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Induction or Reduction of Catecholamine Enzymes

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Regulation of Catecholamine Turnover by Variations of Enzyme Levels

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I. Introduction

RECENTLY, evidence that alterations in enzyme levels might be important factors in the regulation of catecholamine turnover has accumulated. This type of regulation differs from that due to the feed-back mechanisms discussed in the previous section. Changes in the levels of enzymes involved in the biosynthesis and metabolism of catecholamines (catecholamine enzymes) (table 1) are characterized by the following findings:

1. The activity *in vitro* of the enzymes is altered (increased or decreased) without activators, inhibitors or variations of cofactors being responsible for the changes. For instance, the induction of tyrosine hydroxylase caused by reserpine or phenoxybenzamine (see below) leads to an increase in the activity *in vitro* of this enzyme (3, 21, 22, 36, 37, 39, 42). In contrast, the enhanced tyrosine hydroxylation by a "classical" feed-back mechanism (as for example during short-term electrical stimulation of adrenergic nerves (1, 44)) is not reflected by a change in the enzyme activity *in vitro*.

2. Inhibitors of protein and nucleic acid synthesis act against the increase of enzyme activity *in vivo*. It has, indeed, been shown that cycloheximide, puromycin (the specificity of which has recently been questioned (31)) and actinomycin D prevent the activation of the catecholamine turnover induced by factors which bring about an enhanced synthesis of catecholamine enzymes by way of increased neuronal activity (20) (see below).

3. Changes in the rate of synthesis of the enzymes may be detected by experimental methods. Thus, it has been shown by immunoabsorption that the synthesis of dopamine-β-hydroxylase is accelerated 3- to 4-fold during drug-induced neuronal stimulation of the adrenal medulla (14).

It is likely that fluctuations in enzyme levels are responsible for the long-term regulatory mechanisms of catecholamine turnover, whereas short-term regulation

TABLE 1

Catecholamine enzymes

Tyrosine hydroxylase
Dopa decarboxylase
Dopamine-β-hydroxylase
Phenylethanolamine-N-methyltransferase
Monoamine oxidase
Catechol-3-O-methyltransferase

may be controlled by changes in the activity of the enzymes without any alteration in their tissue levels ("classical" feed-back). Some factors leading to variations in the levels of catecholamine enzymes will now be briefly reviewed.

II. Hormones

Adrenocorticotrophic hormone (ACTH) and glucocorticoids exert a profound influence on the enzymes involved in catecholamine turnover. For instance, hypophysectomy leads to a marked decrease of phenylethanolamine-N-methyltransferase (PNMT) in the adrenal medulla, the decreased enzyme levels being restored by ACTH and large doses of glucocorticoids (46). Tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) are also diminished by hypophysectomy and can be restored, at least partly, by ACTH, but not by glucocorticoids (24, 45). The increase of adrenal TH, DBH and PNMT seen in situations of chronic stress (15, 17) is partly due to hormonal influences, probably mainly ACTH, although neuronal factors are also involved (see below) (20).

Among other hormones, insulin has been observed to increase TH and also DBH in the adrenal medulla; this effect, however, is probably due to neuronal activation of the adrenal medulla, since insulin does not influence adrenals devoid of their nerve supply (27, 29, 43).

III. Neuronal Influence

Changes in neuronal influence on catecholamine-containing structures, such as the sympathetic nervous system and the adrenal medulla, can also affect the level of catecholamine enzymes. The following different experimental methods for modifying neuronal activity have been used: direct stimulation of nervous structures such as electrical stimulation of the hypothalamus (20) and brain electroshock (26), artificial stress situations such as immobilization stress (17, 37) and cold exposure (32, 35), changes in psychosocial conditions (2), and the application of various drugs. The latter activate the sympathoadrenal or the adrenal system probably by a reflex mechanism, this being due to several actions of the drugs as, for example, interference with the catecholamine storage by reserpine, blockade of *alpha*-adrenergic receptors by phenoxybenzamine, or destruction of the peripheral sympathetic nerve endings by 6-hydroxydopamine (20).

An *increase of neuronal activity* causes an elevation of TH in the central nervous system and of TH and DBH in the sympathetic ganglia, the sympathetically innervated organs (heart and salivary glands) and the adrenal

medulla. In the latter, PNMT is also increased (3, 10, 15–17, 20–23, 26, 32, 35–39). It is of special interest that changes in catecholamine enzymes may be due to psychological factors. Thus, psychosocial stimulation was shown to increase markedly the activity of PNMT, monoamine oxidase and TH in the adrenals of mice (2).

The rise of enzyme levels is not only prevented by inhibitors of protein and nucleic acid synthesis (see above), but also by isolating the tissues in which the enzyme is synthesized from nerve impulses. Thus, decentralization of the superior cervical ganglion or the use of ganglionic blocking agents prevents the drug-induced increase of TH and DBH in this ganglion (21, 25, 36). Similarly, the increase in adrenal PNMT due to reserpine, 6-hydroxydopamine and hypothalamic stimulation and the enhancement by drugs of TH in this organ are blocked by adrenal denervation (15, 16, 20, 28, 37, 38).

Induction of catecholamine synthesizing enzymes has even been demonstrated *in vitro*. In fact, in sympathetic ganglia maintained in organ culture, an elevated external concentration of K^+ causes an increase of TH activity which can be blocked by cycloheximide (30).

A diminution of catecholamine enzymes as a possible consequence of a *decreased neuronal activity* has also been observed, though less frequently than the increase of enzymes after neuronal activation. For instance, mice raised in isolation showed diminished PNMT and TH activity of the adrenals which is thought to be due to a decrease in enzyme synthesis (2).

It is conceivable that some changes in catecholamine enzyme levels due to neuronal influences are mediated by acetylcholine. In fact, this hormone has been demonstrated to enhance markedly the activity of TH in the intact and denervated adrenal glands of insulin-treated and reserpinized rats. The effect of acetylcholine on DBH, however, is less pronounced (27, 28).

IV. L-Dopa

Prolonged administration of high doses of L-dopa to rats leads to a diminished activity of TH in the adrenals; this seems to be due to a decrease in the levels of this enzyme. L-Dopa also prevents the reserpine-induced rise in adrenal TH. These effects are probably connected with an increased formation of noradrenaline from the precursor L-dopa, the latter being converted to the amine without passing through the rate-limiting step, namely tyrosine hydroxylation (11, 12). An enhanced synthesis and release of noradrenaline may diminish the hydroxylation of tyrosine by a negative feed-back mechanism and lead to a diminution of TH synthesis if the feed-back lasts for a prolonged period of time.

V. Nerve Growth Factor

This factor which enhances growth and differentiation of sympathetic neurons has a profound effect on some catecholamine enzymes. Thus, treatment of newborn rats with nerve growth factor for 10 days causes in the superior cervical ganglion a selective induction of TH and DBH which is much more pronounced than the increase in volume of the ganglion. Other enzymes involved in cate-

cholamine metabolism, like dopa decarboxylase and monoamine oxidase, rise only in proportion to the increase of ganglionic volume (34). It is of interest that a non-catecholamine enzyme, choline acetyltransferase, is also induced by the nerve growth factor. This induction is thought to be due to an indirect, retrograde effect on the preganglionic cholinergic nerve terminals brought about by an increased growth and differentiation of the adrenergic neurons (40).

VI. Other Factors

A. 6-Hydroxydopamine in brain

The long-lasting diminution of noradrenaline in sympathetically innervated organs and brain induced by 6-hydroxydopamine is attributed to a degeneration of noradrenergic nerve terminals as demonstrated by electron microscopy (fig. 1B) (19). It has been suggested that this effect arises from an accumulation in the nerve terminals of 6-hydroxydopamine, a strong reducing agent, this leading to denaturation of biological macromolecules (41). It is of interest that relatively small doses of 6-hydroxydopamine injected into the cerebral ventricles of rats cause biochemical changes in the brain without detectable ultrastructural damage (5, 6). For instance, a single intraventricular application of 200 μg of 6-hydroxydopamine leads to a long-lasting decrease of noradrenaline and TH activity measured *in vitro* in various brain parts. The diminution in activity of

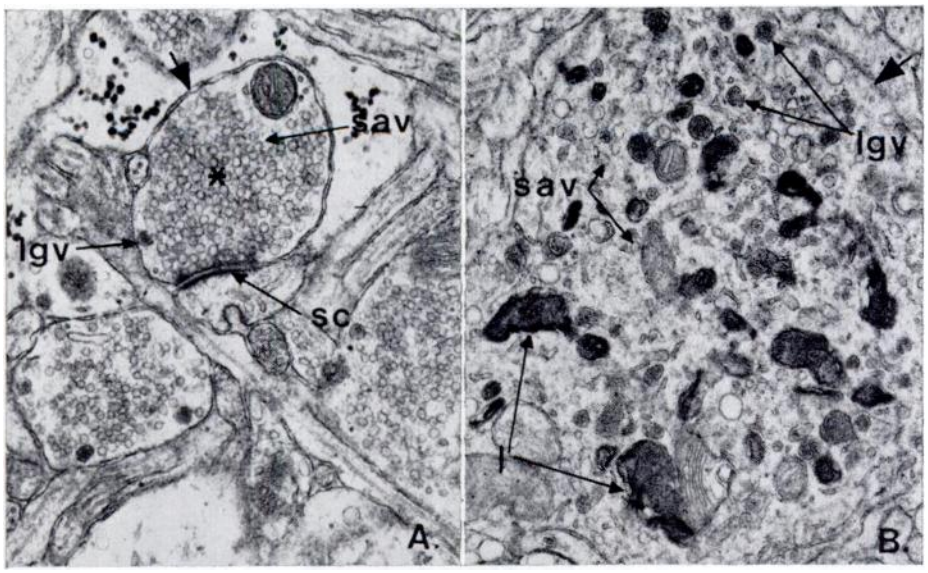

FIG. 1. Ultrastructural aspects of the hypothalamus periventricularis of rat 48 hr after 200 μg (A) and 2 × 200 μg (B) of 6-hydroxydopamine. Large arrow, neuronal cell membrane delineating terminal (A) or preterminal (B) neuronal profiles; l, lysed mitochondria; lgv, large granular vesicles; sav, small agranular vesicles; sc, synaptic contact; *, normal nerve terminal. × 18,000. Notice the presence of ultrastructural damage in figure B, but not in A. (6)

the enzyme parallels that of noradrenaline in the medulla oblongata and the rest of the brain. In the hypothalamus, however, the decrease of noradrenaline is more pronounced than that of TH (fig. 2) (7). On electron microscopy, no ultrastructural changes are to be seen in the brain (fig. 1A) (41). The decrease of TH after small doses of 6-hydroxydopamine might be the first manifestation of damage induced by the drug before changes become apparent on electron microscopy. It remains to be elucidated whether a diminution of enzyme levels due to the formation of metabolites such as 6-hydroxydopamine plays a role under physiological and pathophysiological conditions or during drug therapy, as with high doses of L-dopa.

B. Tyrosine aminotransferase

Tyrosine aminotransferase (L-tyrosine: 2-oxyglutarateaminotransferase, TAT) might be involved in regulating the availability of the catecholamine precursor, tyrosine. The enzyme has been shown to be influenced by several agents (18), among them being catecholamines with a biphasic effect (8, 9). Thus, in adrenalectomized as in hypophysectomized rats, noradrenaline causes an initial increase in hepatic TAT activity followed by a fall below control levels. The initial rise in levels of the enzyme is blocked by cycloheximide, thus indicating dependence on protein synthesis. The suppressing effect of noradrenaline, which appears mainly at night, the time of peak enzyme levels, seems to be due to the interference of noradrenaline with pyridoxal 5′-phosphate, a cofactor of TAT. As a consequence, the synthesis of the apoenzyme might be decreased. A rise of TAT activity by various catecholamines has also been demonstrated in normal animals (fig. 3). Simultaneously, the plasma tyrosine levels decrease, and the urinary excretion of parahydroxyphenylpyruvic acid, resulting from transamination of tyrosine, increases (fig. 4) (4). It has been shown that brain 5-

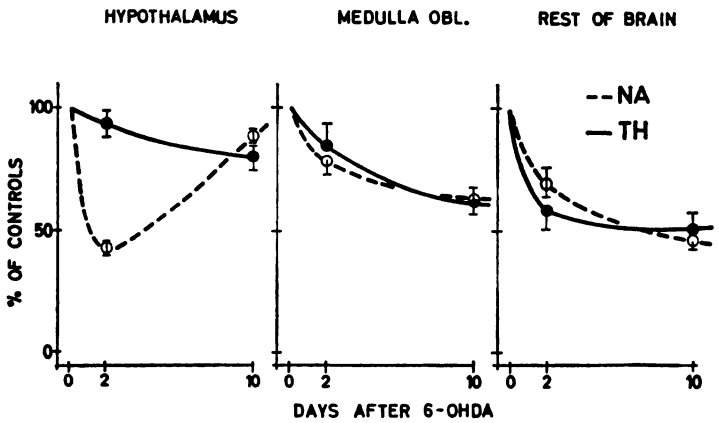

FIG. 2. Effect of 6-hydroxydopamine (6-OHDA) on noradrenaline (NA) content and tyrosine hydroxylase (TH) activity in various brain parts of rats; 200 μg of 6-OHDA were injected into the right lateral ventricle of the brain. NA content and TH activity are indicated in percent of controls (= 100). Averages and S.E. of 3 to 8 experiments (7).

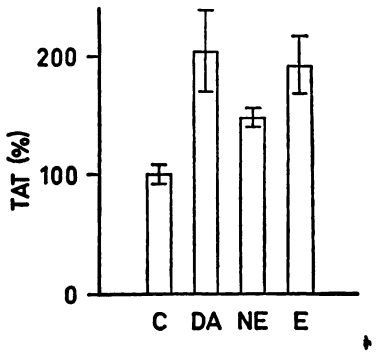

FIG. 3. Effect of various catecholamines on activity of tyrosine aminotransferase (TAT) measured in the supernatant of liver homogenates of rats. One dose of 100 μg/kg of dopamine (DA), L-noradrenaline (NE) or L-adrenaline (E) was injected intraperitoneally 120, 90 and 30 minutes, respectively, before decapitation. The values are indicated in percent of normal controls (C = 100%) and represent averages with S.E. of 3 experiments each performed with a pool of 3 livers (4).

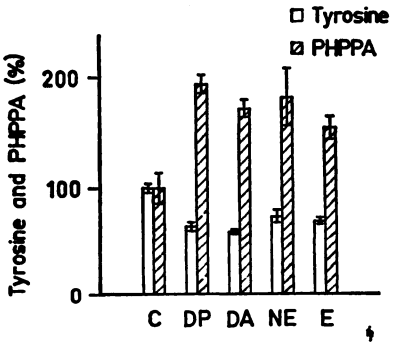

FIG. 4. Effect of intraperitoneal injections of L-dopa and catecholamines on tyrosine levels in blood plasma and urinary elimination of *p*-hydroxyphenylpyruvic acid (PHPPA) in rats. Doses: L-dopa (DP) 200 mg/kg; dopamine (DA) 100 mg/kg (single doses); L-noradrenaline (NE) twice 100 μg/kg (with an interval of 1 hr); L-adrenaline (E) twice 100 μg/kg (tyrosine) or twice 50 μg/kg (PHPPA). Time of measurement after first injection: DP, DA 60 min, NE, E 90 min (tyrosine); DP, DA, NE 120 min, E 240 min (PHPPA). The values are indicated in percent of normal controls (C = 100) and represent averages with S.E. of 3 to 5 experiments (4).

hydroxytryptamine (5-HT) levels depend on physiological changes in the plasma tryptophan concentration (13). By analogy, variations in the level of TAT and, as a consequence, in the plasma concentration of the catecholamine precursor, tyrosine, may be involved in the regulation of catecholamine metabolism in nerve tissue.

VII. Concluding Remarks

The above mentioned findings indicate that the levels of catecholamine enzymes are regulated by several factors which may differ in their importance

from enzyme to enzyme. For instance, hormonal influences probably predominate in the regulation of PNMT, whereas in the case of TH, neuronal impulses are more important. DBH seems to be controlled by both these factors to a similar extent (20). The fact that neuronal impulses may change the macromolecular composition of the postganglionic adrenergic neuron is of interest not only with regard to the mechanism of catecholamine regulation, but also in other respects as, for example, the retention of information in the central nervous system and the development of drug tolerance not related to drug-metabolizing enzymes (33).

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