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The Effects of Interruption of the Ventral Noradrenergic Pathway on the Proestrous Discharge of Prolactin in the Rat (39717)

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Introduction. Considerable evidence has accrued to indicate the participation of catecholaminergic pathways in the control of gonadotropin and prolactin release (1). Dopamine clearly functions as an inhibitor of prolactin release, either by causing the discharge of a peptidic prolactin-inhibiting factor (PIF) which then inhibits prolactin or by inhibiting prolactin by a direct action on the gland after its release into hypophyseal portal vessels. The evidence for a role for norepinephrine in the control of prolactin has been fragmentary. Restoration of norepinephrine synthesis in animals in which catecholamine synthesis had been inhibited by α-methyl paratyrosine resulted in a rise in plasma prolactin, which suggested that norepinephrine might play a stimulatory role to enhance prolactin release (2). In later experiments, intraventricular injection of norepinephrine prevented a decline in prolactin which occurred in saline-injected controls, which would also be consistent with a norepinephrine-induced stimulation of prolactin release (3). The cell bodies of most noradrenergic neurons which terminate in the hypothalamic region are located in the brain stem (4). Consequently, in order to determine the importance of noradrenergic pathways in the control of gonadotropin and prolactin release, we have begun experiments in which these pathways are interrupted by injection of 6-hydroxydopamine. This agent selectively destroys catecholaminergic neurons (5).

To study the effect of interruption of the ventral noradrenergic pathway on the release of prolactin which occurs on proestrus, 6-hydroxydopamine was injected into the ventral noradrenergic tract on the morning of proestrus and its effect on the proestrous discharge of prolactin was studied.

Material and methods. Adult virgin female rats of the Sprague–Dawley strain (Simonsen Laboratories, Gilroy, Calif.) were housed in group cages under conditions of controlled temperature (24°) and lighting (lights on 5:00 AM–7:00 PM). Only rats which had shown two or more consecutive regular 4- or 5-day cycles were used for experiments. The animals were given free access to water and Purina laboratory chow. On the morning of proestrus, the animals were placed in a stereotaxic instrument and 6-hydroxydopamine (2 μl containing 20 μg dissolved in 0.9% NaCl, solution acidified to pH 4.5 with ascorbic acid) was microinjected bilaterally into the ventral noradrenergic tract at the level of the interpeduncular nucleus and medial lemniscus (4). One-milliliter blood samples were withdrawn just prior to operation, at 5:00 PM, and on the following morning. The animals were lightly anesthetized with ether prior to bleeding.

At sacrifice, on the following morning, the hypothalamic region was rapidly frozen in liquid propane and freeze-dried for a period of 6 days. Afterwards, the freeze-dried hypothalami were treated with paraformaldehyde (70% relative humidity) at 80° during a period of 1 hr according to the Falck–Hillarp procedure (6, 7). All pieces were embedded in paraffin *in vacuo* and the blocks were sectioned in slices 10 μm thick. The fluorescence microscopy was performed with a Zeiss microscope equipped with a dark field condenser and a HBO-200 high pressure mercury lamp. The brain stems were fixed in 10% neutral formalin, cut in serial sections at 10 μm, and stained with hematoxylin–eosin to determine the localization of lesions. On the basis of the decline in hypothalamic catecholaminergic

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fluorescence and the location of the lesions, the animals were grouped into those with complete lesions of the noradrenergic tract and those with incomplete lesions.

Results. In control animals there was an elevation of prolactin above the morning values on the afternoon of proestrus in every case, and the mean increase was highly significant (Table I). Lesions which nearly completely or completely interrupted the ventral noradrenergic tract on the basis of a study of their effect on the hypothalamic catecholamine fluorescence and/or the localization of their lesions in the brain stem blocked the afternoon elevation of prolactin in every case. Localization of the effective lesions in the region of the interpeduncular nucleus is illustrated (Fig. 1). In fact, in those animals with effective lesions by these criteria the prolactin titers declined in the afternoon of proestrus in every animal, and the decline was highly significant.

In another group, the lesions were either misplaced, or asymmetrical and were associ-

TABLE I. THE EFFECT OF LESIONS IN THE VENTRAL NORADRENERGIC TRACT ON THE PROESTROUS DISCHARGE OF PROLACTIN.

	Plasma prolactin (ng/ml)				
	AM	PM	Difference	Fluorescence	Lesions
Controls (N = 13)[a]	62 ± 7.6[b]	139 ± 19	79 ± 21*		
Effective lesions (N = 13)					
B-12	89	15	−74	MD[c]	+[d]
8A	37	4	−33	MD	+
19A	133	55	−78	0[e]	+
23A	80	13	−67	0	n.a.[f]
101A	50	8	−42	n.a.	+
119A	43	7	−36	0	n.a.
126A	52	11	−41	0	+
102A	28	6	−22	n.a.	+
106A	37	5	−32	MD	+
109A	58	3	−55	0	+
123A	21	2	−19	MD	n.a.
125A	29	10	−19	n.a.	+
127A	51	18	−33	0	+
	54 ± 8.5	12 ± 3.8	−42 ± 5.6**		
Incomplete lesions (N = 9)					
B1	40	45	5	n.a.	Too far lateral
B4	25	77	52	−[g]	Too far lateral and dorsal
B11	59	90	31	−	Asymmetric dorsal
B8	75	81	6	n.a.	Asymmetric dorsal
134A	79	160	81	−	n.a.
150A	59	55	−4	−	n.a.
172A	24	103	79	−	Asymmetric; too far lateral
141A	48	40	−8	−	n.a.
154A	30	58	28	Almost normal	Asymmetric
	49 ± 6.9	79 ± 12	30 ± 11***		

[a] N, number of rats.
[b] Mean ± SE.
[c] MD, marked decrease in hypothalamic catecholaminergic fluorescence.
[d] +, properly placed 6-OHDA lesions.
[e] 0, absent hypothalamic catecholaminergic fluorescence.
[f] n.a., not available for study.
[g] −, slight or asymmetric decrease in hypothalamic catecholaminergic fluorescence.
* $p < 0.01$.
** $p < 0.001$.
*** $p < 0.05$.

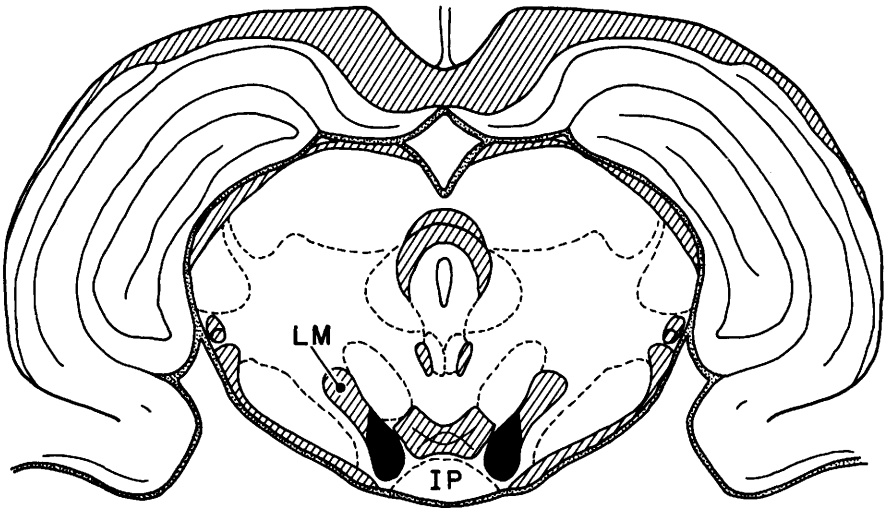

FIG. 1. Location of areas of destruction common to effective lesions in the ventral noradrenergic tract which are seen to lie within the medial lemniscus (LM) just dorsal and lateral to the interpenduncular nucleus (IP).

TABLE II. THE EFFECT OF LESIONS IN THE VENTRAL NORADRENERGIC TRACT ON THE PROESTROUS DISCHARGE OF LH.

	Plasma LH (ng/ml)		
	AM	PM	Difference
Controls (N = 13)[a]	3.6 ± 0.9[b]	43.1 ± 9.3	41.8 ± 10.0*
Effective lesions (N = 13)	2.6 ± 0.5	4.7 ± 1.4	2.1 ± 1.3
Incomplete lesions (N = 9)	5.5 ± 1.0	4.4 ± 1.3	1.2 ± 1.3

[a] N = number of rats.
[b] Mean ± SE.
* $p < 0.01$.

ated with little or no decrease in the catecholamine fluorescence. In these cases, the situation was intermediate between that in the control animals and that in those with effective lesions. The afternoon prolactin values were increased above those in the morning in all but two of the nine animals, but the increase was small except in four cases. The mean increase was significant statistically ($p < 0.05$).

There was an increase in plasma LH in all of the control animals on the afternoon of proestrus in comparison with the values during the morning (Table II). This increase was blocked by the effective lesions but was also blocked by the ineffective lesions, which did not produce a complete block of the rise in prolactin.

Discussion. The results of the present study indicate that effective lesions of the ventral noradrenergic tract which would diminish the release of norepinephrine from the axon terminals within the hypothalamus prevent the rise in prolactin which normally occurs on the afternoon of proestrus in the rat. These lesions appear to have a specific action since lesions which were misplaced or were asymmetrical were associated with some increase in the levels of this hormone. Similarly, these lesions blocked the increase in LH which occurs on the afternoon of proestrus; however, in this instance, the poorly placed lesions were also effective, which raises the question as to whether or not the inhibition was a specific one. Subsequent experiments, in which the effect of these lesions on the progesterone-induced LH release in ovariectomized, estrogen-primed animals was studied, suggest that this is a specific effect since, in that series of experiments, injections of the ascorbic acid diluent into the ventral noradrenergic tract

did not block the rise in LH (8).

A hypothesis which can explain the ability of these lesions to block the proestrous discharge of gonadotropins and prolactin is that on the afternoon of proestrus there is an increased firing rate of neurons of the ventral noradrenergic tract which project to the preoptic anterior hypothalamic region. These neurons synapse there with LH-releasing hormone (LHRH) neurons in the case of LH and prolactin-releasing factor (PRF) neurons in the case of prolactin to stimulate a discharge of these neurohormones, which then provokes the rise in LH and prolactin, respectively. Indeed, prolactin-releasing activity has been extracted from the suprachiasmatic region (9), a region which is rich in noradrenergic terminals, presumably derived largely from the ventral noradrenergic tract (4, 10, 11). Similarly, in the case of LHRH, noradrenergic terminals presumably end in juxtaposition to LHRH neurons in the preoptic anterior hypothalamic area. Inhibitors of catecholamine synthesis have been shown to block the rise in plasma LH following electrochemical stimulation of the preoptic anterior hypothalamic region (12), and this blockade is reversed by administration of drugs which restored norepinephrine but not dopamine synthesis.

In the case of prolactin, which is under dual hypothalamic control by both PRF and PIF, it is also possible to explain the results on the basis of noradrenergic inhibition of PIF release. Further work will be necessary to distinguish between these two possibilities.

It must be recognized that these are acute lesions and that the 6-hydroxydopamine injections destroyed other neurons in addition to the fibers of the noradrenergic tract (13). Consequently, we cannot be certain at the present time that the results are due to the deficiency in norepinephrine which was produced by the lesions. The results are presumably not related to interruption of dopaminergic axons since dopaminergic terminals within the hypothalamus arise from the intrahypothalamic tuberoinfundibular dopaminergic tract (4, 10) which would not be affected by these brain stem lesions. Additional studies with lesions in other loci along the ventral noradrenergic tract and with chronic lesions to eliminate the possible stress effects of acute lesions are needed.

An alternate hypothesis to explain the results would be that tonic discharge of the ventral noradrenergic pathway is required to maintain the central excitatory state of the PRF neurons and that the failure of prolactin release following destruction of the tract is simply related to the reduced central excitatory state. Again, further experimentation will be necessary to determine whether an actual increase in impulse transmission in the ventral noradrenergic tract occurs on the afternoon of proestrus or whether tonic activity in this tract is all that is required for the release of prolactin and LH to occur on proestrus.

Completeness of lesions was judged by their location in a region known to contain the noradrenergic tract at the level of the interpeduncular nucleus (4). In addition, the decline in cathecholaminergic fluorescence was used as an index of completeness of lesions. It is realized that this is not quantitative and some reduction in the fluorescence could be missed; however, when the fluorescence is dramatically reduced or absent, we believe this is certainly an index of interruption of the tract. Furthermore, in subsequent experiments, we have actually measured a reduction in norepinephrine in the hypothalamus following similar 6-hydroxydopamine-induced lesions (Martinovic and McCann, unpublished).

Summary. 6-Hydroxydopamine was microinjected bilaterally into the ventral noradrenergic tract at the level of the interpeduncular nucleus. Effective injections into the tract as indicated by the lesions induced and by a decline in the catecholamine fluoresence in the anterior hypothalamus were associated with a complete blockade of the rise in prolactin which occurs on the afternoon of proestrus and, in fact, a decline in prolactin ensued. Lesions which were asymmetrical or missed the ventral noradrenergic tract had only a slight effect in inhibiting the release of prolactin on the afternoon of proestrus. All lesions were capable of blocking the release of LH on the afternoon of proestrus. It is postulated that increased impulse traffic along the ventral noradrenergic

tract induces the proestrous discharge of prolactin and LH or that, alternatively, tonic activity in this tract is required for this release to occur.

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