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COMMENTARY

REGULATION OF PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE

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Catecholamines play a critical role, both as hormones and as neurotransmitters, in a variety of essential psychologic and physiologic processes. In recent years, the regulation of the enzymes involved in catecholamine biosynthesis has been an area of vigorous scientific inquiry. This work, involving several laboratory groups, has yielded important information of direct significance to the enzymes, and relevant as well to our understanding of other critical cellular processes.

The history of science is dotted with examples where investigations began in one area, only to end up in quite another. In keeping with this fashion, the studies from our laboratory, which began some years ago by investigating the genetic determinants of catecholamine levels, have carried us into some relatively uncharted areas dealing with the control of enzyme synthesis and degradation. The problem has turned out to be an exceedingly complex one. In an effort to gain some control over it, we have focused our attention on dopamine β-hydroxylase (DBH) and phenylethanolamine *N*-methyltransferase (PNMT), the terminal enzymes in noradrenaline and adrenaline biosynthesis respectively. This report will concentrate primarily on PNMT.

Phenylethanolamine *N*-methyltransferase is the terminal enzyme in epinephrine biosynthesis. PNMT catalyzes the methylation of norepinephrine to form epinephrine, a reaction in which *S*-adenosylmethionine participates as the methyl donor. A wide variety of β-hydroxylated phenylethylamine derivatives are substrates for the enzyme. PNMT occurs in a very limited tissue distribution; high concentrations of the enzyme are localized in the adrenal medulla, while very low amounts are found in discrete neonatal sympathetic ganglia and in isolated brainstem neuronal tracts. Purified bovine PNMT is a monomeric protein of a molecular weight of 37,000–40,000 daltons. The enzyme is extremely rich in glutamic acid, a property it shares with dopamine β-hydroxylase.

Genetic studies

Our genetic work began innocently enough with the observation that the adrenal gland levels of PNMT varied over a nearly 4-fold range among several inbred mouse strains [1]. To determine if these differences were genetically determined, mouse strains with the highest and lowest PNMT activity were mated and the F_1 progeny was analyzed. The results of this inquiry showed that the steady-state levels of PNMT were genetically determined. Due to the limited breeding analysis, however, it was not possible to specify how many genes were involved in regulating PNMT levels, nor could we explain the biochemical mechanism of gene control on PNMT [2].

Subsequent studies resolved both these questions. Using two related sublines of the Balb/c inbred mouse strain, we found that PNMT levels were twice as high in one line (Balb/cJ) as in the other (Balb/cN). A detailed breeding analysis of the F_1, F_2 and bidirectional backcross lines derived from the matings of these two strains clearly established that steady-state adrenal PNMT levels were under the control of a single autosomal codominant gene locus [3, 4].

Steady-state enzyme levels reflect a balance between the rate of enzyme synthesis and the rate of enzyme destruction by intracellular proteases. Precedents for a genetic determinant on enzyme synthesis and on enzyme degradative rate have been described by other investigators [5]. Knowing this, we investigated the gene control on PNMT synthesis and degradation. This was done by preparing an antibody to the enzyme and then determining the rate of enzyme synthesis and degradation after pulse-labeling mice with radioactive amino acids. The antibody was used to isolate the radiolabeled PNMT.

Using this technique, we showed that the difference in PNMT levels between high-PNMT and low-PNMT mouse sublines was due to a difference in the rate of PNMT degradation. The strain with low PNMT levels had half the number of PNMT molecules because the enzyme was being degraded twice as rapidly. Insofar as we can determine, it was the action of this protease which was genetically determined in the mouse system we were studying.

This finding was the first demonstration of the importance of enzyme degradation as a regulatory process for a neurotransmitter-synthesizing enzyme. The fact that a genetic control existed on steady-state PNMT levels and was expressed by an altered rate of proteolysis led us to question further the importance of enzyme degradation as a regulatory mechanism.

Glucocorticoid control of enzyme synthesis and degradation

Previous work had shown that the steady-state levels of both PNMT [6] and DBH [7] were controlled by adrenal glucocorticoids and by splanchnic

nerve impulses to the adrenal medulla. The mechanism of glucocorticoid control appeared to be different from that of neuronal control. If normal rats were given reserpine, which reflexively increased splanchnic neuronal activity, DBH and PNMT levels increased nearly 3-fold. In contrast, administration of glucocorticoids to normal rats had no effect on the levels of DBH and PNMT, even though hypophysectomy reduced DBH and PNMT levels profoundly, and the enzyme levels could be restored to normal by ACTH or glucocorticoid treatment.

Using a combined radiolabeling and immunoprecipitation technique, we showed that splanchnic nerve firing induced DBH synthesis without altering the rate of DBH degradation [8]. This effect is a trans-synaptic one, involving release of acetylcholine from the splanchnic neuron. In contrast, hypophysectomy caused a marked acceleration in DBH degradation, but had no effect on the rate of DBH synthesis. The accelerated proteolysis of DBH caused by hypophysectomy was reversed by the administration of ACTH or glucocorticoids. Since these drugs were ineffective in increasing DBH levels in normal rats, we speculated that the action *in vivo* of glucocorticoids was to restore DBH steady-state levels by inhibiting enzyme proteolysis, and that under steady-state conditions this inhibition was at a maximal rate.

There appeared, then, to be two distinct controls on DBH activity. One control was neuronally mediated, involved the action of acetylcholine on an adrenal medullary cell receptor, and resulted finally in an induction of *de novo* DBH synthesis. The outcome was a doubling or tripling of DBH over steady-state levels. The second control was hormonally mediated, appeared to involve a glucocorticoid inhibition of DBH proteolysis, and acted to control only steady-state DBH levels.

Recently we have begun a similar line of studies on the mechanism of glucocorticoid control of PNMT. We have found that hypophysectomy causes a dramatic reduction in immunotitratable PNMT levels through a mechanism which involves increased PNMT proteolysis. Glucocorticoid administration to hypophysectomized rats reverses the increase in PNMT proteolysis and restores enzyme levels to normal. Insofar as we can determine, the mechanism of glucocorticoid control on PNMT and DBH steady-state levels is the same, in that glucocorticoids inhibit *in vivo* proteolysis of both enzymes.

Regulation of PNMT proteolysis

Inquiry into the mechanism of glucocorticoid control of PNMT proteolysis has disclosed some fascinating findings. In an effort to study the proteolytic process further, we discovered that the thermal stability of PNMT (measured as the half-life of the enzyme at 50°) was profoundly reduced in adrenal preparations from hypophysectomized animals. This was a very interesting finding, since it provided us with an independent confirmation that both the proteolytic stability *in vivo* and the thermal stability *in vitro* of the enzyme were reduced by hypophysectomy. Mixing of supernatant preparations from control rats with those of hypophysectomized animals restored PNMT thermal stability in the latter preparations, while dialysis or freezing and thawing reduced it. Treatment of hypophysectomized animals with glucocorticoids restored PNMT thermal stability along a time course similar to that of the restoration of PNMT levels.

Subsequent work disclosed the existence of a freeze–thaw labile, dialyzable substance which was present in the adrenals of normal rats, and which conferred thermal stability on PNMT. This material had an absorption maximum at 264 nm, and appeared to act by binding directly to PNMT. The material, dubbed stabilizing factor, could be separated from the enzyme by immunoadsorbtion of the enzyme-stabilizing factor complex followed by elution of the stabilizing factor. Addition of purified stabilizing factor to an adrenal supernatant fraction from hypophysectomized rats completely restored thermal stability of PNMT. Treatment of hypophysectomized rats with glucocorticoids or ACTH restored PNMT thermal stability and stabilizing factor levels toward normal.

Paper chromatographic procedures suggested the endogenous stabilizing factor migrated with *S*-adenosylmethionine. Addition of *S*-adenosylmethionine to PNMT from hypophysectomized rats restored the thermal stability of the enzyme toward control values. Addition of other PNMT substrates had a slight stabilizing effect but by far the most dramatic stabilization was seen with *S*-adenosylmethionine.

In carrying these studies further, we have since found that the stability of PNMT to proteolysis *in vitro* by trypsin is similarly dependent on an endogenous stabilizing factor, and that *S*-adenosylmethionine markedly stabilizes PNMT against tryptic proteolysis. It seems, therefore, that tryptic stability, thermal stability, and, by inference, proteolytic stability *in vivo* of PNMT are dependent on an endogenous stabilizing factor which may be *S*-adenosylmethionine. We speculate that the action of this stabilizing factor is to stabilize PNMT against denaturation or proteolysis by binding to the enzyme and altering its tertiary conformation in such a way that the enzyme is less vulnerable to destruction. While this mechanism remains only partially elucidated at this time, there is some evidence in favor of it.

The verification of this hypothesis and its generalizability to other catecholamine synthetic enzymes known to be under glucocorticoid control, such as DBH, obviously awaits further investigation. However, it now seems quite reasonable to conclude that proteolytic activity *in vivo* plays an important role in the regulation of steady-state levels of DBH and PNMT, and that specific mechanisms to control proteolytic vulnerability of these enzymes exist. It further seems apparent that intracellular proteolysis is an important aspect of the regulation of enzyme levels and that it is an area well worth investigating in our attempts to understand cellular control mechanisms.

Regulation of PNMT synthesis

Like DBH, PNMT is also responsive to trans-synaptic induction by acetylcholine and by drugs

which release ACh, such as reserpine. We have obtained preliminary evidence that this process takes place via induction of *de novo* PNMT synthesis. Trans-synaptic regulation has been thought to be of less importance in the regulation of PNMT than for either DBH or tyrosine hydroxylase, since PNMT responds much less dramatically to reserpine treatment than the other two enzymes. This seems, however, to be a problem of rate rather than of magnitude of response. In our hands, PNMT reaches peak levels after reserpine which are quite similar to those reached by tyrosine hydroxylase or DBH (250 per cent of control), but as a slower rate. Thus, it appears likely that trans-synaptic induction is as critical a component of PNMT regulation as it is for tyrosine hydroxylase or DBH. The apparent specificity of trans-synaptic induction, however, seems temporal in nature, and is due to the differential rates of response of the enzymes to reserpine. This is consistent with the argument advanced by Berlin and Schimke [9] that the specificity of an enzyme to an inducing stimulus is governed by the rate at which the enzyme is synthesized.

Biologic importance of the dual modes of PNMT and DBH regulation

It is striking that the neural induction of enzyme synthesis and the hormonal inhibition of enzyme proteolysis are regulatory mechanisms which control both DBH and PNMT. What is the significance *in vivo* of a dual regulation of enzyme synthesis and degradation? Biologically, we conceptualize the importance of this system to be in the adaptation of the animal to stress. We see the system as functioning in the following way: glucocorticoids act as the principal regulator of steady-state DBH and PNMT levels by inhibiting enzyme degradation, while nerve impulses stimulate enzyme synthesis and play only a secondary role in the maintenance of steady-state enzyme levels. Although the two regulatory systems work at different points, the consequence of their joint action would be to control the availability of catecholamines. In times of stress, splanchnic nerve firing results in increased DHB and PNMT synthesis, while the glucocorticoid outflow from increased ACTH release ensures maximal inhibition of DBH and PNMT degradation. Under resting conditions, the two systems still act in concert; basal neuronal stimuli are needed for glucocorticoids to maintain steady-state DBH and PNMT levels [10]. When glucocorticoid production is reduced, inhibition of DBH and PNMT degradation is lifted and enzyme levels fall. Thus, the interaction of the two systems ensures that the availability of glucocorticoids and of catecholamines is controlled in tandem. Since the role of the adrenal is in the "fight or flight" response, a linkage between production of cortical and medullary hormones would be an important adaptive capability for the organism.

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