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Antagonistic Behavioral Effects of Calcitonin and Amphetamine in the Rat

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TWERY, M. J., B. KIRKPATRICK, M. H. LEWIS, R. B. MAILMAN AND C. W. COOPER. *Antagonistic behavioral effects of calcitonin and amphetamine in the rat.* PHARMACOL BIOCHEM BEHAV **24**(5) 1203–1207, 1986.—Using an automated testing apparatus, the hypermotility induced by amphetamine had previously been found to be inhibited by intracerebroventricular (ICV) administration of salmon calcitonin (CT). The present study used a computer-supported direct observational method to characterize further the interactions of CT and amphetamine. After treatment with amphetamine (1.5 mg/kg, IP), the incidence of rearing, nose poking, and locomotion was reduced in rats that were pretreated with 85 pmol salmon CT ICV; the incidence of sniffing and grooming remained unchanged. CT-induced dyskinesia, a unique consequence of central CT treatment, was attenuated but not abolished by administration of amphetamine. These results support the premise that a compound with receptor recognition characteristics similar to those of salmon CT may act as a neurotransmitter-modulator in the central nervous system.

Calcitonin Amphetamine Dyskinesia Locomotor activity Motor function

INTRACEREBROVENTRICULAR (ICV) administration of salmon calcitonin (CT) has a number of effects on rats, including decreases in food and water consumption [12, 19, 32] and an attenuated response to painful stimuli [25]. Our previous studies showed that ICV salmon CT inhibits amphetamine-induced locomotion, as measured by an automated counting device, and causes dyskinetic movements [30,31]. The present study was designed to investigate in more detail the interaction between calcitonin and amphetamine.

METHOD

Male, Sprague-Dawley rats (175–200 g) obtained from Charles River (Wilmington, MA) were administered salmon CT or vehicle ICV using the method of Popick [28], which has been validated in other studies [16, 21, 32]. Salmon CT (gift from Armour Pharmaceutical) was dissolved in 1 mM HCl 0.15 M NaCl; a dose of 85 or 8.5 pmol CT (ca. 300 or 30 ng, respectively) was delivered in a 10 μl volume. After 30 min, each rat was placed under an inverted, clear Plexiglas cage (21×37 cm) with a wire mesh floor. Illumination was provided by overhead fluorescent lighting, and a white noise background was generated electronically.

The effect of salmon CT on amphetamine-stimulated behavior was studied by systematically recording and analyzing observational data for selected behaviors using the method of Lewis *et al.* [20]. Observers were not aware of treatment condition (calcitonin or vehicle). During each 60 min session, 10 equally spaced, 1 min observations were made of each rat. Each 1 min observation period consisted of four consecutive 15 sec scoring intervals. A proportion was then generated for each observation period based on the number of intervals in which a behavior was seen (0, 1, 2, 3 or 4) divided by the total number of intervals (4). This is expressed as % occurrence in that 1 min observation period. The transform p′=2*arcsin[sqrt(p)] was used to stabilize the variances [20], and results were transformed prior to statistical analysis using a two-factor analysis of variance for repeated measures.

The behavioral categories scored included those that occur with drug-free rats as well as unusual behaviors induced by drug treatment. We have described in detail [20] the behaviors for which scores could be entered: asleep; inactive; rearing; grooming; locomotion; circling; sniffing; licking; gnawing; body gnawing; nose poking; tongue protrusion; or dyskinesia. These behavioral categories describe virtually all behaviors observed with these treatments. In some cases, the occurrence of a particular behavior was at such a low incidence that data are not presented. It should be noted that the scoring method used could accomodate unexpected behaviors, such as myoclonus, if they did occur [20].

Interobserver reliability was examined to indicate the adequacy of behavioral definitions. Data collected simulta-

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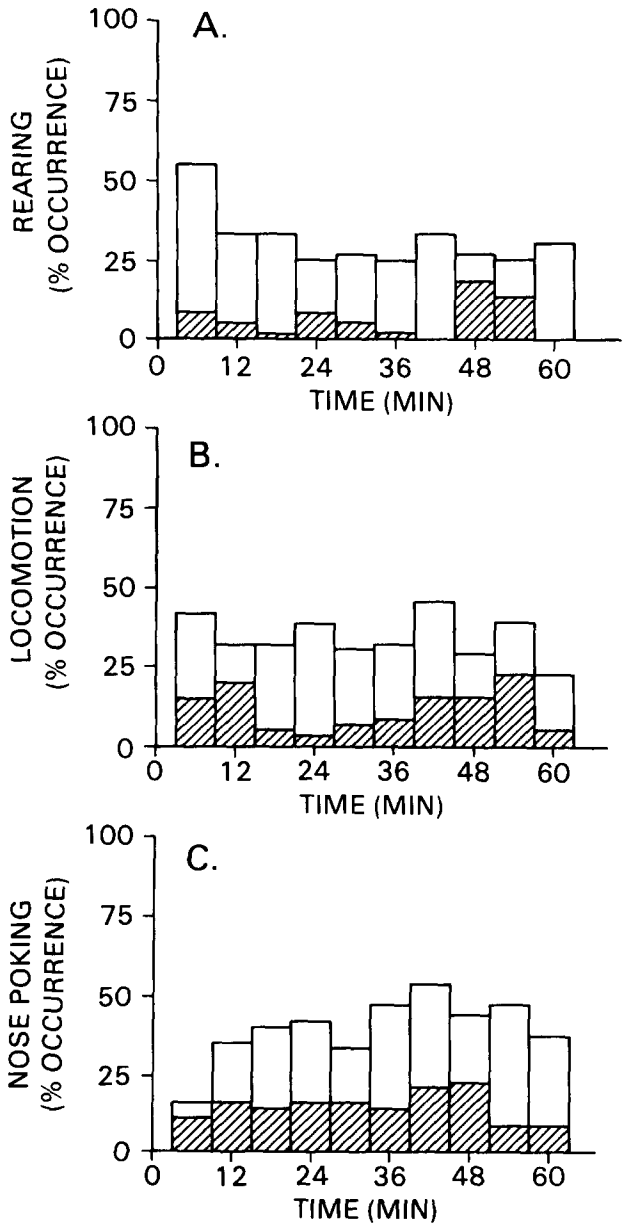

FIG. 1. The effects of salmon CT pretreatment on the incidence of amphetamine-induced rearing (A); locomotion (B); and nose poking (C). Bars represent the mean incidence of rearing over a one minute observation period. Vehicle (unshaded bars) or 85 pmol of salmon CT (Bachem Corp.) was administered ICV 2.5 hr prior to treatment with 1.5 mg/kg amphetamine SC (15 rats/group). Analysis of variance for repeated measures revealed the following significant effects: rearing: (1A): effect due to treatment, $F(1,28)=20.8$, $p<0.001$; locomotion (1B): effect due to treatment, $F(1,28)=13.4$, $p<0.01$; nose poking (1C): effect due to treatment, $F(1,28)=7.27$, $p<0.05$; and time, $F(9,252)=2.28$, $p<0.05$.

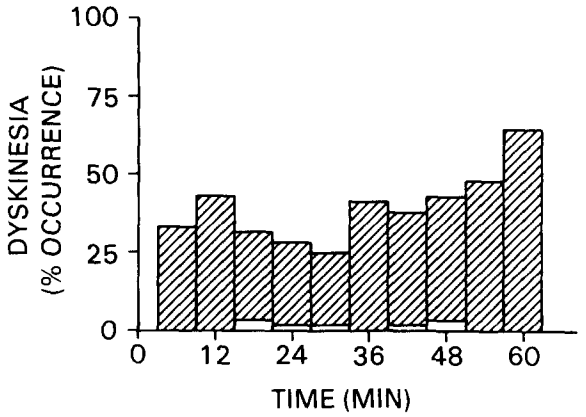

FIG. 2. The inability of amphetamine to block dyskinesia induced by prior (2.5 hr) treatment with salmon CT. Bars represent the mean incidence of dyskinesia over a one minute observation perior. Vehicle (unshaded bars) or 85 pmol of salmon CT (shaded bars; Bachem Corp.) was administered ICV 2.5 hr prior to treatment of all rats with 1.5 mg/kg amphetamine (15 rats/group). Analysis of variance for repeated measures revealed a significant effect due to treatment, $F(1,2)=32.2$, $p<0.0001$, time, $F(9,252)=2.50$, $p<0.01$, and treatment by time interaction, $F(9,252)=3.4$, $p<0.01$. One factor analysis of variance for simple main-effects indicated a significant effect due to treatment in all 10 periods of observation.

neously by two independent observers were compared for each observation session. Interobserver scoring agreement was summarized by the kappa statistic for reliability. Kappa is less sensitive than simple percentage of agreement to the frequency and ease with which either occurrence or nonoccurrence can be scored [13].

Animals were observed routinely for two distinct 60 min sessions. The first 60 min observation session followed a 30 min pretreatment with either salmon CT or vehicle, and habituation behavior was recorded. Sixty min after the first observation session (150 min after treatment with either salmon CT or vehicle ICV), the rats were administered d-amphetamine (1.5 mg/kg, Sigma Chemical Co.) intraperitoneally (IP) which had been dissolved in sterile water (0.75 mg/ml). Ten minutes after amphetamine treatment, a second 60 min observation session recorded the effect of salmon CT on amphetamine-stimulated activity. In a separate experiment, the animals were administered CT or vehicle ICV, followed by amphetamine IP within a few seconds. The lengths of these observation periods were arbitrarily selected to provide sufficient numbers of intervals during time periods when the drugs were believed to be active.

RESULTS

Amphetamine increased the incidence of rearing, grooming, locomotion and nose poking when compared to animals treated with vehicle alone (data not shown). The rats treated with 85 pmol dose of salmon CT had significantly less amphetamine-stimulated rearing, locomotion, and nose poking throughout most of the observation session (Fig. 1). However, the CT reduced sniffing only at 60 min (55%, $p<0.01$) and did not affect grooming at all (data not shown).

Calcitonin causes a syndrome we have termed "dyskinesia," consisting of tail flicking, limb and trunk choreiform movements, and head shaking [30]. Because these behaviors occur together, often almost simultaneously,

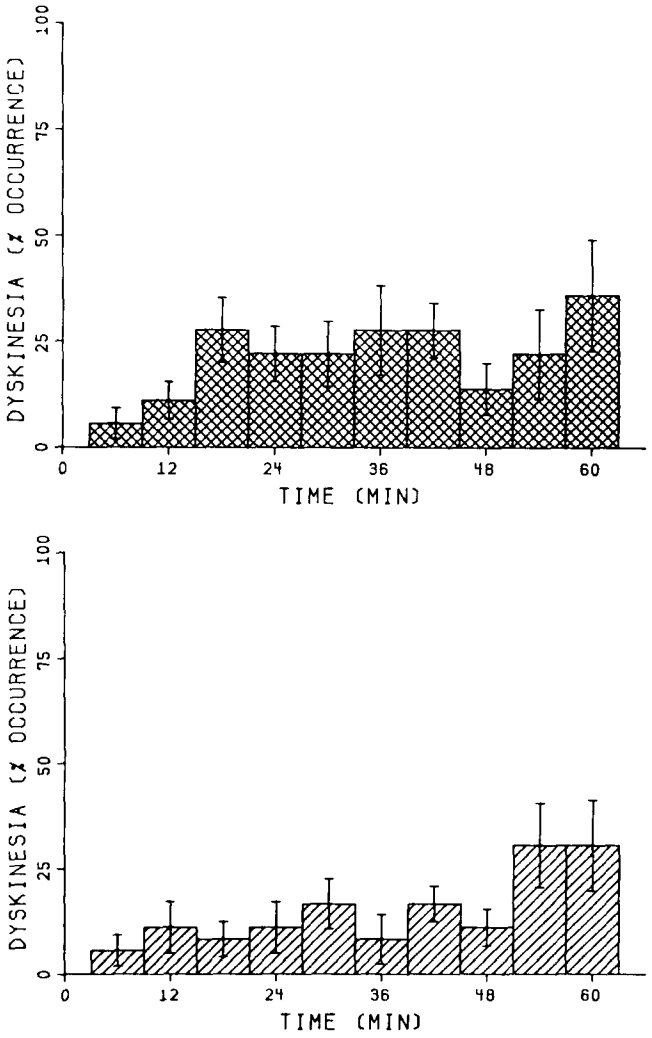

FIG. 3. The inability of simultaneous administration of amphetamine to block the incidence of salmon CT-induced dyskinesia. Bars represent the mean±SE incidence of dyskinetic activity over a 1 min observation period. All animals received 85 PMOL salmon CT (Bachem Corp.) ICV and either vehicle (cross-hatched bars) or 1.5 mg/kg amphetamine IP (shaded bars) 10 min prior to observations (9 rats/group). Analysis of variance for repeated measures revealed a significant effect due to time, $F(9,144)=3.68$, $p<0.001$.

in calcitonin-treated rats, we have lumped them together under the term dyskinesia [30]. Although the frequency and severity of dyskinesia appeared to decrease after treatment with amphetamine, the incidence of dyskinesia remained greater for animals treated with 85 pmol salmon CT than vehicle-treated controls ($p<0.001$; Fig. 2). In animals pretreated with 8.5 pmol salmon CT, a decrease in grooming ($p<0.05$; data not shown) was observed, but there were no changes in the incidence of nose poking, sniffing, locomotion, rearing or dyskinesia. Finally, nearly simultaneous treatment with salmon CT followed by amphetamine also failed to block the dyskinesia (Fig. 3), although again there appeared to be a decrease in the dyskinesia's frequency.

During habituation, values for kappa ranged from +0.85 (grooming) to +0.94 (locomotion). For the period of observation following amphetamine treatment, values ranged from +0.69 (sniffing) to +0.91 (rearing). These high values demonstrate that the scoring criteria were sufficiently defined and were being systematically applied.

DISCUSSION

Our results show that salmon CT treatment decreased the incidence of rearing and locomotion, both during habituation and after stimulation by amphetamine. These findings are consistent with our previous studies, which showed that both mammalian and nonmammalian forms of CT suppressed amphetamine-stimulated locomotor activity measured by a circular photocell apparatus [31]. These effects do not reflect simple sedation or gross motor impairment, as evidenced by the finding that centrally administered salmon CT affects only certain categories of behavior: the incidence of sniffing and nose poking during habituation, and the occurrence of sniffing and grooming elicited by amphetamine, were not materially reduced by CT treatment.

Because automated measuring devices may miss certain important behavioral effects, systematic observations by raters blind to treatment condition (active drug or vehicle) can provide important information. For instance, our previous work with automated devices showed that calcitonin depressed amphetamine-induced locomotion, but could not reveal the presence of dyskinesia, attenuation of the dyskinesia by amphetamine, or the other behavioral effects of amphetamine combined with calcitonin that we have reported here.

Centrally administered CT has several effects, including analgesia, the suppression of food and water intake, decreased gastric acid secretion, and reduced secretion of several pituitary hormones [2, 3, 12, 18, 22, 25–27, 32]. The pharmacological effects of centrally administered salmon CT may be due to an interaction with specific binding sites for an endogenous CT-like substance located within the CNS. Evidence for this hypothesis includes the presence in the brain of high affinity binding sites [9, 11, 14, 17], salmon CT-like immunoreactivity [4, 6, 7, 10], and gene products structurally similar to CT [1,29].

Other lines of evidence suggested that the behavioral interaction of amphetamine and CT might be due to a direct interaction of CT with dopamine receptors. For instance, dopamine has a crucial role in the locomotor response to low doses of amphetamine [5,15], and nigrostriatal and other dopamine neurons are involved in a variety of motor and postural functions [8]. Furthermore, Nicoletti *et al.* [24] reported that CT potentiates the cataleptic response to haloperidol. However, radioligand binding studies with rat striatal membranes found that CT failed to displace the binding of either a dopaminergic agonist ([^{3}H]-dopamine) or a dopaminergic antagonist ([^{3}H]-spiperone) [30,33]. Moreover, dopamine and other monoamines do not antagonize the binding of [^{125}I]-salmon CT to brain tissue [23]. Taken together, the evidence suggests that CT does not act directly via dopamine receptors. Peptide neuromodulators may have complex relationships to classical neurotransmitters. Similar complexity may be found when the other central effects of CT, such as analgesia or suppression of food and water consumption, are assessed.

The site at which CT interacts with the motor system is as yet unknown. High affinity, stereospecific recognition sites for CT have been found in the striatum and midbrain [11,17]; it is possible that occupation of the purported receptors in these regions accounts for the motor effects of CT when administered ICV. However, our previous work also

showed a suppression of amphetamine's locomotor effects when small doses of CT (6.4 μg/kg) were administered subcutaneously [31]. Salmon CT, a peptide with 32 amino acids, probably does not cross the blood-brain barrier readily. After intracardial administration, CT binds to recognition sites in the area postrema, subfornix, median eminence, and lamina terminalis of the organum vasculosum [34]; when administered subcutaneously, CT might exert its influence at these sites, where the blood-brain barrier is not so restrictive to the passage of large molecules as in other areas of the brain. The fact that these structures are not thought to have an important role in motor function weakens this hypothesis. Salmon CT could produce an effect on motor function by interaction with receptors in these sites only if events in other structures were modulated. It is also possible, though unlikely, that CT administered subcutaneously may have an effect on motor function secondary to its peripheral effects, such as hypocalcemia.

In summary, these data confirm that calcitonin can alter amphetamine-induced behaviors, but that only some topographies (e.g., locomotion, rearing and nose poking) are affected. In concert with other data, it appears that the actions of calcitonin apparently occur without direct effects on dopamine synaptic transmission. This suggests that a calcitonin-like peptide and dopamine may not interact directly as is known to occur with dopamine and neurotensin, or dopamine and opioid peptides. However, the relatively low doses of calcitonin needed for these effects are consistent with the involvement of high affinity recognition sites [9, 11, 14, 17] such as receptors for an endogenous neurotransmitter-modulator. The loci of these recognition sites, and their relationship to specific neurochemical messengers, remain to be elucidated.

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