MOLECULAR PHARMACOLOGY, 8, 511-520

Regulation of Adrenal Phenylethanolamine *N*-Methyltransferase Activity in Three Inbred Mouse Strains

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(Received May 30, 1972)

SUMMARY

The regulation of phenylethanolamine *N*-methyltransferase (EC 2.1.1) has been studied in the adrenal glands of three inbred mouse strains: DBA/2J, C57BL/Ka, and CBA/J. Although the pituitary gland is involved in the regulation of enzyme activity in each strain, the mechanism of regulation differs between strains. In the DBA/2J strain, both glucocorticoid and phenoxybenzamine administration as well as cold exposure were effective in increasing enzyme activity. These results suggest that the DBA/2J strain enzyme is under both glucocorticoid and neuronal control. In the C57BL/Ka strain, only cold exposure and ACTH were effective in phenylethanolamine *N*-methyltransferase induction. Exogenous glucocorticoid administration had no effect on enzyme activity. Hypophysectomy both reduced control enzyme levels and abolished the enzyme response to cold exposure. We therefore propose that ACTH exerts a regulatory effect on the enzyme without the mediation of adrenal glucocorticoids. No evidence for neuronal control of phenylethanolamine *N*-methyltransferase activity has been found in the C57BL/Ka strain. In the CBA/J strain, hypophysectomy virtually abolished the enzyme activity. Activity could be partially restored by ACTH or dexamethasone administration. Aminoglutethimide blocked the enzyme response to ACTH but not to dexamethasone, suggesting that the enzyme response to ACTH is mediated via glucocorticoids. No evidence for neuronal control of phenylethanolamine *N*-methyltransferase could be found in the CBA/J strain. The half-life of the enzyme was estimated to be 1 hr in the DBA strain, 3 hr in the C57BL/Ka strain, and 7 hr in the CBA/J strain. The rate of increase of enzyme activity following induction is 10-fold greater in the DBA/2J strain than in either the C57BL/Ka or CBA/J strains. These results suggest that there are qualitative as well as quantitative differences in genetic regulation of phenylethanolamine *N*-methyltransferase activity.

INTRODUCTION

Phenylethanolamine *N*-methyltransferase (EC 2.1.1) is the enzyme involved in the biosynthesis of epinephrine (1). This enzyme is localized primarily in the mammalian adrenal medulla (1), although much smaller amounts have been reported in the brain (2-4). In the rat, phenylethanolamine *N*-methyltransferase is under the control of adrenal glucocortocoids (5) and, to a lesser

This work was supported by Public Health Service Grant MH 16,632 from the National Institute of Mental Health and Contract N00014-67-A-0112-0027 with the Office of Naval Research.

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extent, by impulses from the splanchnic nerves to the adrenal medulla (6). Enzyme activity declines following hypophysectomy, and can be restored with either ACTH, hydrocortisone, or dexamethasone (5). The increase in enzyme activity is blocked by protein synthesis inhibitors, suggesting that its induction is mediated via glucocorticoids (5). The time course of enzyme elevation after dexamethasone follows a hyperbolic profile in the rat, suggesting that dexamethasone induction is achieved by increasing the rate of synthesis of the enzyme rather than by inhibiting its degradation (7). The half-life of phenylethanolamine *N*-methyltransferase in the hypophysectomized rat has been estimated to be 6 days (7).

Phenylethanolamine *N*-methyltransferase activity cannot be elevated in the intact rat by chronic glucocorticoid administration (5, 7) or by conditions which acutely activate the pituitary-adrenal axis (8). Extraordinary conditions of prolonged ACTH increase, such as implantation of an ACTH-secreting tumor (9), unilateral adrenalectomy (10), or chronic intermittent restraint stress (11), are required before an increase in enzyme activity is seen.

Conditions which activate the splanchnic nerves to the adrenal medulla also cause an elevation of phenylethanolamine *N*-methyltransferase activity in the intact rat. 6-Hydroxydopamine, an agent which destroys sympathetic nerve endings, causes splanchnic nerve activation, inducing enzyme activity (12). Similar effects are observed after chronic reserpine administration (7, 13). In the intact rat, the enzyme response to 6-hydroxydopamine or reserpine occurs slowly over a time course similar to that observed in situations of chronic pituitary-adrenal activation.[3] These considerations have led to the suggestion that the control systems operant on epinephrine synthesis in the rat are related to adaptation to chronic stress (10).

During the course of our work in inbred mouse strains (14, 15), it became apparent that the rate of increase of phenylethanolamine *N*-methyltransferase was extremely rapid in the mouse (16). Differences in the rates of response between inbred mouse strains were also observed. Further investigation demonstrated that the transferase may be controlled by different mechanisms in different mouse strains.

[3] R. D. Ciaranello and J. D. Barchas, unpublished observations.

MATERIALS AND METHODS

Animals

Mice of the DBA/2J and CBA/J strains were purchased from Jackson Memorial Laboratory, Bar Harbor, Maine, and C57BL/Ka strain animals were obtained from the Department of Radiology, Stanford Medical Center. Unless otherwise indicated, all experiments were performed on male mice weighing 20–25 g at the time of study. All animals were housed in a soundproof facility on a cycle of 13 hr light–11 hr dark, and were maintained on diets of Purina laboratory chow (4% fat) and water ad libitum. Mice were hypophysectomized by the parapharyngeal route. Since hypophysectomized mice have difficulty in maintaining body temperature, both hypophysectomized and sham-operated mice were maintained in a controlled environment at 27° and 37% humidity on a cycle of 13 hr light–11 hr dark. The animals were studied at intervals following surgery as indicated under RESULTS. The authenticity of hypophysectomy was verified by inspection of the sella turcica at autopsy.

Cold exposure studies were carried out in a cold room maintained at 4°. Animals were housed in bedding-free, stainless steel cages (12 × 7 × 6 inches) in groups no larger than three.

Drugs

Dexamethasone phosphate (Decadron) was obtained from Merck Sharp & Dohme. Phenoxybenzamine hydrochloride (Dibenzyline) was the generous gift of Smith Kline & French Laboratories. Dibutyryladenosine cyclic 3,5-monophosphate (dibutyryl cyclic AMP) was purchased from Sigma. ACTH (Acthar gel) was obtained from Armour and Company. Cycloheximide (Actidione) was obtained from the Upjohn Company. Pentolinium tartrate (Ansolysen) was obtained from Wyeth Laboratories. Aminoglutethi-

mide was the generous gift of Dr. Joan Vernikos-Danellis, NASA–Ames Research Laboratories, Moffett Field, Cal. NSD-1055 (Brocresine) was the generous gift of Lederle Laboratories, Pearl River, N.Y.

Enzyme Assays

Phenylethanolamine *N*-methyltransferase activity was measured by a modification of the method of Axelrod (1) described by Deguchi and Barchas (4). Mouse adrenals were homogenized either singly or in pairs in ice-cold 0.32 M sucrose (0.3 ml/gland). *S*-[*methyl*-^{14}C]Adenosylmethionine (47.1 mCi/mmole, New England Nuclear; 54 mCi/mmole, Amersham-Searle) was used as methyl donor at a concentration of 7 μM. The assay was otherwise identical with the procedure previously described.

Monamine oxidase activity was estimated by the technique of Wurtman and Axelrod (17), using [^{14}C]tryptamine (8.8 mCi/mmole, New England Nuclear) as substrate.

Adrenal tyrosine hydroxylase was measured by a previously described modification (14) of the method of Levitt.

Statistical Tests

Results were analyzed using the two-tailed Student *t*-test.

RESULTS

Effects of cold exposure on phenylethanolamine N-methyltransferase activity. To determine whether acute stress would elevate phenylethanolamine *N*-methyltransferase activity, animals of the three strains were subjected to cold exposure. After 3 hr the DBA/2J strain responded with a 1.32-fold increase in enzyme activity (Table 1). Figure 1 shows the time course of the enzyme response to cold exposure in the C57BL/Ka and CBA/J strains. Although basal activities differed (14, 15), the rates of increase (units per adrenal pair per hour) were identical in the C57BL/Ka and CBA/J strains. These results suggested that the enzyme responds rapidly to exogenous stimuli in these mouse strains. Accordingly, studies were undertaken to determine the mechanism of control of phenylethanolamine *N*-methyltransferase in each strain.

TABLE 1

Effects of cold exposure in DBA/2J mice

Groups of nine DBA/2J mice were exposed to cold for 3 hr and then killed. The adrenal glands were removed, and their phenylethanolamine *N*-methyltransferase activity was determined (see MATERIALS AND METHODS). Enzyme units are the formation of 1 nmole of *N*-[*methyl*-^{14}C]phenylethanolamine per hour, and are means and standard errors.

Treatment	Phenylethanolamine *N*-methyltransferase	*p*
	unit/adrenal pr	
Control	0.268 ± 0.010	
3-hr cold exposure	0.352 ± 0.015	<0.001

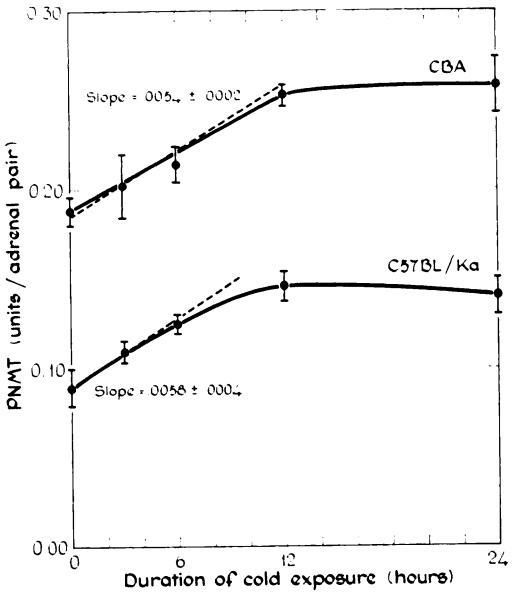

FIG. 1. *Effects of cold exposure in C57BL/Ka and CBA/J mice*

Groups of nine mice were exposed to cold for various times, after which they were immediately killed and their adrenal glands were removed. The dashed line drawn through the linear portion of each curve was obtained by linear regression analysis. A computer program which derives the slope of this line and its standard error was used to calculate the rate of increase of the enzyme in each strain. A significant ($p < 0.001$) elevation above control was seen at 3 hr and thereafter in the C57BL/Ka strain, and at 12 hr ($p < 0.001$) and 24 hr ($p < 0.01$) in the CBA strain. PNMT, phenylethanolamine *N*-methyltransferase.

TABLE 2

Effects of dexamethasone treatment on phenylethanolamine N-methyltransferase activity in inbred mouse strains

Dexamethasone (5 mg/kg) or phenoxybenzamine (10 mg/kg) was administered intraperitoneally to groups of mice. All dilutions were prepared freshly in 0.9% NaCl and discarded immediately after use to ensure maximum potency of the drugs. Control animals received an equivalent volume of NaCl. Untreated controls (not shown here) were also studied; they were no different from the NaCl-treated animals. Enzyme activity units are the means and standard errors of 8–10 animals/group.

Strain	Treatment	Phenylethanolamine *N*-methyltransferase	*p*
		unit/adrenal pr	
DBA/2J	Control	0.243 ± 0.016	
	3-hr dexamethasone	0.320 ± 0.014	<0.01
C57BL/Ka	Control	0.143 ± 0.010	
	3-hr dexamethasone	0.149 ± 0.009	NS[a]
	12-hr dexamethasone	0.121 ± 0.005	<0.05
	24-hr dexamethasone	0.127 ± 0.011	NS
DBA/2J	Control	0.173 ± 0.011	
	Phenoxybenzamine	0.217 ± 0.014	<0.02
C57BL/Ka	Control	0.125 ± 0.003	
	Phenoxybenzamine	0.110 ± 0.006	NS

[a] Not significant.

Effects of dexamethasone and phenoxybenzamine injections. To learn whether exogenous glucocorticoid administration would elevate enzyme activity, groups of DBA/2J and C57BL/Ka mice were treated with dexamethasone. The DBA/2J strain responded with a 1.3-fold increase after 3 hr of steroid treatment (Table 2). The C57BL/Ka strain failed to respond to dexamethasone despite repeated demonstrations of enzyme elevation by cold exposure. Increasing the dose of dexamethasone to 40 mg/kg did not elevate the enzyme.

To determine whether the transferase activity was influenced by reflex neuronal stimuli, groups of DBA/2J and C57BL/Ka mice were treated with the *alpha* adrenergic blocking agent phenoxybenzamine. This agent causes systemic hypotension which results in reflex activation of the nerves to the adrenal medulla (18). Only the DBA/2J strain responded to this drug (Table 3), suggesting that both hormonal and neuronal stimuli affect phenylethanolamine *N*-methyltransferase activity in this strain. The C57BL/Ka animals showed no response to phenoxybenzamine at 10 mg/kg or 20 mg/kg.

Regulation of Phenylethanolamine N-methyltransferase in C57BL/Ka Strain

Role of pituitary and exogenous ACTH. Although phenylethanolamine *N*-methyltransferase in the C57BL/Ka strain did not respond to either phenoxybenzamine or dexamethasone, it showed a clear-cut response to cold exposure. Recent reports (19, 20) have emphasized the possible role of ACTH in directly regulating catecholamine enzyme activity. Accordingly, the levels of phenylethanolamine *N*-methyltransferase and its response to cold exposure were tested in mice which had been hypophysectomized 5 days earlier. As shown in Fig. 2, hypophysectomy reduced the enzyme levels to approximately two-thirds of the intact control values; the increase in activity seen after cold exposure was abolished in hypophysectomized animals. To test whether exogenous ACTH would affect enzyme activity, 1 IU of ACTH in 1% gelatin was injected subcutaneously into groups of

TABLE 3

Effects of cycloheximide on dexamethasone induction of phenylethanolamine N-methyltransferase activity in DBA/2J mice

Groups of 8–10 DBA/2J mice were treated intraperitoneally with 5 mg/kg of dexamethasone, 50 mg/kg of cyclohexmide, or the two drugs simultaneously, and killed 3 hr later. Results are expressed as means and standard errors.

Treatment	Phenylethanolamine N-methyltransferase	p
	unit/adrenal pr	
Control	0.349 ± 0.011	
Dexamethasone	0.462 ± 0.027	0.01
Cycloheximide	0.373 ± 0.011	NS[a]
Dexamethasone + cycloheximide	0.371 ± 0.025	NS

[a] Not significant

normal C57BL/Ka mice that were killed 6 hr later. ACTH produced a 1.35-fold increase in enzyme activity (control = 0.111 ± 0.005, ACTH-treated = 0.151 ± 0.010 units/adrenal pair; $p < 0.01$). Dibutyryl cyclic AMP (50 mg/kg) had no effect on enzyme activity.

To determine whether ACTH caused induction of phenylethanolamine N-methyltransferase, groups of C57BL/Ka mice were treated with either 1 IU of ACTH, 50 mg/kg of cycloheximide, or the two drugs in combination (cycloheximide followed by ACTH within 10–15 min). The results are shown in Table 4. Cycloheximide itself caused a slight increase in enzyme activity. The combination of cycloheximide and ACTH resulted in a return of the enzyme to control levels. Cycloheximide also blocked the cold-induced rise in phenylethanolamine N-methyltransferase. Both ACTH and cold stress appeared to increase enzyme activity by stimulating protein synthesis.

Effects of aminoglutethimide and pentolinium. ACTH appeared to induce phenylethanolamine N-methyltransferase directly in the C57BL/Ka strain, while neuronal stimuli were not effective. To test these propositions further, groups of C57BL/Ka mice were treated with 100 mg/kg of aminoglutethimide, a blocker of glucocorticoid biosynthesis (21). Another group was treated

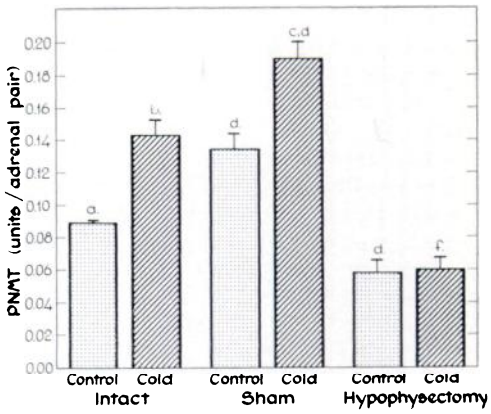

FIG. 2. *Role of pituitary in regulation of phenylethanolamine N-methyltransferase (PNMT) in C57BL/Ka mice*

Groups of C57BL/Ka mice were hypophysectomized 5 days prior to study and maintained in a controlled environment (see MATERIALS AND METHODS). Sham-operated animals were similarly treated. Intact animals were housed in the main animal facility as described in the text. On the day of study, the animals were subjected to cold exposure for 6 hr. This condition was extremely stressful to the hypophysectomized animals, and some appeared so debilitated that the animals were killed earlier than the intended 6 hr. The enzyme values are the means and standard errors for groups of the following sizes: intact—nine animals in each group; sham-operated—five animals in each group; hypophysectomized—six controls, five cold-stressed. *a*, $p < 0.01$ difference from appropriate nonstressed control (sham or intact); *b*, $p < 0.001$ difference from appropriate nonstressed control (sham or intact); *c*, $p < 0.01$ difference from nonstressed sham; *d*, $p < 0.001$ difference from nonstressed intact; *f*, $p < 0.011$ difference from cold-stressed intact.

with 10 mg/kg of pentolinium tartrate, a ganglionic blocking agent. Some of these animals were then exposed to cold for 6 hr. Table 5 shows that neither of these agents blocked the effects of cold exposure. Aminoglutethimide, which causes compensatory ACTH hypersecretion, caused a significant increase in enzyme activity. Pentolinium neither blocked the response to cold nor depressed basal enzyme levels.

Regulation of phenylethanolamine N-methyltransferase in CBA/J Mice

Role of pituitary. Phenylethanolamine N-methyltransferase in the CBA/J strain

TABLE 4

Effects of cycloheximide on ACTH- or cold stress-mediated rise in phenylethanolamine N-methyltransferase in C57BL/Ka mice

Groups of eight or nine C57BL/Ka mice were treated subcutaneously with 1 IU of ACTH or intraperitoneally with 50 mg/kg of cycloheximide, or with cycloheximide followed by ACTH. Six hours following drug administration the mice were killed and their phenylethanolamine *N*-methyltransferase activities were determined. In the cold stress experiment groups of nine C57BL/Ka mice received 50 mg/kg of cycloheximide intraperitoneally. One hour later they were subjected to cold exposure for 6 hr. A second groups was subjected to cold exposure for 6 hr without drug treatment. Unstressed animals served as controls. Results are expressed as means and standard errors.

Treatment	Phenylethanolamine *N*-methyltransferase	*p*
	unit/adrenal pr	
Control	0.122 ± 0.008	
ACTH	0.163 ± 0.006	<0.001
Cycloheximide	0.145 ± 0.005	<0.02
ACTH + Cycloheximide	0.147 ± 0.010	NS[a]
Control	0.125 ± 0.003	
Cold stress	0.156 ± 0.013	<0.02
Cold stress + cycloheximide	0.127 ± 0.005	NS

[a] Not significant.

showed a statistically significant response to cold exposure only after 12 hr (Fig. 1). To study the role of the pituitary gland in this strain, animals were hypophysectomized and their phenylethanolamine *N*-methyltransferase levels were determined 8 days after surgery. At this time enzyme levels were reduced markedly (Table 6). To assess the importance of ACTH and glucocorticoids in maintenance of enzyme activity, groups of hypophysectomized CBA/J mice were treated with ACTH or dexamethasone. Another group of hypophysectomized CBA/J mice was treated with 10 mg/kg of phenoxybenzamine. Animals were killed 12 hr later. Both ACTH and dexamethasone were effective in partially restoring enzyme levels, while phenoxybenzamine had no effect (Table 6).

Locus of action of ACTH in CBA/J mice. Both ACTH and dexamethasone stimulated phenylethanolamine *N*-methyltransferase activity in hypophysectomized CBA/J mice. ACTH and dexamethasone might act independently, or the enzyme response to ACTH might be mediated through adrenal glucocorticoids. To distinguish between these alternatives, groups of normal CBA/J mice were treated with aminoglutethimide and ACTH or dexamethasone. As shown in Table 7, aminoglutethimide completely blocked the enzyme response to ACTH but had no effect on the response to dexametha-

TABLE 5

Effects of aminoglutethimide or pentolinium on cold stress induction of phenylethanolamine N-methyltransferase in C57BL/Ka mice

Groups of nine C57BL/Ka mice received 100 mg/kg of aminoglutethimide or 10 mg/kg of pentolinium as described under RESULTS. All probability values are compared with unstressed controls. Results are expressed as means and standard errors.

Treatment		Phenylethanolamine *N*-methyltransferase	*p*
Stress	Drug		
		unit/adrenal pr	
None	None	0.090 ± 0.001	
Cold	None	0.125 ± 0.005	<0.001
None	Aminoglutethimide	0.137 ± 0.008	<0.001
Cold	Aminoglutethimide	0.121 ± 0.006	<0.01
None	Pentolinium	0.088 ± 0.005	
Cold	Pentolinium	0.124 ± 0.003	<0.001

TABLE 6

Effects of hypophysectomy and drug treatment on phenylethanolamine N-methyltransferase activity in CBA/J mice

Groups of five or six CBA/J mice were studied. Eight days after surgery, hypophysectomized animals received 1 IU of ACTH in 8% gelatin subcutaneously, 10 mg/kg of phenoxybenzamine intraperitoneally, or 5 mg/kg of dexamethasone intraperitoneally, and were killed 12 hr later. Results are expressed as means and standard errors.

Treatment	Phenylethanolamine N-methyltransferase
	unit/adrenal pr
Intact control	0.188 ± 0.009
Sham hypophysectomy	0.163 ± 0.010[a]
Hypophysectomy	0.025 ± 0.004[b]
Hypophysectomy + ACTH	0.058 ± 0.007
Hypophysectomy + dexamethasone	0.074 ± 0.006[b]
Hypophysectomy + phenoxybenzamine	0.030 ± 0.006[a]

[a] $p < 0.001$ less than sham-hypophysectomized.
[b] $p < 0.001$ greater than untreated hypophysectomized.

TABLE 7

Effects of aminoglutethimide on dexamethasone- and ACTH-mediated increase in phenylethanolamine N-methyltransferase in CBA/J mice

Groups of six or seven mice received 100 mg/kg of aminoglutethimide followed 15–30 min later by the subcutaneous injection of 2 IU of ACTH in 16% gelatin or intraperitoneal injection of 5 mg/kg of dexamethasone. Other animals received ACTH or dexamethasone alone. Animals were killed 12 hr after drug administration. Results are expressed as means and standard errors.

Treatment	Phenylethanolamine N-methyltransferase
	unit/adrenal pr
Control	0.254 ± 0.021
Aminoglutethimide	0.264 ± 0.024
ACTH	0.334 ± 0.012[a,b]
ACTH + aminoglutethimide	0.236 ± 0.010
Dexamethasone	0.331 ± 0.018[c]
Dexamethasone + aminogluthethimide	0.358 ± 0.007[d]

[a] $p < 0.01$ greater than control.
[b] $p < 0.001$ greater than ACTH + aminoglutethimide.
[c] $p < 0.02$ greater than control.
[d] $p < 0.001$ greater than control.

sone. These data were interpreted to mean that the pathway of stimulation was ACTH → glucocorticoids → phenylethanolamine *N*-methyltransferase, and that ACTH acted via glucocorticoids in the induction of the enzyme.

Effects of hypophysectomy and drug treatment on other adrenal medullary enzymes in CBA/J strain. To learn whether other adrenal enzymes involved in catecholamine metabolism would be affected by hypophysectomy, the activities of tyrosine hydroxylase and monoamine oxidase were determined. Tyrosine hydroxylase is the initial and rate-limiting step in catecholamine biosynthesis, while monoamine oxidase is the principal enzyme involved in intraneuronal catecholamine inactivation. The activity of both enzymes was depressed following hypophysectomy (Table 8). Tyrosine hydroxylase activity could be partially restored by ACTH administration, but not by either dexamethasone or phenoxybenzamine. Monoamine oxidase activity was also depressed by hypophysectomy, but could be restored by ACTH administration to levels not statistically different from the control values. Phenoxybenzamine resulted in a further depression of enzyme activity below those found after hypophysectomy.

Regulation of Phenylethanolamine N-methyltransferase Activity in DBA/2J Mice

Effects of cycloheximide. Glucocorticoids are known to induce the synthesis of a number of enzymes. To learn whether induction of phenylethanolamine *N*-methyltransferase occurred in the steroid-responsive DBA/2J strain, groups were treated with either dexamethasone, cycloheximide (an antibiotic inhibitor of protein synthesis), or a combination of the two agents simultaneously. Cycloheximide (Table 3) completely blocked the increase after dexametha-

TABLE 8

Effects of hypophysectomy and drug treatment on adrenal enzyme activity in CBA/J mice

Groups of CBA/J mice were treated as follows. Sham-hypophysectomized and hypophysectomized animals were housed as previously described. Hypophysectomized animals were treated subcutaneously with 1 IU of ACTH in 8% gelatin, intraperitoneally with 10 mg/kg of phenoxybenzamine, or subcutaneously with 5 mg/kg of dexamethasone. The animals were killed 12 hr later and their adrenals were removed. Glands were homogenized in 0.32 M sucrose. Monoamine oxidase determinations were performed on the homogenates (16). Tyrosine hydroxylase activity was estimated by the previously described modification of the method of Levitt (14). Monoamine oxidase units are the formation of 1 nmole of [^{14}C]indoleacetic acid per hour. Tyrosine hydroxylase units are the formation of 1 nmole of [^{14}C]dihydroxyphenylalanine per hour. Results are expressed as means and standard errors.

Treatment	No. of animals	Tyrosine hydroxylase	Monoamine oxidase
		units/adrenal pr	
Sham hypophysectomy	6	2.76 ± 0.21	20.76 ± 0.82
Hypophysectomy	5	1.97 ± 0.15[a]	15.50 ± 0.37[b]
Hypophysectomy + ACTH	6	2.30 ± 0.26	17.93 ± 0.79[c]
Hypophysectomy + phenoxybenzamine	5	2.03 ± 0.16[d]	12.18 ± 0.25[e]
Hypophysectomy + dexamethasone	6	1.91 ± 0.16[a]	13.93 ± 0.40[f,g]

[a] $p < 0.01$ lower than sham-hypophysectomized.
[b] $p < 0.001$ lower than sham-hypophysectomized.
[c] $p < 0.02$ greater than hypophysectomized.
[d] $p < 0.001$ lower than sham-hypophysectomized.
[e] $p < 0.001$ lower than hypophysectomized.
[f] $p < 0.02$ lower than hypophysectomized.
[g] $p < 0.01$ greater than phenoxybenzamine-treated.

sone, suggesting that protein synthesis was involved in the enzyme response to the glucocorticoid.

Time course of dexamethasone induction in DBA/2J mice. Dexamethasone was administered to groups of DBA/2J mice, and the animals were killed at various intervals thereafter. The results (Fig. 3) showed an elevation of enzyme activity lasting up to 24 hr after a single injection of the drug. The persistent elevation of the enzyme may possibly be explained by the recent report of decreased glucocorticoid degradation in this strain (22). Enzyme activity reached a plateau about 1.4 times the zero-time value after 2 hr. Regression analysis of the ascending portion of this curve showed a rate of rise of 0.054 ± 0.004 unit/adrenal pair per hour. This rate is 10 times the rate of rise in the C57BL/Ka and CBA/J strains. By the kinetic criteria defined by Berlin and

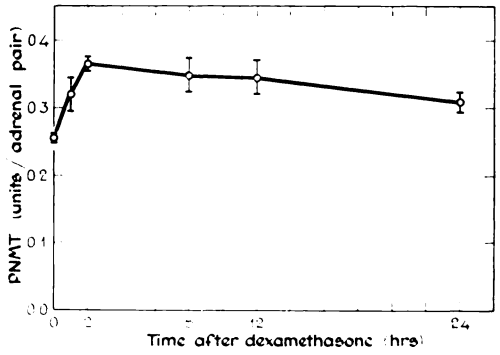

FIG. 3. *Time course of dexamethasone induction of phenylethanolamine N-methyltransferase (PNMT) in DBA/2J mice*

DBA/2J mice were treated intraperitoneally with a single dose of 5 mg/kg of dexamethasone. At various times after drug administration, groups of eight animals were killed. The enzyme value at 1 hr is significantly greater than at zero time ($p < 0.05$) and different from the 2 hr value ($p < 0.05$).

Schimke (23), a half-life of about 1 hr is estimated for phenylethanolamine *N*-methyltransferase in the DBA/2J strain. Similar analysis gives a half-life of about 3 hr for the C57BL/Ka strain and about 7 hr for the CBA/J strain (Fig. 1). Schimke, Sweeney, and Berlin (24) have proposed that the induction of an enzyme along an exponential time course is consistent with increased synthesis of the enzyme rather than inhibition of degradation. These conclusions might be applicable to the curves obtained in Figs. 1 and 3.

DISCUSSION

The finding that short-term exposure to cold resulted in a rapid increase in enzyme activity suggested that the mouse might serve as an excellent model system in which to study the regulation of phenylethanolamine *N*-methyltransferase. Almost all previous studies of this enzyme were done in the rat, in which lengthy periods are required before an effect is observed.

The results above demonstrate considerable heterogeneity in the phenylethanolamine *N*-methyltransferase responses of three inbred mouse strains. All three strains showed a rapid increase in enzyme activity after cold stress. However, the DBA/2J strain responded nearly 10 times more rapidly than the other two. The estimated half-lives range from 1 to 7 hr. Although the pituitary gland plays a role in phenylethanolamine *N*-methyltransferase regulation in each strain, no two strains were identical in their mechanism of regulation.

The DBA/2J strain responded to both glucocorticoids and phenoxybenzamine administration, suggesting both neuronal and hormonal aspects to enzyme control. In a qualitative sense, this strain bears the most resemblance to the rat, in which hormonal and neuronal controls are also operant. Our previous data (14, 15) showed the DBA/2J strain to have almost no measurable adrenal corticosterone. Currently we are investigating further the cold stress response in this strain to determine whether the principal control *in vivo* is exerted via glucocorticoids or nerve impulses.

Both cold exposure and ACTH induced phenylethanolamine *N*-methyltransferase in the C57BL/Ka strain. This effect seemed to be independent of adrenal glucocorticoids, since dexamethasone was ineffective in inducing the enzyme even in extremely high doses, and blockade of glucocorticoid synthesis did not block the enzyme response to cold. Hypophysectomy reduced the enzyme to two-thirds of the controls levels. This was somewhat surprising, since this procedure would be expected to abolish enzyme activity if ACTH were the sole regulator of the enzyme. It is possible that the residual activity represents a genetically distinct pool which is not controlled by ACTH or glucocorticoids. If this notion is true, then evaluation of the biochemical, biophysical, and immunochemical properties of the enzyme in intact and hypophysectomized animals would be fruitful. Another possibility is that some other factor participates in the maintenance of phenylethanolamine *N*-methyltransferase activity in this strain. This hypothetical factor would appear to be involved only in the maintenance of basal enzyme activity, not participating in the response of the enzyme to cold, since hypophysectomy abolished this response. For the same reason, this factor could not be pituitary in origin, nor is it likely to be an ACTH-controlled glucocorticoid. Since ganglionic blockade had no effect on the basal level of the enzyme, this factor would not appear to be neuronal, although this should be proven by adrenal denervation. Until more is learned about the control of phenylethanolamine *N*-methyltransferase in this strain, these notions must be considered speculative.

The principal regulator of phenylethanolamine *N*-methyltransferase in the CBA/J strain appears to be the pituitary-adrenocortical axis. No evidence for a neuronal component of enzyme control could be found in this strain. Hypophysectomy appeared to affect other enzymes in the catecholamine pathway as well. The mechanism of control is unclear for tyrosine hydroxylase and monoamine oxidase, although ACTH appears to be involved.

Inbred mouse strains are the best available system for mammalian biochemical genetic studies. The possibility that the differences in phenylethanolamine *N*-methyltransferase regulation reported here are due to differences in genetic makeup must be considered. While genetic differences in the rates of synthesis (25) and degradation (26) of mouse liver enzymes have been reported, there have been few studies describing qualitative differences in enzyme regulation. Interbreeding the strains studied here should shed light on the genetics of phenylethanolamine *N*-methyltransferase regulation in inbred mouse strains.

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