

# Histamine Elevates Free Intracellular Calcium in Mouse Retinal Dopaminergic Cells via H<sub>1</sub>-Receptors

Renata Frazão,<sup>1</sup> Douglas G. McMahon,<sup>2</sup> Walter Schunack,<sup>3</sup> Proleta Datta,<sup>1</sup> Ruth Heidelberger,<sup>1</sup> and David W. Marshak<sup>1</sup>

**PURPOSE.** Previously, retinopetal axons containing histamine and dopaminergic neurons expressing histamine H<sub>1</sub>-receptor had been localized in mouse retinas using anatomic techniques. The goal of these experiments was to demonstrate that these receptors are functional.

**METHODS.** Dopaminergic cells were acutely isolated from retinas of transgenic mice expressing red fluorescent protein under control of the tyrosine hydroxylase promoter and loaded with the calcium indicator Fura-2.

**RESULTS.** Under control conditions, there were spontaneous oscillations in the levels of free intracellular calcium in dopaminergic cells. These oscillations were abolished in nominally calcium-free extracellular medium and in 1  $\mu$ M tetrodotoxin, findings suggesting that the oscillations were mediated by calcium entry across the plasma membrane in response to sodium-dependent action potentials. Histamine increased the mean free intracellular calcium in the dopaminergic cells by increasing the frequency and/or amplitude of the calcium oscillations. The effects of histamine were dose-dependent and reached maximum at 5  $\mu$ M. With this dose, there was a 65% increase in the mean free intracellular calcium concentration. The histamine H<sub>1</sub>-receptor antagonist, pyrilamine, blocked the effects of 5  $\mu$ M histamine when applied at 50  $\mu$ M. The selective histamine H<sub>1</sub>-receptor agonists, 2-(3-trifluoromethylphenyl) histamine and methylhistaprodifen significantly increased mean free intracellular calcium when applied at 5  $\mu$ M.

**CONCLUSIONS.** Histamine released from retinopetal axons in the mouse retina can elevate intracellular calcium levels in the perikarya of dopaminergic cells via the activation of histamine H<sub>1</sub>-receptors. (*Invest Ophthalmol Vis Sci.* 2011;52:3083–3088) DOI:10.1167/iovs.10.6160

In several mammalian species, the retina receives a projection from histaminergic neurons in the posterior hypothalamus. These include guinea pigs,<sup>1</sup> macaques,<sup>2</sup> rats,<sup>3</sup> and mice.<sup>4</sup> In guinea pigs, rats, and mice, there is evidence suggesting that

dopaminergic cells are among the targets of these retinopetal axons. Histamine inhibits the release of dopamine from guinea pig<sup>5</sup> and rat (Marshak DW, et al. *IOVS* 2008;49:ARVO E-Abstract 5792) retinas, and immunoreactive histamine H<sub>1</sub>-receptor (HR1) has been localized to dopaminergic neurons in rats<sup>6</sup> and mice.<sup>4</sup> In this respect, histaminergic retinopetal axons resemble those containing gonadotropin-releasing hormone in the teleost.<sup>7</sup>

To understand the function of the histaminergic inputs, we studied the effects of histamine on dopaminergic cells in mouse retinas. To ensure that the effects of histamine that we observed were direct, we used acutely isolated neurons. Dopaminergic neurons comprise only a small proportion of the total in the retina, and they have no distinctive morphologic features after dissociation. Therefore, we used a transgenic mouse model in which the dopaminergic neurons express a red fluorescent protein.<sup>7</sup> To monitor the effects of histamine and other agents, we loaded the dissociated cells with a calcium-sensitive dye and monitored levels of free intracellular calcium ([Ca<sup>2+</sup>]<sub>i</sub>) in the fluorescent perikarya.<sup>8</sup> We found that histamine increased the mean [Ca<sup>2+</sup>]<sub>i</sub> level in the perikarya of mouse dopaminergic cells. This effect was dose dependent in the low micromolar range and was mediated by HR1.

## METHODS

All experiments were begun in mid morning, 2 hours after the onset of the light phase of a 12-hour light/12-hour dark cycle. The mice were euthanized by cervical dislocation and the eyes were enucleated, with procedures that adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The eyes were hemisected and the retinas isolated. Each retina was cut into three pieces and stored in Ames medium at 11°C containing 20 mM D-glucose (Sigma-Aldrich, St Louis, MO) and equilibrated with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Retinal pieces were then incubated for 6 minutes at 22°C in low-Ca<sup>2+</sup> saline solution containing (in mM): 138 NaCl, 0.5 CaCl<sub>2</sub>, 0.5 MgCl<sub>2</sub>, 0.4 MgSO<sub>4</sub>, 5 KCl, 0.44 KH<sub>2</sub>PO<sub>4</sub>, 0.34 Na<sub>2</sub>HPO<sub>4</sub>, 10 D-glucose, and 10 HEPES (pH 7.4, 310–315 mOsm), supplemented with 2.7 mM L-cysteine and 30 U/mL papain (Sigma-Aldrich). Each piece of retina was individually triturated in approximately 0.5 mL of normal saline at 11°C with a fire-polished Pasteur pipette. The dissociated cells were plated at low density onto clean glass coverslips (12-545-82-12Cir-1D; Fisher Scientific, Pittsburgh, PA).

The cells were loaded for 45 minutes in darkness at 22°C with 1  $\mu$ M Fura-2 acetoxymethyl ester in saline containing 0.5 mM Ca<sup>2+</sup> (Invitrogen-Molecular Probes, Eugene, OR). Then, the coverslips were mounted on the stage of an inverted microscope with epifluorescent illumination (Carl Zeiss Meditec, GmbH, Oberkochen, Germany) and superfused with HEPES-buffered saline solution in a moist oxygen atmosphere at 22°C. Alternating excitation at 360 and 380 nm was provided by a computer-controlled, monochromator-based system, and the resulting fluorescence signal was captured by a photomultiplier.<sup>8</sup> To minimize phototoxic damage and bleaching of Fura-2, exposure to UV light was limited. During each ~565-ms epoch, a cell was exposed to a total of

From the <sup>1</sup>Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, Houston, Texas; the <sup>2</sup>Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee; and the <sup>3</sup>Institut für Pharmazie, Freie Universität Berlin, Berlin, Germany.

Supported by postdoctoral fellowship 201804/2008-5 from the National Council for Scientific and Technological Development (RF) and National Eye Institute Grants EY06472 (DWM), EY012128 (RH), and EY09256 (DGM) and Core Grant EY10608.

Submitted for publication July 2, 2010; revised November 9, 2010; accepted December 14, 2010.

Disclosure: **R. Frazão**, None; **D.G. McMahon**, None; **W. Schunack**, None; **P. Datta**, None; **R. Heidelberger**, None; **D.W. Marshak**, None

Corresponding author: David W. Marshak, Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, PO Box 20708, Houston, TX 77225; david.w.marshak@uth.tmc.edu.

65 ms of UV illumination: 30 ms of illumination at 360 nm, followed by 35 ms of illumination at 388 nm, followed by 500 ms of darkness. Free intracellular calcium ( $[Ca^{2+}]_i$ ) was determined from the ratio of the emitted fluorescence signals at the two wavelengths.<sup>9</sup> The system was calibrated in vitro using a Ca-EGTA-buffered standard solution.<sup>10</sup>  $K_{effective}$  was  $2.1 e^{-6}$  M,  $R_{min}$  was 0.770, and  $R_{max}$  was 12.587.

The following external solutions were used (in mM). Normal  $Ca^{2+}$  solution: 138 NaCl, 2.0  $CaCl_2$ , 0.5  $MgCl_2$ , 0.4  $MgSO_4$ , 5 KCl, 0.44  $KH_2PO_4$ , 0.34  $Na_2HPO_4$ , 10 D-glucose, and 10 HEPES (pH 7.4; 310–315 mOsm). Nominally 0  $Ca^{2+}$  solution: 138 NaCl, 2.0,  $MgCl_2$ , 0.4  $MgSO_4$ , 5 KCl, 0.44  $KH_2PO_4$ , 0.34  $Na_2HPO_4$ , 10 D-glucose, and 10 HEPES (pH 7.4; 310–315 mOsm). Low- $Ca^{2+}$  solution was made as described earlier.

The dopaminergic cells were identified by their red fluorescence (Fig. 1). Under the conditions used for the physiological experiments, their perikarya were relatively large and round, having average diameters  $17.2 \pm 1.8$  (long axis)  $\times$   $16.2 \pm 1.5$   $\mu m$  (short axis;  $n = 20$ ). Many of the cells had processes with varicosities. The second type of labeled cell, with smaller perikarya,<sup>7</sup> was only rarely observed under

these dissociation conditions and were not studied. The cells that showed swelling or exponential increases in  $[Ca^{2+}]_i$  were not included in the data set. Three cells exhibiting red fluorescence were typically present on each coverslip, but only one cell was studied on each coverslip.

The recording experiments were performed with a 60 $\times$  water-immersion objective (Olympus, Lake Success, NY). The recordings started with a 5-minute baseline period showing  $[Ca^{2+}]_i$  in the presence of normal saline solution. Then, the chamber was superfused with saline varying in calcium concentration or else normal saline plus the drug of interest. To minimize desensitization, histamine and its agonists were applied only once to each cell. Normal saline was also applied alone as a control for perfusion artifacts. The drugs used included histamine (made fresh daily; Sigma-Aldrich), pyrilamine maleate salt (Sigma-Aldrich), 2-(3-trifluoromethylphenyl)histamine dihydrogenmaleate (TFMH),<sup>9,11</sup> methylhistaprodifen dihydrogenoxalate (MH),<sup>10,12</sup> and tetrodotoxin (TTX; Sigma-Aldrich).

The group mean  $[Ca^{2+}]_i$  was calculated over the last 3 minutes of saline or drug application in each cell. Mean  $[Ca^{2+}]_i$  values were typically compared by using paired *t*-tests, when studying the same cells in two different conditions. A repeated-measures ANOVA was used to compare three mean  $[Ca^{2+}]_i$  values in one set of experiments (Prism; GraphPad, San Diego, CA). All the results were expressed as the mean  $\pm$  SD, except where otherwise noted. To determine the effects of histamine on the variance of  $[Ca^{2+}]_i$  during each 3-minute time period, we obtained the variance for each treatment by pooling the sum of all the squared deviations from the mean of each cell and then divided the result by the degrees of freedom. The ratio of the pre- and posttreatment variance in  $[Ca^{2+}]_i$  was analyzed with an *F*-test (Excel; Microsoft, Redmond, WA).

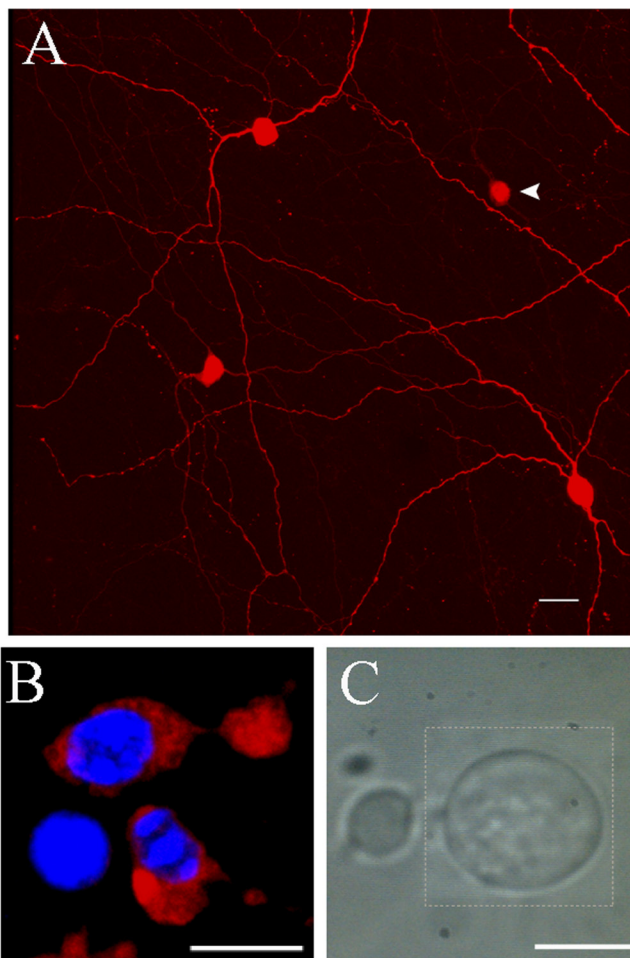
## RESULTS

Isolated dopaminergic cells of the mouse retina showed prominent oscillations in  $[Ca^{2+}]_i$  in normal  $Ca^{2+}$  external solution, consistent with what has been reported in other species.<sup>13,14</sup> These oscillations were dependent on extracellular  $Ca^{2+}$ , as they decreased substantially when the experiment was performed using low- $Ca^{2+}$  solution and were absent in nominally 0  $Ca^{2+}$  (Fig. 2). The average value of  $[Ca^{2+}]_i$  in normal  $Ca^{2+}$  external solution was  $107.9 \pm 29.8$  nM (mean  $\pm$  SD) versus  $68.6 \pm 21.2$  nM ( $n = 6$ ;  $P = 0.0183$ ) in nominally 0  $Ca^{2+}$  external solution.

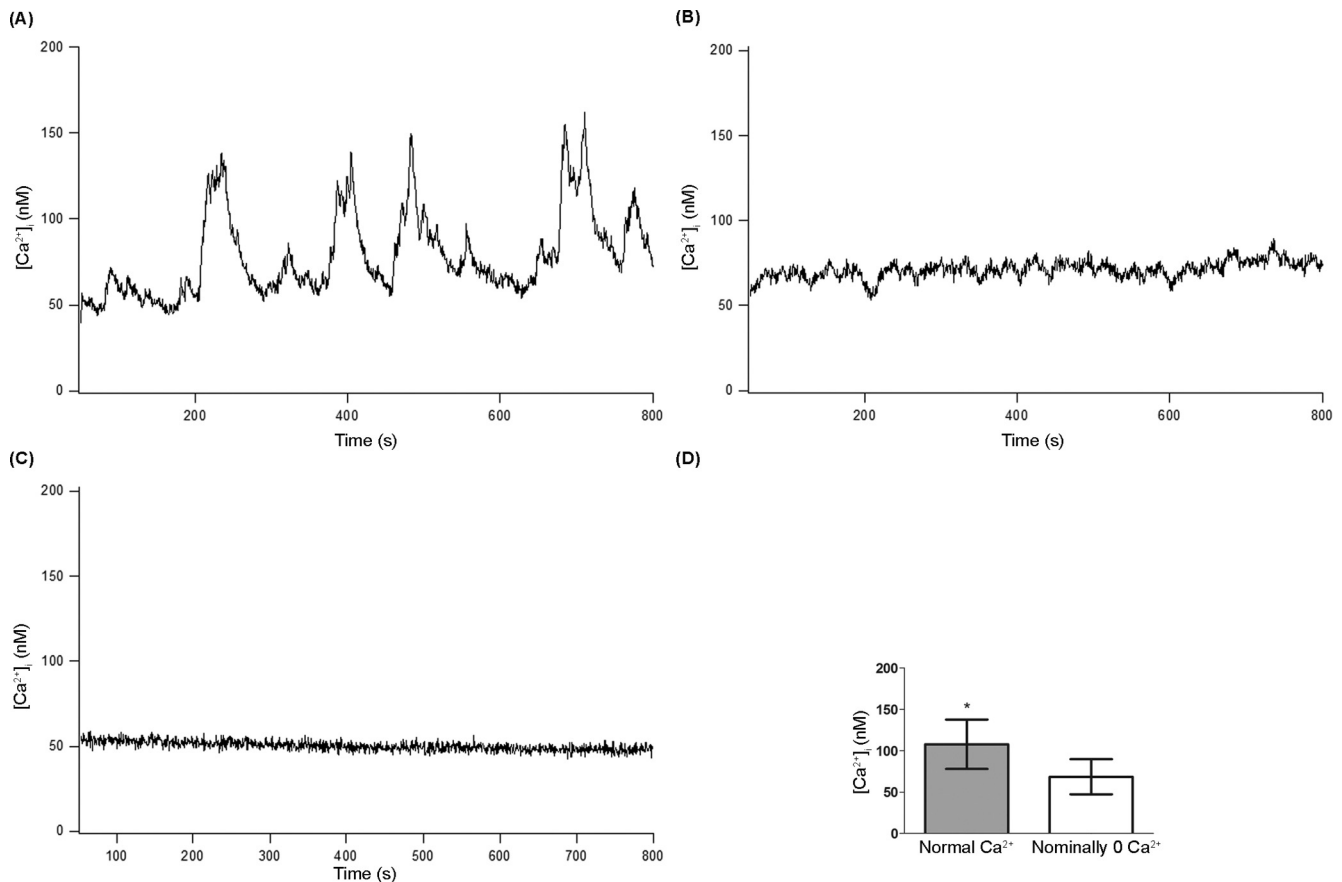
The  $Ca^{2+}$  oscillations were also dependent on voltage-gated  $Na^+$  channels, like those described previously in rats.<sup>14</sup> As shown in Figure 3, superfusion with 1  $\mu M$  TTX in normal  $Ca^{2+}$  external solution completely blocked the  $Ca^{2+}$  oscillations. Accordingly, inhibition of the  $Ca^{2+}$  oscillations by TTX resulted in a significant decrease in the mean  $[Ca^{2+}]_i$  ( $n = 6$ ;  $P = 0.0132$ ).

To test whether histamine application could affect  $Ca^{2+}$  levels in dopaminergic cells, we bath applied the histamine (5  $\mu M$ ). Figure 4A shows a representative response. On average, 5  $\mu M$  histamine elevated the mean  $[Ca^{2+}]_i$  by 65%. The average value of  $[Ca^{2+}]_i$  in normal  $Ca^{2+}$  external solution was  $89.8 \pm 32.2$  nM, whereas the average of  $[Ca^{2+}]_i$  in normal  $Ca^{2+}$  external solution in the presence of 5  $\mu M$  histamine was  $143.9 \pm 57.2$  nM ( $n = 14$ ,  $P = 0.0005$ ). Control experiments with normal saline did not show an increase in mean  $[Ca^{2+}]_i$ , indicating that the rise in  $Ca^{2+}$  observed in the presence of histamine was not due to a perfusion artifact ( $n = 11$ ;  $P = 0.6053$ ).

To determine whether histamine relied on mechanisms similar to those that generated the spontaneous  $Ca^{2+}$  oscillations, we examined the effects of 5  $\mu M$  histamine on dopaminergic neurons in nominally 0  $Ca^{2+}$  external solution. Under these conditions, superfusion with histamine had no effect (not illustrated;  $n = 8$ ;  $P = 0.2628$ ). The  $Na^+$  channel blocker



**FIGURE 1.** Morphology of dopaminergic neurons. (A) A whole mount image of a transgenic mouse retina in which dopaminergic cells express a red fluorescent protein.<sup>7</sup> Three large dopaminergic cells are visible. A smaller red cell of a type that was not examined in this study is also observed (arrowhead). (B) Isolated dopaminergic cells (red) were fixed for 10 minutes in 4% paraformaldehyde and coverslipped in mounting medium containing the nuclear dye 4,6-diamino-2-phenylindole dihydrochloride (DAPI; blue). A stack of nine 0.5- $\mu m$  optical sections was collected with a confocal scanning microscope. (C) An acutely dissociated dopaminergic cell used in an experiment. Dotted box: region of the field from which the emitted Fura-2 fluorescence signal was collected. Scale bar: (A) 20  $\mu m$ ; (B, C) 10  $\mu m$ .



**FIGURE 2.** Effects of external  $Ca^{2+}$  concentration on dopaminergic cells. (A) Oscillations of  $[Ca^{2+}]_i$  levels were observed in normal (2 mM) external  $Ca^{2+}$ . (B) Oscillations were greatly decreased in 0.5 mM  $Ca^{2+}$  and (C) were absent in nominally 0  $Ca^{2+}$ . (D) A statistically significant difference in the  $[Ca^{2+}]_i$  levels was observed between the normal  $Ca^{2+}$  ( $n = 7$ ) and nominally 0  $Ca^{2+}$  ( $n = 6$ ) groups ( $P = 0.0183$ ). Data are the mean  $\pm$  SD.

TTX, which blocked the  $Ca^{2+}$  oscillations, also prevented the histamine-induced rise in mean  $[Ca^{2+}]_i$  in normal  $Ca^{2+}$  (not illustrated;  $n = 7$ ;  $P = 0.1297$ ).

The effects of histamine on mean  $[Ca^{2+}]_i$  were dose dependent. Superfusion of 0.5  $\mu$ M ( $n = 8$ ) or 1.0  $\mu$ M ( $n = 7$ ) histamine produced greater than 20% increases in mean  $[Ca^{2+}]_i$  in five cells; however, these results were not statistically significant. Higher doses of histamine yielded increases in a larger proportion of cells, and these increases were statistically significant. Superfusion of 2  $\mu$ M histamine increased mean  $[Ca^{2+}]_i$  in 66% of the cells analyzed ( $n = 12$ ;  $P = 0.036$ ), 5  $\mu$ M histamine increased mean  $[Ca^{2+}]_i$  in 79% of the cells analyzed ( $n = 14$ ;  $P = 0.0005$ ), and 10  $\mu$ M histamine increased  $[Ca^{2+}]_i$  in 54% of the cells analyzed ( $n = 11$ ;  $P = 0.0067$ ). The effect of histamine was maximum at 5  $\mu$ M; the increase in mean  $[Ca^{2+}]_i$  was 65%, on average, with that dose. The  $EC_{50}$ , calculated by fitting the dose-response curve from 0.1 to 5  $\mu$ M, was  $1.9 \pm 0.5$   $\mu$ M (Fig. 4B).

The variance of the mean  $[Ca^{2+}]_i$  was also significantly greater at histamine doses ranging from 1 to 10  $\mu$ M, a finding suggesting that the oscillations had increased in amplitude, frequency, or both. At 1  $\mu$ M histamine, the variance was 3.7 times that at baseline ( $P < 0.0001$ ), and at 2  $\mu$ M the variance was 3.5 times that at baseline ( $P < 0.0001$ ). The effect on variance was smaller, but statistically significant, at higher doses of histamine: 2.8 times baseline at 5  $\mu$ M ( $P < 0.0001$ ) and 1.9 times baseline at 10  $\mu$ M ( $P < 0.0001$ ).

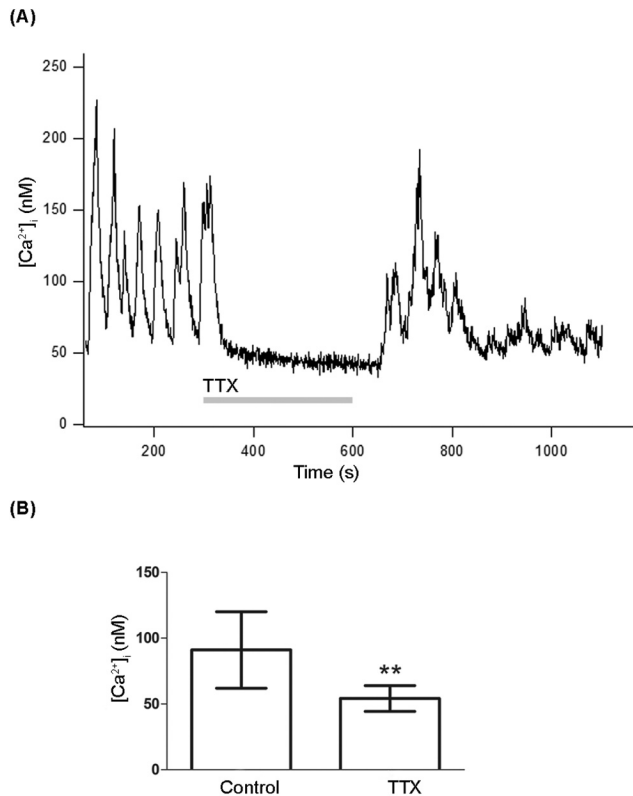
Two lines of evidence indicate that the effects of histamine are mediated by HR1. The HR1 antagonist pyrilamine partially

blocked the effects of 5  $\mu$ M histamine when applied at 10  $\mu$ M ( $n = 7$ ) and completely blocked them at 50  $\mu$ M (Fig. 5). The mean  $[Ca^{2+}]_i$  in the baseline period was  $98.4 \pm 30.6$  nM; with pyrilamine present, it was  $91.9 \pm 33.12$  nM. The mean  $[Ca^{2+}]_i$  in 50  $\mu$ M pyrilamine plus histamine was  $95.3 \pm 42.6$  nM, and in the washout period, it was  $99.5 \pm 26.6$  nM ( $n = 7$ ;  $P = 0.975$ ). The effects of histamine and selective HR1 agonists, applied at 5  $\mu$ M, were also very similar (Fig. 6). TFMH increased the mean  $[Ca^{2+}]_i$  from  $98.5 \pm 21.05$  nM in the baseline period to  $111.9 \pm 15.88$  nM ( $n = 9$ ;  $P = 0.039$ ). The effect of MH was smaller, but statistically significant. The mean  $[Ca^{2+}]_i$  in the baseline period was  $107.5 \pm 36.5$  nM, and in the presence of the drug, it was  $115.8 \pm 37.89$  nM ( $n = 5$ ;  $P = 0.0218$ ). The HR1 agonists had no statistically significant effects at 1  $\mu$ M (not illustrated).

## DISCUSSION

In the absence of external stimulation, acutely dissociated dopaminergic cells of the mouse retina exhibited spontaneous  $Ca^{2+}$  oscillations that were dependent on external  $Ca^{2+}$  and blocked by TTX. It has been shown that most acutely isolated dopaminergic cells from mouse retinas fire  $Na^+$  action potentials spontaneously.<sup>15,16</sup> In addition, their voltage-gated  $Ca^{2+}$  currents ( $I_{Ca}$ ) activate rapidly and show no apparent inactivation.<sup>17</sup> Together, these findings suggest that the observed spontaneous  $Ca^{2+}$  oscillations are a consequence of spontaneous  $Na^+$  channel activity. The resulting depolarization would





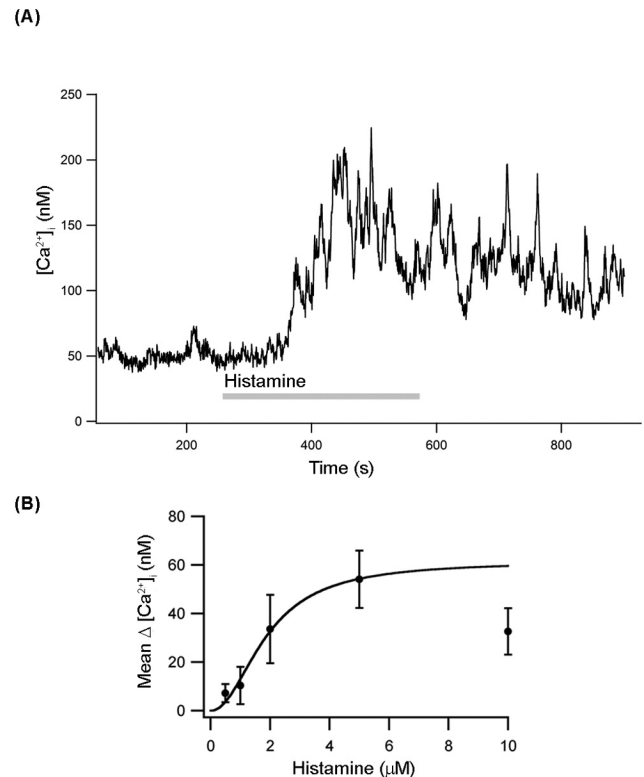
**FIGURE 3.** Superfusion of TTX in normal external  $\text{Ca}^{2+}$  blocked the  $[\text{Ca}^{2+}]_i$  oscillations. **(A)** Horizontal bar indicates when  $1 \mu\text{M}$  TTX was present. **(B)** The decrease in  $[\text{Ca}^{2+}]_i$  was statistically significant ( $n = 6$ ;  $P = 0.0050$ ). Data are the mean  $\pm$  SD.

be expected to activate  $I_{\text{Ca}}$  and promote entry of  $\text{Ca}^{2+}$  from the extracellular space.

These spontaneous oscillations in  $[\text{Ca}^{2+}]_i$  were similar in many respects to those observed in a subset of GABAergic amacrine cells isolated from rat retina.<sup>14</sup> Both types of spontaneous events were highly variable in their temporal patterns and were dependent on voltage-gated  $\text{Na}^+$  currents. They also resembled the “motes” described in amacrine cells isolated from chicken retina. In both instances, the elevations of  $[\text{Ca}^{2+}]_i$  tended to be in clusters, and the underlying mechanisms were sensitive to modulation by GTP binding protein-coupled receptors. Because both types of events were sensitive to the extracellular  $\text{Ca}^{2+}$  concentration, they required  $\text{Ca}^{2+}$  entry.<sup>13,18</sup> However, the  $\text{Ca}^{2+}$  influx in chicken amacrine cells occurred at “hot spots” on the dendrites, whereas the oscillations that we observed in mouse dopaminergic cells occurred in the perikarya.

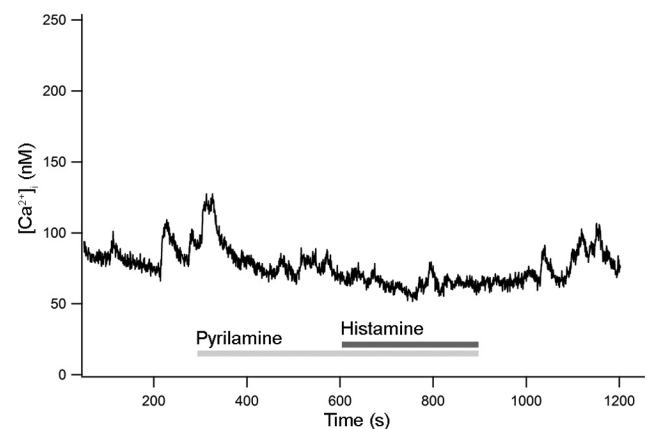
We observed considerable variability in the spontaneous oscillations of  $[\text{Ca}^{2+}]_i$  under control conditions and in the responses to histamine. The same is true of electrical activity of dopaminergic neurons in intact mouse retinas. There, some dopaminergic neurons fire bursts of spikes in darkness, and others fire mainly single spikes. Both the firing rates and the bursting characteristics are quite variable, with blockade of chemical synaptic transmission favoring bursting.<sup>19</sup> Previous studies of acutely isolated mouse dopaminergic cells reported that the spontaneous firing rates were variable and that the intervals between spikes were both regular and irregular.<sup>15,20</sup> Another source of variability may arise from the heterogeneity in responses to photopic light stimuli that mouse dopaminergic amacrine cells exhibit in situ.<sup>19</sup>

A main finding of this study was that low micromolar concentrations of histamine increased the mean  $[\text{Ca}^{2+}]_i$  in the

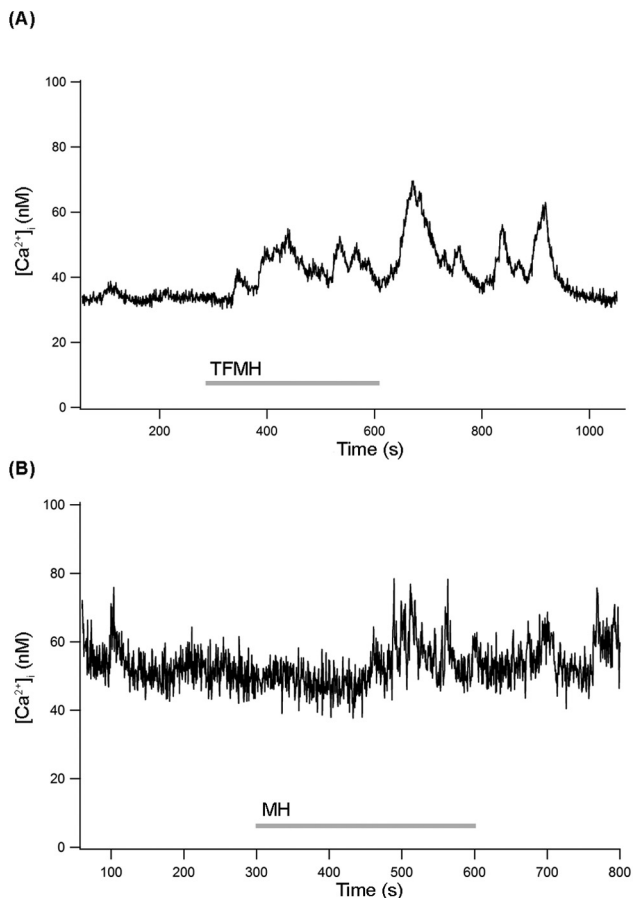


**FIGURE 4.** Superfusion of histamine in normal external  $\text{Ca}^{2+}$  increased mean  $[\text{Ca}^{2+}]_i$ . **(A)** Horizontal bar indicates the presence of  $5 \mu\text{M}$  histamine. **(B)** An increase in the concentration of histamine is associated in an increase in the mean  $[\text{Ca}^{2+}]_i$ . Curve is fitted from  $0.5$  to  $5 \mu\text{M}$  histamine and is given by  $\Delta[\text{Ca}^{2+}]_i = \Delta[\text{Ca}^{2+}]_{i,\text{max}}/[1 + (K_{1/2}/\text{histamine})^n]$ , where  $\Delta[\text{Ca}^{2+}]_{i,\text{max}} = 61 \pm 12 \text{ nM}$ ,  $K_{1/2} = 1.9 \pm 0.5 \mu\text{M}$ , and  $n = 2.1 \pm 0.8$ . Error bars,  $\pm$ SEM.

perikarya of dopaminergic cells from mouse retina. Histamine also increased the variance in the mean  $[\text{Ca}^{2+}]_i$ . Like the spontaneous  $\text{Ca}^{2+}$  oscillations, these effects were dependent on external  $\text{Ca}^{2+}$  and blocked by TTX. Together, the data suggest that histamine potentiates  $\text{Ca}^{2+}$  signaling by enhancing spontaneous  $\text{Ca}^{2+}$  oscillations. The molecular mechanism by which histamine potentiates  $\text{Ca}^{2+}$  signaling is not yet clear. One possibility is that histamine modulates ion channel activity. Alternatively, it may act downstream of ion channels, per-



**FIGURE 5.** Superfusion of  $5 \mu\text{M}$  histamine (dark bar) in the presence of  $50 \mu\text{M}$   $\text{H}_1$ -receptor antagonist pyrilamine (light bar) in normal external  $\text{Ca}^{2+}$  had no effect on mean  $[\text{Ca}^{2+}]_i$  ( $P = 0.9753$ ).



**FIGURE 6.** Superfusion of  $H_1$ -receptor agonists in normal external  $Ca^{2+}$  increased mean  $[Ca^{2+}]_i$ . (A) TFMH 5  $\mu$ M; (B) MH. Horizontal bars indicate when the drugs were present.

haps on  $Ca^{2+}$  clearance or on other signaling pathways.  $HR1$  effects mediated via TRP (transient receptor potential) channels and calcium exchangers have been described previously.<sup>21</sup>

Consistent with a receptor-mediated process, the effect of histamine on the mean  $[Ca^{2+}]_i$  of dopaminergic neurons was dose dependent. There were increases in the proportion of cells responding, as well as the amplitude of the responses, as the dose was increased up to 5  $\mu$ M. The half-maximum dose for this effect was approximately 2  $\mu$ M. The mean  $[Ca^{2+}]_i$  was also significantly elevated at 10  $\mu$ M, but there appeared to be a plateau, or even a decline in mean  $[Ca^{2+}]_i$  at this dose, the highest tested. One explanation for the decline may be desensitization, as described previously for  $HR1$ .<sup>22</sup> The doses of histamine used in this study were comparable to those used in recent studies of histamine effects on neurons, or lower. For example, Lundius et al.<sup>23</sup> used 3 to 20  $\mu$ M histamine in a study of hypothalamic neurons, and Kajihara et al.<sup>24</sup> used 100  $\mu$ M histamine in a study of primary sensory neurons.

Three types of G-protein-coupled receptors for histamine,  $HR1$ ,  $HR2$ , and  $HR3$ , have been described in the central nervous system.<sup>25</sup> Two lines of evidence indicate that the elevation in  $Ca^{2+}$  signal observed in the presence of histamine is mediated by  $HR1$ . The specific  $HR1$  antagonist, pyrilamine, had no effect on the dopaminergic cells when applied alone, but it blocked the responses to 5  $\mu$ M histamine partially when applied at 10  $\mu$ M and completely at 50  $\mu$ M. Another indication that the effect of histamine was mediated by  $HR1$  is that mean  $[Ca^{2+}]_i$  was increased by specific agonists of this receptor,

TFMH and MH, applied at 5  $\mu$ M.<sup>9-12</sup> These findings are consistent with the localization of  $HR1$  on dopaminergic retinal neurons of rodents by immunolabeling techniques.<sup>4,6</sup>

Histamine inhibits the release of dopamine into the superfusate when applied to rat and guinea pig retinas (Marshak DW, et al. *IOVS* 2008;49:ARVO E-Abstract 5792).<sup>5</sup> In guinea pig retinas, this inhibition is mediated by  $HR3$ ,<sup>25</sup> and the same may be true in mouse retinas, because mRNA for  $HR3$  is present.<sup>4</sup> In macaques, inhibitory  $HR3$  are localized to the dendrites of ON bipolar cells.<sup>6,22</sup> ON bipolar cells provide excitatory input to dopaminergic cells in mice,<sup>26,27</sup> and if ON bipolar cells of mice also express  $HR3$ , this site would be one where histamine acts to inhibit dopamine release in the intact retina.

It is also possible that the  $HR1$ -mediated elevation in  $[Ca^{2+}]_i$  that we observed in the perikarya of isolated mouse dopaminergic cells in response to histamine would have a net inhibitory effect on dopamine release. Acutely isolated dopaminergic cells release dopamine from large, dense-cored vesicles, and this release is  $Ca^{2+}$ -dependent.<sup>24</sup> The temporal pattern of these events is similar to that of the oscillations in  $[Ca^{2+}]_i$  that we observed under control conditions, and like the oscillations, they are sensitive to 1  $\mu$ M TTX. These vesicles also contain GABA,<sup>28</sup> and GABA is inhibitory to dopaminergic cells.<sup>18</sup> The dopamine they contain would also be inhibitory because dopaminergic neurons express D2 autoreceptors.<sup>29</sup> A third possibility is that the elevated  $[Ca^{2+}]_i$  produced by histamine via  $HR1$  has effects on the dopaminergic neurons that are unrelated to neurotransmitter release. Intracellular calcium also influences many other cellular processes, such as metabolism and gene expression.<sup>30</sup>

In summary, these results provide direct evidence that dopaminergic neurons are among the targets of retinopetal axons. Because dopamine is the major neuromodulator in the retina, affecting virtually all types of neurons,<sup>31</sup> it greatly amplifies the effects of inputs from the brain to the retina.

### Acknowledgments

The authors thank Samuel M. Wu and Richard B. Clark for valuable discussions, Alice Chuang for help with the statistical analysis, and Alejandro Vila and Carolina Rangel for technical assistance.

### References

- Airaksinen MS, Panula P. The histaminergic system in the guinea pig central nervous system: an immunocytochemical mapping study using an antiserum against histamine. *J Comp Neurol*. 1988; 273:163-186.
- Gastinger MJ, O'Brien JJ, Larsen NB, Marshak DW. Histamine immunoreactive axons in the macaque retina. *Invest Ophthalmol Vis Sci*. 1999;40:487-495.
- Gastinger MJ, Barber AJ, Khin SA, McRill CS, Gardner TW, Marshak DW. Abnormal centrifugal axons in streptozotocin-diabetic rat retinas. *Invest Ophthalmol Vis Sci*. 2001;42:2679-2685.
- Greferath U, Kambourakis M, Barth C, Fletcher EL, Murphy M. Characterization of histamine projections and their potential cellular targets in the mouse retina. *Neuroscience*. 2009;158:932-944.
- Weber B, Schlicker E. Modulation of dopamine release in the guinea-pig retina by G (i)- but not by G (s)- or G (q)-protein-coupled receptors. *Fundam Clin Pharmacol*. 2001;15:393-400.
- Gastinger MJ, Barber AJ, Vardi N, Marshak DW. Histamine receptors in mammalian retinas. *J Comp Neurol*. 2006;495:658-667.
- Zhang DQ, Stone JF, Zhou T, Ohta H, McMahon DG. Characterization of genetically labeled catecholamine neurons in the mouse retina. *Neuroreport*. 2004;15:1761-1765.
- Wan QF, Vila A, Zhou ZY, Heidelberger R. Synaptic vesicle dynamics in mouse rod bipolar cells. *Vis Neurosci*. 2008;25:523-533.
- Zingel V, Leschke C, Schunack W. Developments in histamine  $H_1$ -receptor agonists. *Prog Drug Res*. 1995;44:49-85.

10. Čarman-Kržan M, Bavec A, Zorko M, Schunack W. Molecular characterization of specific H1-receptor agonists histaprodifen and its Na-substituted analogues on bovine aortic H1-receptors. *Naunyn-Schmiedeberg Arch Pharmacol*. 2003;367:538-546.
11. Pertz HH, Elz S, Schunack W. Structure-activity relationships of histamine H1-receptor agonists. *Mini Rev Med Chem*. 2004;4:935-940.
12. Elz S, Kramer K, Pertz HH, et al. Histaprodifens: synthesis, pharmacological in vitro evaluation, and molecular modeling of a new class of highly active and selective histamine H1-receptor agonists. *J Med Chem*. 2000;43:1071-1084.
13. Borges S, Lindstrom S, Walters C, Warriar A, Wilson M. Discrete influx events refill depleted Ca<sup>2+</sup> stores in a chick retinal neuron. *J Physiol*. 2008;586:605-626.
14. Firth SI, Feller MB. Dissociated GABAergic retinal interneurons exhibit spontaneous increases in intracellular calcium. *Vis Neurosci*. 2006;23:807-814.
15. Feigenspan A, Gustincich S, Bean BP, Raviola E. Spontaneous activity of solitary dopaminergic cells of the retina. *J Neurosci*. 1998;18:6776-6789.
16. Gustincich S, Feigenspan A, Wu DK, Koopman LJ, Raviola E. Control of dopamine release in the retina: a transgenic approach to neural networks. *Neuron*. 1997;18:723-736.
17. Xiao J, Cai Y, Yen J, et al. Voltage-clamp analysis and computational model of dopaminergic neurons from mouse retina. *Vis Neurosci*. 2004;21:835-849.
18. Crousillac S, Colonna J, McMains E, Dewey JS, Gleason E. Sphingosine-1-phosphate elicits receptor-dependent calcium signaling in retinal amacrine cells. *J Neurophysiol*. 2009;102:3295-3309.
19. Zhang DQ, Zhou TR, McMahon DG. Functional heterogeneity of retinal dopaminergic neurons underlying their multiple roles in vision. *J Neurosci*. 2007;27:692-699.
20. Puopolo M, Hochstetler SE, Gustincich S, Wightman RM, Raviola E. Extrasynaptic release of dopamine in a retinal neuron: activity dependence and transmitter modulation. *Neuron*. 2001;30:211-225.
21. Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. *Physiol Rev*. 2008;88:1183-1241.
22. Yu YC, Satoh H, Wu SM, Marshak DW. Histamine enhances voltage-gated potassium currents of ON bipolar cells in macaque retina. *Invest Ophthalmol Vis Sci*. 2009;50:959-965.
23. Lundius EG, Sanchez-Alavez M, Ghochani Y, Klaus J, Tabarean IV. Histamine influences body temperature by acting at H1 and H3 receptors on distinct populations of preoptic neurons. *J Neurosci*. 2010;30:4369-4381.
24. Kajihara Y, Murakami M, Imagawa T, Otsuguro K, Ito S, Ohta T. Histamine potentiates acid-induced responses mediating transient receptor potential V1 in mouse primary sensory neurons. *Neuroscience*. 2010;166:292-304.
25. Schlicker E, Kathmann M. Modulation of in vitro neurotransmission in the CNS and in the retina via H3 heteroreceptors. In: Leurs R, Timmerman H, eds. *The Histamine H3 Receptor: a Target for New Drugs*. Amsterdam: Elsevier; 1998:13-26.
26. Dumitrescu ON, Pucci FG, Wong KY, Berson DM. Ectopic retinal ON bipolar cell synapses in the OFF inner plexiform layer: contacts with dopaminergic amacrine cells and melanopsin ganglion cells. *J Comp Neurol*. 2009;517:226-244.
27. Contini M, Lin B, Kobayashi K, Okano H, Masland RH, Raviola E. Synaptic input of ON-bipolar cells onto the dopaminergic neurons of the mouse retina. *J Comp Neurol*. 2010;518:2035-2050.
28. Hirasawa H, Puopolo M, Raviola E. Extrasynaptic release of GABA by retinal dopaminergic neurons. *J Neurophysiol*. 2009;102:146-158.
29. Veruki ML. Dopaminergic neurons in the rat retina express dopamine D2/3 receptors. *Eur J Neurosci*. 1997;9:1096-1100.
30. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*. 2003;4:517-529.
31. Witkovsky P. Dopamine and retinal function. *Doc Ophthalmol*. 2004;108:17-40.