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Extracellular dopamine concentration in the retina of the clawed frog, *Xenopus laevis*

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ABSTRACT **Dopamine reaches targets in the outer retina of the clawed frog (*Xenopus laevis*) by diffusion from a network of dopaminergic cells and processes located predominantly at the junction of inner nuclear and inner plexiform layers. We obtained values for the steady-state release, uptake, and extracellular concentration of dopamine in the retina by a combination of HPLC (with electrochemical detection), scintillation spectroscopy, and fast-scan cyclic voltammetry. Vitreal concentrations of dopamine varied from 564 ± 109 nM in light-adapted eyes near the time of subjective dawn to 156 ± 12 nM in dark-adapted eyes. The data are consistent with a simple model for steady-state dopamine diffusion from an appropriately sited thin-sheet source. This model was used to generate a profile of extracellular dopamine concentration as a function of retinal depth. The model predicted an increase in the dopamine concentration from the vitreous to the layer of dopaminergic cells, remaining constant from that layer to the distal tips of the photoreceptors. This prediction was borne out by comparing fast-scan voltammetric measures of dopamine at the distal tips of the receptors with the vitreal concentrations determined by HPLC using electrochemical detection.**

In the vertebrate retina, dopamine has been implicated in a host of processes, including modulation of neural circuitry (1–4), light and dark adaptation (5), photomechanical movements (6), and circadian rhythmicity (7). The sole source of dopamine in the retina is a subclass of amacrine or interplexiform cell (8). Thus in all vertebrates except fishes, the great majority of dopaminergic processes are confined to the inner retina (9), yet there is clear evidence that outer retinal cells—i.e., photoreceptors, horizontal cells, and cells of the retinal pigment epithelium, located 10–100 μm from the layer of dopaminergic neurons—have receptors for dopamine (10–12). On this basis it has been postulated that dopamine reaches these targets by diffusion (11), thus exemplifying a "local hormone" or volume transmission mode of communication (13).

The motivation for the present study was to answer the central questions raised by this diffusion hypothesis: what is the extracellular concentration in the vicinity of the outer retinal cells, and how well does this concentration correspond to the thresholds of the dopamine-dependent mechanisms revealed by application of exogenous dopamine to the intact tissue?

METHODS

Clawed frogs (*Xenopus laevis*) were obtained from Nasco (Ft. Atkinson, WI) and housed in a 300-gallon aquarium on a 12 hr/12 hr light/dark cycle (lights on at 6 a.m.) for at least 3 weeks before use. Room illumination by fluorescent bulbs located 2 m above the tank provided about 30 μW/cm² at the surface of the water. Dark-adapted eyes were obtained from frogs kept overnight in a light-tight container and sacrificed under dim red light.

To measure vitreous dopamine, dark- or light-adapted eyes were flash frozen on dry ice immediately after enucleation, and the frozen vitreous bodies were dissected free of adherent tissues under a microscope. A 10-μl aliquot of deionized water containing 0.004% ascorbate was added to each sample. The isolated vitreous bodies were stored at −80°C until analyzed by HPLC with electrochemical detection (ED) using an ESA (Bedford, MA) model 5100 coulometric electrochemical detector equipped with a model 5011 dual-electrode high-sensitivity cell. The working potential for the coulometric–amperometric analytical cell was +0.05 V for dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC), preceded by a −0.22-V reduction cell. The system was fitted with an ESA HR-80 column run at ambient temperature. The mobile phase was 50 mM monobasic sodium phosphate to which 0.008% EDTA, 0.003% sodium octyl sulfate, 0.0232% heptanesulfonic acid, and 8–10% methanol were added. The pH was 3.06 and the flow rate was 1.5 ml/min.

To estimate the rate of dopamine release, eyecups were incubated in 200 μl of Ringer solution (14) with 0.009% ascorbate in room light, at room temperature, in a 95% O_2/5% CO_2 atmosphere. After 30 min an aliquot was removed and flash frozen until analyzed by HPLC–ED as described above.

For uptake studies, [^{3}H]dopamine (45.7 Ci/mmol; 1 Ci = 37 GBq) was obtained from New England Nuclear. Aliquots were evaporated to dryness under a stream of N_2 and resuspended in *Xenopus* Ringer solution, pH 7.4. The posterior pole of an eyecup was incubated for 20 min in Ringer solution with or without the uptake inhibitor nomifensine (10 μM) on a rotating table (65 rpm) at room temperature in room light. The eyecup then was incubated for 20 min in Ringer solution with [^{3}H]dopamine at 2 nM to 5 μM. After several washes in ice-cold Ringer solution, the retina was dissected free and dissolved in Protosol (New England Nuclear). The dissolved tissue was suspended in Aquasol (New England Nuclear) containing 1% glacial acetic acid and the radioactivity was measured in a Beckman LT 3801 scintillation spectrometer. Counts were corrected to dpm by using an external standard and then converted to concentration by reference to the specific activity. Nomifensine was a gift from Hoechst–Roussel; ouabain was purchased from Sigma. Specificity of uptake sites was tested by autoradiography. Eyecups were incubated with [^{3}H]dopamine for 30 min and then processed for autoradiography by standard methods (15).

Fast-scan cyclic voltammetry (16–18) was used to measure extracellular dopamine. Preparation of Nafion-coated carbon fiber microelectrodes (CFVMs) has been described (16). The voltage scan rate was 900 V/sec from −0.4 V (holding

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Abbreviations: ED, electrochemical detection; CFVM, carbon fiber microelectrode; DOPAC, 3,4-dihydroxyphenylacetic acid.

potential) to +1.0 V vs. Ag/AgCl with a sampling interval of 100 msec. CFVMs were calibrated before and after tissue measurements with dopamine and DOPAC solutions; post-calibrations were used to calculate extracellular concentration in the tissue. Some electrodes also were calibrated with norepinephrine. Although the voltammograms for dopamine, DOPAC, and norepinephrine were similar, the electrodes were 100–1000 times more sensitive to dopamine than to DOPAC and 2 times more sensitive to dopamine than to norepinephrine.

RESULTS

Model for Extracellular Dopamine Distribution in the Retina. Immunocytochemistry (19, 20) of the dopaminergic cells in *Xenopus* retina has revealed that the cells and processes are distributed fairly evenly in the horizontal plane at a density of 25 cells per mm^2. The arbors of adjacent cells overlap, creating a dense network of fine processes in the distal-most sublamina of the inner plexiform layer. In this model we assume that dopamine efflux occurs solely from a thin sheet located 25 μm from the vitreous. Dopamine is removed from the extracellular space by reuptake into dopaminergic cells and their processes and by diffusion into the vitreous.

Since the sheet of dopaminergic processes extends throughout the retina we can assume that dopamine diffuses only in the direction normal to the retinal surface. The concentration of dopamine at any point x(cm) and time t(sec) is $C(x, t)$ (mol·liter^{-1}). The value of $x = 0$ occurs at the retina/vitreous interface. Dopamine is released at a distance, a (0.0025 cm), from the vitreous with a flux Q (mol·cm^{-2}·sec^{-1}) and recaptured with flux U (mol·cm^{-2}·sec^{-1}). In general, U will be a function of concentration obeying Michaelis–Menten kinetics (21), but in the steady state U will take a single value and we can combine the efflux and uptake into a single term for net efflux, $P = Q - U$ (mol·cm^{-2}·sec^{-1}).

The released dopamine enters a restricted extracellular space defined by a volume fraction α. In many brain regions α is about 0.21 (22), but in the retina there is evidence from impedance measurements that α is much smaller (average value of $\alpha = 0.074$; ref. 23). After release into the extracellular space, the diffusion of dopamine is hindered by the obstructions imposed by cells and their processes and the influence of these factors is characterized by the tortuosity, λ (22). The effect is to reduce the diffusion coefficient, D (cm^2·sec^{-1}), that would obtain in a free aqueous medium, by a factor of λ^2. Tortuosity has not been measured in the retina but has been taken to be 1.55 (23). The value of D for dopamine at the temperature of these experiments is 5.8×10^{-6} cm^2·sec^{-1} (16).

To complete the model we define the boundary conditions. We assume that the retinal pigment epithelium is functionally impermeable, so that all dopamine which fails to be recaptured by reuptake leaves the retina at the vitreous surface. In the steady state the flux at this surface must equal the net efflux at the cell layer and, furthermore, the concentration at this surface must be constant and equal to the concentration in the vitreous body, C_v (mol·liter^{-1}).

These considerations enable the steady-state dopamine distribution to be described by

$$C = C_v + \frac{P\lambda^2}{\alpha D}x, \quad x < a; \qquad C = C_v + \frac{P\lambda^2}{\alpha D}a, \quad x \geq a. \quad [1]$$

Dopamine Concentration in the Vitreous Body (C_v). Fig. 1 shows the chromatograms of DOPAC and dopamine standards compared with those obtained from vitreous samples. These peaks were clearly resolved from that of norepinephrine, which also was identified in the vitreous (Fig. 1*B*). The concentrations of dopamine and DOPAC in the vitreous body varied as a function of lighting conditions and time of day (Fig. 2). Vitreous samples harvested from eyes exposed to light for 3 hr (6–9 a.m., $n = 8$) had 564 ± 109 nM dopamine (mean ± SE) and 311 ± 29 nM DOPAC, whereas eyes taken from animals in the same batch, but dark-adapted overnight and sacrificed at 9 a.m. ($n = 9$) had 156 ± 12 nM dopamine and 41 ± 4 nM DOPAC. Dopamine and DOPAC concentrations were 231 ± 16 nM and 121 ± 15 nM, respectively, in eyes from which the vitreous was removed under light-adapted conditions at various times between 10 a.m. and 4 p.m. ($n = 25$). These data indicate that dopamine turnover and release are stimulated by light and are higher shortly after subjective dawn compared to later periods in the day, even when the light level is kept constant during the later period.

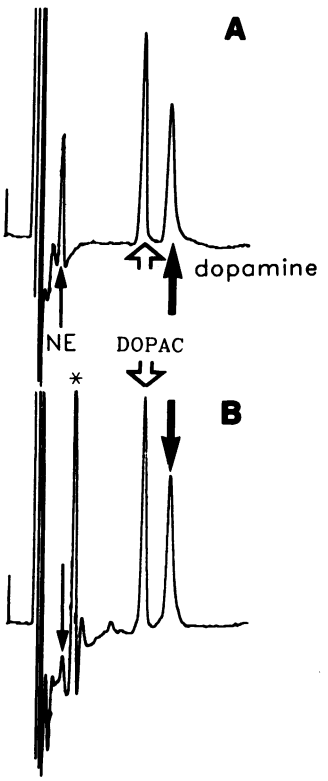

FIG. 1. (*A*) Chromatogram generated by a mixture of standards: norepinephrine (NE, thin arrow), DOPAC (open arrow), and dopamine (thick arrow), each at 100 pg/20 μl. (*B*) Chromatogram of a vitreous sample. Note the close correspondence between standard and experimental DOPAC and dopamine peaks. There is relatively little norepinephrine in the vitreous sample. A prominent additional peak (asterisk) is not matched by any of the three catecholamines in *A*.

Net Dopamine Release by *Xenopus* Eyecups ($Q - U$). Dopamine overflow was measured from light-adapted eyes between 3 and 4 p.m. The rate of release was $2.5 \pm 0.8 \times 10^{-16}$ mol of dopamine per retina per second and $3.6 \pm 0.8 \times 10^{-16}$ mol of DOPAC per retina per second (mean ± SE; $n = 6$). For a hemispherical *Xenopus* retina of diameter 4.5 mm, the dopamine release is equivalent to a mean value for ($Q - U$) of 8.1×10^{-15} mol·cm^{-2}·sec^{-1}. The retinal content of dopamine and DOPAC was estimated by HPLC from retinal homogenates at 7.5×10^{-12} mol and 1.7×10^{-12} mol, respectively, indicating that the turnover of dopamine in light-adapted eyes is about 2% per minute.

[^{3}H]Dopamine Uptake by the Retina (U). The rate of dopamine accumulation by the *Xenopus* retina was estimated from the uptake of [^{3}H]dopamine (Fig. 3). The data points

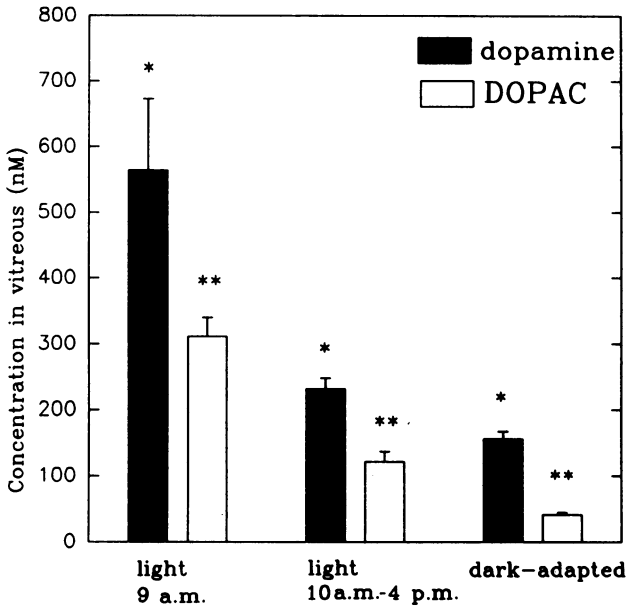

FIG. 2. Vitreous concentrations of dopamine (filled bars) and DOPAC (open bars) as a function of time of day and lighting conditions. The left-hand pair represent eyes exposed to 3 hr of room light between 6 and 9 a.m. (mean ± SE, $n = 8$). The right-hand pair give the comparable values from eyes dark-adapted overnight until 9 a.m. ($n = 9$). The central pair give dopamine/DOPAC concentrations from light-adapted eyes obtained at various times from 10 a.m. to 4 p.m. ($n = 25$). For dopamine, the mean values are statistically different at the 95% confidence level (single asterisk), and for DOPAC, at the 99% confidence level (double asterisks).

were fitted by the Michaelis–Menten function (solid line), from which K_m was estimated at 1.8 μM and V_{max} at 5.8 × 10^{-16} mol per retina per second. Dopamine uptake per unit time is approximately a linear function of bath concentration in the range 5–500 nM. Uptake was reduced to about 50% control by exposure to either 10 μM nomifensine or 100 μM ouabain. Autoradiograms (Fig. 4) indicated that the ^{3}H label was concentrated over the plexus of fine fibers in sublamina 1 of the inner plexiform layer, indicating that [^{3}H]dopamine was taken up selectively by dopaminergic fibers.

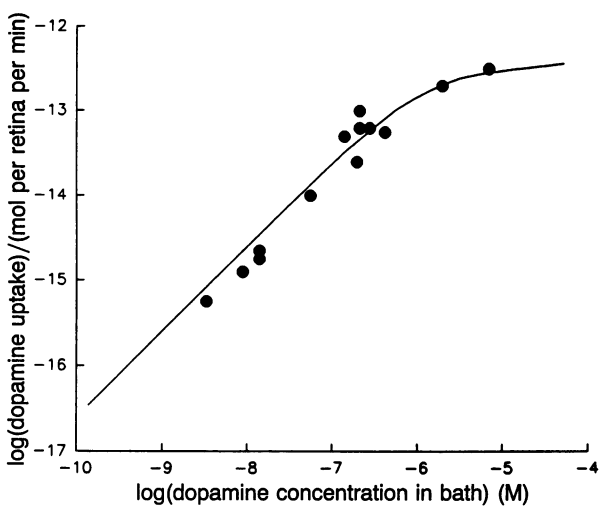

FIG. 3. Uptake of dopamine by the *Xenopus* retina as a function of its concentration in the bath. Total uptake was computed from retinal radioactivity, as explained in *Methods*. The Michaelis–Menten equation $V/V_{max} = [C]/([C] + K_m)$ fits the data for $V_m = 5.8 \times 10^{-16}$ mol per retina per second and $K_m = 1.8$ μM.

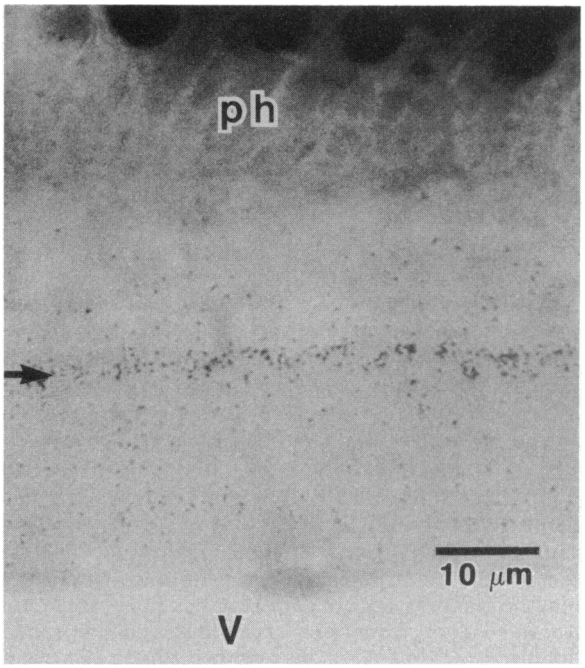

FIG. 4. [^{3}H]Dopamine autoradiography of a semithin vertical section of *Xenopus* retina showing radioactive grains concentrated over sublamina 1 of the inner plexiform layer (arrow). V, vitreous; ph, photoreceptor layer.

With these experimental values we can evaluate expression (1). Since both our voltammetric and efflux measures were obtained in the afternoon under light-adapted conditions, the corresponding value for vitreous dopamine concentration, $C_v = 231$ nM (Fig. 2, 10 a.m. to 4 p.m.). As noted above, the net flux $(Q - U) = P = 8.1 \times 10^{-15}$ mol·cm^{-2}·sec^{-1}. Thus, from Eq. 1, $C = 231 + 45{,}100x$ nM, $x < a$; $C = 344$ nM, $x > a$. The accuracy of this prediction was tested by fast-scan cyclic voltammetry.

Voltammetric Studies. In our initial experiments, eyecups were pinned to the wax bottom of the perfusion chamber through which Ringer solution flowed at 1.5 ml/min. Reference signals were obtained in the solution above the eyecup. Then the electrode was positioned adjacent to the retina/choroid interface at the cut edge of the eyecup, where a large voltammogram with an oxidation peak characteristic of catecholamines was recorded. By reference to the dopamine calibration curve, this signal was equivalent to a local concentration of 734 ± 154 nM (range, 310–2130 nM; $n = 13$). It was noted, however, that when the CFVM remained positioned at the edge of the eyecup while the retina was progressively detached mechanically, the voltammogram was virtually unaffected. Further experiments showed that the choroid generated a large voltammogram, as did the isolated iris. On the other hand, the apical surface of the retinal pigment epithelium, which could be approached by the CFVM after the retina either was folded back or stripped entirely from the eyecup, generated no voltammetric signal.

Both iris and choroid are known to be innervated by sympathetic, noradrenergic fibers (24, 25). Since norepinephrine and dopamine generate nearly identical voltammograms (26), it was probable that norepinephrine was responsible for the large contaminating response. As noted above, norepinephrine was identified by HPLC–ED (see Fig. 1) in vitreous samples from light-adapted eyes and found to be present at 111 ± 13 nM ($n = 7$). To minimize contamination from this source of norepinephrine, we utilized isolated retinas placed receptor-side-up in the perfusion chamber. The interval between dissection and voltammetric scan was 2–5 min. When

the CFVM closely approached or touched the receptor surface we obtained voltammograms with a catecholamine oxidation peak at +0.5 V and an earlier peak near +0.3 V (Fig. 5 *Lower*) which reflects the CFVM sensitivity to pH (16). Rapidly metabolizing tissues typically are more acidic than the bath pH (27). Fig. 5 *Upper* illustrates the calibration voltammogram when the same CFVM was exposed to 500 nM dopamine. We obtained 24 voltammograms from 11 light-adapted retinas, between 12 and 4 p.m. The mean value was 283 ± 22 nM (±SE).

DISCUSSION

Our main finding is that the dopamine levels found experimentally in the extracellular space of the *Xenopus* retina are much higher than in dopamine-rich regions of mammalian brain (28–30). Correspondingly, extracellular DOPAC concentration in striatum is about 1000-fold higher than that of dopamine, but this ratio is about 1 in the retina. We suggest that the basis of this difference is the geometry of the dopaminergic cells. In striatum, dopaminergic terminals form a dense and uniform plexus throughout, whereas in the retina, the great majority of dopaminergic fibers are confined to a thin stratum. Thus, most released dopamine is recaptured in striatum but can more readily diffuse away from the release sites in the retina.

A second main finding is that exposure to light greatly increases dopamine production and turnover in the *Xenopus* retina, compared with that measured in dark-adapted animals. Our data agree qualitatively with those of Boatright *et al.* (31), who reported that 1 hr of exposure to light increased dopamine overflow 2.4-fold over dark-adapted controls. We found that DOPAC levels also were increased about 2-fold by exposing the eye to light. Thus, light increases the absolute level of dopamine while decreasing the dopamine/DOPAC ratio (3.8 in the dark to 1.8 in the light), indicating that synthesis and turnover may be separately regulated. This is suggested by the data of Wulle *et al.* (32), who examined

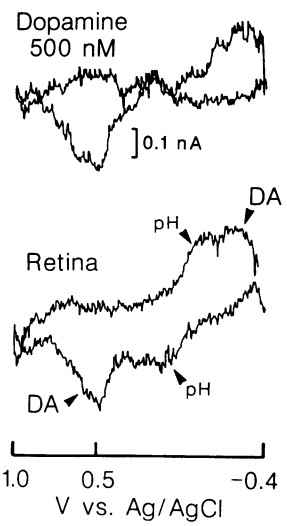

FIG. 5. Dopamine voltammograms. Fast-scan cyclic voltammograms from *in vitro* calibration with 500 nM dopamine (*Upper*) and from the isolated retina (*Lower*). Scan rate was 900 V/sec. Voltammograms were obtained by subtracting a background voltammogram from the scan recorded after addition of dopamine to Ringer solution or after closely approaching the photoreceptor surface of the retina. The oxidation potential for dopamine at this electrode was +0.5 V vs. Ag/AgCl. The additional oxidation and reduction peaks in the retinal voltammogram (arrowheads) reflect an artifact from the more acidic pH of the retina compared to the superfusion medium. By reference to the calibration, the retinal dopamine concentration was 320 nM. DA, dopamine.

retinal dopamine and DOPAC content as well as tyrosine hydroxylase activity in a cichlid fish. They noted that changes in these measures were greatest around times of altered light conditions. Our model for dopamine diffusion predicted that, under steady-state conditions, the concentration of dopamine would increase from the vitreous to the layer of dopaminergic cells and then remain constant to the photoreceptor layer. When tested under the same lighting conditions and time of day, voltammetric measures of dopamine concentration in outer retina were indeed higher than those found in the vitreous by HPLC. We note two possible sources of error in our measurements. First, norepinephrine may have contributed to the voltammetric peak. Given the differential sensitivity of the CFVM to dopamine and norepinephrine and the vitreous concentration of norepinephrine of about 100 nM, this error is maximally 50 nM. Second, when the retina is isolated from the retinal pigment epithelium and immersed in a bath, there will be an efflux of dopamine (and norepinephrine) from the retina. Further analysis of the diffusion model (not described here) indicates that, over sufficient time, the dopamine level in the photoreceptor layer will fall to a low value, so that our voltammetric sampling of this region could underestimate the true dopamine concentration.

We return now to the central questions of this study, posed in the Introduction, whether changes in the extracellular concentration of dopamine correspond to the thresholds of dopamine-dependent mechanisms. We confine ourselves to studies of amphibian retinas and to outer retinal targets of dopamine. Iuvone (33) showed that 1 μM dopamine, acting through a D_2 receptor, inhibited by 80% the nocturnal increase of *N*-acetyltransferase activity. The end product of *N*-acetyltransferase activity is melatonin, a potent inhibitor of dopamine release (34). Cahill and Besharse (35) found that 100–1000 nM dopamine suppressed melatonin release in proportion to concentration and also was effective in phase shifting a circadian clock. The pharmacological characteristics of these responses also implicated a D_2 receptor. Dearry *et al.* (36) studied dopamine-dependent retinomotor movements in the bullfrog *Rana catesbeiana*. Cone contraction was effected through a D_2 mechanism whereas pigment dispersal in retinal pigment epithelial cells had the characteristics of a D_1 dopamine receptor-dependent response. In either case, however, 1 μM dopamine elicited 50–80% of the maximum response. Recent electrophysiological studies of dopamine-induced shifts in rod–cone inputs to horizontal cells in the *Xenopus* retina (37) showed that threshold effects were achieved with 0.5 μM dopamine. Both D_1 and D_2 receptors were implicated. Collectively these studies are quite consistent in indicating that changes in extracellular dopamine concentration in the range 0.1–1.0 μM would alter the degree of activation of several D_1 and D_2 dopamine-dependent responses in outer retina. It is very significant that this concentration range is exactly that which our measurements indicate occurs in the *Xenopus* retina.

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1. Hedden, W. L., Jr., & Dowling, J. E. (1978) *Proc. R. Soc. London B* **201**, 27–51.
2. Gerschenfeld, H. M., Neyton, J., Piccolino, M. & Witkovsky, P. (1982) *Biomed. Res.* **3**, Suppl., 21–32.
3. Knapp, A. G. & Dowling, J. E. (1987) *Nature (London)* **325**, 437–439.
4. Witkovsky, P., Stone, S. & Besharse, J. C. (1988) *Brain Res.* **449**, 332–336.

5. Witkovsky, P. & Shi, X.-P. (1990) *Visual Neurosci.* **5,** 405–413.
6. Dearry, A. & Burnside, B. (1988) in *Dopaminergic Mechanisms in Vision*, eds. Bodis-Wollner, I. & Piccolino, M. (Liss, New York), pp. 109–135.
7. Besharse, J. C. & Iuvone, P. M. (1983) *Nature (London)* **305,** 133–135.
8. Ehinger, B. (1982) *Retina* **2,** 305–321.
9. Witkovsky, P. & Schutte, M. (1991) *Visual Neurosci.* **7,** 113–124.
10. Piccolino, M., Neyton, J. & Gerschenfeld, H. M. (1984) *J. Neurosci.* **4,** 2477–2488.
11. Witkovsky, P., Alones, V. & Piccolino, M. (1987) *J. Neurocytol.* **16,** 55–67.
12. Dearry, A. & Burnside, B. (1986) *J. Neurochem.* **46,** 1006–1021.
13. Fuxe, K. & Agnati, L. F., eds. (1991) *Volume Transmission in the Brain* (Raven, New York).
14. Stone, S. & Witkovsky, P. (1984) *J. Physiol. (London)* **353,** 249–264.
15. Hendrickson, A. & Edward, S. B. (1978) in *Neuroanatomical Research Techniques*, ed. Robertson, R. T. (Academic, New York), pp. 242–290.
16. Rice, M. E. & Nicholson, C. (1989) *Anal. Chem.* **61,** 1805–1810.
17. Armstrong-James, M. & Millar, J. (1979) *J. Neurosci. Methods* **1,** 279–287.
18. Millar, J., Stamford, J. A., Kruk, Z. L. & Wightman, R. M. (1985) *Eur. J. Pharmacol.* **109,** 341–348.
19. Schutte, M. & Witkovsky, P. (1991) *J. Neurocytol.* **20,** 195–207.
20. Zhu, B. & Straznicky, C. (1991) *Anat. Embryol.* **184,** 33–45.
21. Snyder, S. H. & Coyle, J. T. (1969) *J. Pharmacol. Exp. Ther.* **165,** 78–86.
22. Nicholson, C. & Phillips, J. M. (1981) *J. Physiol. (London)* **321,** 225–257.
23. Karwoski, C. J., Frambach, D. A. & Proenza, L. M. (1985) *J. Neurophysiol.* **54,** 1607–1619.
24. Haggendal, J. & Malmfors, T. (1965) *Acta. Physiol. Scand.* **64,** 58–66.
25. Armstrong, P. B. & Bell, A. I. (1968) *Am. J. Physiol.* **214,** 566–573.
26. Baur, J. E., Kristensen, E. W., May, L. J., Wiedemann, D. J. & Wightman, R. M. (1988) *Anal. Chem.* **60,** 1268–1272.
27. Chesler, M. & Chan, C. Y. (1988) *Neuroscience* **27,** 941–948.
28. Gonon, F. G. & Buda, M. J. (1985) *Neuroscience* **13,** 765–774.
29. Louilot, A., LeMoal, M. & Simon, H. (1986) *Brain Res.* **397,** 395–400.
30. Leviel, V., Gobert, A. & Guibert, B. (1989) *Brain Res.* **499,** 205–213.
31. Boatright, J. H., Hoel, M. J. & Iuvone, P. M. (1989) *Brain Res.* **482,** 164–168.
32. Wulle, I., Kirsch, M. & Wagner, H.-J. (1990) *Brain Res.* **515,** 163–167.
33. Iuvone, P. M. (1986) *Life Sci.* **38,** 331–342.
34. Dubocovich, M. (1983) *Nature (London)* **306,** 782–784.
35. Cahill, G. M. & Besharse, J. C. (1991) *J. Neurosci.* **11,** 2959–2971.
36. Dearry, A., Edelman, J. L., Miller, S. & Burnside, B. (1990) *J. Neurochem.* **54,** 1367–1378.
37. Krizaj, D. & Witkovsky, P. (1992) *Soc. Neurosci. Abstr.* **18,** 840.