Effects of melatonin on ionic currents in cultured ocular tissues

ADAM RICH,1,2 GIANRICO FARRUGIA,1,2 AND JAMES L. RAE1,3
1Division of Gastroenterology and Hepatology and Departments of 2Physiology and Biophysics and 3Ophthalmology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

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Effects of melatonin on ionic currents in cultured ocular tissues. Am. J. Physiol. 276 (Cell Physiol. 45): C923–C929, 1999.—The effects of melatonin on ionic conductances in a cultured mouse lens epithelial cell line (α-TN4) and in cultured human trabecular meshwork (HTM) cells were measured using the amphotericin perforated patch whole cell voltage-clamp technique. Melatonin stimulated a voltage-dependent Na⁺-selective current in lens epithelial cells and trabecular meshwork cells. The effects of melatonin were observed at 50 pM and were maximal at 100 µM. Melatonin enhanced activation and inactivation kinetics, but no change was observed in the voltage dependence of activation. The results are consistent with an increase in the total number of ion channels available for activation by membrane depolarization. Melatonin was also found to stimulate a K⁺-selective current at high doses (1 mM). Melatonin did not affect the inwardly rectifying K⁺ current or the delayed rectifier type K⁺ current that has been described in cultured mouse lens epithelial cells. The results show that melatonin specifically stimulated the TTX-insensitive voltage-dependent Na⁺ current by an apparently novel mechanism.

sodium channels; trabecular meshwork; epithelium; electrophysiology

THE SYNTHESIS AND SECRETION of melatonin (N-acetyl-5-methoxytryptamine) by the pineal gland and the retina occur at night, and recent reviews give excellent summaries (10, 13, 16, 17, 21, 27). Circulating melatonin can modulate several physiological processes and may alter the timing of circadian rhythms (4, 8). The effects of melatonin on circadian rhythms are likely mediated by high-affinity receptors located in the suprachiasmatic nucleus (27). High-affinity receptors have also been identified in the inner plexiform layer of the retina as well as the hypophysial pars tuberalis (17). Three specific melatonin receptor subtypes have been identified: Mel₁₅ [dissociation constant (Kd) = 20–40 pM] in the suprachiasmatic nucleus and pars tuberalis, Mel₁₆ (Kd = 160 pM) in the retina, and Mel₃c (Kd = 20–60 pM). However, the Mel₃c receptor has not been cloned in mammals (27). Receptor activation results in inhibition of adenylate cyclase by a pertussis toxin-sensitive G protein (17, 34). The decrease in intracellular cAMP mediates aggregation of pigment granules in frog skin, photoperiodic regulation of prolactin secretion by the hypophysial pars tuberalis, and vasoconstriction of rat cerebral artery (4, 34). However, the signaling mecha-

isms coupling melatonin receptor activation to neuronal activity in the suprachiasmatic nucleus or to the inhibition of dopamine release in the retina remain unknown (16).

There is evidence that melatonin modulates ionic conductances in several preparations. Melatonin suppresses the timing of neurons in the suprachiasmatic nucleus in vitro (19). Recent experiments have shown that melatonin stimulates a K⁺-selective conductance through a receptor-mediated mechanism (15). The activity of several ionic conductances in cultured cells shows circadian patterns, but the signaling mechanisms are unknown. For example, a tetraethylammonium-sensitive K⁺ conductance in the optic nerve of mollusk is lowest before dawn and increases at dusk (20). A Ca²⁺-permeable cation-selective conductance in cultured avian pineal gland cells is spontaneously active at night, coincident with melatonin secretion (9).

Melatonin is lipophilic and thus has access to cytosolic, mitochondrial, and nuclear compartments. In the cytosol, melatonin has been shown to act as a Ca²⁺/calmodulin antagonist and therefore may directly affect Ca²⁺ signaling mechanisms (10, 13). Melatonin-calmodulin binding has been shown to affect tubulin polymerization and alter the cytoskeleton in Madin-Darby canine kidney and NIE-115 cells (3). Addition of melatonin has also been shown to stimulate the Ca²⁺-ATPase in cardiomyocytes (6). Melatonin is a powerful free radical scavenger and may act ubiquitously as an intracellular antioxidant (26). A high-affinity binding site has been identified in the nucleus (Kd = 10–9 M), where melatonin may regulate gene transcription or may prevent oxidative damage to DNA (2). Thus the effects of melatonin may result from binding to high-affinity receptors in the plasma membrane, cytosol, or the nucleus, leading to changes in gene expression or altering second messenger levels (cAMP and Ca²⁺). The effects of melatonin may also result from nonspecific interactions related to the antioxidant activity of melatonin or from direct binding to ion channels.

Melatonin synthesis and secretion by the retina have been well characterized, as well as inhibition by melatonin of dopamine release in the retina, but the target receptors and the mechanisms by which melatonin alters physiological function are not as well understood. Because retinal melatonin does not significantly contribute to circulating melatonin levels, a local effect on ocular tissues, such as modulation of neuronal transmission or excitability, or regulation of intraocular pressure is possible. Intraocular pressure varies diurnally, decreasing at night, and this has been associated with the circadian fluctuations in melatonin concentration in plasma or aqueous humor (18). Plasma

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Melatonin levels have also been correlated with intraocular pressure in humans, and intraocular pressure decreased after oral administration of melatonin (29). However, the basic mechanism for these observations is not known.

We used the patch-clamp technique to measure the effects of melatonin on ionic currents in two different cell types, a mouse lens epithelial cell line (α-TN4) and a human trabecular meshwork cell line (HTM). We have found that melatonin specifically stimulates the Na+ current in cultured mouse lens epithelial cells by a novel mechanism (33). Voltage-gated Na+ channels are present in most excitable cells and can be divided into two types: TTX-sensitive and TTX-insensitive. TTX-present in most excitable cells and can be divided into novel mechanism (33). Voltage-gated Na+ channels have a TTX-sensitive and TTX-insensitive Na+ channels, found in nerve and skeletal muscle, have a TTX-Kd of 1–20 nM (12). TTX-insensitive Na+ channels have a TTX-Kd of 0.5–10 μM. This difference in binding affinity results from a change in a single residue within the TTX-binding domain (5). The Na+-selective channel in mouse lens epithelial cells is the TTX-insensitive type. Melatonin was also found to stimulate a Na+ current in cultured HTM cells, suggesting that the effects were not unique to the Na+ current in α-TN4 cells, but that melatonin is Na+ channel specific. Finally, melatonin did not affect the inwardly rectifying K+ conductance or the delayed rectifying type K+ conductance in α-TN4 cells but did stimulate an outward K+ current in HTM cells at very high doses.

MATERIALS AND METHODS

Cell culture and isolation. Experiments were performed on two types of cultured cells: an immortalized transgenic lens epithelial cell line (α-TN4) kindly provided by Dr. Paul Russell, and cultured HTM cells kindly provided by Dr. Richard Seftor. α-TN4 cells were cultured according to a standard protocol (28). HTM cells were cultured following a previously published protocol (30). Because cultured cells may change phenotype after repeated passages, only cells from the third or fourth passage were used for these experiments. Cells were dissociated using a trypsin low-Ca2+ solution (0.5%) and by gentle trituration with a Pasteur pipette. The cell suspension was centrifuged at 180 g for 5 min and resuspended in an enzyme-free Ringer solution. Centrifugation was repeated, and the cells were resuspended in a Ringer solution containing 5 mM glucose and stored at room temperature. Experiments were performed during the next 6 h. Three to six drops of the cell suspension were placed in a 300-μl acrylic recording chamber with a glass coverslip bottom. The cells were allowed to settle for ~30 min. The bath was perfused with several volumes of NaCl Ringer solution to wash away unattached cells and debris.

Patch electrodes for whole cell recordings were made from Kimble KG-12 glass (Garner Glass, Claremont, CA) and pulled on a Sutter Instruments (Novato, CA) P80 microelectrode puller. Tips were coated with Dow Corning (Midland, MI) Sylgard no. 184 and fire polished under direct observation, to a final resistance of 3–5 MΩ. Most whole cell recordings were made using the amphotericin perforated patch technique, since this technique results in stable values for the access resistance and maintains cytoplasmic integrity (23). The electrodes were mounted in a polycarbonate holder connected to an Axopatch 200 patch voltage-clamp amplifier (Axon Instruments, Foster City, CA) and positioned immediately adjacent to the cell membrane. Slight suction resulted in a gigahm seal for the majority of cells. Amphotericin usually partitioned into the membrane isolated in the membrane tip within 15 min, resulting in access resistance ranging from 6 to 20 MΩ. Data were recorded with the use of a modified IBM-AT computer using a TL1 Labmaster interface (Axon Instruments) and driven by pCLAMP software (version 6.0, Axon Instruments), allowing voltage-clamp protocols with concomitant digitization of the membrane currents. Whole cell current records were collected at 10 kHz or faster and filtered at 2 kHz with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA). Voltage step protocols were repeated twice, and the resulting currents at each voltage were averaged to produce the final records. Each current-voltage record was corrected for the offset potential resulting from a combination of the liquid junction potential and the Donnan potential produced by the mismatch of pipette and cellular anions. Previous studies have shown that K+ selective currents reverse between 7 and 12 mV when the bath and pipette solutions contain equal K+ concentrations (23). Therefore, the current-voltage relationships were corrected by 10 mV. Capacity transients were adjusted during recording. Whole cell currents were usually stable during experiments lasting up to 1 h while using the perforated patch technique or the whole cell technique. The records were not leak subtracted or modified otherwise. Data were analyzed using pCLAMP software (Axon Instruments) or using custom-written macros in Excel (Microsoft, Redman, WA). Final plots were prepared using SigmaPlot (Jandel, Madera, CA). Data are reported as means ± SE, and statistical significance was determined using Student’s t-test.

Normal Ringer solution contained (in mM) 4.74 KCl, 149.2 NaCl, 2.5 CaCl2, and 5 HEPES, resulting in a final osmolality of 293 mosM. For KCi Ringer solution, KCi was substituted for NaCl. The pipette filling solution contained (in mM) 125 KMeSO3, 20 KCl, 5 HEPES, and 2 EGTA, resulting in a final osmolality of 289 mosM. All solutions were adjusted to a final pH of 7.35. Flufenamic acid, melatonin, fluoxetine (Prozac), quinidine, and diltiazem were purchased from Sigma (St. Louis, MO).

RESULTS

Effects of melatonin on whole cell currents in cultured mouse lens epithelial cells. Three current types have been previously described in α-TN4 cells: an inwardly rectifying K+ selective current (Iiir), an outwardly rectifying K+ selective current (Iiir), and a Na+ selective transient inward current (INa) (7, 22, 33). Typical whole cell currents recorded in NaCl Ringer solution during a series of voltage steps from −150 to +80 mV are shown in Fig. 1A. The three current types are apparent in this experiment. Hyperpolarizing voltage steps activated Iiir, an inwardly directed K+ current that activates rapidly and inactivates very slowly during the voltage command. Depolarizing voltage steps positive to −60 mV activated INa. This inward current reached a maximum very rapidly and completely inactivated within 20 ms. Depolarizing voltage steps positive to approximately −20 mV activated Iir. This outward K+ current activated more slowly compared with INa or Iiir and persisted for the entire duration of the voltage pulse. The effects of bath perfusion with 100 μM melatonin in NaCl Ringer solution for this experiment are shown in Fig. 1B. An increase in INa was evident immediately
after bath perfusion with melatonin. $I_{ir}$ and $I_{dr}$ were not affected. Current recordings and access resistance were verified to be stable for 5–10 min after obtaining whole cell access. For some experiments $I_{Na}$ exhibited rundown, a decrease with time, and these experiments were not included in the analysis. Bath perfusion with normal Ringer solution did not alter $I_{Na}$; in 11 separate experiments $I_{Na}$ averaged $-129 \pm 38$ pA before and $-130 \pm 39$ pA ($P = 0.46$) after perfusion with Ringer solution.

The peak inward current and steady-state current-voltage relationships for this experiment are plotted in Fig. 2. Figure 2A shows that $I_{Na}$ began to activate at approximately $-50$ mV and reached the maximum of $-238$ pA at $-20$ mV (circles). Perfusion with 100 µM melatonin (squares) increased peak $I_{Na}$ to $-308$ pA. An increase in $I_{Na}$ was observed in 13 out of 16 cells. The average current was $-288 \pm 97$ pA in Ringer solution and $-374 \pm 112$ pA ($n = 16$, $P = 0.003$) after bath perfusion with 100 µM melatonin. The effects of melatonin were reversible; $I_{Na}$ averaged $-373 \pm 176$ pA ($n = 5$, $P = 0.33$) after the wash with Ringer solution. Melatonin did not appear to shift the voltage dependence of $I_{Na}$, because the voltage at the peak current was unaltered. The steady-state current-voltage relationship is shown in Fig. 2B. These currents were quantified as the average of 20 data points near the end of the voltage step. Neither $I_{ir}$, measured as the inward current evoked during voltage steps more hyperpolarized than $-90$ mV, nor $I_{dr}$, measured as the outward current evoked by step depolarizations, was affected by melatonin. In six experiments $I_{ir}$ measured $-108 \pm 29$ and $-99.5 \pm 28$ pA ($P > 0.05$) and $I_{dr}$ measured $47.5 \pm 18$ and $55.5 \pm 20$ pA ($P > 0.05$) before and after 100 µM melatonin, respectively. The resting membrane potential ($E_{m}$) measured $-16.0 \pm 3.7$ mV under control conditions and $-25.0 \pm 8.5$ mV ($P > 0.05$) after bath perfusion with 100 µM melatonin. These results show that $K^{+}$-selective currents in this preparation are unaffected by melatonin.

The site of action for melatonin may be a direct interaction with the $Na^{+}$ channel or with specific melatonin receptors in the plasma membrane. Melatonin stimulates a Ca$^{2+}$-dependent ATPase pump in rat cardiac myocytes with a $K_{d}$ of 28 ng/ml (0.12 µM) (6). If melatonin stimulated the Ca$^{2+}$-ATPase pump in $\alpha$-TN4 cells, then intracellular Ca$^{2+}$ levels should decrease. However, fluorescence experiments utilizing the Ca$^{2+}$-sensitive ratiometric dye fura 2 showed that melatonin (100 µM) did not alter the intracellular free Ca$^{2+}$ concentration (data not shown, $n = 4$). Melatonin was found to selectively stimulate $I_{Na}$ in $\alpha$-TN4 cells, suggesting a tightly coupled mechanism, as opposed to a more general cell signaling mechanism that would be expected to modulate several ionic conductances in different cell types.

Stimulation of $I_{Na}$ by melatonin may result from a change in the time course of activation or inactivation. Several toxins such as TTX specifically alter Na$^{+}$ currents, and these effects may be characterized by alterations in channel gating. The kinetics of $I_{Na}$ were examined using a faster sampling rate (20 kHz) and a pulse protocol that focused on the voltages where $I_{Na}$ was activated (Fig. 3). Close inspection of the current records in Fig. 3 shows that activation and inactivation kinetics were faster after melatonin (Fig. 3B) compared with control records (Fig. 3A). This is more clearly seen
in Fig. 3, inset, where the peak currents, before and after melatonin, were plotted on an expanded time scale. Activation time was measured as time from the start of the depolarizing voltage step to time when \( I_{\text{Na}} \) peaked. The inactivation time course was well fit with a single exponential function \( \tau_{\text{inactivation}} \). The time to peak and \( \tau_{\text{inactivation}} \) were determined at test voltages where \( I_{\text{Na}} \) was well resolved for four experiments (Fig. 4). Melatonin (100 µM, squares) decreased activation time as well as \( \tau_{\text{inactivation}} \) (Fig. 4B).

Dissociation constants for melatonin binding receptors range from 20 to 160 pM (27). The dose dependence of melatonin on \( I_{\text{Na}} \) is shown in Fig. 5. These data show that melatonin stimulated \( I_{\text{Na}} \) at doses consistent with receptor binding. Figure 5 also shows further stimulation of \( I_{\text{Na}} \) by melatonin at doses four orders of magnitude greater than the reported dissociation constants. A maximal effect was observed at 100 µM, and 1 mM melatonin inhibited \( I_{\text{Na}} \).

Whole cell currents measured in HTM cells. Whole cell currents were also measured in cultured HTM cells. Typical whole cell currents recorded in NaCl Ringer solution are shown in Fig. 6A. Cells were held at −90 mV and stepped from −120 to 30 mV for 40 ms in 10-mV increments. The most prominent current under these conditions was a rapidly activating inward current that completely inactivated within 20 ms. The peak inward current and steady-state current-voltage relationships for this experiment are shown in Fig. 6B. Inward current began to activate at approximately −20 mV and reached the maximum of −384 pA at 10 mV in this cell. \( I_{\text{Na}} \) averaged −538 ± 79 pA in seven separate experiments. The observed current activated at voltages of −20 mV depolarized compared with \( I_{\text{Na}} \) measured in α-TN4 cells. The steady-state current-voltage relationship, quantified as the average of 20 data points near the end of the voltage step, was very small compared with \( I_{\text{Na}} \). Input resistance, calculated as the slope of a straight line fit to the four steady-state current values recorded during hyperpolarizing voltage steps and constrained to go through the origin, was 11.6 ± 2.0 GΩ (n = 18). \( E_{\text{Na}} \) was −39.2 ± 5.2 mV (n = 15). The peak inward current was identified as Na⁺ selective based on several criteria. First, the only ions that have electrochemical gradients that would drive inward current from −20 to 40 mV are Na⁺ and Ca²⁺. At these potentials K⁺ would move outward \( (E_k = −87.9 \text{ mV}) \) and Cl⁻ would move inward \( (E_{Cl} = −53 \text{ mV}) \), both resulting in outwardly directed current records. Reducing the concentration of Na⁺ in the bath solution from 149.2 to 75 mM (while maintaining constant osmolarity with mannitol replacement) resulted in a decrease in the inward current from 465 ± 141 to −228 ± 64 pA \( (n = 7, P = 0.014) \), and replacing extracellular Na⁺ with K⁺ completely abolished the current (data not shown). Extracellular Ca²⁺ concentration was constant during these experiments, indicating...
that the inward current was not carried by Ca\textsuperscript{2+}. Furthermore, replacing extracellular Ca\textsuperscript{2+} with 80 mM Ba\textsuperscript{2+}, which enhances inward current through L type Ca\textsuperscript{2+} channels, blocked the fast inward current (data not shown), indicating that the fast inward current is not carried by Ca\textsuperscript{2+}. Depolarizing the holding potential reduced the inward current, identical to voltage-dependent inactivation of I\textsubscript{Na} described in \textalpha{}-TN4 cells (33). The inward current in HTM cells also shared pharmacological properties with I\textsubscript{Na} that we have observed in the \textalpha{}-TN4 cell line: fluoxetine, diltiazem, flufenamic acid, and quinidine all blocked I\textsubscript{Na} (data not shown). These data are all consistent with the hypothesis that the fast inward current is Na\textsuperscript{+} selective.

The effects of bath perfusion with melatonin in NaCl Ringer solution are shown in Fig. 7. Bath perfusion with 100 \textmu{}M melatonin (Fig. 7B) stimulated I\textsubscript{Na} similar to the effects observed in \textalpha{}-TN4 cells. Higher levels of melatonin (1 mM) also activated a prominent outward current (I\textsubscript{out}) in this preparation (Fig. 7C).

The current-voltage relationship for this experiment is shown in Fig. 8. I\textsubscript{Na} (Fig. 8A) activated at −20 mV and reached a maximum of −720 pA at 0 mV. Bath perfusion with 100 \textmu{}M melatonin increased I\textsubscript{Na} to −1,044 pA. Melatonin increased I\textsubscript{Na} in six out of six separate experiments from −500 ± 82 pA to −620 ± 110 pA (P = 0.04). Bath perfusion with 1 mM melatonin resulted in a decrease in I\textsubscript{Na} to −469 pA for the experiment shown and I\textsubscript{Na} averaged −415 ± 91 (n = 7). The effects of melatonin were reversible: I\textsubscript{Na} averaged −505 ± 84 (P = 0.44) after bath perfusion with Ringer solution after melatonin.

It is difficult to determine whether the decrease in the peak inward current after 1 mM melatonin resulted from inhibition of I\textsubscript{Na} or from stimulation of I\textsubscript{out}. Close inspection of the current records in Fig. 7 shows that activation of I\textsubscript{out} overlaps with I\textsubscript{Na}. Therefore, an increase in I\textsubscript{out} would be reflected as a decrease in I\textsubscript{Na}. The effects of melatonin on I\textsubscript{out} are shown in Fig. 8B. Bath perfusion with 100 \textmu{}M melatonin increased outward current from 12 to 176 pA and hyperpolarized E\textsubscript{m} from −50 mV to −68 mV. Perfusion with 1 mM melatonin resulted in a decrease in I\textsubscript{Na} (2 separate experiments from inhibition of I\textsubscript{Na} or from stimulation of I\textsubscript{out}).

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increased $I_{\text{out}}$ to 685 pA and further hyperpolarized $E_m$ to $-75$ mV. These results suggest that $I_{\text{out}}$ was highly $K^+$ selective because the reversal potential for $K^+$ under these conditions was calculated to be $-86$ mV.

The apparent similarity between $I_{\text{out}}$ in HTM cells and the $K^+$ current ($I_K$) in rabbit corneal epithelial cells (see Fig. 1 in Ref. 11) led us to test the effects of agents that selectively inhibit $I_K$ in the corneal epithelium such as diltiazem (1 mM), quinidine (1 mM), and fluoxetine (100 µM) (11, 24, 25). These agents were all effective blockers of $I_{\text{out}}$: quinidine resulted in an $81 \pm 8\%$ ($n = 3$) decrease, fluoxetine resulted in a $92\%$ ($n = 1$) decrease, and diltiazem resulted in a $39 \pm 27\%$ ($n = 2$) decrease in $I_{\text{out}}$. These results are consistent with the presence of a delayed rectifier type $K^+$ current in HTM cells.

**DISCUSSION**

Voltage-gated $Na^+$ channels are found in nerve cells, skeletal muscle, and smooth muscle and support excitability in these cell types. Cardiac muscle, Purkinje fibers, and denervated skeletal muscle also express voltage-gated $Na^+$ channels that support impulse propagation. $Na^+$ channels from these tissues are distinct in that one type is TTX sensitive ($K_d \approx 10^{-9}$ M) and the other is TTX insensitive ($K_d \approx 10^{-6}$ M). Functional expression of TTX-insensitive $Na^+$ channels has also been observed in the lens epithelium and corneal endothelium (33). We have shown that melatonin stimulated $I_{Na}$ in cultured human lens epithelial cells and in cultured HTM cells at doses comparable with the reported $K_d$ for receptor binding (20–160 pM) (17). Melatonin did not affect the other ionic conductances that have been characterized in lens epithelial cells, including an inward rectifying $K^+$-selective current ($I_{\text{in}}$) and an outward delayed rectifier type $K^+$-selective current ($I_{\text{out}}$) (7). We also did not observe any effects on the delayed rectifier $K^+$-selective currents in two types of freshly dispersed cells that are commonly used in the laboratory: rabbit corneal epithelial cells or smooth muscle cells from the canine jejunum (unpublished results). At high doses (1 mM) melatonin also stimulated an outward $K^+$ current in cultured HTM cells.

Melatonin increased the peak $Na^+$ current amplitude and the kinetics of activation and inactivation (Figs. 3 and 4). The current-voltage relationship appears unaffected. The most simple gating diagram for $Na^+$ channels exhibits three connected conformational states: a nonconducting resting state, a conducting open state, and a nonconducting inactivated state. A number of toxins specifically alter $Na^+$ channel gating. The most familiar may be the guanidinium toxins, TTX and saxitoxin, commonly called $Na^+$ channel blockers, which act by stabilizing the inactivated state (31). Several other toxins increase $I_{Na}$. The effects of melatonin are most similar to $\alpha$-scorpion toxin, a small water-soluble polypeptide, which prolongs action potential duration (31). Lipophilic toxins, which are thought to act at sites within the lipid layer, increase the activation rate like melatonin (31). However, lipophilic toxins also change the voltage dependence of activation. In cultured neuroblastoma cells $\alpha$-toxin slowed the rate of inactivation and thereby increased peak $I_{Na}$ (12). It is possible that melatonin increased peak $I_{Na}$ due to enhanced recovery from an inactivated state, thereby recruiting channels for activation.

HTM cells function to maintain the composition of the aqueous humor outflow pathway in the juxtacanicular region of the eye. Regulation of the outflow facility through the trabecular meshwork is likely to play an important role in determining intraocular pressure. This hypothesis is supported by studies that correlate relaxation of the trabecular meshwork with an increase in ocular outflow (35). Cultured bovine trabecular meshwork cells have recently been shown to express maxi-$K^+$ channels that are activated by cGMP (32). In smooth muscle cells, activation of $K^+$-selective channels leads to membrane hyperpolarization and relaxation (14). However, little is known about ion channels in HTM cells. We have advanced these results and shown that cultured HTM cells also express $Na^+$ channels and delayed rectifier type $K^+$ channels. Ionic currents with similar pharmacology have been characterized in other ocular tissues, but molecular studies are necessary to unambiguously identify these channels (22).

The lens epithelium transports fluid and salt to preserve lens integrity. The physiological role of $I_{Na}$ is not clear. One possibility, assuming that a small window current exists at the resting membrane potential, is that $I_{Na}$ provides a $Na^+$ inflow pathway that interacts with the $Na^+-K^+$ pump or that $I_{Na}$ may serve as a cellular signal in response to $Ca^{2+}$ depletion (33). The physiological role for $Na^+$ channels in HTM cells must also depend on a small but significant channel open probability at the resting membrane potential. In this case $I_{Na}$ may be involved in modulating the tone of HTM cells and thereby modulate outflow resistance and intraocular pressure.

Melatonin appears to be a novel activator of voltage-gated TTX-insensitive $Na^+$ channels. We have shown that melatonin increases peak $I_{Na}$ and speeds activation and inactivation kinetics and increases peak $I_{Na}$ in two types of cultured cells, $\alpha$-TN4 and HTM. Because melatonin was effective at doses that are similar to the reported $K_d$ for the cloned melatonin receptors, it is possible that these effects are receptor mediated, but a direct interaction between melatonin and $Na^+$ channels cannot be ruled out. Although a great deal is known about the regulation of melatonin synthesis and the diurnal variation of melatonin in the bloodstream, much less is known about its physiological roles. This study shows that alterations in ion channel gating may be considered as a final effector of the actions of melatonin.

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Address for reprint requests and other correspondence: A. Rich, Dept. of Physiology and Biophysics, Mayo Clinic and Mayo Founda-
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