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Anatomical Mapping of the Rat Hypothalamus for Calcitonin-Induced Anorexia

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DE BEAUREPAIRE, R. AND W. J. FREED. *Anatomical mapping of the rat hypothalamus for calcitonin-induced anorexia.* PHARMACOL BIOCHEM BEHAV 27(1) 177–182, 1987.—The primary physiological function of calcitonin, a peptide hormone secreted by the thyroid gland, is to modulate plasma calcium concentrations. Calcitonin also has several effects on the central nervous system including an inhibition of feeding behavior. In the present study synthetic salmon calcitonin (15 ng in 0.3 microliter) was found to produce a marked suppression of eating when infused in several hypothalamic areas. The greatest inhibition was produced by infusions into the paraventricular nucleus of the hypothalamus, the perifornical area and several areas on the floor of the hypothalamus. A less marked inhibition of eating was produced by infusions in the nucleus accumbens. Infusions in the olfactory tubercule, the ventrolateral hypothalamus, the medial forebrain bundle and the posterior nucleus of the hypothalamus had no effect. It is concluded that the anorectic effects of calcitonin on the central nervous system are mediated by several hypothalamic and extrahypothalamic sites.

Calcitonin Hypothalamus Eating behavior Obesity Anorexia Peptides
Nucleus accumbens septi Paraventricular hypothalamus Perifornical area Supraoptic area
Intracerebral infusions

THE primary physiological function of calcitonin, a peptide hormone secreted by the C-cells of the thyroid gland, is probably to modulate plasma calcium concentrations through its actions on bone and kidney [2, 7, 15]. Calcitonin has also been found to inhibit eating [11, 12, 22, 32, 43, 44] and elevated calcitonin concentrations have been found in pituitary and thyroid of genetically obese rats [5,24]. In addition, calcitonin has been reported to influence gastric acid secretion [25], pain perception [30], hormone secretion [3, 4, 21, 28, 33], and behavioral activity [4, 27, 41], and to produce dyskinetic movements [42].

Synthetic salmon calcitonin is one of the most potent anorectic agents known: dosages of less than 10 micrograms per kilogram body weight, administered orally or intraperitoneally, markedly inhibit eating for 24 hours or more in rats and monkeys [11, 12, 22, 32, 43, 44]. Calcitonin also inhibits eating when administered into the rat cerebral ventricles, in dosages on the order of 100-fold smaller than those required to inhibit eating when administered peripherally [11, 12, 22]. This suggests that the eating suppressant effect of calcitonin is mediated by the central nervous system, and raises the possibility that calcitonin serves as an endogenous messenger which acts on the brain to inhibit eating behavior. The specific sites of action of calcitonin within the brain are unknown. Although calcitonin binding sites are found in the hypothalamus of the rat [9, 14, 26, 29, 34], calcitonin is apparently not produced in the rat brain [1,35]. It is possible, however, that calcitonin produced in the periphery or by the pituitary [6, 8, 10] enters the brain in the hypothalamus, where it inhibits eating through an effect on hypothalamic neurons. We have therefore determined whether small amounts of calcitonin can inhibit eating when locally injected into various hypothalamic regions.

METHOD

Experiments were performed on male Sprague Dawley rats (Zivic-Miller Laboratories, Inc.) housed in individual clear plastic cages with wire mesh floors in a room maintained on 12 hours dark/light cycle (light 8 a.m–8 p.m.) and temperature controlled (20 degrees C). When the animals were 10 to 15 weeks old, weighing 300 to 400 grams, they were anesthetized with Chloropent (Fort Dodge Laboratories) and bilaterally implanted with chronic 10 mm long 24 gauge steel guide cannulae terminating 2 mm above several hypothalamic sites. All of the injection sites were 2 mm below the end of the guide cannula and always more than 2 mm below the ventricle, so that the injection cannula did not cross cerebrospinal fluid when it was removed after the injection. Injection sites were the paraventricular nucleus (PVH, n=28), the perifornical area (n=15), the anterior hypothalamic area (n=8), the floor of hypothalamus (n=6), the ventrolateral hypothalamus (n=10), the ventromedial nucleus of the hypothalamus (n=3), the posterior hypotha-

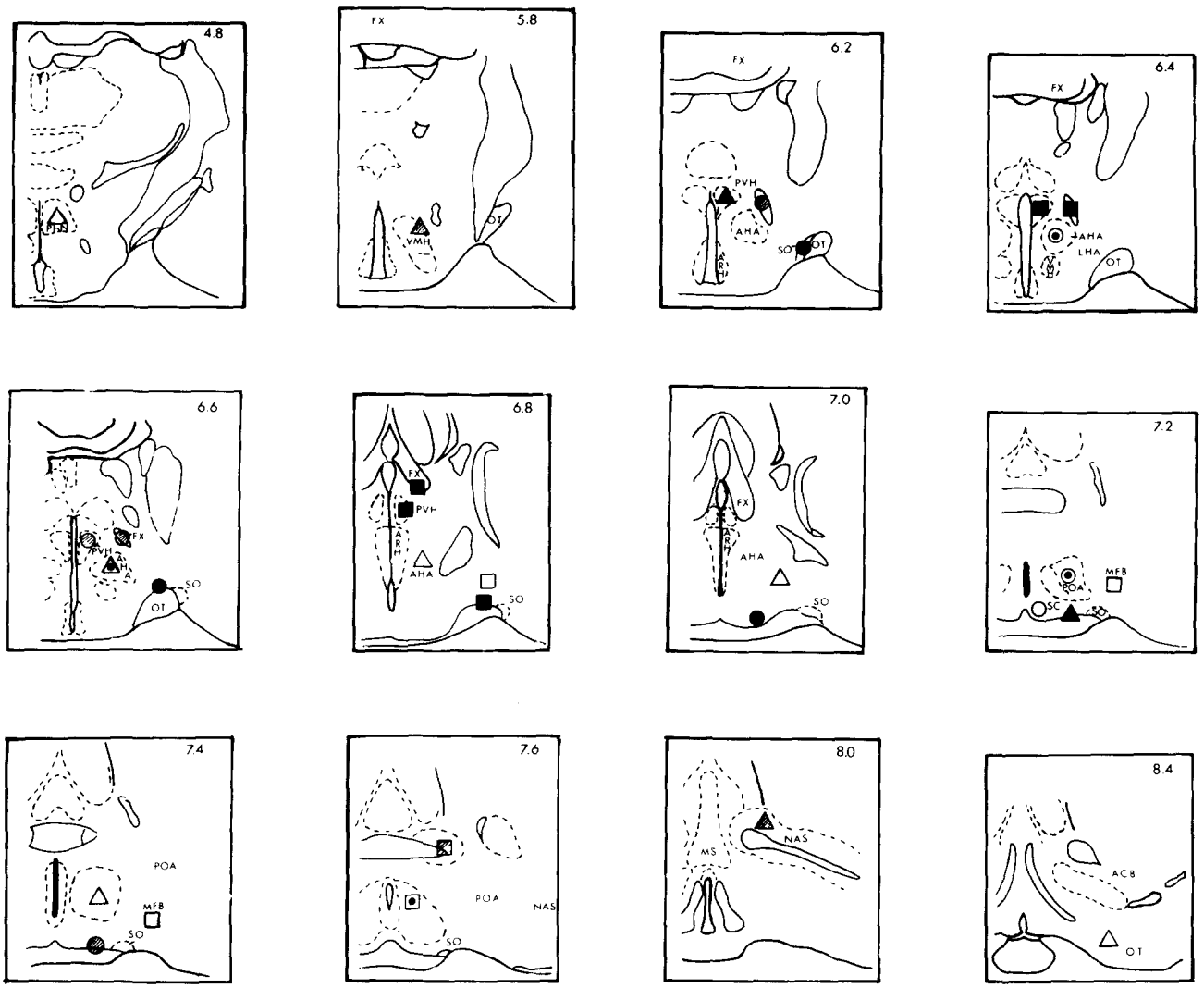

FIG. 1. Approximate locations of sites of calcitonin infusions according to the atlas of Pellegrino, Pellegrino and Cushman [31]. The number of animals infused at each site is indicated by the *shape* of the symbol, and the degree of effect is indicated by the *content* of the symbol. Open symbols indicate an inhibition of eating of less than 15%. Symbols containing a dot indicate an inhibition of eating of 16–50%. Shaded symbols indicate a 51–80% inhibition of eating. Filled symbols indicate an inhibition of eating of greater than 80%. Circles indicate that 1 to 2 animals were tested at the site indicated, triangles indicate that 3 to 4 animals were tested at the site indicated, and squares indicate that 5 to 9 animals were tested at the site indicated. Numbers indicate Pellegrino *et al.* frontal planes.

lamic nucleus (n=4), the supraoptic area, primarily the area adjacent to the internal part of the supraoptic nucleus, above the optic chiasm (n=7), the retrochiasmatic supraoptic nucleus (n=2), the preoptic area (n=11), the median forebrain bundle (n=13), the nucleus accumbens (n=13), the suprachiasmatic nucleus (n=2), and the olfactory tract (n=3). Two weeks after surgery the animals were trained over 10 days to a 24 hour feeding schedule so that they received a moist mash (1 kg rat chow to 1 liter of water) for 45 minutes per day. The mash was placed into individual heavy glass dishes, and the dishes were placed in the cages at 8:30 p.m. every day and removed at 9:15 p.m. so that the animals were eating during darkness. The dishes of food were weighed every day before and after the meal during adaptation, testing and post-testing. Water was always provided continuously ad lib. The animals maintained or increased their body weight on this feeding regimen.

Synthetic salmon calcitonin (courtesy of Armour Pharmaceutical Company) dissolved in 1% gelatin in normal saline, was infused through a 33 gauge cannula connected to a Hamilton syringe pushed by a syringe pump in an amount of 15 ng in 0.3 μl delivered in 30 seconds. The food was given 45 minutes after infusion and the intake during the following 45 minutes was recorded. The percentage decrease in eating as compared to the previous day was used as data. When an infusion in one side had no effect another infusion was performed a week later on the other side. Food intake was also measured for a few days after the infusion before the animal was sacrificed. At the conclusion of the testing the animals were given a 0.3 μl injection of 10% Evans Blue through the same cannulas used for the calcitonin injection, and perfused with 10% buffered formalin following an overdose of chloral hydrate. Brains were frozen and sectioned at 80 μm and the point of injection of the calcitonin was verified. In 10 animals

TABLE 1

LOCALIZATION OF THE ANORECTIC EFFECT OF CALCITONIN

Brain Region	Food Eaten Before Infusion (Means ± SEM)	Percentage Decrease in Eating After Calcitonin Infusions*	p†	t
		Regions Where Eating Was Inhibited		
Paraventricular Nucleus	33 ± 1.8 g	89 ± 2.1% (n=18)	<0.001	15.07
Perifornical Area	32 ± 1.8 g	87 ± 2.6% (n=15)	<0.001	14.42
Supraoptic Area	35 ± 2.9 g	89 ± 5.4% (n=7)	<0.001	10.69
Floor of the Hypothalamus	34 ± 2.9 g	80 ± 5.6% (n=6)	<0.001	6.97
Supraoptic Retrochiasmatic Nucleus	35 ± 4.0 g	95 ± 5.0% (n=2)	N.S.	6.09
Nucleus Accumbens	34 ± 1.5 g	73 ± 3.3% (n=13)	<0.001	14.31
Ventromedial Nucleus	36 ± 4.6 g	69 ± 4.0% (n=3)	<0.01	10.26
Preoptic Area	35 ± 2.3 g	16 ± 8.0% (n=11)	<0.05	2.23
		Regions Where Eating Was Not Inhibited		
Anterior Hypothalamic Area	34 ± 2.0 g	12.5 ± 8.1% (n=8)	N.S.	1.67
Ventral Lateral Hypothalamus	34 ± 2.3 g	3.6 ± 2.4% (n=10)	N.S.	1.28
Suprachiasmatic Nucleus	35 ± 6.0 g	3.5 ± 3.5% (n=2)	N.S.	0.50
Olfactory Tract	35 ± 1.2 g	1 ± 5.5% (n=3)	N.S.	0.33
Median Forebrain Bundle	33 ± 1.9 g	−2.2 ± 2.4% (n=13)	N.S.	0.76
Posterior Hypothalamic	29 ± 3.2 g	−5.5 ± 9.0% (n=4)	N.S.	1.37
		Vehicle Injections		
Paraventricular Nucleus	33 ± 2.9 g	3.5 ± 9.0% (n=10)	N.S.	1.25

*Negative numbers indicate an increase.

†Two-tailed t-test for matched pairs; comparison of amount of food eaten on the day before calcitonin injection as compared to the day of calcitonin infusion.

infusions were done with vehicle alone and for these infusions the position of the cannula was verified by the same method.

RESULTS

The sites where calcitonin produced the greatest inhibition of eating (80% or more) were the PVH, the perifornical area, and several sites on the floor of the hypothalamus, including the supraoptic decussation and large areas over the optic tract and the optic chiasma (Fig. 1). Effectiveness of the injections was not clearly related to proximity to the supraoptic nucleus itself. Anteriorly the posteromedial part of the nucleus accumbens produced a 73% decrease in eating. Anorexia was not produced in several sites including the medial forebrain bundle, the ventrolateral hypothalamus, the olfactory tubercule, the suprachiasmatic nucleus and the posterior hypothalamic nucleus. Three of the infusions were located at the superior part of the ventromedial nucleus, producing a 79% anorexia, but these infusions were very close to the fornix so that diffusion to the perifornical area was a possibility. Thus it is unclear whether the ventromedial nucleus itself was sensitive to calcitonin. Infusions into the preoptic area and into the anterior hypothalamic area produced decreased eating in only some animals.

The amounts of food eaten on the day prior to infusion and the percentage decrease after calcitonin infusions are shown for each anatomical region in Table 1. The supraoptic retrochiasmatic nucleus is included as an area where calcitonin inhibited eating. Even though eating was decreased by 95% by infusions in this area, this decrease was not significant due to the small number of animals tested. Because of the small size of this region, only two cannula tips were found in this nucleus. Other related placements (see Fig. 1 sections 6.6 thru 7.2) also markedly decreased eating. Infusions into the preoptic area decreased eating by a mean of 16% (Table 1). This decrease was statistically significant ($p<0.05$), but examination of the data revealed that the infusions substantially decreased eating (i.e., by 32 to 54 percent) in four of the 11 rats, while the other rats were not affected (mean of less than 1% increase). The mean 12.5% decrease produced by infusions in the anterior hypothalamic area was not statistically significant. It may be that the occasional decreases produced by injections in these regions were due to diffusion to other nearby nuclei. Animals infused into the PVH (n=10) with vehicle showed no change in their eating behavior. The difference between the inhibition of eating induced by PVH infusions of calcitonin as compared to infusions of vehicle was statistically significant, $p<0.001$, $t(26)=21.11$, two-tailed

TABLE 2
AMOUNTS OF CALCITONIN REQUIRED TO INHIBIT EATING IN RATS*

Route of Administration	Absolute Dosage (units per rat)	Duration of Measurement	Percentage Inhibition of Eating	Reference
Subcutaneous	20.	24 hours	61%	[12]
Intraventricular	0.02	24 hours	38%	[12]
Intraventricular	0.02	30 minutes	40%	[11]
Paraventricular Hypothalamus	0.07	45 minutes	89%	present study
Perifornical Area	0.07	45 minutes	87%	present study
Supraoptic Retrochiasmatic Nucleus	0.07	45 minutes	95%	present study

*One unit=213 ng of synthetic salmon calcitonin or 10 μg of synthetic human calcitonin.

t-test for independent groups. There was no overlap between the groups; the *maximum* inhibition of eating induced by vehicle was 22%, and the *minimum* inhibition of eating induced by calcitonin was 66%. In essentially all of the animals, food intake returned to baseline levels on the day after infusions.

The radius of spread of Evans's blue dye was usually about 0.5 mm, with a somewhat greater spread of 0.5 to 2.0 mm dorsally along the cannula tract. Thus injections into the lateral ventromedial hypothalamus, for example, could have reached the perifornical area.

DISCUSSION

The role of the hypothalamus in the regulation of feeding behavior has been studied extensively. Infusions of norepinephrine in the paraventricular nucleus, the perifornical area, the supraoptic nucleus, and the medial preoptic area increase feeding [19,20]. In the paraventricular nucleus, infusions of opiates or of neuropeptide Y increase feeding [18,37] while infusions of neurotensin [36] and corticotropin releasing factor [18] decrease eating behavior. Cholecystokinin injections in the medial hypothalamus [23] and bombesin injections in the lateral hypothalamus also produce decreases in food intake [38]. The nucleus accumbens may also have a role in the regulation of feeding behavior, as lesions of this area with 6-hydroxydopamine produce increased food comsumption [17].

It has been well established that calcitonin binding sites occur in relatively high concentrations in the hypothalamus [9, 14, 26, 29, 34]. It has also been reported that calcitonin inhibits calcium uptake by rat hypothalamus *in vitro* [16,22]. Also several other effects of calcitonin are associated with hypothalamic functions. For example, calcitonin is very potent in inhibiting gastric acid secretion when administered into the cerebral ventricles [25]. The central nervous system control of gastric secretion appears to be mainly hypothalamic since intrahypothalamic injections of gastrin stimulate gastric acid secretion [40]. Thus calcitonin and gastrin may have reciprocal roles in the hypothalamic regulation of gastric acid secretion. Intraventricular calcitonin has been found to decrease prolactin secretion, and this effect was blocked by lesions of the median eminence [3,28]. Thus regulation of prolactin secretion may be a third hypothalamic effect of calcitonin. Calcitonin alters growth hormone, thyroid stimulating hormone, and luteinizing hormone release [4, 21, 33] and these effects could also be hypothalamic. In the present study, calcitonin infusions into the hypothalamus were found to markedly inhibit eating. Taken together these data suggest that the hypothalamus is a secondary site of action of calcitonin, and that at least one of the consequences of an interaction of calcitonin with several hypothalamic nuclei is an inhibition of eating.

The 15 ng dosage of calcitonin which was found to inhibit eating in the present study was well below the minimum dosage of calcitonin which was required to inhibit eating after SC or intraventricular injection (Table 2, cf. [11,12]). This suggests that the hypothalamus may be the site through which systemically-administered calcitonin acts to inhibit eating. The fact that calcitonin may not be produced by the brain [1,35], however, raises an issue of what is the endogenous substance which interacts with calcitonin-sensitive hypothalamic nuclei. One possibility is that peripherally or pituitary derived calcitonin enters the brain in the hypothalamus, either through a reverse portal blood vessel system or due to the increased permeability of the blood-brain barrier in the hypothalamus [6, 8, 10]. Immunoreactive calcitonin has, in fact, been measured in the hypothalamus, albeit in low concentrations [10]. It is perhaps more probable that another endogenous substance such as calcitonin gene-related peptide interacts with these sites, since an interaction of calcitonin and gene-related peptide at receptor sites has recently been found [13]. Calcitonin gene-related peptide has also been shown to have some anorectic properties when injected into the cerebral ventricles [39].

The results of the present experiment are consistent with the hypothesis that calcitonin or a related peptide interacts directly with neurons in hypothalamic nuclei, particularly the PVH, the perifornical area, and certain areas in the floor of the hypothalamus including the supraoptic area. This interaction results in an inhibition of eating, and possibly other effects as well. Previous studies have indentified the hypothalamus as a possible new target organ for the hormone calcitonin [9, 14, 16, 26, 29, 34], while other studies have suggested that modulation of eating behavior may be secondary physiological effect of calcitonin [12, 22, 43, 44]. The present study supports both hypotheses and suggests an association between the two. Specifically, these results are consistent with the hypothesis that one consequence of an interaction of calcitonin with the hypothalamus is an inhibition of eating behavior.

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