Proceedings of the National Academy of Sciences
Vol. 65, No. 1, pp. 58–62, January 1970

Phase Difference in the Induction of Tyrosine Hydroxylase in Cell Body and Nerve Terminals of Sympathetic Neurones

Hans Thoenen,* Robert A. Mueller,† and Julius Axelrod

LABORATORY OF CLINICAL SCIENCE, NATIONAL INSTITUTE OF MENTAL HEALTH, BETHESDA, MARYLAND

Communicated by Seymour S. Kety, November 10, 1969

Abstract. The induction of tyrosine hydroxylase in the nerve terminals of the rat heart by reserpine lags behind that in the stellate ganglion by two to three days. Cycloheximide given three days after reserpine blocks the further rise of the enzyme in the nerve terminals. The increase in tyrosine hydroxylase activity of the lumbar ganglion is as marked as that in the stellate ganglion. The increase of enzyme activity in the sciatic nerve after reserpine administration resembles that found in the heart nerve terminals. Determination of enzyme activity in segments of sciatic nerves indicates a two-day lag and then a proximal-distal transport of enzyme, but the apparent rate is not sufficient to account for the increase in enzyme in the nerve terminals. These findings are compatible with the local synthesis of induced tyrosine hydroxylase in the nerve terminals rather than the peripheral movement of the completed enzyme.

In previous studies, it has been shown that tyrosine hydroxylase can be induced both in the adrenal medulla and sympathetic ganglia by drugs which cause a reflex increase in the activity of the sympathetic nervous system.[1, 2] Tyrosine hydroxylase is a critical enzyme in the biosynthesis of norepinephrine because it is rate limiting in formation of the sympathetic neurotransmitter, noradrenaline.[3] Kinetic data[4] and the fact that the increase in the activity of this enzyme can be blocked by inhibition of protein synthesis[5] indicate that it represents an increase in enzyme protein rather than changes in inhibitor or activator concentration or the allosteric exposure of "hidden" enzyme sites. The present experiments were designed to investigate whether the increase in tyrosine hydroxylase also occurs in the nerve terminals and to investigate the cell body of the sympathetic neurones to see whether an axonal transport of the enzyme occurs. We therefore compared the time course of the increase in tyrosine hydroxylase activity in the stellate ganglion with that in the nerve terminals of the rat heart after treatment of rats with reserpine. The sympathetic innervation of the rat heart originates predominantly from the stellate ganglion, and it has previously been shown that tyrosine hydroxylase is entirely confined to the sympathetic nerves of the rat heart.[1] In addition, we have studied the changes in tyrosine hydroxylase activity occurring in lumbar ganglia

and in consecutive segments of the sciatic nerve from one to three days after administration of reserpine.

Methods. Male Sprague-Dawley rats weighing 100–125 gm were obtained from Hormone Assay Laboratories (Chicago, Ill.). The animals were injected with reserpine either in single doses of 5 mg/kg or in repeated doses of 2.5 mg/kg. The animals were killed by a blow on the head at different time intervals after the administration of the drug. The stellate or lumbar ganglia were rapidly removed, homogenized in 0.5 ml ice cold 0.25 *M* sucrose, and centrifuged at 27,000 × *g* for 10 min. Both right and left sciatic nerves of two animals were removed from the intervertebral foramina to the distal hindlimb, combined, and divided in five equal segments approximately 1 cm in length. Thus, the corresponding segments of four nerves were pooled, homogenized in 0.5 ml of ice cold 0.25 *M* sucrose, and centrifuged at 27,000 × *g* for 10 min. The hearts were cooled on cracked ice, blotted, minced using an Arbor tissue press, and centrifuged for 20 min at 105,000 × *g*. To remove compounds interfering with the determination of tyrosine hydroxylase, aliquots of 50–100 μl of the supernatant heart press juice were passed over Sephadex G-10 columns (3-cm length) at 4°C, equilibrated with 0.25 *M* sucrose. Protein-containing portions of the effluent from the Sephadex columns and the supernatant fraction of ganglia and nerves were assayed for tyrosine hydroxylase according to a method of Levitt *et al.*,[6] with modifications described in detail previously.[4] Aliquots of the supernatant fractions of nerve or ganglia and the column effluents from hearts were analyzed for protein content by the method of Lowry *et al.*[7]

Results. Tyrosine hydroxylase activity of the stellate ganglion was already increased by 40 per cent ($p < 0.01$) 24 hours after the administration of reserpine (Fig. 1). Enzyme activity further increased and reached a maximum three

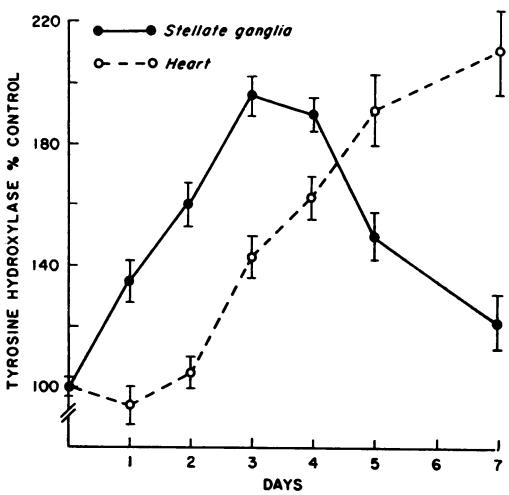

FIG. 1.—Time course of increase in tyrosine hydroxylase in stellate ganglia and heart after reserpine administration. Each value represents the mean ±SE (brackets) of 12 to 42 observations. Animals received 5 mg/kg reserpine subcutaneously at zero time and were killed at the indicated time intervals. All enzyme assays contained 0.74 mM 6,7-dimethyl-5,6,7,8-tetrahydropteridine·HCl·$5H_2O$; ganglia assays contained 9.8–21.3 μM and heart assays contained 0.31–0.45 μM ditritiotyrosine (1.3–4.2 μc).

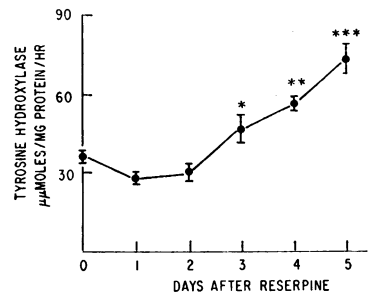

FIG. 2.—Time course of increase in axonal tyrosine hydroxylase after reserpine. All animals received 5 mg/kg reserpine subcutaneously at zero time. Each point represents the mean ±SE (brackets) of 4 to 8 determinations. All enzyme assays were performed using 0.37 mM pteridine cofactor and 6.0 μM 3,5-ditritiotyrosine. *, $p < 0.05$; ** $p < 0.01$; and ***, $p < 0.001$.

days after giving reserpine. After three days, the tyrosine hydroxylase activity progressively fell and was no longer significantly elevated at seven days. In contrast to the ganglia, the heart tyrosine hydroxylase did not begin to rise until the third day after reserpine and the maximum activity was achieved on the seventh day. Preliminary experiments indicate that the heart enzyme gradually decreases after seven days, but is still 60 per cent higher than the control values two and three weeks after reserpine. The difference in time course of the initial rise in enzyme activity in stellate ganglion and heart could not be changed by repeated administration of 2.5 mg/kg of reserpine for four days. A proximo-distal axoplasmic flow of protein has previously been shown by Livett *et al.* in the sympathetic splenic nerve of the cat.[8] If it is assumed that the lag between the rise in the enzyme activity in the stellate ganglion and the heart is the result of a similar axoplasmic transport of tyrosine hydroxylase from the stellate ganglion to the nerve terminals, blockade of protein synthesis between the third and the fourth day after reserpine administration should not have any effect on the appearance of the preformed enzyme in the heart. However, cycloheximide, an inhibitor of protein synthesis, given between the third and fourth day resulted in a significantly lower level of enzyme activity on the fourth day after reserpine (Table 1). These results suggest that synthesis of tyrosine hydroxylase occurs in the nerve terminals of the heart.

To examine the selective changes in tyrosine hydroxylase in the axon as well as the cell body, rats were given reserpine (5 mg/kg) and the enzyme was examined in the lumbar ganglia and five segments of the sciatic nerve. Like the superior cervical and stellate ganglia, the tyrosine hydroxylase activity of the lumbar ganglia was elevated by about 80 per cent two days after reserpine. Tyrosine hydroxylase was found in all segments of the sciatic nerve (40 $\mu\mu$moles product formed per milligram protein per hour). No increase in the average enzyme activity was found in the sciatic nerve until the third day after reserpine (Fig. 2).

When consecutive segments of the sciatic nerves were examined for enzyme activity, it was found that in control animals, the activity was greater in proximal segments than in distal segments (Fig. 3). One day after reserpine administration the mean tyrosine hydroxylase activity of all five segments was slightly below control values. Two days after reserpine only the two most distal seg-

TABLE 1. *Effect of cycloheximide on the reserpine-initiated increase in ganglia and heart tyrosine hydroxylase.*

	Ganglia (mμmoles/mg protein/hr)	Heart ($\mu\mu$moles/mg protein/hr)
Control	1.77 ± 0.16	0.71 ± 0.08
Cycloheximide	1.56 ± 0.31	0.85 ± 0.05
Reserpine, 3 days	3.67 ± 0.37	1.28 ± 0.10
Reserpine, 4 days	3.51 ± 0.21	1.40 ± 0.06
Reserpine and cycloheximide 4 days	3.05 ± 0.24	1.16 ± 0.09*

Ganglia and heart enzyme assays were performed using 0.74 mM 6,7-dimethyl-5,6,7,8-tetrahydropteridine·HCl·$5H_2O$ as cofactor; ganglia and heart enzyme was assayed using 12.6 μM 3,5-ditritiotyrosine (1.5–3 μc) and 0.33 μM ditritiotyrosine (2–4 μc), respectively. Animals received 5 mg/kg reserpine subcutaneously at the indicated time before being killed. Cycloheximide 1.5 mg/kg was given subcutaneously 24, 16, and 8 hr before removal of the heart and ganglia.

* $P < 0.05$ when compared with reserpine, four days.

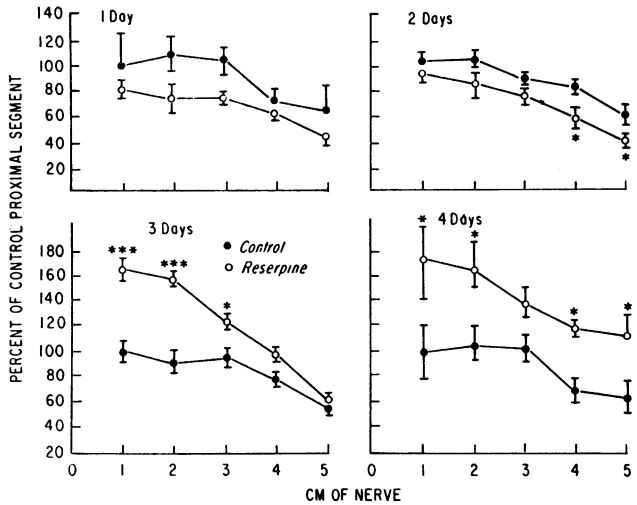

FIG. 3.—Time course of proximodistal appearance of tyrosine hydroxylase in the sciatic nerve after reserpine. All treated animals received 5.0 mg/kg reserpine subcutaneously at the indicated time intervals before use. Each point represents the mean ±SE (brackets) of 4 to 6 determinations; each determination was made on pooled similar segments of four sciatic nerves from two animals. All assays were performed using 0.37 mM pteridine cofactor. Assays on samples from day 1 and day 4 contained 6.0 μM, day 2 = 11.4 μM, and day 3 = 9.0 μM 3,5-ditritiotyrosine. All values are expressed as a percentage of the most proximal segment of control nerves. The velocity in μμmoles/mg protein/hr of this reference point is 40 on day 1, 42 on day 4, 95 on day 2, and 81 on day 3.

ments were significantly below control, and the third day the three proximal segments were significantly above control, while the last two had reached control values. At four days after reserpine, all five segments were greater than control values, and the shape of the curve of enzyme activity resembled that of sciatic nerves from untreated rats.

Discussion. The present experiments have shown that the rise of the tyrosine hydroxylase activity in the nerve terminal in rat heart after the administration of reserpine lags behind the increase in the stellate ganglion by two to three days. The enzyme in the nerve terminals of the heart reaches its highest level at five to seven days, at which time the enzyme activity in the stellate ganglia has already begun to decline. The enzyme activity in the heart remains elevated above control values for at least three weeks after reserpine, while that in the stellate ganglia returns to control levels by about seven days. These differences in the time course of the rise and decline in tyrosine hydroxylase activity after reserpine could be explained by an initial increase in this enzyme in the nerve cell body and subsequent transport of the enzyme to the distal parts of the nerve. However, cycloheximide, an inhibitor of protein synthesis, lowers the enzyme activity in the heart nerve terminals when given after the tyrosine hydroxylase activity has already begun to increase in the heart. The action of cycloheximide on tyrosine hydroxylase in nerve terminals could not be due to interference with axoplasmic flow. Several investigators have demonstrated that inhibition of protein synthesis by this compound does not affect

axoplasmic flow.[10, 11] Thus it appears that the induced enzyme is being synthesized in the nerve terminals.

Although there is rapid initial increase in the activity of tyrosine hydroxylase in the cell body (lumbar ganglia) after reserpine, a proximo-distal gradient of enzyme activity develops in the axon (sciatic nerve) only after several days. If the rate of increase of enzyme activity in any single 1-cm segment of nerve is related to the rate of enzyme transport, the apparent proximo-distal velocity is about 2–3 cm/day. The amount of noradrenaline stored in the terminals of sympathetic nerves contained within the sciatic nerve has been estimated as 0.92 μg,[9] more than that present in the heart, and there probably are at least as many sympathetic fibers in the two sciatic nerves as in the two cardiac nerves which run from the stellate ganglion to the heart. The total amount of enzyme in 1 cm of both sciatic nerves is only about 4 per cent in the entire heart. Since the enzyme content of the heart can double in three days (day 2 to day 5), a rapid rate of transport of tyrosine hydroxylase would have to be present if the enzyme increase in the nerve terminals were solely the result of transport from the cell body. Our results indicate that axonal transport of tyrosine hydroxylase would account for no more than 12 per cent of that which appears in the terminals. Furthermore, it would be difficult to reconcile a rapid rate of enzyme transport with the observed lag period of two days in the axon and nerve terminals.

It would seem likely that the time lag in appearance of increased enzyme in the nerve terminals after reserpine is not due to slow axonal transport of the enzyme, even though such a slow transport is discernible. The slow rate of transport of tyrosine hydroxylase observed here is consistent with the inability of other investigators to detect an accumulation of tyrosine hydroxylase above a constriction of the sympathetic splenic nerve,[12] although an accumulation of noradrenaline and dopamine β-oxidase has been readily detected.[9, 13] The lag in increased enzyme activity in the nerve terminals may be attributable to the time of transit from the cell body of information regulating the rate of enzyme synthesis or degradation.

* Visiting scientist, Hoffmann-LaRoche, Basel, Switzerland.

† Present address: Department of Anesthesia, Northwestern University Medical School, Chicago, Ill.

[1] Mueller, R. A., H. Thoenen, and J. Axelrod, *Science*, **163**, 468 (1969).

[2] Thoenen, H., R. A. Mueller, and J. Axelrod, *Nature*, **221**, 1264 (1969).

[3] Levitt, M., S. Spector, A. Sjoerdsma, and S. Udenfriend, *J. Pharmacol. Exptl. Therap.*, **148**, 1 (1965).

[4] Mueller, R. A., H. Thoenen, and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, in press.

[5] Mueller, R. A., H. Thoenen, and J. Axelrod, *Molec. Pharmacol.*, in press.

[6] Levitt, M., J. W. Gibb, J. W. Daly, M. Lipton, and S. Udenfriend, *Biochem. Pharmacol.*, **16**, 1313 (1967).

[7] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[8] Livett, B. G., L. B. Geffen, and L. Austin, *Nature*, **217**, 278 (1968).

[9] Dahlström, A., and J. Häggendal, *Acta Physiol. Scand.*, **67**, 278 (1966).

[10] Peterson, R. P., R. M. Hurwitz, and R. Lindsay, *Exptl. Brain Res.*, **4**, 138 (1967).

[11] Barondes, S. H., *J. Neurochem.*, **15**, 343 (1968).

[12] Laduron, P., and F. Belpaire, *Nature*, **217**, 1155 (1968).

[13] Livett, B. G., L. B. Geffen, and R. A. Rush, *Biochem. Pharmacol.*, **18**, 923 (1969).