MOLECULAR PHARMACOLOGY, 14, 1212-1219

Retinal Tyrosine Hydroxylase: Comparison of Short-term and Long-term Stimulation by Light

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(Received March 27, 1978)
(Accepted July 19, 1978)

SUMMARY

IUVONE, P. M., GALLI, C. L. & NEFF, N. H. (1978) Retinal tyrosine hydroxylase: Comparison of short-term and long-term stimulation by light. *Mol. Pharmacol.*, **14**, 1212-1219.

Dopamine (DA) is a putative neurotransmitter within some retinal amacrine neurons. Exposure of rats to light for either 15 min or 96 hr increases retinal tyrosine hydroxylase activity. Concomitant with the increase of enzyme activity is a 4-fold increase of DA formation. The molecular mechanism for the increased enzyme activity for the two exposures to light is apparently different. Short-term exposure to light decreases the K_m but not the V_{max} of the enzyme for the pteridine cofactor, while 96 hr of exposure to light increases the V_{max} for tyrosine but has no significant effect on the K_m for tyrosine or cofactor. Enzyme activity of rats exposed to 15 min of light decreases to the level found in 96 hr dark-adapted rats within 30 min of darkness, while more than 6 hr of darkness are required after 96 hr of light exposure. Our studies are consistant with the hypothesis that retinal DA formation is modulated by different molecular mechanisms depending on the duration of exposure of light. Short-term exposure to light activates tyrosine hydroxylase while long-term exposure to light results in the formation of more active molecules of enzyme.

INTRODUCTION

Dopamine[1] is a putative neurotransmitter in some amacrine neurons of retina (1, 2). Exposure to light increases the release of DA from the perfused eye (3) and enhances the rate of formation of DA in retina (4, 5). Furthermore, there is a circadian rhythm for retinal tyrosine hydroxylase in rats kept on a 12 hr light-dark cycle, with enzymatic activity being highest during the light phase of the cycle (5). Retinal amacrine neurons, therefore, can serve as a relatively simple physiological system for evaluating the biochemical changes that accompany activation of dopaminergic neurons, as well as provide valuable data on the retinal processing of visual information.

We have compared the effects of brief (5 min-3 hr) and prolonged (96 hr) exposure to light on the activity and kinetic properties of tyrosine hydroxylase and on the concentration and rate of formation of DA by amacrine neurons.

METHODS

Materials. L-Tyrosine [1-^{14}C], 54 mCi/mmol, was obtained from New England Nuclear, Boston, Mass. Reduced nic-

[1] Abbreviations used are: DA, dopamine; AMPT, alpha-methyl-p-tyrosine methyl ester; $DMPH_4$, 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine.

0026-895X/78/0146-1212$02.00/0

otinamide adenine dinucleotide phosphate, catalase and AMPT were purchased from Sigma Chemical Co., St. Louis, Mo. Pyridoxal phosphate and $DMPH_4$ were obtained from Calbiochem, La Jolla, Ca. Pentafluoropropionic anhydride was purchased from Pierce Chemical Co., Rockford, Ill. All other reagents were obtained in the purest form available.

Animals. Male Sprague-Dawley rats (Zivic-Miller, Allison Park, Pa.), 150–200 g, were housed in groups of 5–6 in clear plastic cages with perforated metal tops. They had free access to food and water. Room light for either light-adapted rats (exposed to continuous light for 96 hr) or short-term exposure to light was provided by overhead fluorescent lamps. Light intensity inside the cages was approximately 400 lux. Dark-adapted rats (exposed to continuous dark for 96 hr) were housed in similar cages in a room with a double-door entrance which permitted access to the room without light-leakage.

Rats were decapitated and the retinas dissected and frozen within 5 min. Dissection of the eyes was performed as follows: an incision was made at the border of the cornea and sclera; the lens and vitreous body were removed and then the retina was separated from the choroid and removed with a small spatula. The approximate weight of the retina after freezing was 8 mg. Killing, dissecting and freezing of retinas from dark-adapted rats were performed under a red photographic safety light (Kodak Wratten filter No. 1).

Tyrosine hydroxylase assay. Within 24 hr after killing the rats, frozen retinas were homogenized with ice-cold 0.05 M Tris-acetate, pH 6.0, containing 0.2% Triton X-100 (65 μl buffer/retina) in glass-teflon homogenizers. Homogenizing tubes were wrapped in aluminum foil when retinas from dark-adapted rats were processed. Homogenates were centrifuged at 20,000 $\times$ *g* for 15 min at 4° and the supernatant used for the assay of tyrosine hydroxylase.

Tyrosine hydroxylase activity was measured by the decarboxylase-coupled assay of Waymire *et al.* (24) as modified by Zivkovic *et al.* (7, 8). Unless otherwise noted, the concentration of L-tyrosine was 0.1 mM and the concentration of pteridine cofactor was 0.4 mM. Dihydropteridine reductase, purified through the second ammonium sulfate precipitation according to Kaufman (9), was included in the incubation mixture to regenerate reduced cofactor. Unless otherwise noted the assay pH was 6.4. Duplicate samples were incubated for 5 min at 37° before the addition of tyrosine, after which the incubation was continued for 30 min. The reaction was stopped by adding 0.5 ml of 10% trichloroacetic acid. Tubes were kept at 37° for an additional 2 hr for radioactive CO_2 collection on Whatman No. 1 paper presoaked with 0.1 ml "NCS" (Amersham/Searle, Arlington Heights, Ill.). The radioactivity on the paper was counted in Econofluor, 10 ml (New England Nuclear) containing 10% methanol.

The protein content of the supernatants were determined by the method of Lowry *et al.* (10).

Catecholamine assays. DA and norepinephrine were determined by gas chromatography-mass spectrometry (11). Briefly, frozen retinas were homogenized with 1.0 N HCl (0.1 ml/retina) and centrifuged for 15 min at 20,000 $\times$ *g* at 4°. A portion of the clear supernatant was dried under vacuum and pentafluoropropionyl derivatives of the endogenous catecholamines and deuterated-DA and deuterated-epinephrine, added as internal standards, were prepared as described by Koslow *et al.* (11). A Finnigan Model 3200 gas chromatograph-quadrupole mass spectrometer with programmed ion monitor and fitted with a 5 m 3% OV-17 (Supelco, Bellefonte, Pa.) glass column was used for analysis. The ion fragments monitored were: m/e 428 DA; m/e 434 deuterated-DA; m/e 590 norepinephrine; and m/e 592 deuterated-epinephrine.

Dopamine turnover. The turnover of retinal DA was determined by examining the rate of decline of endogenous dopamine after inhibiting catecholamine synthesis with AMPT. The concentration of dopamine in retina was measured 0, 30, and 60 min after an intraperitoneal injection of AMPT (300 mg/kg). The fractional rate constant and calculated rate of DA formation were determined as described by Brodie *et al.* (12).

RESULTS

Retinal tyrosine hydroxylase activity, assayed with subsaturating concentrations of $DMPH_4$, rapidly increased when dark-adapted rats were placed in the light (Fig. 1). Within 5 min enzyme activity was apparently increased maximally and remained so for 180 min. Tyrosine hydroxylase activity apparently remained elevated when rats were kept in light for 96 hr (Fig. 1). In subsequent studies we evaluated whether the apparent kinetic mechanisms for the increase of enzyme activity were similar in dark-adapted rats exposed to light for 15 min and in rats exposed to light for 96 hr.

Kinetic characteristics of tyrosine hydroxylase after exposure to light for 15 min. Exposure of dark-adapted rats to light for 15 min significantly decreased the apparent K_m of tyrosine hydroxylase for $DMPH_4$, but had no significant influence on the V_{max} (Fig. 2 and Table 1). There were no light-dark differences in the apparent K_m for tyrosine but the apparent V_{max} was higher in the rats exposed to 15 min of light when measured in the presence of 1.5 mM $DMPH_4$, a suboptimal concentration (Table 2).

Kinetic characteristics of tyrosine hydroxylase after exposure to light for 96 hr. The apparent V_{max} with respect to both tyrosine (Table 2) and $DMPH_4$ (Fig. 3 and Table 1) was higher in light than in dark-adapted rats. There was no light-dark difference in the K_m of the enzyme for either substrate or the cofactor.

The time-course for the onset of the V_{max} change was determined by measuring the activity of tyrosine hydroxylase in the presence of 4.0 mM $DMPH_4$, a saturating concentration of cofactor, after exposure to constant light or dark for, 1, 2, 3, or 4 days (Fig. 4). After one or two days of exposure

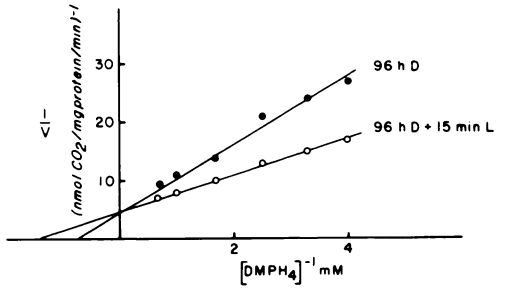

FIG. 2. *Reciprocal plot of retinal tyrosine hydroxylase activity versus $DMPH_4$ concentration after short-term light exposure*

Rats were exposed to dark for 96 hr (●——●) or dark for 96 hr followed by light for 15 min (○——○). Tyrosine hydroxylase was assayed as described in MATERIALS AND METHODS in the presence of 0.1 mM tyrosine. The best-fit lines were determined by least-squares linear regression analysis of the data from at least 5 experiments.

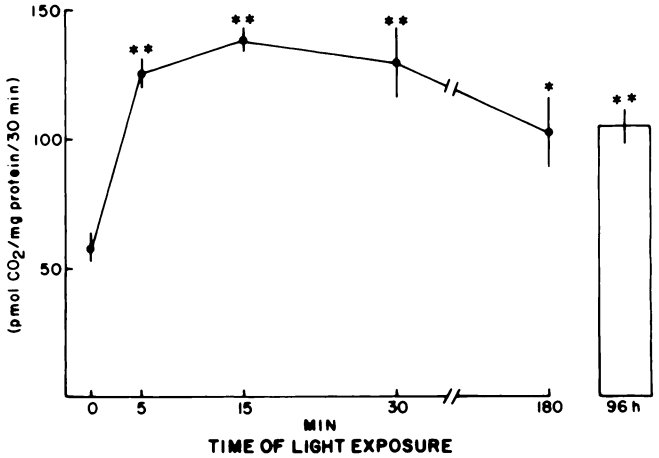

FIG. 1. *Activation of retinal tyrosine hydroxylase by light*

Rats were placed in the dark for 96 hr and then exposed to light for the times indicated. The histogram shows the activity after exposure to light for 96 hr. Data expressed as mean ± the standard error of the mean for 5 duplicate determinations. * $p < 0.025$; ** $p < 0.01$; Dunnett's *post hoc* test.

TABLE 1

Apparent retinal tyrosine hydroxylase kinetics for $DMPH_4$ in dark- and light-adapted rats and dark-adapted rats exposed to light for 15 min

Lighting	$DMPH_4$ kinetics[a]	
	K_m	V_{max}
	mM	*nmol CO_2/mg prot/30 min*
Dark 96 hr	1.4 ± 0.2	0.24 ± 0.02
Dark 96 hr + light 15 min	0.70 ± 0.09[b]	0.22 ± 0.02
Light 96 hr	1.0 ± 0.1	0.31 ± 0.01[b]

[a] Values, mean ± S.E.M., were determined from double reciprocal plots fitted by least-squares linear regression analysis for three separate studies. The concentration of tyrosine was 0.1 mM.

[b] $p < 0.05$ (Students' *t*-test, 2-tailed) when compared with corresponding 96 hr in dark group.

TABLE 2

Apparent retinal tyrosine hydroxylase kinetics for tyrosine in dark- and light-adapted rats and dark-adapted rats exposed to light for 15 min

Lighting	Tyrosine kinetics[a]	
	K_m	V_{max}
	mM	*nmol CO_2/mg prot/30 min*
Dark 96 hr	0.14 ± 0.01	0.23 ± 0.02
Dark 96 hr + light 15 min	0.13 ± 0.02	0.33 ± 0.02*
Light 96 hr	0.13 ± 0.02	0.54 ± 0.08*

* $p < 0.02$ (Student's *t*-test, 2-tailed) when compared with Dark 96 hr group.

[a] Values, mean ± S.E.M., were determined from double reciprocal plots fitted by least-squares linear regression analysis for three separate studies. The concentration of $DMPH_4$ was 1.5 mM.

there were no significant differences in enzyme activity but by the third day the activity from the light-exposed retinas was greater than that from the dark-exposed retinas. The magnitude of this difference continued to increase on the fourth day of constant light and dark. Over the three days of darkness, from the end of day 1 to the end of day 3, the enzyme activity decreased 17%, but this effect was not statistically significant. Over the same period of constant light, the enzyme activity was significantly increased by 86%.

Concentration of DA and its apparent rate of formation in retina. The concentration of DA in the retina was about 50% higher in 96 hr light-adapted rats than in 96 hr dark-adapted rats (Table 3). Norepinephrine was not detected in the retina. Our limit of sensitivity was about 0.5 pmol/retina.

The decline of DA appeared exponential in both light- and dark-adapted rats after treatment with AMPT. However, the decline was faster in light-adapted rats (Table 3). The rate of DA formation, calculated by multiplying the fractional rate constant by the steady-state concentration, was approximately 4-fold higher in the light-adapted animals.

Some properties of tyrosine hydroxylase after exposure to light. Light-adapted animals or dark-adapted animals exposed to light for 15 min were placed in the dark to study the time required for the enzyme to return to values found for dark-adapted rats (Fig. 5). The increase of enzyme activity after exposure to light for 15 min was completely reversed after about 30 min in the dark. In contrast, more than 6 hr was required for the enzyme activity of the 96 hr light-adapted animals to return to values found for the dark-adapted rats.

Tyrosine hydroxylase activity was assayed at pH 6.4 and 7.2 in the three experimental groups of animals (Table 4). At pH 6.4, which was the standard pH of our as-

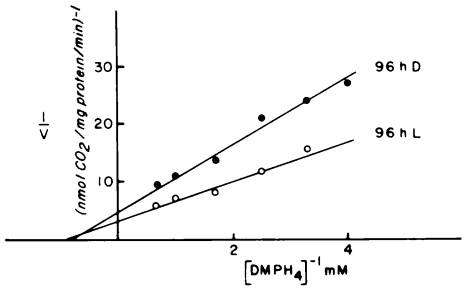

FIG. 3. *Reciprocal plot of retinal tyrosine hydroxylase activity versus $DMPH_4$ concentration after long-term light exposure*

Rats were exposed to light (○——○) or dark (●——●) for 96 hr. Tyrosine hydroxylase activity was assayed as described in MATERIALS AND METHODS in the presence of 0.1 mM tyrosine. The best-fit lines were determined by least-squares linear regression analysis of the data from at least 4 experiments.

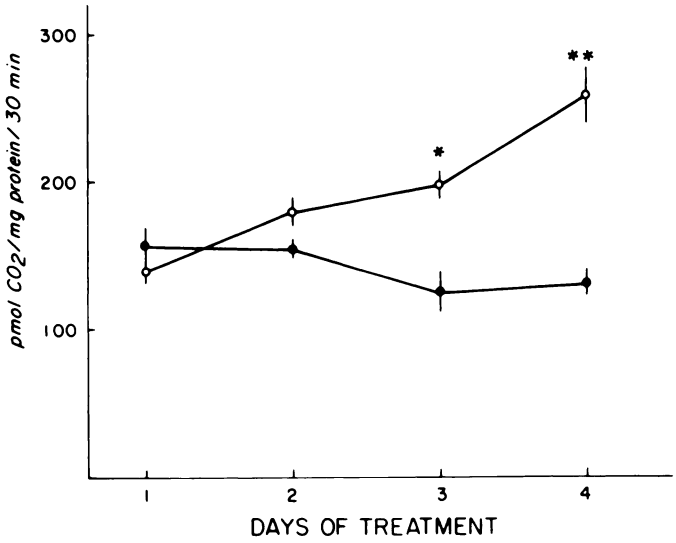

FIG. 4. *Prolonged light exposure enhances retinal tyrosine hydroxylase activity measured with a saturating concentration of $DMPH_4$*

Rats were exposed to light (○——○) or dark (●——●) for 1–4 days prior to sacrifice. Retinal tyrosine hydroxylase activity was measured as described in MATERIALS AND METHODS in the presence of 0.1 mM tyrosine and 4.0 mM $DMPH_4$. Statistical analysis by the Neuman-Keuls method. * Light day 3 vs. dark day 3, $p < 0.01$, and light day 3 vs. light day 1, $p < 0.05$; ** Light day 4 vs. dark day 4, $p < 0.01$, and light day 4 vs. light day 1, $p < 0.01$.

TABLE 3

Light-dark differences in retinal dopamine metabolism

Rats, 15 per group, were placed in light or dark for 96 hr. Alpha-methyl-p-tyrosine methylester (300 mg/kg, i.p.) was injected 0, 30, or 60 min prior to killing. Retinal DA was determined as described in MATERIALS AND METHODS. The fractional rate constant and calculated rate of formation were determined as described by Brodie *et al.* (12).

Condition	Dopamine steady-state concentration	Half-life	Fractional rate constant	Calculated rate of formation
	pmol/retina ± S.E.M.	*min*	*(min^{-1} ± S.E.M.) × 10^3*	*pmol/retina/min*
Light	22 ± 1	46	15.0 ± 0.3	0.33
Dark	15 ± 1*	137	5.0 ± 0.4*	0.075

* $p < 0.01$.

says, exposure of dark-adapted rats to light for 15 min increased enzyme activity by about 50% over dark-adapted rats. The enzyme activity for light-adapted animals, however, was about 140% higher than in dark-adapted rats. At pH 7.2, 15 min of exposure to light increased enzyme activity by about 190%. In contrast to enzyme activity at pH 6.4, there were no significant differences between enzyme activity for dark- and light-adapted rats. Enzyme activity for all the experimental groups was higher when measured at pH 6.4 than at pH 7.2.

DISCUSSION

DA-containing amacrine neurons of retina form synapses with bipolar and ganglion cells. They are therefore in a strategic position to modulate the transfer of visual information to the brain. Electrophysiological and biochemical studies have provided evidence that amacrine neurons are activated by light. For example, exposure to light results in synaptic depolarization (13), increased tyrosine hydroxylase activity and increased release (3) and turnover of DA (5) by amacrine neurons. DA apparently

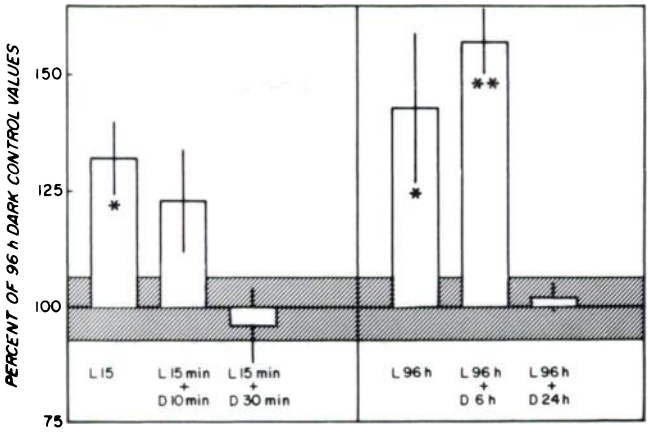

FIG. 5. *Dark-induced decrease in retinal tyrosine hydroxylase activity after short- and long-term light exposure*

Rats were exposed to dark for 96 hr followed by light for 15 min (L15) and returned to darkness for 10 min (L15 + D10) or 30 min (L15 + D30). Other rats were exposed to light for 96 hr (L96) and returned to darkness for 6 hr (L96 + D6) or 24 hr (L96 + D24). Tyrosine hydroxylase activity was measured as described in MATERIALS AND METHODS in the presence of 0.1 mM tyrosine and 0.4 mM $DMPH_4$. Data are expressed as the mean and S.E.M. of the increase in enzyme activity relative to rats exposed to dark for 96 hr. The hatched area represents the corrected S.E.M. of the activity of the rats exposed to 96 hr of dark, which was 61 ± 4 pmol CO_2/mg prot/30 min. Statistical analysis (Dunnett's *post hoc* test) was performed on the actual data. * $p < 0.025$; ** $p < 0.01$.

TABLE 4

Retinal tyrosine hydroxylase activity at pH 6.4 and 7.2 for dark- and light-adapted rats and dark-adapted rats exposed to light for 15 min

Retinas from two rats were pooled, homogenized in 270 μl of buffer at either pH 6.4 or 7.2, and assayed as described in MATERIALS AND METHODS. Values presented are the means ± S.E.M. for 5 separate assays. The concentrations of tyrosine and $DMPH_4$ were 0.1 mM and 0.4 mM, respectively.

Lighting	Tyrosine hydroxylase activity (pmol CO_2/mg prot/30 min)	
	pH 6.4	pH 7.2
Dark 96 hr	52 ± 6	21 ± 3
Dark 96 hr + light 15 min	77 ± 6*	60 ± 2*
Light 96 hr	123 ± 22*	27 ± 4

$p < 0.01$ (Student's *t*-test, 2-tailed) when compared with corresponding Dark 96 hr group.

decreases the activity of ganglion cells (14). Moreover, retina contains a DA-sensitive adenylate cyclase (15) which responds to drugs similarly to the DA-sensitive adenylate cyclase of the striatum (16). DA, therefore, meets many of the requirements for a neurotransmitter role in the retina.

DA, but not norepinephrine, was detected in retinal extracts. It is therefore assumed that the tyrosine hydroxylase activity measured in these experiments is derived primarily from dopaminergic neurons.

Both short-term (5) and long-term exposure to light increase retinal tyrosine hydroxylase activity and DA turnover. However, the molecular mechanisms involved in the increases in enzyme activity after short-term and prolonged light exposure are different. Short-term light exposure decreased the apparent K_m of tyrosine hydroxylase for $DMPH_4$ but did not influence the V_{max}. In contrast, long-term light exposure had no significant effect on the apparent K_m for $DMPH_4$ but increased the apparent V_{max}. Both treatments increased the apparent V_{max} with respect to tyrosine but had no effect on the K_m for this substrate. A suboptimal concentration of $DMPH_4$ was used in these studies, therefore the increased V_{max} for tyrosine in the short-term activated retinas was probably due to the decreased K_m for $DMPH_4$. However, the increased V_{max} in the long-term activated retinas may represent a true increase in the maximal velocity of the reaction under op-

timal conditions since the K_m for $DMPH_4$ was not significantly altered in this preparation.

Tyrosine hydroxylase activity after short-term light exposure was reversed within 30 min when the animals were placed in the dark. In contrast, tyrosine hydroxylase activity in the light-adapted rats required more than 6 hr to return to the activity of dark-adapted animals.

The increase in tyrosine hydroxylase activity after short-term exposure to light was greater when measured at pH 7.2 than at pH 6.4. In contrast, the increase in enzyme activity after prolonged exposure to light was greater at pH 6.4 than at pH 7.2. These findings taken together with the aforementioned findings suggest that the molecular mechanisms for the increase of enzyme activity after short- and long-term exposure to light are different.

The rate of formation of DA increased by about the same extent after short- and long-term exposure to light, even though the mechanisms for enhanced enzyme activity are apparently different (5). Both exposure schedules increase DA formation about 4-fold. Moreover, both increase the rate of loss of DA after treatment with AMPT when compared with dark-adapted animals, an indication that amacrine neurons are activated (17) by short or prolonged exposure to light.

The kinetic and enzymic property changes we found for the retina, as well as similar studies reported for other catecholamine-containing systems, provide the bases for the following hypothetical model. Enhanced tyrosine hydroxylase activity following rapid changes in neuronal activity may be the consequence of enzyme activation resulting in a decrease of the K_m for the pteridine cofactor without a change in the number of active enzyme molecules. Enhanced tyrosine hydroxylase activity following prolonged increases in neuronal activity appears to result in more active enzyme molecules, as indicated by the increased V_{max} of the reaction. The end result is enhanced DA formation. Activation of tyrosine hydroxylase is apparently only a provisional state which proceeds to an increase in the amount of active enzyme when amacrine neurons are continually activated by light. Approximately 3 days of stimulation are required for the increase in V_{max} to develop as indicated by the observation that there were no differences in enzyme activity measured in the presence of saturating concentrations of cofactor until 3 days of constant light.

Incubating tyrosine hydroxylase from striatal tissue under phosphorylating conditions results in apparent kinetic changes that are similar to those we observed after short-term exposure to light (18, 19). Moreover, the effect of phosphorylating conditions on tyrosine hydroxylase activity is greater when measured at pH 7 than at pH 6 (20, 21). These findings suggest that phosphorylation of tyrosine hydroxylase or some allosteric effector may be involved in the short-term activation of DA-containing neurons.

The kinetic changes found after short-term light activation of tyrosine hydroxylase resemble those seen after activation of nigrostriatal DA-containing neurons by neuroleptic drugs (7, 8) or direct electrical stimulation of catecholamine-containing neurons (22, 6).

The increase in the apparent V_{max} of tyrosine hydroxylase after prolonged activation of the retinal DA-containing neurons may represent an induction of the enzyme or may represent a delayed activation of existing enzyme similar to that reported to occur in locus coeruleus 3 days after oxotremorine treatment (23).

In conclusion, we have shown that light increases retinal tyrosine hydroxylase activity and that the characteristics of the activity increase depend on the duration of exposure to light and by inference amacrine neuron activation. The initial response to increased amacrine neuron activity is activation of tyrosine hydroxylase; there is a change in the K_m of tyrosine hydroxylase for its pteridine cofactor, possibly by an allosteric mechanism. More active enzyme molecules may accumulate after prolonged increases in neuronal activity, making it unnecessary for the enzyme to remain in the activated state. Both responses to light exposure enhance the formation of DA by the amacrine neurons.

REFERENCES

1. Haggendal, J. & Malmfors, T. (1965) *Acta Physiol. Scand.*, **64,** 58–66.
2. Malmfors, T. (1963) *Acta Physiol. Scand.*, **58,** 99–100.
3. Kramer, S. G. (1971) *Invest. Ophthalmol.*, **10,** 438–452.
4. Da Prada, M. (1977) *Adv. Biochem. Psychopharmacol.*, **16,** 311–319.
5. Iuvone, P. M., Galli, C. L., Garrison-Gund, C. K. & Neff, N. H. (1978) *Science,* in press.
6. Weiner, N., Lee, F.-L., Barnes, E. & Dreyer, E. (1977) in *Structure and Function of Monoamine Enzymes* (Usdin, E., Weiner, N. & Youdim, M. B. H., eds.), pp. 109–148, Marcel-Dekker, New York.
7. Zivkovic, B., Guidotti, A. & Costa, E. (1974) *Mol. Pharmacol.*, **10,** 727–735.
8. Zivkovic, B., Guidotti, A., Revuelta, A. & Costa, E. (1975) *J. Pharmacol. Exp. Ther.*, **194,** 37–46.
9. Kaufman, S. (1962) *Meth. Enzymol.*, **5,** 809–816.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193,** 265–275.
11. Koslow, S. H., Cattabeni, F. & Costa, E. (1972) *Science,* **176,** 177–180.
12. Brodie, B. B., Costa, E., Dlabac, A., Neff, N. H. & Smookler, H. H. (1966) *J. Pharmacol. Exp. Ther.*, **154,** 493–498.
13. Werblin, F. S. & Dowling, J. E. (1969) *J. Neurophysiol.*, **32,** 339–355.
14. Ames, A. III & Pollen, D. A. (1969) *J. Neurophysiol.*, **32,** 424–442.
15. Brown, J. H. & Makman, M. H. (1972) *Proc. Nat. Acad. Sci. U.S.A.*, **69,** 539–543.
16. Spano, P. F., Gogoni, S., Hofmann, M., Kumakura, K. & Trabucchi, M. (1977) *Adv. Biochem. Psychopharmacol.*, **16,** 307–310.
17. Gordon, R., Spector, S., Sjoerdsma, A. & Undenfriend, S. (1966) *J. Pharmacol. Exp. Ther.*, **153,** 440–447.
18. Harris, J. E., Morgenroth, V. H. III, Roth, R. H. & Baldessarini, R. J. (1974) *Nature* **252,** 156–158.
19. Lovenberg, W., Bruckwick, E. A. & Hanbauer, I. (1975) *Proc. Nat. Acad. Sci. U.S.A.*, **72,** 2955–2958.
20. Goldstein, M., Bronaugh, R. L., Ebstein, B. & Roberge, C. (1976) *Brain Res.*, **109,** 563–574.
21. Lloyd, T. & Kaufman, S. (1975) *Biochem. Biophys. Res. Commun.*, **66,** 907–913.
22. Murrin, L. C., Morgenroth, V. H. III & Roth, R. H. (1976) *Mol. Pharmacol.*, **12,** 1070–1081.
23. Lewander, T., Joh, T. H. & Reis, D. J. (1977) *J. Pharmacol. Exp. Ther.*, **200,** 523–534.
24. Waymire, J. C., Bjur, R. & Weiner, N. (1971) *Anal. Biochem.*, **43,** 588–600.