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Regional Localization of the Antagonism of Amphetamine-Induced Hyperactivity by Intracerebral Calcitonin Injections

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DE BEAUREPAIRE, R. AND W. J. FREED. *Regional localization of the antagonism of amphetamine-induced hyperactivity by intracerebral calcitonin injections.* PHARMACOL BIOCHEM BEHAV 27(1) 183–186, 1987.—Calcitonin receptors are found in the brain, and intracerebral infusions of calcitonin can produce behavioral effects. Among these behavioral effects are decreases in food intake and decreases in amphetamine-induced locomotor activity. In previous experiments we found that decreases in food intake were induced by local administration of calcitonin into several hypothalamic sites and into the nucleus accumbens. In the present experiment calcitonin decreased locomotor activity when locally injected into the same sites where it decreases food intake. The areas where calcitonin is most effective in decreasing locomotor activity are located in the hypothalamus and nucleus accumbens, suggesting that these areas are the major sites of action of calcitonin in inhibiting amphetamine-induced locomotor activity.

Calcitonin Amphetamine Intracerebral injections Locomotor activity

MANY neuropeptides increase or decrease locomotor activity when injected intracerebrally [3, 12–14, 16]. Calcitonin, a tridecapeptide secreted by the C cells of the thyroid gland, can also decrease amphetamine-induced hyperactivity when infused into the cerebral ventricles [4,17]. In previous experiments we found that calcitonin could decrease food intake when infused into the lateral cerebral ventricle [6,7] and that anorexia was also induced by local injections into several sites in the hypothalamus, including the paraventricular nucleus of the hypothalamus (PVH), the perifornical area (PFA) and areas on the floor of the hypothalamus [2]. Infusions of calcitonin into the nucleus accumbens septi (NAS) also decreased food intake [2]. Since calcitonin infusions in the cerebral ventricles decrease amphetamine-induced locomotor activity [4,17] we have studied amphetamine-induced locomotor activity after infusions of calcitonin into several hypothalamic and extra hypothalamic sites, to explore the relationship between calcitonin-induced alterations in locomotor activity and feeding behavior.

METHOD

Male Sprague Dawley rats weighing 250 to 400 grams (8 to 12 weeks old) were used. They were housed in a room maintained on a 12 hour dark-light cycle and temperature controlled at 20 degrees C. Animals were anesthetized with Chloropent (Fort Dodge Laboratories) and bilaterally implanted with chronic stainless steel guide cannulae (24 gauge, 10 mm long) terminating 2 mm above nine different sites using the following coordinates according to the Pellegrino, Pellegrino and Cushman atlas [11]: the PVH (ant. 6.6, lat. 0.6, deep 8.0) (n=7), the PFA (n=7) (ant. 6.6, lat. 1.0, deep 8.0), the stria medullaris (ant. 6.6, lat. 1.3, deep 7.0) (n=3), the lateral hypothalamus (ant. 6.6, lat. 1.5, deep 8.7) (n=4), the floor of the hypothalamus over its anterior, suprachiasmatic, part (ant. 7.4, lat. 1.2, deep 9.8) (n=6) and the floor of the hypothalamus over its posterior part (ant. 6.2, lat. 1.0, deep 9.8) (n=3), the nucleus accumbens over its medial part (ant. 9.0, lat. 1.0, deep 7.0) (n=6), the striatum (ant. 9.0, lat. 1.5, deep 5.0) (n=4) and an area in the posterior hypothalamus situated between the internal parts of the zona incerta, the medial lemniscus and the mammilothalmic tract, and the external part of the nucleus reuniens (zona incerta internal) (ant. 5.6, lat. 1.7, deep 8.2) (n=7). The head was oriented in the Pellegrino *et al.* [11] position, with the bite bar 5.0 mm above the interauricular line. The sites of infusion are shown in Fig. 1.

Testing was initiated two weeks after surgery. Each animal was tested twice on the same side using the same infusion cannula (except if the guide cannula used for the first infusion became obstructed, the contralateral side was used for the second infusion). For one test each animal received an infusion of calcitonin, and for the other the animals received vehicle according to a counterbalanced schedule.

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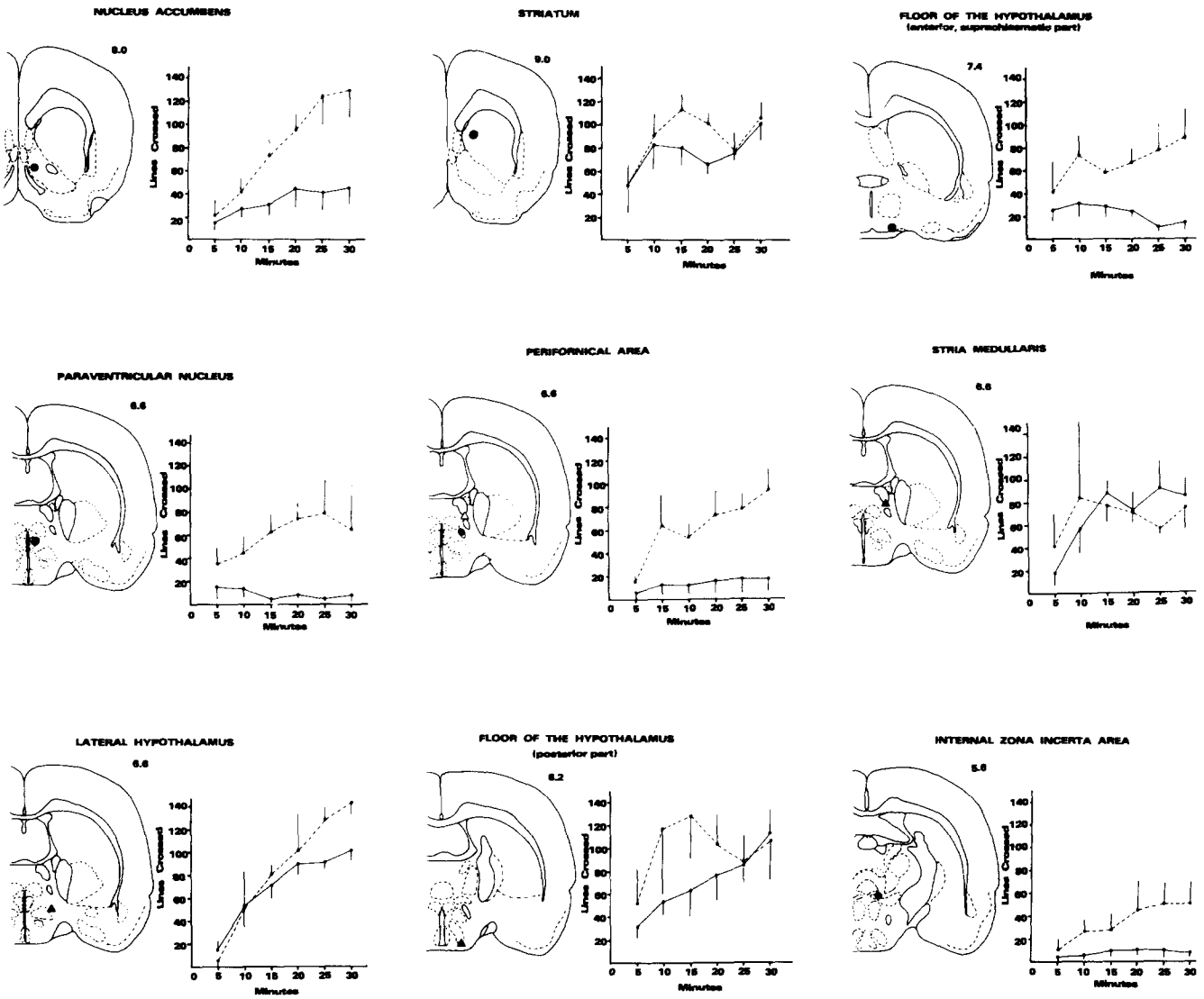

FIG. 1. Effects of calcitonin on locomotor activity for each of the nine infusion sites. For each infusion site, the point of infusion is shown on the left. Numbers indicate frontal planes according to the Pellegrino *et al.* [11] atlas. The graphs show the effect of calcitonin at each site. Numbers of lines crossed (mean±SEM) are shown for each group of animals after infusion of vehicle (dotted lines) or calcitonin (solid lines) as a function of time. Some standard error bars have been omitted for clarity.

Synthetic salmon calcitonin (courtesy of Armour Pharmaceutical Company) was dissolved in 1% gelatin in normal saline and infused in an amount of 50 ng in 0.5 μl. Control infusions consisted of 0.5 μl of vehicle. Solutions were infused through a 33 gauge cannula connected to a Hamilton syringe over 60 seconds using a syringe pump. The infusion cannula was removed 30 seconds after the end of the infusion.

For 30 minutes before infusion each animal was habituated to an open field. Thirty minutes after infusion each animal was given 1.5 mg/kg of d-amphetamine IP and then tested in the open field for 30 minutes. The open field was one meter square with lines on the floor spaced every 0.3 m. The number of lines crossed was recorded every minute by the observer. The two tests (calcitonin and saline) were separated by a one week interval, and tests were always conducted at the same hour of the day.

The day after the second infusion the animals were sacrificed (perfused with 10% buffered formalin after an overdose of chloral hydrate), the brain was removed, fixed in 10% formalin solution, frozen, sectioned at 80 μm, and the point of injection was verified. No dye was injected, as the cannula tract was always clearly visible.

RESULTS

Calcitonin produced substantial decreases in amphetamine-stimulated locomotor activity when infused into the PVH, the PFA, the NAS, the floor of the hypothalamus above the optic chiasma and the area surrounding the internal part of the zone incerta (Fig. 1). Calcitonin in these areas reduced locomotor activity by 50% or more as compared to the saline infusions (Table 1). The most effective sites were the PVH where an 84% inhibition of

TABLE 1

DECREASES IN AMPHETAMINE-INDUCED LOCOMOTOR STIMULATION AFTER LOCAL INJECTIONS OF CALCITONIN IN VARIOUS BRAIN REGIONS

Brain Region	Percentage Change in Activity (Mean ± SEM)*	p†
Stria Medullaris	+7.2 ± 13%	N.S.‡
Corpus Striatum	−8.6 ± 15%	N.S.
Floor of Hypothalamus, Posterior	−10 ± 26%	N.S.
Lateral Hypothalamus	−16 ± 3.1%	$p<0.05$ $t(2)=6.36$
Nucleus Accumbens	−59 ± 9.8%	$p<0.01$ $t(4)=6.69$
Zona Incerta, Internal	−63 ± 14%	$p<0.02$ $t(5)=1.76$
Floor of Hypothalamus, Anterior	−65 ± 2.7%	$p<0.01$ $t(4)=7.33$
Perifornical Area	−81 ± 8.9%	$p<0.001$ $t(5)=8.17$
Paraventricular Nucleus	−84 ± 4.5%	$p<0.01$ $t(5)=4.51$

*Negative numbers indicate decreases.
†Two-tailed t-tests for related samples.
‡N.S.=Not significant.

activity was produced, followed by the PFA (81%). In three sites, the corpus striatum, the stria medullaris, and the posterior part of the floor of the hypothalamus there was no inhibition of activity (Table 1). A slight (16%) but significant decrease in activity was induced by infusions in the lateral hypothalamus. Although the lateral hypothalamus is close to the PFA, infusions into the stria medullaris, which is also very close to the PFA, did not significantly decrease locomotor activity. This diffusion of the injected calcitonin to other structures was probably minimal.

DISCUSSION

Calcitonin decreased amphetamine-induced hyperactivity when infused into several hypothalamic and extrahypothalamic sites, where calcitonin also decreases food intake. In previous experiments [2], calcitonin has been found to be most potent in decreasing food intake when infused into the PVH and the PFA, followed by the area over the optic tract and to a lesser extent, the NAS. When infused into the lateral hypothalamus calcitonin slightly decreased amphetamine-induced locomotor activity, but did not produce anorexia. The areas of the lateral hypothalamus which were tested for anorexia were, however, more anterior, in the median forebrain bundle. In these previous experiments we did not test for anorectic effects of calcitonin when infused into the striatum, the stria medullaris, the posterior part of the floor of the hypothalamus, or the internal part of the zona incerta. The lack of an effect of calcitonin in the stria medullaris, striatum, and posterior floor of the hypothalamus, and the minor effect in the lateral hypothalamus must be considered to be tentative findings because of the small numbers of animals that were tested.

Although relatively small dosages of calcitonin were used in the present study, any diffusion into the cerebral ventricles could have resulted in a much wider diffusion. All of the sites injected, with the exception of the stria medullaris, were more than 2 mm below the guide cannula, so that no ventricles were crossed between the point of injection and the tip of the guide cannula when the injection cannula was removed after the infusion. The only site where the cannula tract could have crossed the ventricle was the stria medullaris, but no effect was observed in that area. The most rostral tip of the lateral ventricle also comes very close to the site of the cannula tip for the nucleus accumbens site that was used in the present experiment [15]. It is therefore possible that the effects observed in the nucleus accumbens were due, in part, to diffusion into the ventricular system. Otherwise, the effects of calcitonin do not seem to be related to diffusion into the cerebrospinal fluid.

Therefore the areas from which changes in locomotor activity and feeding behavior can be obtained are anatomically similar. Some structures in the hypothalamus may have a prominent role in the regulation of locomotor activity. The role of the paraventricular-perifornical area appears to be the most pronounced. For example, in other experiments calcium channel inhibitors were found to produce pronounced hyperactivity when infused into the PVH [1]. It is also possible that the effects observed in the present study are related to some of the other reported effects of intracerebral calcitonin, such as the production of dyskinetic movements [4] and analgesia [10].

The mechanisms by which this decrease in locomotor activity is produced are unknown. It would not appear that the dopamine system is primarily involved because calcitonin did not have behavioral activity in the striatum, which contains high concentrations of dopamine. Dopamine-containing cells have been described in the PVH, but not in the PFA or over the optic tract [8]. Dopamine is thought to have an important role in the regulation of locomotor activity in the NAS. The ability of calcitonin to decrease locomotor activity following infusion into the NAS has previously been described by Everist and his colleagues [5], who also found calcitonin receptors in the NAS. Calcitonin receptors have also been visualized in all areas of the hypothalamus where we detected an effect of calcitonin on locomotor activity and feeding behavior [9]. In conclusion, significant decreases in amphetamine-induced locomotor activity can be obtained from localized intracerebral infusions of synthetic calcitonin, particularly into the perifornical area, the paraventricular nucleus, the floor of the hypothalamus, the zona incerta, and the nucleus accumbens.

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