Effect of Dopamine on the Biological Activity and *in Vitro* Release of ACTH and FSH[1] (34639)

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(Introduced by W. F. Ganong)

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Considerable evidence is accumulating suggesting that catecholamines play a role in the regulation of anterior pituitary hormone secretion. In addition to the widespread distribution of noradrenergic nerve terminals in the hypothalamus, there is a particularly prominent group of dopaminergic nerve endings in the median eminence (1). Stressful stimuli, such as electric shock, hemorrhage, and hypoglycemia which stimulate ACTH secretion, cause a decrease in brain norepinephrine content (2), and norepinephrine turnover in hypothalamic nerve terminals is increased under the influence of immobilization stress (3). Furthermore, reserpine, a catecholamine-depleting agent, stimulates ACTH secretion (4), whereas, amphetamine, a catecholamine-releasing agent, inhibits ACTH secretion (5).

Alterations in hypothalamic catecholamines also appear to be correlated with changes in gonadotropin and prolactin secretion. Increased fluorescence of the dopaminergic fibers in the median eminence has been observed in pseudopregnant and lactating rats (1). Castration of male and female rats, which increases FSH and LH secretion (6), results in altered ratios of hypothalamic content of norepinephrine and dopamine (7). Alterations in brain norepinephrine turnover have been reported in castrated male and female rats (8). Finally, drugs such as reserpine and α-methyltyrosine which are known to deplete brain catecholamines (9, 10) have been shown to delay the onset of puberty (11), inhibit ovulation (12, 13), induce pseudopregnancy (12), and cause increased prolactin secretion and lactation (14) in the female rat.

There are several ways that brain monoamines may affect the secretion of anterior pituitary hormones. Possible mechanisms include serving as transmitters in neural pathways providing input to neurons secreting releasing factors; mediation of presynaptic inhibition on the terminals of such neurons; altering flow in the hypothalamic–hypophyseal portal blood vessels; or direct action on the anterior pituitary cells. This paper reports results of a series of experiments in which the possibility of a direct action of dopamine on the pituitary was tested by incubating anterior pituitary tissue with dopamine *in vitro* and measuring the amount of ACTH and FSH in the incubation medium.

Methods. Anterior pituitary glands were obtained from 200–250 g male Sprague-Dawley rats (Berkeley Pacific Company) that were sacrificed by decapitation. Four halves of hemisected rat anterior pituitaries were preincubated in 25-ml Erlenmeyer flasks containing 3 ml of medium 199 for 20–30 min at pH 7.4 and 37° under constant gassing with 95% O_2–5% CO_2. This preincubation medium was then discarded, replaced by fresh medium, and the incubation was continued for 6 hr under identical conditions. If hypothalamic extract was used in the incubation, it was added after the preincubation period. At the end of the incubation period, the medium was decanted, immediately frozen, and stored at —20° until subsequent assay for ACTH and FSH.

Paired flasks containing anterior pituitary halves and hypothalamic extract were incu-

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bated with or without added dopamine hydrochloride in a dose of 20 or 200 μg/anterior pituitary (*i.e.*, 40 or 400 μg/3 ml of medium) for the 6-hr period. Similar experiments were performed without hypothalamic extract. In addition, medium containing rat ACTH and FSH was incubated with or without dopamine hydrochloride in a concentration of 400 μg/3 ml. This medium was a pool from previous preincubations.

The effect of preincubation of anterior pituitaries with dopamine on ACTH and FSH release was studied in a further series of *in vitro* experiments. In these experiments, dopamine hydrochloride, 200 μg/anterior pituitary, was added to the medium during the initial preincubation, and this incubation was carried out for 30 min. After this period, the medium was discarded and the pituitaries were incubated with or without hypothalamic extract for a subsequent 6-hr period in medium 199 alone.

Hypothalamic extract was prepared from adult Sprague-Dawley rats of both sexes, sacrificed by decapitation. Hypothalamic fragments were homogenized in cold 0.1 *N* HCl (0.2 ml/fragment) and the suspension centrifuged at 12,000*g* at 4° for 40 min. The supernatant was decanted, frozen, and stored at −20° overnight. Just prior to use in the incubation system, the extract was thawed, neutralized with 1 *N* NaOH and diluted with medium 199 to a concentration of 1 hypothalamus/ml. In all experiments using hypothalamic extract, the amount added was 0.5 hypothalamic equivalent / pituitary.

ACTH was assayed by the method of Nelson and Hume (15), with results expressed as 17-hydroxycorticosteroid outputs in micrograms per minute in one adrenal vein from the 4th to the 14th minute after injection in each hypophysectomized assay dog. Standard and each unknown were assayed at two dose levels in the same dog. In addition, fresh dopamine and dopamine that had been previously incubated for 6 hr was added to standard ACTH and immediately injected into assay dogs. FSH was assayed in 21-day-old female Sprague-Dawley (Holtzman) rats by a modification of the method of Steelman and Pohley (16). Results are expressed as the weights of both ovaries in the assay rats. The ACTH standard was commercial ACTH, 40 U/vial. The FSH standard was NIH FSH-S-3.

Results. As shown in Table I, the amount of ACTH in the medium after 6 hr of incubation of pituitary tissue and hypothalamic extract was not affected by the addition of 20 μg of dopamine/pituitary, but was reduced by the addition of 200 μg of dopamine. However, a comparable amount of dopamine also reduced the biological activity of rat ACTH when incubated without pituitary tissue (Table II). When the ACTH standard was mixed with dopamine that had been incubated for 6 hr, or with fresh dopamine, there was no reduction in the adrenal-stimulating effect

TABLE I. Assay of ACTH Released from Anterior Pituitaries Incubated with Hypothalamic Extract and Dopamine.

Incubation medium	Treatment of assay dog	17-Hydroxycorticosteroid output (μg/min)
—	ACTH standard, 2 mU	0.4[a]
	8 mU	3.9[a]
Pituitary and hypothalamic extract	1 AP eq[b]	3.0
	4 AP eq	7.5
Pituitary and hypothalamic extract plus 20 μg of dopamine	1 AP eq	2.7
	4 AP eq	8.2
Pituitary and hypothalamic extract plus 200 μg of dopamine	1 AP eq	0
	4 AP eq	0.5

[a] Mean of 2 values.

[b] AP eq = anterior pituitary equivalent which is contained in 1.5 ml of incubation medium.

TABLE II. Assay of Rat ACTH Incubated with Dopamine.

Incubation medium	Treatment of assay dog	17-Hydroxycorticosteroid output (μg/min)
—	ACTH standard, 2 mU	7.8[a]
	8 mU	20.8[a]
Rat ACTH	Medium, 3 ml	3.5
	12 ml	8.0
Rat ACTH + dopamine, 133 μg/ml	Medium, 3 ml	0
	12 ml	0
Dopamine, 133 μg/ml	ACTH standard, 8 mU + Medium, 3 ml	18.6
	12 ml	20.5
—	ACTH standard, 8 mU + 3 ml of dopamine, 133 μg/ml	19.7

[a] Mean of 2 values.

of the ACTH (Table II). These observations suggest that neither dopamine nor an incubation product of dopamine are interfering with the ACTH assay. When pituitary tissue was preincubated for 30 min in medium containing 200 μg of dopamine/pituitary, then incubated in fresh medium that did not contain the amine, the amount of ACTH at the end of the 6-hr incubation period was not reduced (Table III). This was true in the presence as well as the absence of hypothalamic extract.

Similar results were obtained with FSH. Two hundred μg of dopamine/pituitary, but not 20 μg, reduced the amount of FSH in the medium after incubation in the presence of hypothalamic extract (Table IV). However, there was also less FSH biological activity in the medium when rat FSH was incubated with dopamine without pituitary tissue (Table V). Preincubation with dopamine for 30 min failed to reduce the amount of FSH

TABLE III. Effect of Preincubation with Dopamine on the Release of ACTH by Anterior Pituitaries.

Incubation medium	Treatment of assay dog	17-Hydroxycorticosteroid output (μg/min)
Exp. 1.		
—	ACTH standard, 2 mU	3.2[a]
	8 mU	10.2[a]
Pituitary	1 AP eq[b]	1.5
	4 AP eq	6.7
Pituitary preincubated with 200 μg of dopamine	1 AP eq	3.2
	4 AP eq	7.2
Exp. 2.		
—	ACTH standard, 2 mU	0.1[a]
	8 mU	3.2[a]
Hypothalamic extract plus pituitary	1 AP eq	1.8
	4 AP eq	8.3
Hypothalamic extract plus pituitary preincubated with 200 μg of dopamine	1 AP eq	2.6
	4 AP eq	8.8

[a] Mean of 2 values.

[b] AP eq = anterior pituitary equivalent which is contained in 1.5 ml of incubation medium.

released into the medium following subsequent incubation without the amine (Table VI). This was true in the presence as well as the absence of hypothalamic extract.

An interesting incidental finding was the formation of a heavy black granular precipitate in the medium whenever pituitary tissue was incubated with 200 μg of dopamine/pituitary. It was observed to a lesser extent with 20 μg of dopamine/pituitary and only slightly when dopamine was incubated with medium containing hormones but not pituitary tissue.

TABLE IV. Assay of FSH Released from Anterior Pituitaries Incubated with Hypothalamic Extract and Dopamine.

Treatment of assay rat	Ovarian wt of assay rats (mg: mean ± SE)
Exp. 1	
FSH standard (μg)	
50	109.7 ± 11.2
100	154.3 ± 8.4
200	199.6 ± 9.3
Incubation medium	
Pituitary and hypothalamic extract	132.8 ± 9.8
Pituitary and hypothalamic extract + 20 μg of dopamine	155.6 ± 9.6
Exp. 2	
FSH standard (μg)	
25	63.9 ± 4.7
50	86.8 ± 9.1
100	166.2 ± 15.7
200	266.2 ± 28.8
Incubation medium	
Pituitary and hypothalamic extract	145.7 ± 15.9
Pituitary and hypothalamic extract + 200 μg of dopamine	65.6 ± 4.2

TABLE V. Assay of Rat FSH Incubated with Dopamine.

Treatment of assay rat	Ovarian wt of assay rats (mg: mean ± SE)
FSH standard (μg)	
50	109.7 ± 11.2
100	154.3 ± 8.4
200	199.6 ± 9.3
Incubation medium	
Rat FSH	220.9 ± 12.0
Rat FSH + dopamine, 133 μg/ml	70.6 ± 5.2

TABLE VI. Effect of Preincubation with Dopamine on the Release of FSH by Anterior Pituitaries.

Treatment of assay rat	Ovarian wt of assay rats (mg: mean ± SE)
Exp. 1	
FSH standard (μg)	
50	72.5 ± 7.5
100	136.6 ± 11.2
200	185.0 ± 15.4
Incubation medium	
Pituitary	81.8 ± 6.3
Pituitary preincubated with 200 μg of dopamine	81.6 ± 7.4
Exp. 2	
FSH standard (μg)	
50	104.4 ± 6.4
100	160.1 ± 14.5
200	214.8 ± 24.6
Incubation medium	
Hypothalamic extract + pituitary	147.5 ± 9.8
Hypothalamic extract + pituitary preincubated with 200 μg of dopamine	166.7 ± 15.3

Discussion. These experiments indicate that dopamine, when incubated with rat ACTH and FSH, destroys the biological activities of these hormones. This hormone degradation occurred only if dopamine was present in high concentration and in spatial proximity with the hormone for a considerable period of time. It is probable that the hormones were degraded by dopamine or an incubation product via an oxidation–reduction reaction. During the course of incubating anterior pituitaries with dopamine, black granules were formed. Vanderwende described the formation of a similar pigment during incubation of whole brain extracts with dopamine (17). He suggested that the

pigment was dopamine–melanin, since the pigment had the same visual appearance, spectral properties and solubility properties as melanin granules formed from the oxidation of dopamine by hydroxyl ions or tyrosinase. Our data do not indicate that inactivation by dopamine is a physiologic process by which hormones are broken down within the pituitary. However, the data do suggest that *in vitro* experiments on the effect of dopamine on pituitary secretion should be interpreted with caution because of hormone inactivation. While this manuscript was being prepared for publication, a similar effect of dopamine on FSH was reported (18).

There is evidence that pituitary cells take up catecholamines and are capable of forming dopamine (1). However, no evidence was obtained in the present study for any direct effect of dopamine on pituitary cells. ACTH and FSH release were unaffected by 20 μg of dopamine/pituitary, and preincubation of pituitaries with 200 μg of dopamine/pituitary failed to affect the ability of the pituitary to release ACTH and FSH upon subsequent incubation without the amine. Furthermore, dopamine did not appear to alter the pituitary response to hypothalamic extract. *In vivo* experiments in the dog have suggested that catecholamines may participate in an inhibitory system regulating ACTH secretion (19, 20). The present experiments do not support a direct action on the pituitary in the mediation of this inhibitory effect of catecholamines on ACTH secretion.

Summary. Pituitary tissue and rat pituitary extracts were incubated with dopamine *in vitro*. The results indicate that dopamine chemically inactivates ACTH and FSH when incubated in high concentration with these hormones for prolonged periods. The data fail to support a direct effect of dopamine on the pituitary release of ACTH and FSH, and dopamine does not alter the pituitary response to hypothalamic extract.

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