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Tyrosine Administration Enhances Dopamine Synthesis and Release in Light-Activated Rat Retina

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With 1 Figure

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Summary

Exposure of dark-adapted albino rats to light (350 lux) significantly elevated retinal levels of the dopamine metabolite dihydroxyphenyl acetic acid during the next hour; their return to a dark environment caused dihydroxyphenyl acetic acid levels to fall. Retinal dopamine levels were increased slightly by light exposure, suggesting that the increase in dihydroxyphenyl acetic acid reflected accelerated dopamine synthesis. Administration of tyrosine (100 mg/kg, i.p.) further elevated retinal dihydroxyphenyl acetic acid among light-exposed animals, but failed to affect dopamine release among animals in the dark. These observations show that a physiological stimulus—light exposure—can cause catecholaminergic neurons to become tyrosine-dependent; they also suggest that food consumption may affect neurotransmitter release within the retina.

Introduction

The rate at which neurons synthesize the catecholamine neurotransmitters depends upon the activity of the enzyme tyrosine hydroxylase (TH) which catalyzes the conversion of tyrosine to dopa (*Udenfriend*, 1966). The enzyme's activity is diminished in the presence of its catecholamine end products and increased when the

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firing frequency of the neuron containing it is accelerated (*Weiner et al.*, 1977).

TH activity can also be affected by tissue tyrosine concentrations (*Wurtman et al.*, 1974; *Wurtman et al.*, 1981; *Gibson* and *Wurtman*, 1977) which determine the saturation of the enzyme with this substrate. The extent to which catecholaminergic neurons respond to having more tyrosine also depends to a major extent on their apparent firing frequencies: for example, in their resting state, nigrostriatal neurons fail to synthesize or release more dopamine when rats receive tyrosine (*Wurtman et al.*, 1981); however, when the animals receive a treatment that accelerates nigrostriatal firing, the neurons become highly responsive to additional tyrosine. To date, all of the experimental manipulations that have caused TH to become tyrosine-dependent have used drugs or lesions. We now show that a physiologic stimulus—exposure of rats to environmental lighting—which selectively activates particular dopaminergic neurons, can also cause these neurons to become tyrosine-dependent.

The neurons examined were the dopaminergic amacrine cells present in the inner nuclear layer of the retina; their processes extend to the inner plexiform layer where they synapse with other amacrine cells (*Ehinger*, 1977). Like TH in other tissues, the retinal enzyme exists in two states—a basal state in dark-adapted retinas, and an activated state in light-exposed retinas (*Iuvone et al.*, 1978 a; *Iuvone et al.*, 1978 b; *Iuvone et al.*, 1979) Brief exposure to light activates the enzyme so that its activity doubles within 15 min when rats are placed under 400 lux of fluorescent light (*Iuvone et al.*, 1978 b). Light adaption also results in increases in dopa accumulation after decarboxylase inhibition (*Proll et al.*, 1982 a; *Proll* and *Morgan*, 1982 b; *daPrada*, 1977) and increases in DA content (*Nichols et al.*, 1967). We find that such light exposure also allows tyrosine administration to affect retinal dopamine's synthesis and release.

Methods

Animals and Drugs

Male Sprague-Dawley rats (150–200 g; Charles River Breeding Laboratories, Wilmington, MA) housed in hanging metal cages had free access to food (Charles River Rat and Mouse Formula) and tap water and were exposed to light from noon to midnight (Vita-Lite, 300 $\mu W/cm^2$; Duro-Test Corp, North Bergen, NJ; 11–33 lux illumination inside rat cages). Animals were acclimatized to the lighting schedule for at least 1 week before experimentation. On the experimental day, rats were dark-adapted for 11 hours and then exposed to direct lighting (~ 350 lux) for 30 or 60 min as noted.

Except as noted, tyrosine was administered (100 mg/kg) as the l-amino acid (Aldrich Chemical Co., Milwaukee, WI) in a saline based slurry; it was injected intraperitoneally 30 min after exposure to light.

Animals were decapitated and blood was collected from the neck wound. Serum was assayed for tyrosine fluorometrically (*Waalkes* and *Udenfriend,* 1957). At time of killing, eyes were quickly removed and frozen on dry ice. In dark-adapted rats, experiments were conducted under a dim red light (GE F40R, showing no illumination on a light meter).

Dissection and Assays

Retinas were dissected from the frozen eyes on an ice-cold glass plate. The cornea was sliced off with a razor blade and the lens and frozen vitreous extruded by mild pressure with a blunt forceps. Retinas were removed and transferred with a microspatula to Eppendorf tubes containing 0.1 N perchloric acid and an internal standard, dihydroxybenzylamine (4 ng; Sigma Chemical Co., St. Louis, MO). Samples were sonicated (Cell Disruptor W-225R, Heat Systems-Ultrasonics; Plainview, NY), and centrifuged (Eppendorf 5413; Brinkman, Westbury, NY) and the supernatant fluids were assayed for dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) using high performance liquid chromatography with electrochemical detection (BioAnalytical Systems, West Lafayette, IN [*Felice et al.,* 1978]). The mobile phase consisted of 0.05 M sodium phosphate buffer (pH 5.0) containing 0.25 mM sodium octyl sulfate, 0.1 mM EDTA and 4% methanol. Standards (DA-HCl, DOPAC and dihydroxybenzylamine-HBr) were obtained from Sigma Chemical Co. or Regis Chemical Co. (Morton Grove, IL), and high pressure liquid chromatography grade solvents from the J. T. Baker Co. (Phillipsburg, NJ) or Burdick and Jackson (Muskegon, MI).

Results

Light Activation of Retinal Dopamine Neurons

In rats exposed to light (350 lux for 30 min), retinal DOPAC increased from 0.99 ± 0.10 to 3.74 ± 0.39 ng/pair ($p < 0.01$) and retinal DA from 4.11 ± 0.17 to 5.42 ± 0.46 ng/pair. Serum tyrosine was unaffected by light; its concentration was 19.9 ± 0.4 μg/ml in dark-exposed rats vs 17.2 ± 0.7 in light-exposed animals.

Iuvone et al. found that retinal TH was activated 15 min after previously dark-adapted rats were exposed to 400 lux of light. We similarly observed a time-dependent increase in retinal DOPAC over a 60-min period of exposure to 350 lux (Table 1), from 1.6 to 2.7 ng/pair; the increase became significant at 30 min. Retinal DA also tended to increase, but these changes did not attain statistical significance. As before, serum tyrosine concentration was unaffected by the light.

When rats adapted to light for 12 hours were placed in the dark for 2 hours, retinal DOPAC fell from 3.4 to 1.9 ng/pair ($p < 0.01$). DA levels were not significantly different (4.7 ng/pair in light-adapted retinas vs 3.8 ng/pair in dark-adapted retinas).

Effect of Tyrosine on Retinal DOPAC Accumulation

As described above, exposure of rats to light increased retinal DOPAC significantly (Table 2). This increase was enhanced by administration of tyrosine (100 mg/kg): DOPAC levels rose from 2.8 to 4.0 ng/pair ($p < 0.01$) in light-exposed animals but failed to change at all among animals kept in darkness (1.5 vs 1.6 ng/pair) (Table 2). In this experiment, light significantly elevated retinal DA (from 2.9 to

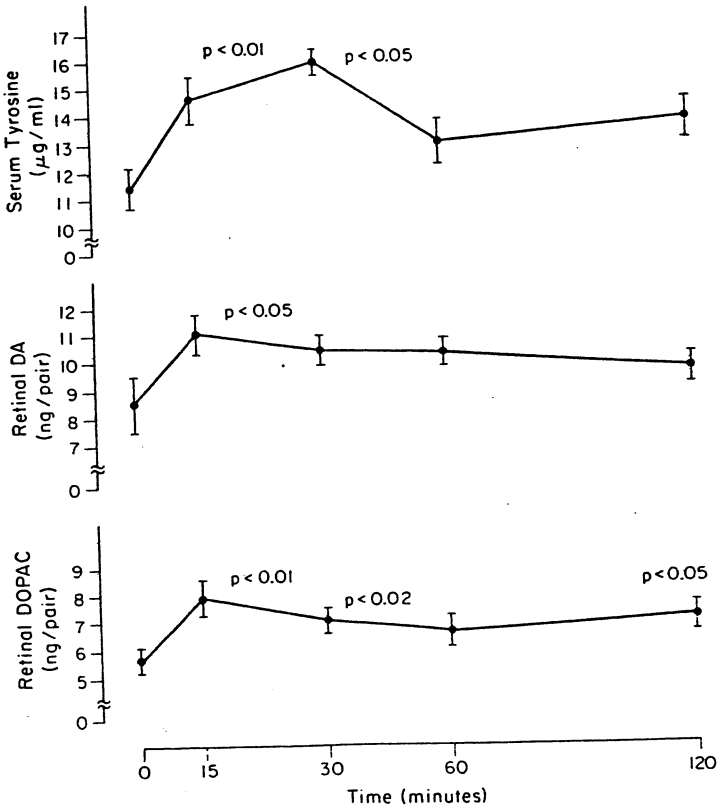

Fig. 1. Effect of tyrosine on increases in catechols caused by light exposure. Groups of five male Sprague-Dawley rats were injected intraperitoneally with a suspension of L-tyrosine (100 mg/kg in saline) and decapitated 15, 30, 60, or 120 min later. Injections were given between the 8th and 9th hour of the daily light period. Retinal DA and DOPAC were measured by HPLC with electrochemical detection (*Felice et al.*, 1978). Serum tyrosine was measured fluorimetrically (*Waalkes* and *Udenfriend*, 1957). Data are presented as ng/pair of retinas ± S.E.M.

Table 1. *Effect of light exposure on retinal DA and DOPAC*

Duration of exposure (min)	DA (ng/pair)	DOPAC (ng/pair)
0	4.2±0.3	1.6±0.2
1	4.3±0.1	1.5±0.1
2	4.4±0.3	1.7±0.2
5	4.8±0.4	2.2±0.1
15	4.5±0.4	2.1±0.1
30	5.6±0.7	2.7±0.4*
60	5.4±0.2	2.7±0.3*

Groups of six male Sprague-Dawley rats were dark-adapted for 11 hours and then exposed to light (350 lux) for varying amounts of time. Retinas were assayed for DA and DOPAC using an HPLC method with electrochemical detection (*Felice et al.*, 1978). Data are expressed as means ± S.E.

* $p < 0.05$ compared with zero-time controls, by one-way analysis of variance using the Neuman-Keuls test.

4.7 ng/pair, $p < 0.05$); tyrosine administration had no further effect on DA levels, although serum tyrosine rose significantly in both dark-adapted (40%) and light-exposed rats (33%).

In a separate study, retinal tyrosine levels in rats given tyrosine as the ethyl ester (115 mg/kg); Sigma) were increased by 60% among dark-adapted animals (from a mean of 1.47 μg/pair to 2.34 μg/pair) and by 66% among light-exposed animals (1.56 vs 2.59 μg/pair); serum tyrosine concentrations increased by 50 and 75%, respectively.

Among rats injected with tyrosine during the 8th hour of the daily light period and killed at intervals thereafter, retinal DOPAC

Table 2. *Effect of tyrosine on increase in retinal catechols caused by light exposure*

		DA (ng/pair)	DOPAC (ng/pair)
Dark	Saline	2.9±0.2	1.5±0.3
	Tyrosine	2.6±0.3	1.6±0.3
Light	Saline	4.7±0.4*	2.8±0.1*
	Tyrosine	4.3±0.5*	4.0±0.5†

Groups of six male Sprague-Dawley rats were dark-adapted for 11 hours and then exposed to 1 hour of light (350 lux) before killing. Thirty min before decapitation, animals were injected with tyrosine (100 mg/kg, i.p.) or its vehicle.

* $p < 0.05$ differs significantly from either dark group.

† $p < 0.01$ differs significantly from dark groups and from light-exposed controls.

Data are expressed as means ± S.E.; statistical differences were determined by two-way analysis of variance and the Neuman-Keuls test.

levels were significantly elevated after 15 min and at all times examined thereafter (30, 60 and 120 min). Retinal DA was significantly elevated only at 15 min (Fig. 1). Serum tyrosine levels peaked 30 min after its administration and remained elevated for 2 hours.

Discussion

These findings extend earlier indications that light exposure activates retinal dopaminergic amacrine cells. (Similar observations were recently reported [*Frucht et al.*, 1982] showing that the light-induced increases in DA and DOPAC are dependent on the presence of viable photoreceptor cells rather than on a direct effect of light on the amacrine cells.)

We have further shown that light exposure, a physiological input, allows the dopaminergic amacrine cells of the rat's retina to synthesize more dopamine when provided with additional tyrosine. When animals were dark-adapted, the levels of DOPAC, a major dopamine metabolite in retina, were low, and failed to increase after systemic tyrosine administration (Table 2). When animals were exposed to room lighting for 30 min or more, retinal DOPAC levels were elevated; moreover, increases in serum tyrosine that failed to elevate retinal DOPAC in dark-adapted rats did elevate the DA metabolite in these light-exposed rats (Table 2).

Iuvone et al. (1978 a; 1978 b; 1979) have shown that light exposure activates the TH in retinal amacrine cells, initially increasing the enzyme's affinity for its tetrahydrobiopterin cofactor and subsequently also increasing the enzyme's v_{max}. Since treatments that activate TH also cause neurons to synthesize more catecholamines after tyrosine administration (*Gibson* and *Wurtman*, 1977; *Wurtman et al.*, 1981; *Scally et al.*, 1977; *Sved* and *Fernstrom*, 1981, *Melamed et al.*, 1980), the most likely explanation for our findings is that, as a consequence of light exposure, retinal TH became activated and thus dependent on the availability of its amino acid substrate. This increased dependence could reflect greater saturation with the enzyme's cofactor (similar to the changes in saturation observed after activation with neuroleptic drugs (*Zivkovic et al.*, 1974) or simply greater utilization of the amino acid.

In most experiments we did not measure retinal tyrosine levels but instead monitored plasma tyrosine. In the single study in which both tissues were assayed, their tyrosine levels did change in parallel after tyrosine administration. Within the central nervous system, tyrosine levels depend not only on plasma tyrosine but also on

plasma concentrations of several other large neutral amino acids (*e.g.*, leucine, isoleucine, valine, phenylalanine and tryptophan [*Fernstrom* and *Faller*, 1978]) which compete with tyrosine for transport across the blood-brain barrier. It is not known whether a similar competitive carrier system mediates the uptake of circulating tyrosine into the retina.

Plasma tyrosine levels increase markedly in rats (and humans) consuming high-protein meals: the plasma tyrosine/large neutral amino acids ratio in humans is lowered after such meals, but in rats it is elevated (*Fernstrom* and *Faller*, 1978; *Fernstrom et al.*, 1979). Hence, regardless of whether retinal tyrosine levels depend on plasma tyrosine or on its ratio to other large neutral amino acids, it seems likely that they will be influenced by food consumption which may in turn affect dopaminergic transmission in the retina. Physiologic consequences of such alterations await characterization.

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