 0.05$, Dunnett's test for multiple comparison).

haloperidol. The second experiment was devoted to the study of motor activity of rats riceiving ICV injection of saline or r-CGRP together with SC injection of saline or apomorphine. Again, the dose of 1 ng/rat r-CGRP was used to antagonize apomorphine-induced hypermotility. Biochemical assay was performed in another group of rats after ICV injection of saline or r-CGRP.

Statistical Analysis

Statistical differences were calculated with the two-way analysis of variance (ANOVA) and the post-hoc Dunnett's test for multiple comparison. A p level of 0.05 or less was accepted as indicative of a significant difference.

RESULTS

As expected, the cataleptic score of animals injected IP with haloperidol was higher than that of controls (Fig. 1). A single ICV injection of r-CGRP at the dose of 100 ng/rat also induced a higher cataleptic score as compared to that found after ICV injection of saline. This effect was not present at the dose of 1 ng/rat. However, the lower ineffective dose of r-CGRP was able to potentiate haloperidol-induced catalepsy. A significant interaction behavior/treatment was found with the ANOVA test, $F(4, 28)$, $p < 0.05$.

Both doses induced hypomotility in the open field test (Fig. 2). In contrast, apomorphine-injected rats showed an increase in locomotor activity as compared to control animals. Moreover, apomorphine-induced hypermotility was significantly reduced by ICV injection of 1 ng/rat r-CGRP. A significant interaction was found also in this case, $F(4, 28)$, $p < 0.05$.

No significant changes of striatal DA or DOPAC concentrations were observed following r-CGRP injection at the doses of 1 and 100 ng/rat (Table 1).

DISCUSSION

Evidence exists that r-CGRP interferes with some behavioral activities such as eating (10) and pain threshold (3). It has been suggested that these effects involve various central mechanisms, that is, cerebral calcium metabolism (20) and the release of neurotransmitters (7). Another possibility is that r-CGRP interferes with CT binding sites (18).

The present results demonstrate that r-CGRP, after single a central administration, may induce catalespy and hypomotility in the rat. In addition, the peptide may potentiate haloperidol-induced catalepsy and antagonize, at least in part, apomorphine-induced hypermotility. These results might be

TABLE 1

EFFECTS OF ICV INJECTION OF r-CGRP AT DIFFERENT DOSES ON DOPAMINE (DA) AND DEHYDROXYPHENYLACETIC ACID (DOPAC) CONTENT IN THE STRIATUM

		Striatum	
Drug	Dose (ng/rat)	DA (ng/g f.t.)	DOPAC (ng/g f.t.)
Saline	–	7122.12 ± 212.90	1495.30 ± 550.40
r-CGRP	1	6961.60 ± 315.30	1285.30 ± 630.90
r-CGRP	100	6760.90 ± 380.00	1310.80 ± 390.83

f.t., fresh tissue.

related to an interference of the peptide with DA neurotransmission in selected brain areas. It is well established, in fact, that apomorphine-induced hypermotility is due to the dopaminergic action of apomorphine, whereas haloperidol induces catalepsy by antagonizing central DA receptors (16). This hypothesis is supported by the evidence that catalepsy induced by central acute injection of r-CGRP is diminished in animals chronically pretreated with apomorphine, probably due to a downregulation of DA receptors in the brain (Clementi, unpublished). However, it should be noted that our biochemical data are not consistent with a direct effect of CGRP on DA release and/or turnover in the nigrostriatal system. In fact, no change in striatal DA or DOPAC concentration was observed following intraventricular r-CGRP injection. However, we cannot rule out the possibility that r-CGRP interferes with DA receptors without affecting primarily DA release and/or turnover.

As another possibility, this peptide may induce changes in DA activity by influencing different neurotransmitter systems. Accordingly, some effects exerted by r-CGRP, such as reduction of plasma prolactin levels (7) and of growth hormone secretion (18), have been related to the interference of the peptide with specific neurotransmitter pathways.

It is known that CT potentiates haloperidol-induced catalepsy and reduces apomorhine-induced hypermotility (12). Since a cross-reactivity of r-CGRP on neuronal CT receptors has been recently demonstrated, we can also speculate that r-CGRP may elicit its behavioral effects by interacting with receptor binding sites for CT.

In conclusion, r-CGRP after a single central administration induces catalepsy and hypomotility; since no direct evidence shows an interference of the peptide with the dopaminergic neurotransmission, the mechanism of action responsible for these behavioral effects remaines unknown.

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