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Calcitonin Depresses Amphetamine-Induced Locomotor Activity[1,2]

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TWERY, M. J., C. W. COOPER AND R. B. MAILMAN. *Calcitonin depresses amphetamine-induced locomotor activity.* PHARMACOL BIOCHEM BEHAV **18**(6) 857–862, 1983.—Synthetic salmon calcitonin (sCT), given subcutaneously (6.4 μg/kg) or intracerebroventricularly (ICV, 30–600 ng), depressed amphetamine-induced locomotor activity in rats by more than 50%. ICV injection of sCT, either three hours or immediately before intraperitoneal amphetamine (1–3 mg/kg), significantly reduced the amphetamine-induced activity. In the absence of amphetamine, sCT had no effect on locomotor activity during the first 100 minutes after treatment. These results show sCT can act centrally to modify drug-induced behavior and may be related to reports of calcitonin receptors and calcitonin-like peptides in the brain.

Calcitonin Amphetamine Locomotor activity

THE putative physiological role of calcitonin is to decrease the blood calcium level through an action on bone. However, high affinity calcitonin binding sites exist in other tissues, including the brain [14, 22, 32, 39]. Furthermore, immunoreactive calcitonin-like peptides have been found in the hypothalamus and pituitary of several mammals including the rat and man [9, 10, 12–15].

Analgesia [1, 16, 25, 34], changes in prolactin release [5, 18, 35], and the inhibition of both drug and stress-induced feeding [24, 28, 29] have been reported to follow direct administration of salmon CT (sCT) into the brain. The present studies demonstrate that sCT can depress amphetamine-induced locomotor activity and address the dose-effect and time course relationships of this action.

METHOD

Animals

Male, Sprague-Dawley rats, 5–10 weeks old and weighing 125–200 g were purchased from ARS (Madison, WI) or Charles River Breeding Labs (Wilmington, MA).

General Procedures

Animals were housed in wire cages at a temperature of 25±1°C with a 12 hr light cycle (lights on 0700–1900 hr). Locomotor activity was estimated using doughnut-shaped cages equipped with six equally spaced photocell detectors [20]. The cages were individually housed in soundproof boxes the interiors of which were dimly lighted by a 7.5 W incandescent bulb. Rats receiving sCT intracerebroventricularly (ICV) were first habituated to the locomotor apparatus for 2 hr prior to treatment. Individual animals were then removed, injected intraperitoneally (IP) with either amphetamine or vehicle and ICV with sCT or vehicle, and immediately returned to their respective locomotor units for an additional hour of post-treatment measurement. Other rats received subcutaneous (SC) injections of sCT or vehicle two hours before receiving amphetamine or vehicle IP. The number of photocell interruptions (counts) during habituation as well as after drug treatment were recorded automatically for each animal.

ICV injections were performed using the method of Popick [38]. Briefly, a semicircular band of metal fitted with an appropriately located guide cannula was placed over the head of the rat relative to preselected anatomical coordinates. A 50 μl Hamilton syringe fitted with a 27 ga., 0.5 in. syringe needle was thrust through the guide cannula, penetrating the calvaria and stopping within the lateral ventricle. Correct configuration of the band and location of the cannula were confirmed in test animals by injecting 10 μl of a dye solution. The rat was sacrificed after 10 minutes and saggital sections were inspected for evidence that the dye had penetrated to the ventricular system. This method of rapid ICV administration also produced results similar to those obtained with cannulas chronically implanted in the right lateral ventricle.

Drug Administration

Synthetic sCT (4700 MRC U/mg) was a gift from Armour Pharmaceutical Co. (Kankakee, IL) and was dissolved in 1 mM HCl:0.15 M NaCl for injection. For SC administration, sCT was injected in a volume of 1 ml/kg body weight. All doses of sCT administered ICV were delivered as a bolus

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injection in 10 μl. With both injection procedures, control animals received vehicle only. d-Amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile distilled water for IP injection (1.5 ml/kg).

Statistical Analysis

Data analyses were based on either total photocell counts for the one hour post-drug period, or the photocell counts for each of twelve consecutive five minute periods. Values are presented as mean±SEM and represent data grouped from several experiments.

When presented as total counts for one hour, analysis of variance (ANOVA) and Duncan's Multiple Range test were used within experiments to determine which treatment levels were different at the $p=0.05$ significance level. These data are graphed in two ways: (1) as photocell counts per hour, (2) as the proportion, (AC-VV)/(AV-VV), where AC is the mean number of photocell counts for rats administered amphetamine and sCT; AV, the mean activity of rats injected with amphetamine IP and vehicle ICV; and VV, the mean activity of rats receiving vehicle IP and ICV. This manipulation corrected for the interexperimental variation in the response to amphetamine and vehicle, permitting a more coherent presentation of data grouped from different experiments. However, statistical comparisons were not made between these experiments due to baseline variability.

The repeated measures ANOVA was considered for analyzing the data consisting of consecutive observations and was determined to be liberally biased. The test produces artificially large F values when markedly different levels of correlation exist between observations in adjacent time periods compared to more distant periods [27]. Consequently, a one factor multivariate ANOVA was used to test these results. This approach provided a more conservative estimate of the differences between treatment levels. The statistical model consisted of an independent variable, treatment, with three levels, and the twelve consecutive time periods (5 minute photocell counts) as dependent variables. Treatment effects were accepted as significant at the $p=0.05$ level.

Two computer programs, the General Linear Model procedure from SAS (release 79.5) and the MANOVA procedure from SPSS (version H, release 9.0) performed all computations.

RESULTS

Peripheral Injection of sCT

Figure 1 shows that SC injection of 6.4 μg/kg sCT depressed amphetamine-induced activity 40 to 50%, $F(3,16)=7.64$, $p=0.0022$. A thirty fold lower dose did not significantly change the locomotor response to amphetamine.

Administration of either 0.21 μg or 6.4 μg/kg of sCT, in the absence of amphetamine, had no effect on locomotor activity during the first 100 minutes after treatment. For example, in one experiment, rats treated with vehicle SC produced 661±111 photocell counts during a 100 minute period, while animals receiving sCT generated 709±154 counts with 0.21 μg/kg and 592±118 counts with 6.4 μg/kg.

ICV Administration of sCT

Figure 2 shows the effect of 10 to 600 ng sCT administered ICV. The 600 ng dose completely suppressed amphetamine-induced activity. A dose of sCT as low as 30 ng significantly decreased the amphetamine-induced activity 40 to 50%, $F(2,27)=3.89$, $p=0.033$.

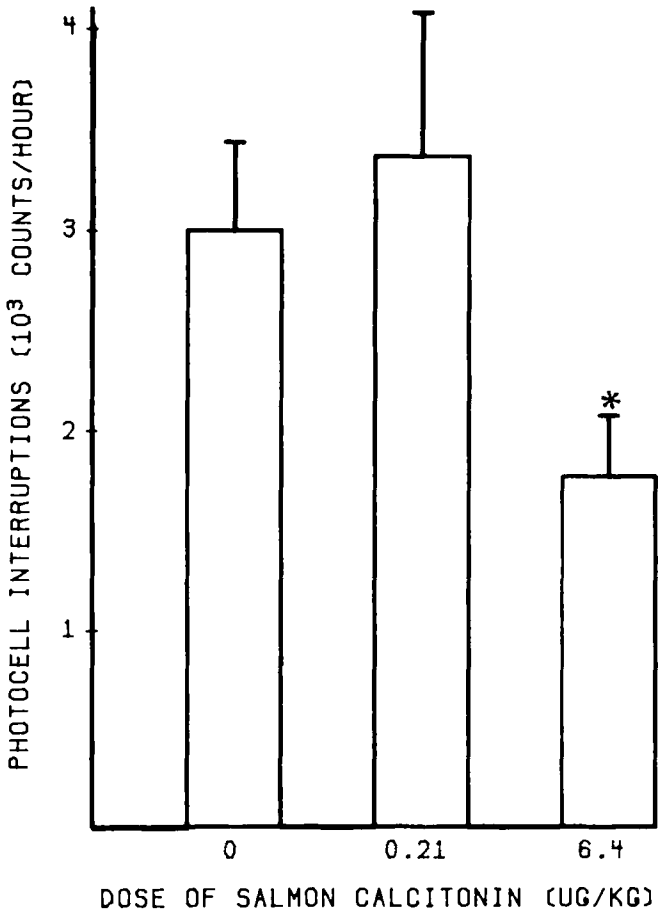

FIG. 1. The effect of sCT on amphetamine-induced activity. Rats were injected with amphetamine (1.5 mg/kg) IP, two hours following SC administration of 0, 0.21, or 6.4 μg/kg sCT. Bars represent mean±SEM (5–7 rats/group). The asterisk indicates a significant difference ($p<0.05$) from the zero dose using Duncan's Multiple Range Test.

Figure 3 illustrates how the locomotor response to 1 mg/kg amphetamine was altered by 300 ng sCT in one experiment. Although sCT treatment decreased the total number of photocell counts, the overall time course of the amphetamine response was not statistically different. This relationship was seen at each dose of amphetamine and sCT tested. However, with larger doses of amphetamine, the maximum number of photocell interruptions occurred 30 to 40 minutes after treatment with or without sCT administration.

In experiments where the dose of amphetamine was varied (Fig. 4), ICV administered sCT (300 ng) decreased the activity produced by 1, 1.5 and 3 mg/kg amphetamine, but not by 10 mg/kg. When sCT was administered without amphetamine, it produced no effect on locomotor activity.

Onset and Duration

The locomotor activity elicited by 1.5 mg/kg am-

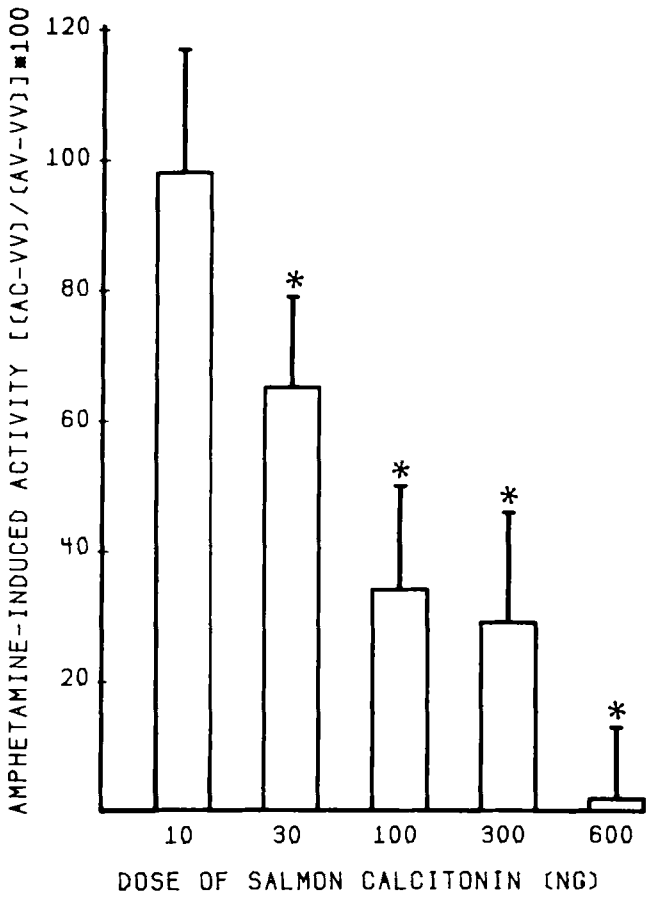

FIG. 2. The effect of 10 to 600 ng sCT, administered ICV, on amphetamine-induced activity. Rats received 1 mg/kg amphetamine IP immediately following ICV administration of sCT. Bars in this and subsequent figures represent the proportion, (AC-VV)/(AV-VV), where AC is the mean number of photocell interruptions for rats administered amphetamine and sCT; AV, the mean activity of rats injected with amphetamine IP and vehicle ICV; and VV, the mean activity of rats receiving vehicle IP and vehicle ICV. A one-way ANOVA was used within experiments (i.e., at each dose) to test the effect of amphetamine and CT treatments. Asterisks indicate doses at which the AV and AC means within experiments were significantly different at the $p=0.05$ confidence level using the Duncan Multiple Range Test. The photocell counts for vehicle control groups (VV) in each experiment were 799±72.4 (600 ng), 1121±288 (300 ng), 417±132 (100 ng), 431±57.4 (30 and 10 ng), N=95 (16–23 rats/bar).

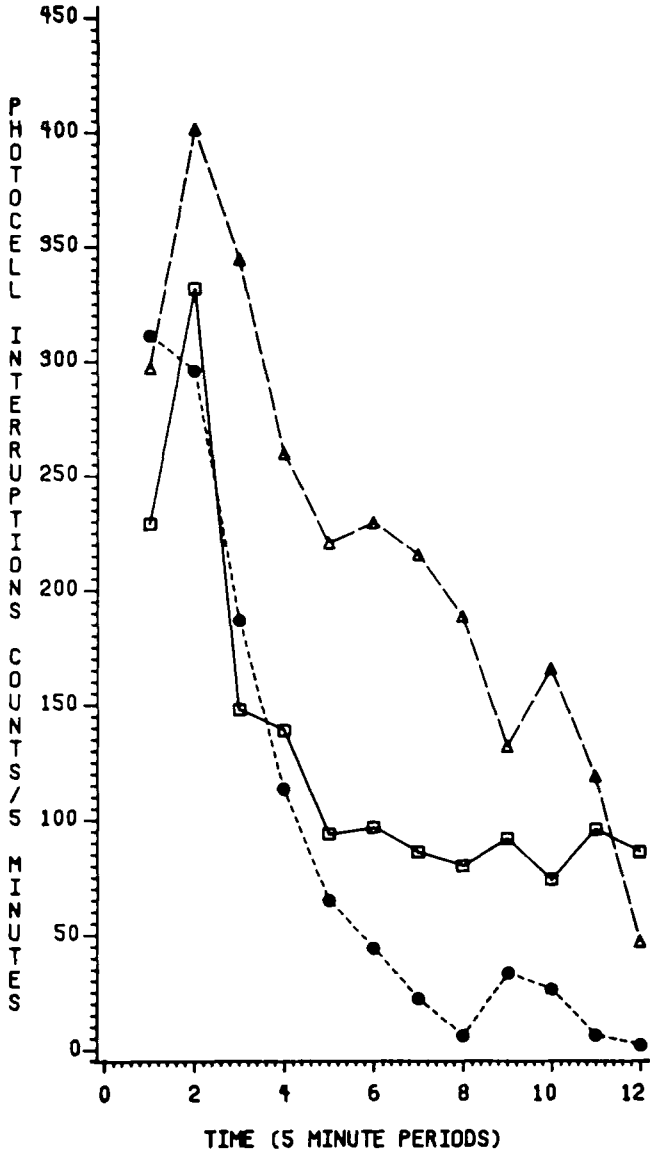

FIG. 3. A representative time course for the response to vehicle alone (···●···), or 1 mg/kg amphetamine IP and vehicle (--△--) or 300 ng sCT (-□-) ICV. Values are the mean number of photocell interruptions accumulated during consecutive five minute periods within each group (4–6 rats/group). Treatment effects were significant (multivariate, $F(2,13)=5.03$, $p<0.025$).

phetamine was depressed by 300 ng sCT (ICV) when sCT was administered either three hours or immediately before amphetamine (Fig. 5). Pretreatment with sCT 18 hours prior to administration of amphetamine increased activity. The duration of the sCT anti-amphetamine-like effect, therefore, appears to be at least three hours, since there was no diminution of the anti-amphetamine-like activity during this time period.

DISCUSSION

Our findings show sCT is a potent and efficacious depressor of amphetamine-induced locomotor activity. The results demonstrate that ICV administration of 30, 100, 300, or 600 ng sCT significantly decreased amphetamine-induced locomotor activity in a manner which appeared to be dose-related (Fig. 2).

The onset of the anti-amphetamine-like property was rapid, being clearly evident 5 to 10 minutes after ICV injection, and persisting for at least three hours (Figs. 3 and 5). Administration of sCT, 18 hr before treatment with amphetamine, actually enhanced the locomotor response. Although the relationship between these contrasting effects is unknown, the ability of sCT to also suppress food consumption [17, 40, 41] may be involved. Starvation can potentiate amphetamine-induced arousal and locomotor activity [3,19],

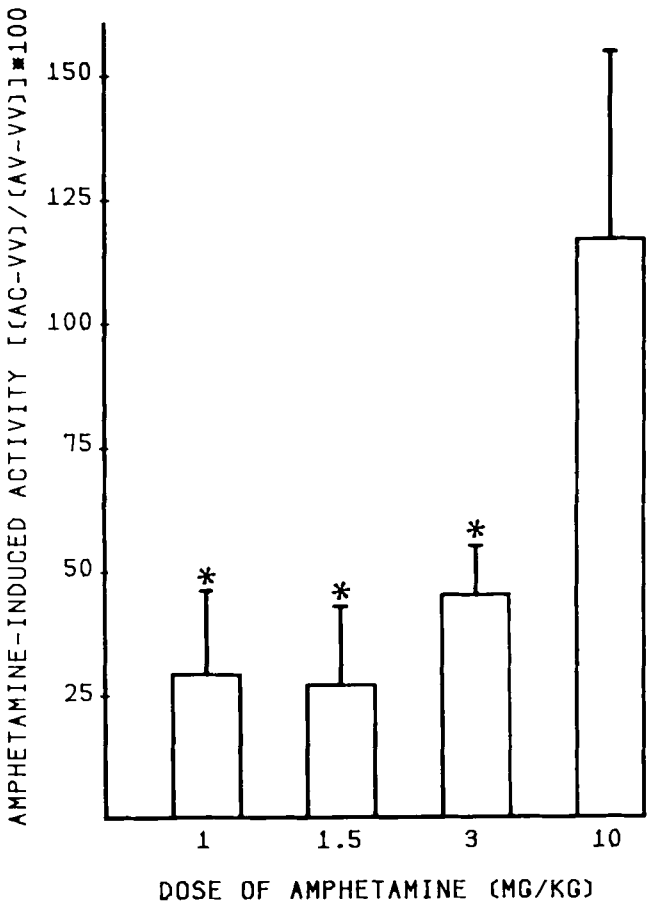

FIG. 4. The anti-amphetamine effect of sCT at doses of amphetamine from 1 to 10 mg/kg. Values are the proportion of amphetamine-induced activity remaining after treatment with sCT (300 ng) ICV (16–20 rats/bar). The photocell counts for vehicle control groups in these experiments were 1121±288 at 1 mg/kg, 837±213 for 1.5 and 3 mg/kg, and 355±62 for 10 mg/kg. *$p<0.05$.

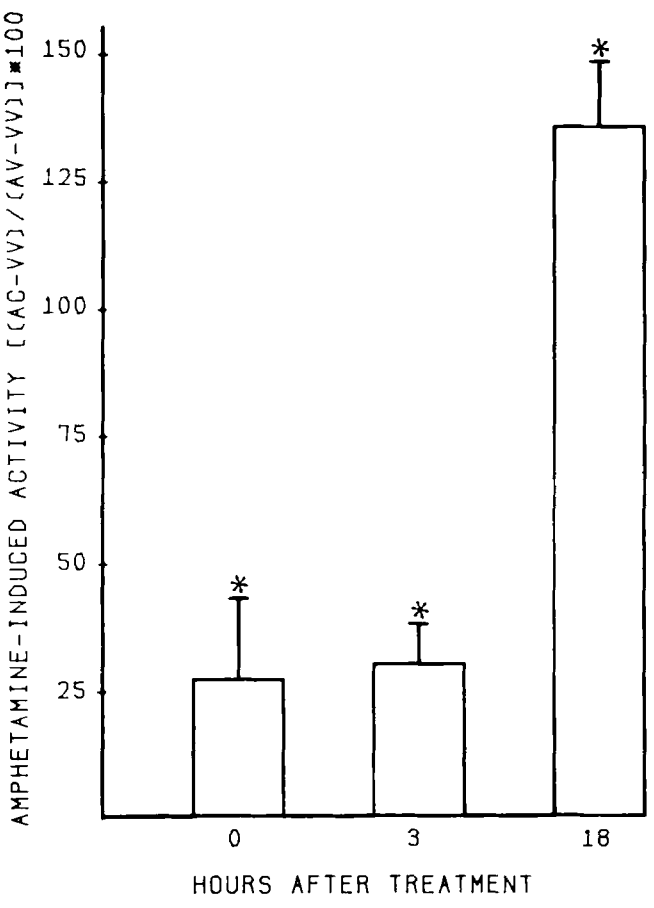

FIG. 5. Onset and duration of the anti-amphetamine effect produced by sCT. Amphetamine (1.5 mg/kg) was administered 0, 3, or 18 hours after ICV infusion of 300 ng sCT. The photocell counts for vehicle control groups were 837±213, 350±147, and 676±54, respectively. Asterisks indicate a significant difference ($p<0.05$ between AV and AC means within experiments, 11–19 rats/bar).

and this has been attributed to an alteration of serotonergic function due to the absence of dietary tryptophan [19]. The significance this mechanism might have in mediating the enhanced response to amphetamine 18 hr following treatment with sCT or whether sCT is more directly involved in this time-course phenomenon (e.g., neurotransmitter release) has not been determined.

Doses of amphetamine (1 to 3 mg/kg) which were sufficient to produce substantial increases in locomotor activity were blocked by sCT. The effect of a larger dose (10 mg/kg), which did not cause any further increases in the number of photocell counts, was not significantly altered by sCT treatment (Fig. 4). It seems likely that sCT does not block all behaviors induced by amphetamine (e.g., locomotion, gnawing, rearing). However, determination of precisely which behavioral activities are affected could not be accomplished using the automated photocell method of estimating locomotor activity which we used in this study.

In the absence of amphetamine, sCT administered SC or ICV produced no change in motor activity which was detectable by the photocell apparatus. This may indicate that physical disablement, following administration of sCT, does not underlie the effects observed.

Several experimental findings suggest sCT acts on the central nervous system to depress amphetamine-induced locomotor activity. Doses of sCT ineffective by SC administration were effective when given ICV (Figs. 1 and 2). Presumably, the higher dose apparently required by the SC route results in a small amount of sCT crossing the blood-brain barrier to reach sensitive areas of the central nervous system. Also, SC administration of 0.21 μg/kg, a dose substantially larger thant hat needed to lower blood calcium [31], did not suppress amphetamine-induced locomotion. This suggests that anti-amphetamine-like activity is probably not related to the classical skeletal actions of calcitonin. Other lines of evidence which suggest sCT may act centrally include the presence of binding sites for calcitonin in several areas of mammalian brain [14, 22, 32, 39] and the detection of an immunoreactive calcitonin-like material in the pituitary and hypothalamus of many species [9, 10, 12–15, 42]. It thus

seems likely that the ability of sCT to depress amphetamine-induced locomotor activity is mediated by the central nervous system.

Since the dopamine system is important for amphetamine-induced motor activity [11, 20, 21], the ability of sCT to attenuate the response to amphetamine suggests an interaction of sCT with dopaminergic systems. However, sCT does not appear to block motor activity produced by the direct-acting dopamine agonist, apomorphine [40]. These data suggest that sCT is affecting presynaptic dopaminergic mechanisms. However, it is unclear whether this occurs directly or through multi-synaptic events involving other chemical messengers, such as serotonin [2, 19, 20].

It is possible that sCT could depress the response to amphetamine by exerting changes in calcium metabolism. In man, parenteral administration of sCT has been reported to increase the calcium concentration in cerebrospinal fluid (CSF) [6]. *In vitro*, the uptake of ^{45}Ca by hypothalamic slices from rat brain has been reported to be decreased by sCT [28]. While neuronal function has been shown to be sensitive to physiological changes in calcium concentration [24] and while changes in CSF calcium are believed to be associated with the symptoms of some behavioral disorders in man (e.g., mania) [6–8], the present findings do not allow us to associate the anti-amphetamine-like activity of sCT with effects on calcium.

How closely the anti-amphetamine-like property of sCT is related to other central nervous system effects purported to follow the administration of calcitonin is unclear. Analgesic effects have been reported in rabbits [1,34] and ICV sCT has been found to alter prolactin release in the rat [18,35]. Morley *et al.*, found that doses of sCT even lower than those required to depress amphetamine-induced locomotion (i.e., 0.45 ng, ICV) can lower gastric acid production and depress tail-pinch induced eating in rats [24,29]. In man, sCT reportedly produces analgesia [16,25], substantially reduces manic symptoms in psychiatric patients [7,8], and suppresses the stimulated release of prolactin [5], growth hormone [26,37], thyroid-stimulating hormone [23], and luteinizing hormone [23]. All studies to date agree in showing that calcitonin is a potent agent when given centrally. Whether this is a pharmacological artifact or whether sCT mimics the action of an endogenous ligand remains to be determined.

Our results show sCT is an effective suppressor of amphetamine-induced activity. This finding, in concert with reports of immunoreactive calcitonin in the pituitary, specific binding sites for calcitonin in brain, and a wide range of behavioral effects following ICV administration of calcitonin, supports the concept of a calcitonin-like molecule acting as a chemical messenger (neurotransmitter or modulator) in the central nervous system.

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REFERENCES

1. Braga, P. C., S. Ferri, A. Santagostino, V. R. Olgiati and A. Pecile. Lack of opiate receptor involvement in centrally induced calcitonin analgesia. *Life Sci* **22:** 971–978, 1978.
2. Breese, G. R., B. R. Cooper and R. A. Mueller. Evidence for involvement of 5-hydroxytryptamine in the actions of amphetamine. *Br J Pharmacol* **52:** 307–314, 1974.
3. Campbell, B. A. and H. C. Fibiger. Potentiation of amphetamine-induced arousal by starvation. *Nature* **233:** 424–425, 1971.
4. Cantalamessa, L., A. Catania, E. Reschini and M. Peracchi. Inhibitory effect of calcitonin on growth hormone and insulin secretion in man. *Metabolism* **27:** 987–992, 1978.
5. Carman, J. S. and R. J. Wyatt. Reduction of serum prolactin after subcutaneous salmon calcitonin. *Lancet* **77:** 1267, 1977.
6. Carman, J. S., E. Crews, A. Bancroft, E. Wyatt, B. Cooper, J. Ledbetter, J. Daniels, S. Monson and W. Bond. Calcium and calcium-regulating hormones in the biphasic periodic psychoses. *J Operational Psych* **11:** 5–17, 1980.
7. Carman, J. S. and R. J. Wyatt. Use of calcitonin in psychotic agitation or mania. *Arch Gen Psychiatry* **36:** 72–75, 1979.
8. Carman, J. S. and R. J. Wyatt. Calcium: Bivalent cation in the bivalent psychoses. *Biol Psych* **14:** 295–336, 1979.
9. Catherwood, B. D. and L. J. Deftos. Presence by radioimmunoassay of a calcitonin-like substance in porcine pituitary glands. *Endocrinology* **106:** 1886–1891, 1980.
10. Cooper, C. W., T.-C. Peng, J. F. Obie and S. C. Garner. Calcitonin-like immunoreactivity in rat and human pituitary glands: histochemical, *in vitro*, and *in vivo* studies. *Endocrinology* **107:** 98–107, 1980.
11. Creese, I. and S. D. Iversen. The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Res* **83:** 419–436, 1975.
12. Deftos, L. J., D. Burton, H. G. Bone, B. D. Catherwood, J. G. Parthemore, R. Y. Moore, S. Minick and R. Guillemin. Immunoreactive calcitonin in the intermediate lobe of the pituitary gland. *Life Sci* **23:** 743–748, 1978.
13. Deftos, L. J., D. Burton, B. D. Catherwood, H. G. Bone, J. G. Parthemore, R. Guillemin, W. Watkins and R. Y. Moore. Demonstration by immunoperoxidase histochemistry of calcitonin in the anterior lobe of the rat pituitary. *J Clin Endocrinol Metab* **47:** 457–460, 1978.
14. Fischer, J. A., P. H. Tobler, M. Kaufman, W. Born, H. Henke, P. E. Cooper, S. M. Sagar and J. B. Martin. Calcitonin: regional distribution of the hormone and its binding sites in the human brain and pituitary. *Proc Natl Acad Sci USA* **78:** 7801–7805, 1981.
15. Flynn, J. J., D. L. Margules and C. W. Cooper. Presence of immunoreactive calcitonin in the hypothalamus and pituitary lobes of rats. *Brain Res Bull* **6:** 547–549, 1981.
16. Fraioli, F., A. Fabbri, L. Gnessi, C. Moretti, C. Santoro and M. Felici. Subarachnoid injection of salmon calcitonin induces analgesia in man. *Eur J Pharmacol* **78:** 381–382, 1982.
17. Freed, W. J., M. J. Perlow and R. J. Wyatt. Calcitonin: inhibitory effect of eating in rats. *Science* **206:** 850–851, 1979.
18. Fujita, T., K. Chihara, J. Iwasaki, Y. Iwasaki, H. Abe and N. Minamitani. Calcitonin, parathyroid hormone, and prolactin secretion. In: *Hormonal Control of Calcium Metabolism*, edited by D. V. Cohn, R. V. Talmage and J. L. Mathews. Amsterdam: Excerpta Medica, 1981, pp. 287–292.
19. Hollister, A. S., G. R. Breese, C. M. Kuhn, B. R. Cooper and S. M. Schanberg. An inhibitory role for brain serotonin-containing systems in the locomotor effects of d-amphetamine. *J Pharmacol Exp Ther* **198:** 12–22, 1976.
20. Hollister, A. S., G. R. Breese and B. R. Cooper. Comparison of tyrosine hydroxylase and dopamine-β-hydroxylase inhibition with the effects of various 6-OHDA treatments on d-amphetamine induced motor activity. *Psychopharmacologia* **36:** 1–16, 1974.
21. Hollister, A. S., B. R. Cooper and G. R. Breese. Evidence for a role of dopamine in amphetamine-induced motor activity and stereotypy. *Pharmacologist* **15:** 254, 1973.

22. Koida, M., H. Nakamuta, S. Furukawa and R. C. Orlowski. Abundance and location of ^{125}I-salmon calcitonin binding site in rat brain. *Jpn J Pharmacol* **30:** 575–577, 1980.
23. Leicht, E., G. Biro and K. F. Weines. Inhibition of releasing-hormone-induced secretion of TSH and LH by calcitonin. *Horm Metab Res* **6:** 410–414, 1974.
24. Levine, A. S. and J. E. Morley. Reduction of feeding in rats by calcitonin. *Brain Res* **222:** 187–191, 1981.
25. Leyland, M. J. and A. Hindley. Calcitonin in the management of malignant bone pain. *Clin Sci* **62:** 43, 1982.
26. Lunetta, M., E. Infantone, D. Spanti and L. Mughni. Effects of synthetic salmon calcitonin administration on gastrin, immunoreactive insulin, and growth hormone release after protein meal in uremic patients. *J Endocrinol Invest* **4:** 185–188, 1981.
27. McCall, R. B. and M. I. Appelbaum. Bias in the analysis of repeated measures designs: Some alternative approaches. *Child Dev* **44:** 401–415, 1973.
28. Morley, J. E. and A. S. Levine. Reduction of feeding in rats by calcitonin. *Brain Res* **222:** 187–191, 1981.
29. Morley, J. E., A. S. Levine and J. Kneip. Muscimol-induced feeding: a model to study the hypothalamic regulation of appetite. *Life Sci* **29:** 1213–1218, 1981.
30. Morley, J. E., A. S. Levine and S. E. Silvas. Intraventricular calcitonin inhibits gastric acid secretion. *Science* **214:** 671–673, 1981.
31. Munson, P. L. Physiology and pharmacology of thyrocalcitonin. In: *Handbook of Physiology*, edited by G. D. Aurbach. Washington, DC: Am Physiol Society 1976, pp. 443–464.
32. Nakamuta, H., S. Furukawa and M. Koida. Specific binding of ^{125}I-salmon calcitonin to rat brain: regional variation and calcitonin specificity. *Jpn J Pharmacol* **31:** 53–60, 1981.
33. Nakhala, A. M. and A. P. N. Majumdar. Calcitonin-mediated changes in plasma tryptophan and brain 5-hydroxytryptamine and acetylcholinesterase activity in rats. *Biochem J* **170:** 445–448, 1978.
34. Pecile, A., S. Ferri, P. C. Braga and V. R. Olgiati. Effects of intracerebroventricular calcitonin in the conscious rabbit. *Experientia* **31:** 332–333, 1975.
35. Pecile, A., V. R. Olgiati, G. Luisetto, F. Guidobono, C. Netti and D. Ziliotto. Calcitonin and control of prolactin secretion. In: *Calcitonin 1980*, edited by A. Pecile. Amsterdam: Excerpta Medica, 1981, pp. 183–198.
36. Perlow, M. J., W. J. Freed, J. S. Carman and R. J. Wyatt. Calcitonin reduces feeding in man, monkey and rat. *Pharmacol Biochem Behav* **12:** 609–612, 1980.
37. Petraliot, A., M. Lunetta, A. Liuszzo, R. A. Mangiafico and C. E. Fiore. Effects of salmon calcitonin on insulin-induced growth hormone release in man. *Horm Metab Res* **11:** 641–642, 1979.
38. Popick, F. E. Application of a new intraventricular injection technique in rat brain norepinephrine studies. *Life Sci* **18:** 197–204, 1976.
39. Rizzo, A. and D. Goltzman. Calcitonin receptors in the central nervous system of the rat. *Endocrinology* **108:** 1672–1677, 1981.
40. Twery, M. J., C. W. Cooper and R. B. Mailman. Effects of calcitonin on the central nervous system. *Abstr Soc Neurosci* **8:** 369, 1982.
41. Twery, M. J., J. F. Obie and C. W. Cooper. Ability of calcitonin to alter food and water consumption in the rat. *Peptides* **3:** 749–755, 1982.
42. Yui, R., Y. Yamada, R. Kayamori and T. Fujita. Calcitonin-immunoreactive neurons in the brain of the Bullfrog, *Rana catesbiana* with special reference to their liquor-contraction and neurosurgery nature. An immunochemical and immunohistochemical study. *Biomed Res* **2:** 208–216, 1981.