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Neurotensin Inhibits LH Release (41235)

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Abstract. The present studies were designed to assess the effect of neurotensin on the release of LH, FSH, and prolactin in long-term castrated female rats. The animals were implanted in the lateral ventricle of the brain with a cannula to allow the administration of either neurotensin or the vehicle. The peptide (30 μg, dissolved in saline) or the control saline solution was injected intraventricularly in a volume of 10 μl following pentobarbital anesthesia. Blood samples were collected at sacrifice 15, 30, and 60 min after injection. A significant decrease of serum LH levels was already present in neurotensin-treated animals at 15 min, and was maintained up to the end of the experiment. This decrease was not accompanied by any change in FSH or prolactin secretion. The results suggest that this tridecapeptide participates in the control of LH release and provide new data on the separate control of the release of the two gonadotropins.

Neurotensin is a linear tridecapeptide which was first isolated from hypothalamic extracts (1, 2). Subsequently its presence has been demonstrated in several other CNS regions (e.g., the thalamus, the cerebral cortex, and the cerebellum) (3). Within the hypothalamus, the highest levels of neurotensin have been found in the median eminence and in the medial and lateral preoptic nuclei (4). Like a number of other CNS peptides, neurotensin is also present in high concentrations in several portions of the gastrointestinal tract (3). This peptide has been shown to possess a large variety of biological effects. These include: effects on smooth muscles of the gastrointestinal tract (1, 5, 6), hypotension, vasodilation (1), and hyperglycemia (7–9) which is probably due to an increase of glucagon release (7, 8), since the peptide has been reported to induce either hyper- (9) or hypoinsulinemia ((7, 8), see also (10) for additional references).

Recent studies indicate that neurotensin may also affect anterior pituitary function. There is a consensus that, in the rat, intravenous or intraventricular injections of the peptide raise circulating levels of GH (11, 12). Divergent results have been reported with regard to the effects of neurotensin on prolactin and gonadotropin secretion. Intravenous injections of the compound have been reported to increase serum levels of prolactin (11, 12), LH, and FSH (13); on the contrary a decrease of serum prolactin and LH has been found following intraventricular injections of the peptide (12).

These discrepancies, and the fact that neurotensin is present in high concentrations in the median eminence, have prompted us to analyze further its effects on gonadotropin and prolactin secretion.

Materials and Methods. Female rats of the Sprague-Dawley strain were housed in a temperature (22°) and light-controlled room (10 hr dark and 14 hr light starting at 06:30). Food and water were available *ad libitum*. Animals were ovariectomized when they reached the weight of 170–180 g. Three weeks later they were implanted, in a lateral ventricle of the brain, with a cannula which was cemented to the skull to allow the administration of neurotensin or of the vehicle into the cerebrospinal fluid. The animals were starved the night preceding the experiment and were anesthetized with pentobarbital (3 mg/100 g body wt, ip) at about 09:00. Different groups of animals were then injected, through the cerebral cannula, either with 30 μg of neurotensin or with saline solution (0.9% NaCl). They were decapitated 15, 30, and 60 min later. A group of controls, bearing a cannula in which no injections were performed was also sacrificed at the beginning of the experiment. Blood was collected from the trunk vessels. Neurotensin (Beckman) was dissolved in saline and injected into the lateral ventricle in a volume of 10 μl. Serum samples were kept frozen at −20°, until the

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radioimmunoassays were performed. LH, FSH, and prolactin were measured, respectively, according to the procedures of Niswender *et al.* (14), Daane and Parlow (15), and Niswender *et al.* (16). The data were statistically analyzed, utilizing the Dunnett test for multiple comparisons after one-way analysis of variance (17).

Results and Discussion. It is apparent from Table I that there was no significant effect of saline injections on serum levels of the three hormones considered. It is also clear that, under the conditions of the present experiment, the intraventricular injection of 30 μg of neurotensin inhibits LH but not FSH and prolactin release. A significant decrease of serum LH was already observed at 15 min and was maintained up to 60 min. With regard to the effects on LH and FSH, the present results agree with those of Vijayan and McCann (12), obtained in ovariectomized freely moving animals. The two groups of data, however, are in total disagreement with those of Makino *et al.* (13) who found a stimulation of the release of the two gonadotropins following intravenous injections of neurotensin into normal rats. Differences in the doses, in the animal preparation, in the way of administration, and in the times of sampling may explain the inconsistency. It must also be underlined at this point that Vijayan and McCann (12) did not observe any effect on LH and FSH secretion of systemically administered neurotensin in ovariectomized rats.

With regard to the effects of neurotensin on prolactin release the results of the present experiments differ from those obtained by McCann's group (12). These authors reported an inhibitory effect of intraventricular neurotensin on prolactin release, which was not confirmed in the present experiment. The discrepancy may probably be accounted for by methodological differences. First of all, the previous authors (12) used nonanesthetized rats and much smaller doses of the peptide (0.5 and 2 μg vs 30 μg) used here. Second, injections into the lateral ventricles were performed in the present study, while Vijayan and McCann (12) administered the peptide directly into the third ventricle; lateral ventricular injections would probably distribute the peptide

TABLE I. EFFECTS OF INTRAVENTRICULAR INJECTIONS OF NEUROTENSIN ON LH, FSH, AND PROLACTIN SERUM LEVELS OF CASTRATED FEMALE RATS

Treatment	Time after injections (min)	N^a	LH (NIH-LH-S 17) (ng/ml)	FSH (NIAMDD-Rat FSH-RP-1) (ng/ml)	Prolactin (NIAMDD-Rat Prolactin-RP-1) (ng/ml)
Controls	0	7	8.94 ± 0.41[b]	1612.25 ± 94.72	20.65 ± 3.15
Controls	15	11	9.28 ± 0.50	1525.33 ± 108.84	25.76 ± 2.54
Neurotensin (30 μg)	15	8	6.01 ± 0.43*	1520.13 ± 90.65	34.61 ± 5.32
Controls	30	10	8.53 ± 0.81	1648.43 ± 127.12	26.12 ± 3.75
Neurotensin (30 μg)	30	8	6.45 ± 0.38*	1889.32 ± 111.90	23.65 ± 2.48
Controls	60	9	9.35 ± 0.39	1712.27 ± 164.13	24.26 ± 3.07
Neurotensin (30 μg)	60	8	5.59 ± 1.01**	1948.68 ± 187.57	27.24 ± 4.12

[a] Number of animals.
[b] Values are means ± SE.
* $p < 0.01$ vs respective controls.
** $p < 0.05$ vs respective controls.

more widely than third ventricular ones, and this might be another factor accounting for the difference in the prolactin responses between the two studies. However, the two groups of data do not support the previous contention that neurotensin given systemically may enhance prolactin release (11, 12). It is possible that the stimulatory effects of systemic injections of neurotensin on prolactin are due to nonspecific factors (metabolic changes, hypotension, etc.) or to stress. It is known that prolactin release is very sensitive to stressful situations (18–20).

The present experiment was not designed to clarify the locus of action of neurotensin. However, the observation that the peptide does not modify gonadotropin release from anterior pituitary tissue incubated *in vitro* (12) suggests that the inhibitory effect on LH release is probably mediated via an action on CNS centers. It is interesting that neurotensin was found to inhibit LH release in the absence of any effect on FSH secretion. This observation points once more to a separate control mechanism for the release of the two gonadotropins (21–25). It is believed that the lack of effect on FSH is not solely due to the relatively more prolonged half-life of this gonadotropin. Even taking this fact into consideration, a decrease of FSH release would have occurred at the 60-min interval.

The present findings do not allow any conclusion on a possible physiological role of neurotensin in the control of LH secretion. The authors do realize that the peptide has been injected in rather elevated doses, which exceed the hypothalamic content of the peptide (as measured by radioimmunoassay) (10). It is obvious, however, that the amounts of the peptide reaching its receptors are considerably lower, due to dilution within the cerebrospinal fluid, degradation, etc.

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