*IMPAIRED SECRETION OF EPINEPHRINE IN RESPONSE TO INSULIN AMONG HYPOPHYSECTOMIZED DOGS**

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Almost all of the epinephrine secreted into the circulation of mammals is synthesized within the adrenal medulla through the action of the enzyme phenylethanolamine-N-methyl transferase (PNMT). This enzyme catalyzes the transfer of a methyl group from S-adenosylmethionine to the amine group of norepinephrine.[1, 2] PNMT activity is stimulated by certain adrenocortical hormones. When rats are hypophysectomized, the activity of the enzyme decreases markedly,[3, 4] but can be restored to normal by the administration of small doses of adrenocorticotropin (ACTH) or very large doses of carbohydrate-active steroids.[5] Since the adrenal medulla is normally perfused with venous portal blood, which has drained through the adrenal cortex,[6] and is thus very rich in steroid hormones, it seems likely that the location of the medulla within the cortical envelope constitutes a physiologic adaptation to maintain normal PNMT activity.[5] The effect of steroid hormones on PNMT activity is probably mediated by the induction of new enzyme.[4]

It has previously been demonstrated that hypophysectomy also lowers the epinephrine content of the rat adrenal.[5] We will now show that the removal of the pituitary leads to an impairment in epinephrine secretion, both basally and in response to insulin-induced hypoglycemia. It is suggested that this effect constitutes one mechanism by which hypophysectomy increases the sensitivity of mammals to insulin.

Methods.—Mongrel male dogs were hypophysectomized by the transbuccal route[7] and maintained for 3–5 months on daily subcutaneous injections of 12.5 mg cortisone (Table 1). Some of the dogs also received ACTH (40 units/day, s.c.) for 4 weeks prior to the measurement of catecholamine secretion. Control dogs were unoperated; they were not treated with cortisone because previous studies[8] had shown that low doses of such steroids inhibit PNMT activity in rats.

Under nembutal anesthesia, one adrenal vein was cannulated as previously described,[9] and blood was collected before and at 10-min intervals after the intravenous injection of insulin. Hypophysectomized animals received 0.1 unit of insulin per kg; some of the control dogs were given 0.3 unit/kg in an effort to produce decreases of blood glucose comparable to those produced in the hypophysectomized animals (Table 1). Two blood samples of approximately 1 ml each were obtained at each experimental point; the collection of each took 20–60 sec. One sample was mixed with sodium fluoride and potassium oxalate and was subsequently assayed for glucose; the other was mixed with heparin, and the plasma was used for determinations of epinephrine and norepinephrine.[10] At the end of the collection period, both adrenals were removed, frozen, and subsequently assayed for epinephrine, norepinephrine, and PNMT.[5, 8]

Results.—As had been expected, the adrenal glands of hypophysectomized dogs weighed considerably less than those of control animals or of hypophysectomized animals treated with ACTH (Table 2). The hypophysectomized animals

TABLE 1. *Experimental animals.*

No.	Date hypophysectomized	Date killed	Weight (kg)	Insulin dose (units/kg)
		Control dogs		
1	——	10/18/66	18.0	0.1
2	——	10/21/66	18.0	0.3
3	——	11/1/66	18.5	0.1
4	——	11/14/66	18.0	0.3
5	——	11/14/66	20.0	0.3
6	——	2/6/67	24.9	0.3
		Hypophysectomized dogs*		
1	5/31/66	10/18/66	21.2	0.1
2	5/31/66	10/20/66	27.5	0.1
3	5/31/66	10/26/66	36.4	0.1
4	5/31/66	11/14/66	32.2	0.1
5	3/23/67	12/8/67	19.1	0.1
6	3/23/67	12/8/67	21.9	0.1
		Hypophysectomized dogs given ACTH†		
1	8/3/67	12/8/67	25.4	0.1
2	3/27/67	12/8/67	26.7	0.1
3	8/3/67	12/8/67	22.8	0.1

* Dogs received cortisone (12.5 mg/day) starting immediately after hypophysectomy.
† Dogs received cortisone (12.5 mg/day) starting immediately after hypophysectomy, and ACTH (40 units/day) starting 11/10/67.

TABLE 2. *Effect of hypophysectomy and ACTH on adrenal weight, body weight, and per cent of total adrenal catecholamine represented by epinephrine.*

Treatment	Adrenal weight (mg)	Body weight (kg)	Adrenal epinephrine (% total catecholamine)
Normal[6]	1130.8 ± 167.9*	19.6 ± 1.1*	72.7 ± 2.4*
Hypophysectomized[7]	689.3 ± 70.6	27.3 ± 2.5	50.6 ± 6.8
Hypophysectomized + ACTH[3]	1313.3 ± 83.3†	24.9 ± 1.1	89.3 ± 3.3‡

All data are expressed as mean ± standard error, and are analyzed by the *t*-test.
* $p < 0.05$ difference from hypophysectomized dogs.
† $p < 0.01$ difference from hypophysectomized dogs.
‡ $p < 0.001$ difference from hypophysectomized dogs.

weighed somewhat more than control dogs (Table 1), probably because the operated animals were 3–5 months older and had been treated each day with cortisone. Hypophysectomy was associated with a marked decrease in adrenal PNMT activity, whether expressed per whole gland or per gram of adrenal tissue (Table 3). ACTH completely restored PNMT activity, raising the ability of the whole adrenal to synthesize epinephrine to a value significantly higher than those observed in glands from unoperated animals.

Hypophysectomy was associated with a decrease in the content of epinephrine per adrenal gland (Fig. 1) and in the per cent of total adrenal catecholamines represented by epinephrine (Table 2). The decrease in epinephrine content was not associated with a comparable increase in adrenal norepinephrine (Fig. 1). (A similar lack of correlation has been observed in the hypophysectomized rat;[5] probably in both species, adrenal norepinephrine exists in several metabolic pools, of which only one serves as the substrate for PNMT.) Treatment with ACTH

TABLE 3. *Effect of hypophysectomy and ACTH on phenylethanolamine-N-methyl transferase activity.*

Treatment	PNMT Activity* mμM/gland	mμM/gm
Normal[6]	123.1 ± 10.4†	122.3 ± 14.0†
Hypophysectomized[7]	28.8 ± 3.5	43.1 ± 4.8
Hypophysectomized + ACTH[8]	190.0 ± 24.9†	147.3 ± 27.4‡

* PNMT activity is expressed as mμM of N-methyl phenylethanolamine produced per hour from phenylethanolamine as previously described.[5, 8]

† $p < 0.001$ difference from hypophysectomized dogs.

‡ $p < 0.01$ difference from hypophysectomized dogs.

increased adrenal epinephrine content (Fig. 1) and the per cent of total adrenal catecholamines represented by epinephrine (Table 2) to such an extent that both functions attained values significantly higher than those observed in unoperated control dogs. Adrenal norepinephrine content was unchanged.

Figure 2 shows the changes in adrenal venous epinephrine, norepinephrine, and glucose in a typical animal from each of the three treatment groups; Figure 3 shows the mean values for epinephrine secretion.

The basal concentration of glucose in adrenal venous blood was somewhat higher in control dogs than in hypophysectomized animals, whether or not the latter had also been treated with ACTH (Table 4). However, all of the animals tested had basal blood glucose values that fell within normal limits. The extent to which blood glucose decreased after insulin treatment did not differ signifi-

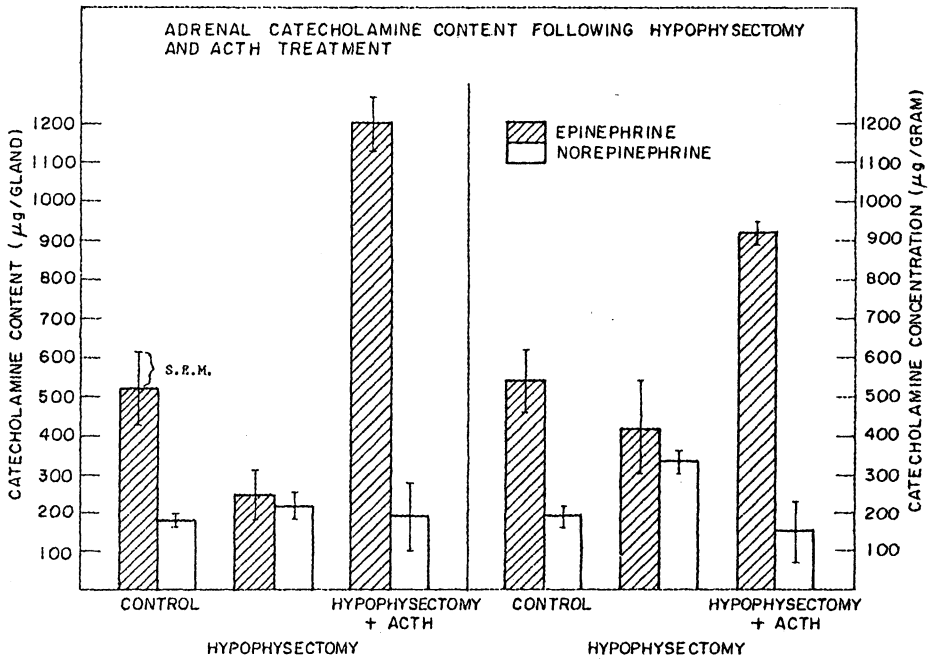

FIG. 1.—Vertical lines represent standard errors of the mean (S.E.M.).

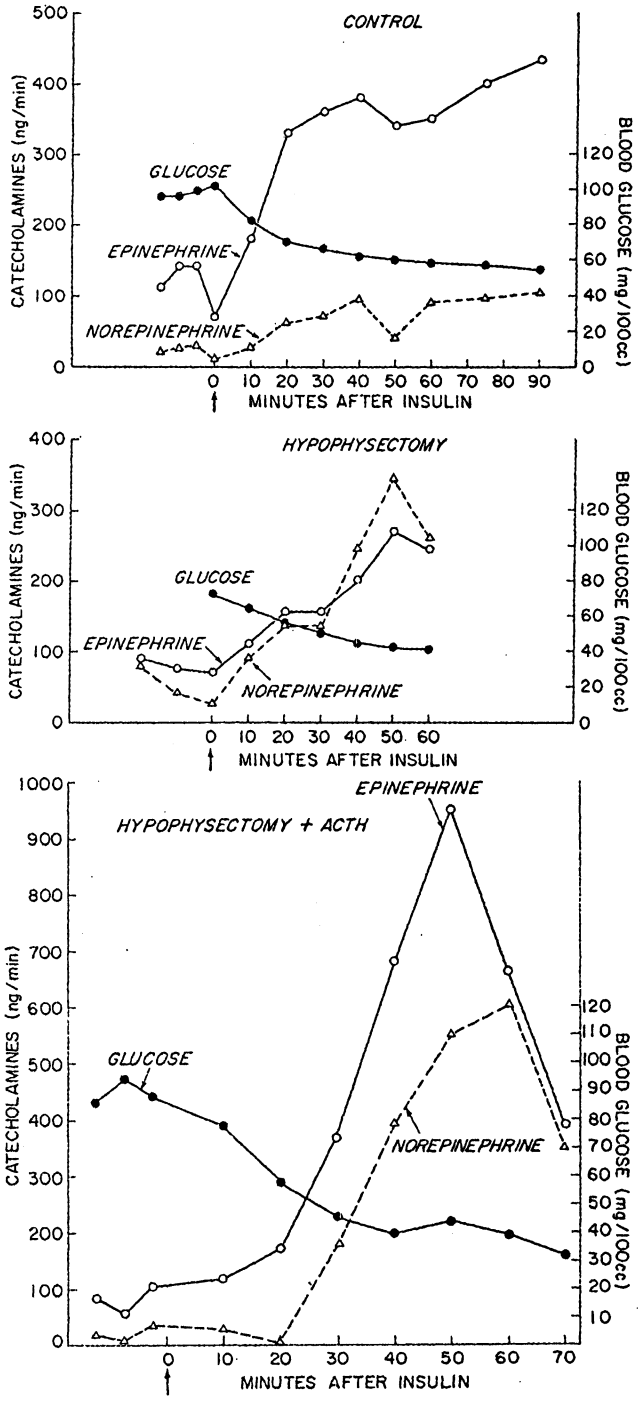

FIG. 2.—Secretion of epinephrine and norepinephrine into adrenal venous blood before and after administration of insulin (0.1 unit/kg). Blood was collected for 30–60 sec every 10 min and assayed for catecholamines and glucose. Animals marked *Hypophysectomy* were hypophysectomized 4 months before study; ACTH (40 units/day) was given subcutaneously.

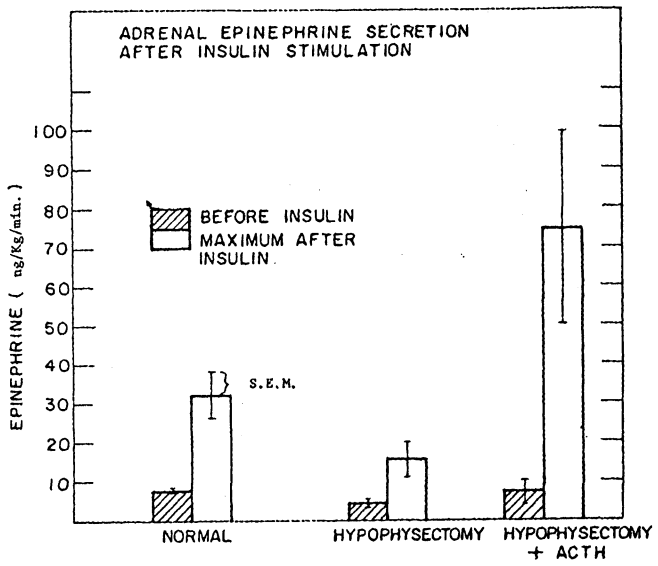

FIG. 3.—Vertical lines represent standard errors of the mean (S.E.M.)

cantly among the three experimental groups, probably because the control animals had been treated with larger doses of insulin (Tables 1 and 4).

Among unoperated dogs, basal epinephrine secretion averaged 7.5 ± 0.01 ng/gland/kg/min and rose to a peak of 31.9 ± 6.1 ng/gland/kg/min in response to insulin hypoglycemia (Fig. 2). The proportion of total blood catecholamine represented by epinephrine was 82.0 ± 4.2 per cent. Among hypophysectomized dogs, basal epinephrine secretion averaged 4.2 ± 0.7 ng/gland/kg/minute and rose to a peak of only 15.6 ± 4.9 ng/gland/kg/min after insulin treatment (Fig. 2). The proportion of total blood catecholamine represented by epinephrine, 62.2 ± 8.3 per cent, was significantly lower in these dogs than in control animals. Treatment with ACTH restored the basal rate of epinephrine secretion to normal (7.2 ± 2.9 ng/gland/kg/min) in hypophysectomized animals. Moreover, the peak concentration of the adrenal venous epinephrine after the injection of insulin was considerably higher than in unoperated control dogs (74.4 ± 24.5 vs. 31.9 ± 6.1 ng/gland/kg/min). The proportion of adrenal venous catecholamine represented by epinephrine in ACTH-treated dogs (69.7 ± 3.5%) was between that of the hypophysectomized and that of the control groups.

TABLE 4. *Fall in blood glucose concentration following insulin treatment in hypophysectomized dogs with and without ACTH treatment.*

Treatment	Control (mg/100 ml)	Per cent decrease
Normal[6]	110.5 ± 4.9*	52.2 ± 3.4
Hypophysectomized[7]	88.6 ± 7.9	52.0 ± 3.3
Hypophysectomized + ACTH[3]	85.7 ± 1.7	60.6 ± 1.7

* $p < 0.05$ difference from hypophysectomized dogs.

Discussion.—It has previously been demonstrated that hypophysectomy is associated with decreases in the activity of the adrenomedullary enzyme PNMT, which synthesizes epinephrine, and in the content of epinephrine in the rat adrenal medulla. The studies reported here show that the pituitary and adrenal cortex also control PNMT activity in the dog. They further reveal that the decline in epinephrine synthesis observed in hypophysectomized mammals is associated with impaired secretion of epinephrine in response to its major physiologic stimulus, hypoglycemia. Adrenal PNMT activity, epinephrine content, and epinephrine secretion remain subnormal when hypophysectomized animals are treated with standard "replacement" doses of carbohydrate-active steroids, even if these doses are sufficient to allow a gain in body weight. However, all of the above functions become normal (or even supranormal, e.g., epinephrine secretion) after long-term treatment with ACTH. These observations are consistent with the hypothesis[5] that PNMT activity is under the direct control of steroid hormones delivered to the medulla via the intra-adrenal portal circulation. Since the concentration of hydrocortisone in this blood is many times higher than that found in the peripheral circulation, it should not be surprising that doses of steroid which are chosen to restore peripheral circulation concentrations to normal do not restore the high concentrations of steroid which are normally available to the adrenal medulla, and thus do not restore PNMT activity.

Pituitary insufficiency in man is frequently associated with extreme sensitivity to the hypoglycemic effects of endogenous and injected insulin.[11] It seems likely that impaired epinephrine secretion is involved in producing this supersensitivity. Hypophysectomized patients have been shown to excrete subnormal amounts of epinephrine into the urine,[12] and the studies described in this report provide direct evidence that hypophysectomy ultimately depresses the secretion of epinephrine into the adrenal venous blood. In several patients with hypopituitarism, supersensitivity to insulin could be treated successfully with ACTH, but not with carbohydrate-active steroids:[13] ACTH might be expected to be the more effective agent because of its greater ability to restore PNMT activity.

Although no data are available on the time course of the development of impairment of epinephrine secretion following hypophysectomy, it seems likely that this effect requires that circulating ACTH be absent for a long time. In order for epinephrine secretion to decrease, there must be a decline in PNMT activity followed by a decrease in adrenal epinephrine content. PNMT activity falls relatively slowly following hypophysectomy in the rat;[4] in the dog, enzyme activity persists at about 20 per cent of the normal rate for as long as five months after hypophysectomy (Table 3). This degree of activity is probably adequate to sustain some epinephrine synthesis. Even if epinephrine synthesis were to stop completely following hypophysectomy, the amine stored in the adrenal would probably require weeks or even months to disappear: the half life of adrenal epinephrine in the rat is of the order of one or two weeks.[14] Hence impairment of epinephrine synthesis probably does not modify insulin sensitivity in the patient with hypopituitarism until his disease has become chronic.

Summary.—Hypophysectomy in dogs is followed by decreases in the activity of the epinephrine-forming enzyme, phenylethanolamine-M-methyl transferase,

and in the content of epinephrine in the adrenal gland. Hypophysectomized dogs also secrete subnormal amounts of epinephrine basally or when made hypoglycemic with insulin. These consequences of hypophysectomy develop in spite of concurrent treatment with "replacement" doses of carbohydrate-active steroids, but can be restored to normal by treatment with ACTH.

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[1] Kirshner, N., and M. Goodall, *Biochim. Biophys. Acta*, **24**, 658–659 (1959).
[2] Axelrod, J., *J. Biol. Chem.*, **237**, 1657–1660 (1962).
[3] Wurtman, R. J., and J. Axelrod, *Science*, **150**, 1464–1465 (1965).
[4] Wurtman, R. J., and J. Axelrod, *J. Biol. Chem.*, **241**, 2301–2305 (1966).
[5] Wurtman, R. J., *Endocrinology*, **79**, 608–614 (1966).
[6] Coupland, R. E., *The Natural History of the Chromaffin Cell* (London: Longmans, Green, and Co., Ltd., 1965), pp. 1–278.
[7] Markowitz, J., in *Experimental Surgery* (Baltimore: Williams and Wilkins, 1949), chap. 24, pp. 325–331.
[8] Wurtman, R. J., E. Noble, and J. Axelrod, *Endocrinology*, **80**, 825–828 (1967).
[9] Hume, D. M., and D. H. Nelson, *Surg. Forum*, **5**, 568–575 (1955).
[10] von Euler, U. S., and F. Lishajko, *Acta Physiol. Scand.*, **51**, 348–356 (1951).
[11] Daughaday, W. H., in *Textbook of Endocrinology*, ed. R. H. Williams (Philadelpha: W. B. Saunders, 1962), chap. 2.
[12] Luft, R., and U. S. von Euler, *J. Clin. Endocrinol.*, **16**, 1017 (1956).
[13] Green, W. L., and S. H. Ingbar, *Arch. Internal Med.*, **108**, 945 (1961).
[14] Udenfriend, S., and J. B. Wyngaarden, *Biochim. Biophys. Acta*, **20**, 48 (1956).