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NEURONALLY DEPENDENT INDUCTION OF ADRENAL PHENYLETHANOLAMINE-*N*-METHYLTRANSFERASE BY 6-HYDROXYDOPAMINE

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Abstract—Destruction of sympathetic nerve terminals with 6-hydroxydopamine (6-HO-DA) produces a small (19 per cent) but statistically significant increase in adrenal phenylethanolamine-*N*-methyltransferase (PNMT) activity in normal and hypophysectomized rats. This increase can be abolished by transection of the splanchnic nerves supplying the adrenal glands. The restitution of the PNMT activity by dexamethasone in hypophysectomized animals is not affected by adrenal denervation. It is concluded that the normal levels of PNMT in the adrenal medulla are maintained by adrenocortical glucocorticoids whereas an increase above the normal level can be produced by a (reflex) increase in splanchnic nerve activity. PNMT activity could not be detected in the superior cervical ganglion of controls or of animals treated with dexamethasone.

THE CONVERSION of norepinephrine to epinephrine is catalyzed by the enzyme phenylethanolamine-*N*-methyltransferase (PNMT).[1, 2] This enzyme is mainly localized in the adrenal medulla[2] and its activity is controlled in mammals by the secretion of glucocorticoids by the adrenal cortex.[3] The PNMT activity declines rapidly after hypophysectomy, but this decrease can be prevented by administration of adrenocorticotropic hormone (ACTH) or glucocorticoids. However, administration of high doses of ACTH or glucocorticoids does not significantly elevate PNMT activity in normal animals.[4, 5]

Recently it has been shown that 2 days after chemical sympathectomy (destruction of sympathetic nerve terminals) by 6-hydroxydopamine (6-HO-DA) a more than 2-fold increase in the activity *in vitro* of adrenal tyrosine hydroxylase occurs.[6] It was assumed that impairment of postganglionic sympathetic nerve function by 6-HO-DA produces a reflex increase in splanchnic nerve activity which induces the enzyme in the adrenal medulla. This assumption was strongly supported by the fact that transection of the splanchnic nerves supplying the adrenal gland or inhibition of protein synthesis completely abolishes the rise in tyrosine hydroxylase.[7] In addition to this increase in adrenal tyrosine hydroxylase, 6-HO-DA causes a smaller but statistically significant elevation of adrenal PNMT activity.[6] It was the purpose of the present experiment to examine whether this increase in PNMT activity after chemical sympathectomy results from an activation of the pituitary–adrenocortical system, or from a transynaptic induction as in the case of tyrosine hydroxylase.[7] The possibility was also

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investigated as to whether the induction of PNMT by dexamethasone in hypophysectomized animals is only the result of a direct effect of this glucocorticoid on the adrenal medulla or whether an increased splanchnic nerve activity is also involved. We wish to report that the rise in PNMT activity produced by 6-HO-DA is still present in hypophysectomized animals and can be abolished by adrenal denervation. In addition, the induction of PNMT by dexamethasone in hypophysectomized animals is unaltered by adrenal denervation.

METHODS

Sprague–Dawley, male rats (normal, hypophysectomized and sham-operated) weighing 110–180 g were obtained from Hormone Assay (Chicagó, Ill.). For individual experiments, the animals were selected so that their weights were within a 20-g range. The normal laboratory diet of the hypophysectomized rats was fortified with oranges.

Adrenal denervation. The nerve fibers leading from the main splanchnic trunk to the left adrenal gland were carefully transected under ether anesthesia. In experiments in which the effect of adrenal denervation on PNMT induction by dexamethasone in hypophysectomized animals was studied, denervation was performed 2 days after hypophysectomy on the day dexamethasone treatment was begun. In experiments in which the effect of 6-HO-DA was examined, splanchnic transection was performed 3 days before giving 6-HO-DA.

Schedule of drug administration. The drugs used in the present study were 6-hydroxydopamine hydrobromide (synthesized by Dr. A. Langmann, Hoffmann-La Roche, Basel) and H-fluoro-16α-methyl-Δ-dihydrocortisol phosphate (dexamethasone). The single doses of 6-HO-DA were given intravenously in 0·2 ml of 0·1 N HCl, followed by 0·5 to 0·7 ml of isotonic saline. Animals with left-side adrenal denervation were injected with 200 mg/kg of 6-HO-DA each at 48 and 24 hr before they were killed. Because of the increased mortality after 6-HO-DA in hypophysectomized animals, both hypophysectomized and sham-operated rats were given only a single dose of of 200 mg/kg 6-HO-DA 8 days after hypophysectomy or sham-operation and 48 hr before they were killed. In experiments in which the effect of adrenal denervation on PNMT induction by dexamethasone was studied, the rats were injected daily for 7 days with 1 mg/kg dexamethasone (intraperitoneally). The first dose of dexamethasone was given immediately after adrenal denervation.

Assay of phenylethanolamine-N-methyl transferase. The animals were killed by a blow on the head and the adrenals were rapidly removed and homogenized in 2 ml of ice-cold 0·25 M sucrose. In experiments in which the innervation of the adrenals was intact, both adrenals were pooled but in denervation experiments, left and right adrenals were homogenized separately in ground-glass homogenizers. Pairs of cervical ganglia were homogenized in 0·5 ml of 0·25 M sucrose. The homogenates were centrifuged for 10 min at 27,000 *g* using a Sorvall RC-2 refrigerated centrifuge. PNMT activity was assayed in the supernatant fraction according to a previously described method,[2] using phenylethanolamine as the substrate.

RESULTS

The effect of hypophysectomy on PNMT induction by 6-HO-DA. In previous studies it has been shown that chemical sympathectomy by 6-HO-DA produces not only a marked increase in adrenal tyrosine hydroxylase but also a consistent although

smaller increase in PNMT activity.[6] In confirmation of these results, pretreatment with 6-HO-DA produced a statistically significant increase in adrenal PNMT of sham-operated rats (Table 1). Ten days after hypophysectomy, the PNMT activity was reduced by 70 per cent control. However, the percentage increase in PNMT activity elicited by 6-HO-DA was about the same in sham-operated (+ 21 per cent) and hypophysectomized (+ 17 per cent) animals.

The effect of adrenal denervation on the increase in PNMT produced by chemical sympathectomy. As shown in Table 2, the small but significant increase in PNMT

TABLE 1. EFFECT OF HYPOPHYSECTOMY ON THE INCREASE IN PNMT ACTIVITY PRODUCED BY 6-HO-DA*

Treatment	Sham-operated	Hypophysectomized
	(mμmoles/pr/hr)	
None	21·1 ± 0·7	8·56 ± 0·33
6-HO-DA	25·6 ± 1·4†	10·0 ± 0·38‡

*The animals were injected with 200 mg/kg 6-HO-DA 8 days after hypophysectomy or sham-operations and killed 48 hr after the administration of 6-HO-DA. PNMT activity is expressed in μmoles product formed per pair of adrenals per hour. The values given represent the mean ± S.E. of five to seven animals.
†$P < 0.01$.
‡$P < 0.001$.

TABLE 2. EFFECT OF ADRENAL DENERVATION ON THE INCREASE IN PNMT ACTIVITY PRODUCED BY 6-HO-DA*

Treatment	Innervated	Denervated
	(mμmoles/adr/hr)	
None	8·05 ± 0·34	7·92 ± 0·66
6-HO-DA	9·30 ± 0·48†	8·40 ± 0·28

*The left adrenal gland was denervated under ether anesthesia 3 days before treatment with 6-HO-DA was begun. The animals were given 200 mg/kg of 6-HO-DA, i.v., 48 and 24 hr before they were killed. The PNMT activity is expressed in mμmoles per pair of adrenals per hour. The values given represent the mean ± S.E. of six animals.
†$P < 0.05$.

activity produced by 6-HO-DA was abolished by adrenal denervation. This indicates that a reflex increase in splanchnic nerve activity after chemical sympathectomy is not only responsible for the increase in adrenal tyrosine hydroxylase,[6, 7] but also for the increase in PNMT.

The effect of adrenal denervation on dexamethasone-induced increase in adrenal PNMT of hypophysectomized rats. Hypophysectomized and sham-operated rats with left-side adrenal denervation were treated with 1 mg/kg dexamethasone given daily intraperitoneally for 7 days. As to be expected from previous experiments,[3,4] dexamethasone, as numerous other glucocorticoids,[8] increase the PNMT activity several-

fold (Table 3). However, no difference in the increase of innervated and denervated adrenals could be detected, indicating that the increase in adrenal PNMT activity of hypophysectomized animals treated with dexamethasone results from a direct effect of the glucocorticoid on the adrenal medulla and that an increase in splanchnic

TABLE 3. EFFECT OF ADRENAL DENERVATION ON THE PNMT INDUCTION IN HYPOPHYSECTOMIZED RATS BY DEXAMETHASONE*

Treatment	Innervated	Denervated
	(mμmoles/adr/hr)	
Hypophysectomized	3·8 ± 0·2	4·2 ± 0·3
Hypophysectomized + dexamethasone	8·9 ± 0·5	8·0 ± 0·5
Sham-operated	10·9 ± 0·3	10·6 ± 0·8
Sham-operated + dexamethasone	9·7 ± 0·4	9·2 ± 0·6

*Adrenal denervation was performed 2 days after hypophysectomy or sham-operation. The animals were treated for 2 days, beginning on the day of denervation, with 1 mg/kg dexamethasone, intraperitoneally. They were killed 24 hr after the last dose. PNMT activity is expressed in mμmoles per adrenal per hour. The values given represent the mean ± S.E. of five to seven animals.

nervous activity is not involved. In accordance with previous experiments[4, 5] dexamethasone did not increase the adrenal PNMT activity in sham-operated rats.

Lack of PNMT activity in the superior cervical ganglion of the rat. Since the superior-cervical ganglion of the rat contains cells which both by fluorescence[9] and electron microscopy[10] resemble the chromaffin cells of the adrenal medulla, we investigated also the PNMT activity of this organ which is embryologically related to the adrenal medulla. In spite of the high sensitivity of the assay which allows the detection of enzyme activities as low as 10 μμmoles product formed per hour, no PNMT activity could be detected in the homogenates of the superior cervical ganglia of either controls or rats treated with 1 mg per kg dexamethasone for 7 days.

DISCUSSION

The present experiments have shown that the increase in adrenal PNMT activity caused by chemical sympathectomy with 6-HO-DA results from a reflex increase in splanchnic nerve activity rather than from an activation of the pituitary–adrenocortical system, since the increase in PNMT activity could be abolished by adrenal denervation, but was still present in hypophysectomized rats. The increase in splanchnic activity could produce an increase in PNMT activity by a direct effect on the adrenal medulla or by an increased synthesis or release of adrenocortical hormones which are delivered to the intra-adrenal portal system and which reach the cells of the adrenal medulla in high concentrations. However, this latter possibility seems to be unlikely since the cells of the adrenal cortex have no autonomic innervation.[11] 6-HO-DA does not alter adrenal catecholamine content,[6] and the rapid decomposition and metabolism of 6-HO-DA precludes interference with the enzyme assay 48 hr later.

The pituitary–adrenocortical system seems to be of primary importance for the maintenance of the normal level of adrenal PNMT activity, since hypophysectomy leads to a drastic decrease within a few days.[3, 4] However, the increase in PNMT activity above the normal level after 6-HO-DA appears to be due to an increased splanchnic nerve activity since it can be blocked by adrenal denervation. In addition, an increase cannot be achieved by very high doses of ACTH or glucocorticoids.[4, 5] The increase in PNMT activity after chemical sympathectomy with 6-HO-DA was considerably smaller than that of tyrosine hydroxylase.[4] On the other hand, the reduction of tyrosine hydroxylase in hypophysectomized animals is much smaller than that of PNMT and cannot be reversed even with high doses of dexamethasone which return the PNMT activity to normal (unpublished observations). It therefore seems that the activity of the adrenal tyrosine hydroxylase is mainly regulated by the activity of the splanchnic nerves and is less sensitive to glucocorticoids than is PNMT. In contrast the normal level of PNMT is maintained by adrenal glucocorticoids, but an increase above the normal level can only be effected by an increase in splanchnic nerve activity.

In bacterial systems the genetic information of enzymes involved in a given metabolic pathway is located in adjacent chromosomal areas forming an operational unit which is governed as a whole by factors determining induction and repression.[12] If the genetic information of the enzymes concerned with epinephrine synthesis in the adrenal medulla is similarly arranged and controlled one would expect that all enzymes involved in epinephrine synthesis would be altered similarly by changes in cell function which affect the genetic level. Neuronal induction of tyrosine hydroxylase[13] and hormonal induction of PNMT[4] are blocked by actinomycin D, which might indicate that changes in transcription are involved in altering the amount of enzyme. Since both the first (tyrosine hydroxylase) and the last (PNMT) enzyme of the metabolic pathway are affected by alterations in both hormonal and neuronal function it is not unlikely that the two enzymes in between (DOPA decarboxylase and dopamine β-hydroxylase) might be similarly regulated. Evidence has already been presented that adrenal dopamine β-hydroxylase activity is influenced by alterations in nerve activity.[14]

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