Steroid Induction of Phenylethanolamine-N-methyl Transferase in Adrenomedullary Explants: Independence of Adrenal Innervation

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ABSTRACT. Unilateral explants of rat adrenal medullas grown in the anterior chamber of the eye contained very low phenylethanolamine-N-methyl transferase (PNMT) activity. When animals were treated with dexamethasone for 7 days, PNMT activity rose more than 10-fold. These findings suggest that the increase in PNMT activity produced by glucocorticoids does not require an intact adrenal innervation. (*Endocrinology* **86:** 1466, 1970)

THE ABILITY of the adrenal medulla to secrete epinephrine depends upon both neural and hormonal factors. The secretory process, *per se*, is under direct neural control: it is triggered by impulses from sympathetic cholinergic nerves that terminate adjacent to the adrenal chromaffin cells (1–3). In contrast, the chemical composition of the secreted material is regulated by hormonal inputs: the ratio of epinephrine to norepinephrine in adrenal venous blood is variable and depends on the concentration of glucocorticoids perfusing the chromaffin cells (4). The conversion of norepinephrine to epinephrine is catalyzed by phenylethanolamine-N-methyl transferase (PNMT) (5, 6), an enzyme induced by natural or synthetic glucocorticoids (7–9). The concentrations of glucocorticoids needed to maintain optimal PNMT activity are high (9); these concentrations are, however, available to the medulla by virtue of a portal vascular system (10) that allows adrenocortical venous blood to perfuse the medulla without prior dilution. If the preferential delivery of glucocorticoids to the medulla is interrupted [*e.g.*, by removal of the pituitary gland (7) or by administration of low doses of dexamethasone (11), which suppress the secretion of endogenous glucocorticoids], PNMT activity declines, as do the ratios of epinephrine to norepinephrine in the adrenal gland (9) and in adrenal venous blood (4).

Surgical denervation of the adrenal medulla has been reported to cause few if any characteristic changes in the histology of the

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TABLE 1. Catecholamine contents and enzyme activities in explanted and intact adrenal medullas

Group	Dexamethasone dose (mg)	Days injected	Explant				Intact			
			PNMT	MAO	Total CA	EPI	PNMT	MAO	Total CA	EPI
A	0	7	0.22± 0.11	0.30± 0.03	1.73± 0.26	0	12.87± 2.00	6.03± 0.80	14.68± 2.42	13.08± 1.72
B	0.1	7	1.49± 0.21*	0.54± 0.36	1.84± 0.42	0.10± 0.07	10.51± 0.52	4.76± 0.85	19.28± 2.04	17.16± 2.16
C	1.0	3	0.66± 0.04*	0.35± 0.12	3.32± 0.65	0.11± 0.08	11.55± 1.13	6.44± 1.28	19.36± 2.00	17.04± 1.84
D	1.0	7	2.38± 0.35*	0.38± 0.11	4.70± 0.73*	0.15± 0.09	11.72± 0.68	6.83± 1.20	18.08± 2.44	15.96± 1.16

Groups of 7 rats in which the left adrenal medulla had been explanted to the anterior chamber of the left eye 21 days previously received daily injections of dexamethasone or its diluent, as indicated above. The tyrosine transaminase activities in livers from the 4 groups of animals were: A: 76.1 ± 6.7 μmoles/g/hr; B: 204.8 ± 10.0 μmoles/g/hr; C: 208.3 ± 14.7 μmoles/g/hr; and D: 199.3 ± 14.1 μmoles/g/hr. Groups B, C and D all differed significantly ($p < 0.01$) from group A.

Enzyme activities are expressed as nmoles/mg protein/hr. Total catecholamine (CA) and epinephrine (EPI) levels are expressed as μg/mg protein.

* $p < 0.01$ differs from explants of untreated rats.

chromaffin cells; this has been taken as evidence that the nerves to these cells fail to exert a trophic influence on them (12, 13). We have attempted to determine whether the activity of PNMT, a specific adrenomedullary protein, is influenced by neural factors as well as by steroid hormones. To examine the changes in PNMT that follow chromaffin cell denervation, we have utilized not surgically denervated whole adrenal glands but fragments of medulla transplanted to the anterior chamber of the eye. This preparation was chosen because it also deprived the chromaffin cells of the high glucocorticoid concentrations that they would receive *in situ*; it thus allowed us to determine whether PNMT activity could be restored in denervated medullary tissue by treatment with exogenous glucocorticoids.

Materials and Methods

Left adrenal glands were removed from Sprague-Dawley female rats; each medulla was dissected free of cortex under a low-power microscope and was inserted into the anterior chamber of the left eye. The animals were then housed in groups of 3 and given access *ad lib.* to Big Red Rat Chow and water. Light (Vita-Lite bulbs, 100 μwatts/cm²) was provided for 12 hr daily. Twenty-one days after surgery, groups of animals were injected with 0.1 mg/kg dexamethasone phosphate for 3 days or with 1.0 mg/kg dexamethasone phosphate for 3 or 7 days. A control group was injected with the diluent for the dexamethasone. All animals were killed 4 hr after the last injection. The medullary explant, the medulla from the intact adrenal, and a section of liver were removed and frozen at −20 C. PNMT and monoamine oxidase (MAO) activities in adrenal samples were assayed by the methods of Wurtman and Axelrod (8, 14); adrenal catecholamines were assayed spectrophotofluorometrically after extraction onto alumina (15, 16). Hepatic tyrosine transaminase activity was measured by a modification of Diamondstone's method (17, 18); tissue protein concentrations were measured by the method of Lowry (19).

Results and Discussion

Transplanted medullary fragments took on a rich vascularization that was visible through the cornea. In general, the amount of tissue present in the anterior chamber at autopsy was smaller than that originally inserted. This decrease probably resulted from resorption of cells that had been damaged during dissection or insertion into the eye. Tissue specimens fixed in paraformaldehyde-glutaraldehyde were stained with Richardson's stain and examined histologically. They revealed extensive vascularization and the presence of typical chromaffin cells as well as fibroblasts and cortical cells.

Adrenal explants from animals that had not received dexamethasone contained much less PNMT or MAO activity per milligram of protein than intact medullas (Table 1). PNMT activity did not change between days 21 and 28 after transplantation in untreated animals. All 3 dexamethasone treatment regimens produced a partial restoration of PNMT activity without elevating MAO activity; explants from animals receiving 1.0 mg/kg of the steroid for 7 days had at least 10 times more PNMT activity per milligram of protein than did explants from untreated animals (Table 1). These observations suggest that the increase in PNMT activity produced by glucocorticoids does not require that the chromaffin cells concurrently receive neural inputs. The elevation of *in situ* adrenal PNMT activity observed in hypophy-

sectomized rats given glucocorticoids is blocked by concurrent treatment with actinomycin D or puromycin (8). Hence, the increase found in ocular transplants very likely reflects the induction of new enzyme protein, and not simply activation of existing enzyme.

The total catecholamine concentration in explants from untreated animals was also very low and consisted entirely of norepinephrine (Table 1). The higher dose of dexamethasone partially restored the total catecholamine concentration, and both doses caused the appearance of some epinephrine in the explants; however, the epinephrine:norepinephrine ratio remained very low. In the absence of data on the rates at which the explants synthesized and released epinephrine and norepinephrine, it is not possible to explain the disparity between the ability of dexamethasone to elevate PNMT activity in explants and its failure to restore their epinephrine levels. The doses of dexamethasone used in this study had no effect on the PNMT activity, MAO activity, or catecholamine content of intact adrenals (Table 1).

A variety of hepatic enzymes are induced by glucocorticoids (20). Since the cells containing these enzymes receive adrenocortical hormones solely from the peripheral circulation, it would be anticipated that their sensitivity to glucocorticoids would be considerably greater than that of adrenomedullary chromaffin cells. The medullary explants in our experimental animals were no longer perfused by an intra-adrenal portal vascular system and thus, like the liver, received glucocorticoids largely from the arterial blood. This allowed us to compare in the same set of animals the dose-response relationships between steroid concentration and the activities of PNMT and an inducible hepatic enzyme, tyrosine transaminase (20). Hepatic cells were much more sensitive to dexamethasone than were explanted chromaffin cells: 0.1 mg/kg of the synthetic glucocorticoid produced a maximal increase in tyrosine transaminase activity, while 1.0 mg/kg did not restore PNMT activity in explants to normal (Table 1). The relative insensitivity of the PNMT-induction mechanism to glucocorticoids remains unexplained.

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