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Involvement of Adenosine 3′,5′-Monophosphate in the Activation of Tyrosine Hydroxylase Elicited by Drugs

Abstract. *Immediately after the injection of reserpine (16 micromoles per kilogram, intraperitoneally), aminophylline (200 micromoles per kilogram, intraperitoneally), and carbamylcholine (8.2 micromoles per kilogram, intraperitoneally), the concentration of adenosine 3′,5′-monophosphate in adrenal medulla of rats is increased severalfold. The three drugs also cause a delayed increase of medullary tyrosine hydroxylase activity. Our results are consistent with the view that an increase of medullary adenosine 3′,5′-monophosphate concentration is involved in the drug-induced increase of tyrosine hydroxylase activity in adrenal medulla. Experiments with tyramine (130 micromoles per kilogram, intraperitoneally) suggest that the increase of tyrosine hydroxylase activity and of adenosine 3′,5′-monophosphate concentrations is independent of an increase in adrenal catecholamine turnover rate.*

Tyrosine hydroxylase (TH) activity of adrenal gland is transsynaptically induced (*1*). Several studies have attempted to explore the molecular basis of this regulation; however, the mechanisms involved are still unclear. Kvetnansky *et al.* (*2*) reported that injections of dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) restored to normal the TH activity of adrenal glands of hypophysectomized rats. Recently, Waymire *et al.* (*3*) reported that the continuous presence of dibutyryl cyclic AMP in neuroblastoma tissue cultures increases the TH activity. Since sodium butyrate also elicits the increase in enzyme activity, one cannot infer that this activation is related to an increase of adenosine 3′,5′-monophosphate (cyclic AMP) without measuring the concentration of this nucleotide. Unfortunately, these measurements were not reported by Waymire *et al.* (*3*).

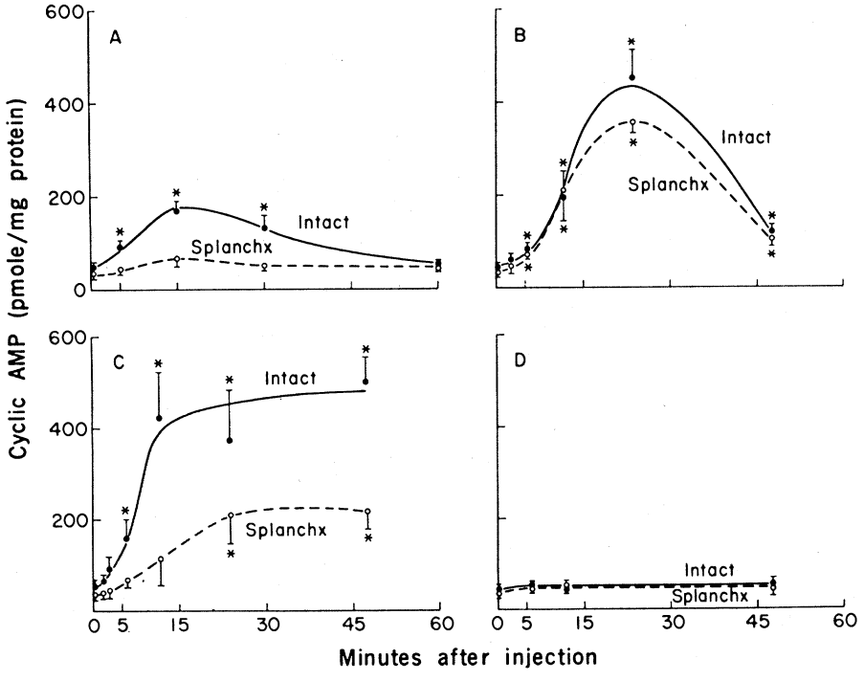

Fig. 1. Cyclic AMP concentration in intact and denervated (*Splanchx*) adrenal medulla of rats receiving intraperitoneally: (A) reserpine (16 μmole/kg), (B) carbamylcholine (8.2 μmole/kg), (C) aminophylline (200 μmole/kg), (D) tyramine (130 μmole/kg). Each point represents the mean values of at least five experiments. The concentrations of cyclic AMP at various times after injection of the solution used for dissolving reserpine or after saline were not different from those of rats not receiving saline. The vertical brackets indicate standard error. *, $P < .05$ compared with control animals.

The present experiments were carried out to verify whether the adenylyl cyclase system of adrenal medulla is involved in the increase of TH activity elicited by drugs. We measured the concentration of cyclic AMP and the activity of TH in intact and denervated adrenal gland of rats receiving various drugs. We studied reserpine because it increases adrenal TH activity (*4*), carbamylcholine because it stimulates adrenal catecholamine secretion (*5*), aminophylline because methylxanthines inhibit phosphodiesterase activity (*6*), and tyramine because it releases catecholamines from peripheral noradrenergic neurons (*7*). The results obtained support the working hypothesis that an increase in concentration of adrenal cyclic AMP is associated with a delayed enhancement of the TH activity.

Sprague-Dawley male rats (Zivic Miller Laboratories, Allison Park, Pa.) (about 180 g) were used. The left splanchnic nerve of these animals was severed 5 days before the experiment. Drugs were injected into monolaterally operated rats, and their effect on endogenous cyclic AMP and TH activity was measured in intact and denervated adrenal medulla. Adrenal medulla was dissected from cortex at about 4°C under a dissecting microscope. The accuracy of the dissection was ascertained by assaying the concentration of catecholamines (*8*) and corticosteroids (*9*). The medulla contained about 95 percent of the catecholamines while the cortex contained about 99 percent of the total corticosteroids present in the adrenal gland.

The concentration of cyclic AMP was measured by the method of Ebadi *et al.* (*10*) as successively modified in our laboratory (*11*). Tyrosine hydroxylase activity was measured by the method of Waymire *et al.* (*12*) with the use of carboxyl-labeled tyrosine (specific activity, 10 μc/μmole).

The intact adrenal medulla of rats receiving intraperitoneally reserpine (16 μmole/kg), carbamylcholine (8.2 μmole/kg), or aminophylline (200 μmole/kg) 6 minutes before being killed contains more cyclic AMP than

the adrenal medulla of rats receiving either saline or tyramine (130 μmole/kg) (Fig. 1). This increase reaches its peak 15 minutes after the injection of reserpine and 24 minutes after the injection of carbamylcholine or aminophylline (Fig. 1). One hour after reserpine or carbamylcholine injection, the concentration of cyclic AMP approaches normal values (Fig. 1). In contrast, the cyclic AMP concentrations in adrenal medulla of rats injected with aminophylline are still maximally increased 48 minutes after the drug injection (Fig. 1). In addition, Fig. 1 shows that in denervated adrenal medulla, the increase of cyclic AMP concentration elicited by carbamylcholine is about the same as that measured in the contralateral intact tissue. However, the cyclic AMP increase elicited by reserpine and aminophylline is smaller in the denervated medulla than in the contralateral intact tissue. Tyramine fails to change the concentration of medullary cyclic AMP (Fig. 1).

From the data in Fig. 1 we have estimated the initial rate of cyclic AMP accumulation. Reserpine elicits different initial rates of cyclic AMP accumulation in intact (11 pmole per milligram of protein per minute) and denervated (3 pmole per milligram of protein per minute) adrenal medulla. Also, the accumulation of cyclic AMP induced by injections of aminophylline depends upon the presence of afferent nerves: medullary cyclic AMP accumulates at a rate of 40 pmole per milligram of protein per minute in intact gland and 8 pmole per milligram of protein per minute in denervated gland. In contrast, the accumulation of cyclic AMP elicited by carbamylcholine proceeds with equal rates in normal and denervated adrenal medulla (25 pmole per milligram of protein per minute).

In another group of animals TH activity of adrenal gland was measured at different time intervals after a single injection of reserpine, aminophylline, carbamylcholine, or tyramine. Reserpine (16 μmole/kg, intraperitoneally) within 24 hours enhances the TH activity of intact adrenal homogenates. The TH activity is increased from 6.4 ± 0.3 to 10 ± 0.5 nmole of dopa per hour per gland. We also confirmed that such an increase fails to occur in denervated adrenal glands (*1*). Aminophylline (200 μmole/kg, intraperitoneally) and carbamylcholine (8.2 μmole/kg, intraperitoneally) (Fig. 2) enhance TH activity in intact adrenal gland. The maximal increase occurs after 24 hours and the activity is still greater than normal at 48 hours (Fig. 2). In denervated adrenal glands, aminophylline fails to produce significant increases of the TH activity, while carbamylcholine increases TH activity equally well in intact and denervated glands (Fig. 2). Tyramine (130 μmole/kg) injected intraperitoneally 24 hours before killing fails to increase the TH activity in both intact and denervated glands.

To investigate whether the increase of TH found in adrenal homogenates was associated with an increase of catecholamine turnover rate in vivo, we compared the turnover rate of adrenal catecholamines 24 hours after injection of saline or aminophylline (*13*). We found that the specific activity of tyrosine (1200 ± 150 disintegrations per minute per nanomole) is altered neither by splanchnicotomy nor by the injection of aminophylline. The specific activity of epinephrine after injection of either aminophylline or saline is 38 ± 3.5 dpm/nmole and 9 ± 1.5 dpm/nmole, respectively; aminophylline injection fails to increase the specific activity of epinephrine in denervated adrenal medulla.

In rats injected with reserpine, aminophylline, or carbamylcholine, the cyclic AMP accumulation in medulla precedes the increase of TH activity. Moreover, the rate of cyclic AMP accumulation and the increase of TH activity is greater in the normal than in the denervated adrenal gland. When the accumulation of cyclic AMP is not curtailed by denervation (rats receiving carbamylcholine), the increase of TH activity is equal in homogenates of intact and denervated adrenal. It is tempting to speculate that the medullary adenylyl cyclase system is involved in the increase of medullary TH activity elicited by drugs.

It has been suggested that the induction of TH activity following reserpine administration is regulated transsynaptically (*1*). If this reflex were elicited by the depletion of tissue catecholamines caused by reserpine, then the early increase in medullary cyclic AMP could not be related to the TH induction, because the catecholamine depletion reaches critical values only 1 or 2 hours after injection of reserpine. However, large doses of reserpine elicit an increase of splanchnic nerve activity immediately after the injection, and this activity remains elevated for about 1 hour (*14*). Since the presence of innervation is required to observe the increase of cyclic AMP and the induction of TH elicited by reserpine, we suggest that the effect of reserpine on the adenylyl cyclase system is transsynap-

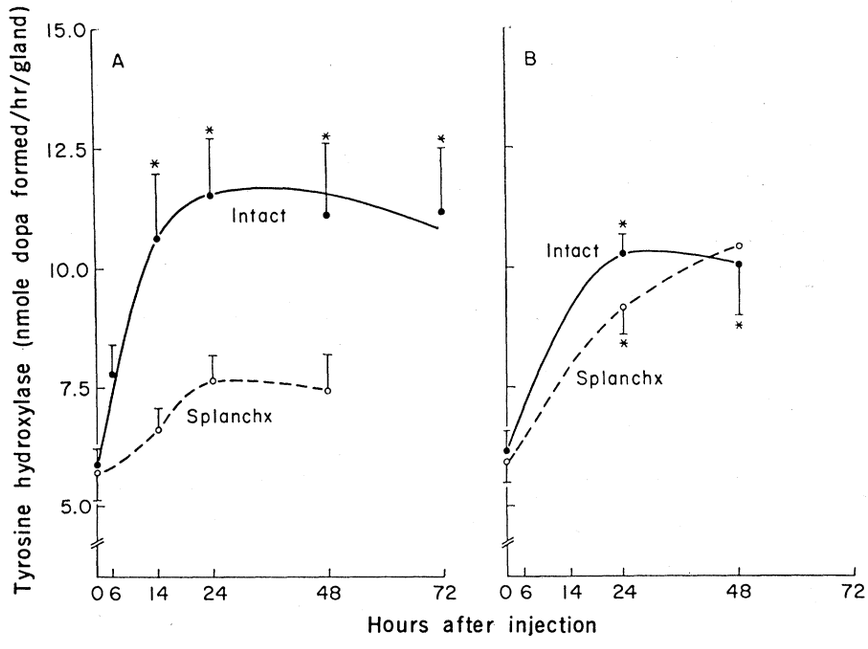

Fig. 2. Tyrosine hydroxylase activity in intact and denervated (*Splanchx*) adrenal glands of rats receiving intraperitoneally: (A) aminophylline (200 μmole/kg) and (B) carbamylcholine (8.2 μmole/kg). Each value is the mean of at least five experiments. Tyrosine hydroxylase activities in adrenal gland at various times after saline were not different from those of rats not receiving saline. The vertical brackets indicate the standard error. *, $P < .05$ compared with control animals.

tically mediated. This suggestion agrees with our finding that reserpine neither reduces phosphodiesterase activity nor increases adenylate cyclase activity when added to adrenal homogenates in concentrations up to $10^{-4}M$ (*15*). Since the splanchnic nerve is cholinergic, the transsynaptic activation of TH implies that acetylcholine may regulate the medullary adenylate cyclase system. Indeed, carbamylcholine, a cholinomimetic drug, increases cyclic AMP concentrations and TH activity in intact and denervated adrenal medulla. The data obtained with aminophylline give further support to the hypothesis that cyclic AMP and TH are both transsynaptically regulated. We tested the inhibition of phosphodiesterase activity by aminophylline in vitro in intact and denervated adrenal medulla and we found that the ID_{50} (dose causing 50 percent inhibition) is equal in both tissues ($10^{-3}M$). Therefore, the accumulation of medullary cyclic AMP elicited by aminophylline injection reflects the in vivo activity of adenylyl cyclase and probably the turnover rate of cyclic AMP in this tissue. Since the rate of medullary cyclic AMP accumulation elicited by aminophylline is greatly reduced by splanchnicotomy, nerve impulses may play a role in the regulation of endogenous cyclic AMP turnover rate in adrenal medulla.

Reserpine (*16*) and carbamylcholine (*5*) release catecholamines from adrenal medulla. It may be inferred that the increase of both cyclic AMP and TH activity elicited by these drugs is in some way related to this release. Several lines of evidence are at variance with the hypothesis that catecholamine release and TH induction are interdependent. Tyramine (130 μmole/kg, intraperitoneally) releases catecholamines from noradrenergic neurons because it reduces by 40 percent the catecholamine concentrations in heart tissue (*17*); although this drug fails to change the steady-state concentrations of adrenal catecholamines it increases their turnover rate and, therefore, it might release catecholamines from adrenal medulla (*17*). However, tyramine increases neither the concentration of cyclic AMP (Fig. 1) nor the TH activity of adrenal glands. Aminophylline changes neither the concentration nor the turnover rate of catecholamines in adrenal medulla (*17*) but it increases cyclic AMP concentration and TH activity of this tissue. Thus, the results reported are consistent with the working hypothesis that an early change in the rate of cyclic AMP accumulation brings about an increase of TH activity. In conclusion, the changing rates of cyclic nucleotide accumulation can be dissociated from a release of adrenal catecholamines but not from the delayed increase of TH activity.

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17. Specific activity of epinephrine, dopamine, and tyrosine was measured (*8*). An injection of [3,5-^{3}H]tyrosine (1 mc/kg, intravenously) was given, and 2 hours later the rats received saline or tyramine (130 μmole/kg, intraperitoneally) or aminophylline (200 μmole/kg, intraperitoneally). They were killed 45 minutes after these injections. Tyramine and aminophylline do not change the steady-state concentration of catecholamines or the specific activity of tyrosine (about 350 dpm/nmole) and dopamine (about 600 dpm/nmole). However, 130 μmole of tyramine per kilogram (intraperitoneally) increases the specific activity of tissue epinephrine (from 53 ± 7 to 77 ± 6 dpm/nmole). At steady state, using the precursor product relationship (*18*), the estimated turnover rate of epinephrine is 0.38 nmole per hour per pair of glands in animals receiving saline and 0.74 nmole per hour per pair of glands in animals receiving tyramine. This dose of tyramine depletes the catecholamine content of heart (from 6.9 ± 0.4 to 4.2 ± 0.6 nmole/g, wet weight, $P < .01$). The turnover rate of adrenal epinephrine of rats receiving aminophylline 45 minutes before is equal to that of controls.
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20 November 1972

Phenylketonuria: Phenylalanine Inhibits Brain Pyruvate Kinase in vivo

Abstract. *The hypothesis that brain damage in phenylketonuria is related to inhibition of pyruvate kinase by phenylalanine was examined in rat brain in vivo. One hour after a single injection of phenylalanine into the rat, the brains were removed and completely frozen in less than a second. The concentration of phenylalanine in the brain was comparable to that found in phenylketonuric patients. Changes in brain glycolytic intermediates were consistent with inhibition of pyruvate kinase in vivo. The inhibition of pyruvate kinase was apparently compensated for by an increase in phosphoenolpyruvate; no decrease in adenosine triphosphate or creatine phosphate was found.*

Phenylketonuria is the most common disorder of amino acid metabolism in man. It is characterized by extraordinarily high levels of phenylalanine and its derivatives in blood and urine (*1*). The basic lesion is the congenital absence of phenylalanine hydroxylase, a hepatic enzyme which converts phenylalanine to tyrosine. Severe mental retardation and motor abnormalities are typical of the untreated disease, but can be prevented by early treatment with a diet low in phenylalanine (*1*). The morphological correlate of the brain damage is a defect in myelination, but the biochemical basis for this abnormality is unclear (*1*).

Weber and co-workers have shown competitive inhibition of purified brain pyruvate kinase (E.C. 2.7.1.40) by phenylalanine (*2*). Phenylalanine also decreased the rate of glycolysis in human and rat brain slices, particularly in those from perinatal rats, in which brain pyruvate kinase activity is relatively low (*2*). These studies have suggested that phenylalanine may have a direct inhibitory effect on energy production in brain, although the relation of the in vitro observations to the mechanism in vivo has not been established (*3*).

We have examined the metabolic effects of hyperphenylalaninemia on