A 5-HT_7 Receptor-Mediated Depolarization in the Anterodorsal Thalamus. II. Involvement of the Hyperpolarization-Activated Current I_h

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ABSTRACT

Previous studies have shown that 5-hydroxytryptamine (5-HT) can modulate the hyperpolarization-activated nonselective cation current (I_h) to elicit a membrane depolarization in neurons. However, the receptor subtype involved in this response remains controversial. In the accompanying study, we have identified a 5-HT_7 receptor-mediated depolarization in the anterodorsal nucleus of the thalamus (ADn). In the present study, we have examined the possible role of I_h in mediating this 5-HT_7 receptor-mediated depolarization. We used the blind tight-seal patch clamp technique to examine the ability of 5-HT to modulate I_h in the ADn. We found that 5-HT induced a shift in the voltage dependence of I_h to more depolarized potentials. The pharmacology of the receptor mediating this effect was consistent with that of a 5-HT_7 receptor. Since the 5-HT_7 receptor is coupled positively to adenylate cyclase, we examined the cAMP dependence of the 5-HT-induced modulation of I_h. Intracellular addition of cAMP mimicked and occluded the 5-HT response. Conversely, in the presence of the protein kinase inhibitors H-8 and staurosporine, ADn neurons still expressed a 5-HT-induced shift in the voltage dependence of I_h. These results suggest that 5-HT regulates I_h in the ADn through a cAMP-dependent but protein kinase A (PKA)-independent mechanism. To determine the contribution of I_h to the 5-HT_7 receptor-mediated depolarization, we used the selective I_h blocker ZD7288. This compound greatly reduced the depolarizing response elicited by activation of 5-HT_7 receptors. We conclude that 5-HT_7 receptors depolarize ADn neurons primarily by increasing I_h through a cAMP-dependent, PKA-independent mechanism.

In the accompanying article (Chapin and Andrade, 2001), we reported that activation of the 5-HT_7 receptor elicits a membrane depolarization and associated inward current in the anterodorsal nucleus of the thalamus (ADn). In many cases, 5-HT-induced depolarizations are elicited through modulation of the hyperpolarization-activated nonselective cation current, I_h (Bobker and Williams, 1989; Pape and McCormick, 1989; Takahashi and Berger, 1990; Larkman and Kelly, 1992; Spain, 1994; Cardenas et al., 1999). Therefore, we tested the possibility that I_h may, at least in part, mediate the depolarization elicited by 5-HT_7 receptors. I_h is a widely distributed nonselective cation current that is activated on hyperpolarization and can display very slow activation kinetics. Under physiological conditions, I_h reverses at approximately −20 to −30 mV (Yanagihara and Irisawa, 1980; Takahashi and Berger et al., 1990; Li et al., 1993) and activates at hyperpolarized potentials with half-activation (V_0.5) values ranging from −92 mV (Larkman and Kelly, 1992) to −75 mV (Banks et al., 1993). Its time constant for activation in a physiological preparation can be slower than 2 s (Pape and McCormick, 1989). I_h is also blocked by ZD7288 with reasonable selectivity and this compound can be used to differentiate it from other currents (BoSmith et al., 1993; Harris and Constanti, 1995). A final characteristic of I_h is its regulation by cAMP. Increased intracellular cAMP shifts the voltage dependence of activation of I_h to more depolarized potentials (DiFrancesco and Tortora, 1991) and, in some cases, increases its maximal conductance (Tokimasa and Akasu, 1990; Accili et al., 1997). Since under physiological conditions I_h is an inward current, these effects result in a greater number of I_h channels open at rest. This change becomes manifested as a membrane depolarization.

I_h-mediated depolarizations signaled through cAMP secondary to the activation of 5-HT receptors have been identified in several brain regions, including thalamic geniculate nuclei (Pape and McCormick, 1989), the brainstem nucleus prepositus hypoglossi (Bobker and Williams, 1989), the me-

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; ADn, anterior dorsal nucleus of the thalamus; I_h, hyperpolarization-activated nonselective cation current; 5-CT, 5-carboxamidotryptamine; 8-OHDPAT, 8-hydroxydipropylaminotetralin; PKA, protein kinase A.
dial nucleus of the trapezoid body (Banks et al., 1993), the cerebral cortex (Spain, 1994), and facial and spinal motor neurons (Takahashi and Berger, 1990; Larkman and Kelly, 1992). However, the specific serotonin receptor subtype involved in these responses remains controversial (Bobker and Williams, 1989; McCormick and Pape, 1990; Takahashi and Berger, 1990; Larkman and Kelly, 1992). Given the involvement of cAMP in regulating Ih, it seems most likely that the receptor involved would belong to the 5-HT4, 5-HT6, or 5-HT7 subtypes. However, this conjecture has not been rigorously tested.

In the preceding study, we identified a serotonin-induced depolarization in the ADn that is mediated by receptors of the 5-HT7 subtype. We hypothesized that part or all of this 5-HT7 receptor-mediated depolarization/inward current could be mediated by modulation of Ih in a cAMP-dependent manner. Therefore, in the present study, we first examined whether 5-HT regulated Ih in the ADn. We then tested for involvement of a 5-HT7 receptor in this response using a pharmacological approach. Finally, we directly examined the role of Ih in mediating the 5-HT7 receptor-induced inward current in this region. From the results of these experiments, we conclude that 5-HT7 receptors depolarize ADn neurons by regulating Ih through a cAMP-dependent, but PKA-independent, mechanism.

Materials and Methods

The methods for preparation of brain slices and electrophysiological recordings were essentially as outlined in the accompanying article (Chapin and Andrade, 2001). To generate Ih activation curves, cells were held at −40 mV. Current-voltage relationships were generated by applying 4.0- to 5.6-s long hyperpolarizing pulses every 10 to 20 s to increasingly hyperpolarized steps (in 5–10 mV increments). Ih was measured by subtracting the instantaneous current from the steady-state current. The amplitude of Ih was normalized to the maximal value.

Data Analysis. Data were analyzed using Origin 6.0 (Microcal Software, Northampton, MA). The voltage activation curve for each cell was fitted to the Boltzmann equation \( \frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp(\frac{V_m - V_{0.5}}{s})} \), where \( I_{\text{max}} \) is the normalized current, \( V_m \) is the command voltage, \( V_{0.5} \) is the half-activation voltage, and \( s \) is the slope factor. The slope factors and the \( V_{0.5} \) values were allowed to vary for analysis. Statistical data were tested using GB-STAT 6.0 or GraphPad Prism. Fitting of the time constant of activation was done using a second-order exponential decay equation \( I = I_o + A_1 e^{-\left(1-x^2\right)\tau^2} + A_2 e^{-\left(1-x^2\right)\tau^4} \) where \( I \) is the current, \( x \) represents time in ms, and \( \tau \) is the time constant. Except where indicated, data are presented as means ± S.E.M.

Results

As illustrated in Fig. 1A, cells of the ADn express an Ih (\( n > 50 \) cells). We characterized this current by applying voltage steps to increasingly hyperpolarized potentials from a holding potential of −40 mV (Fig. 1A). Steps to voltages negative to −60 mV revealed a slowly developing inward relaxation. This is the physiological profile expected for Ih. We confirmed that this slowly developing inward current corresponded to Ih by its sensitivity to ZD7288.

Ih in the ADn displays many common characteristics of this current found in other regions. We determined the voltage activation of this current in the ADn by measuring Ih at each voltage tested and normalizing these to the greatest magni-

\[ \frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp(\frac{V_m - V_{0.5}}{s})} \]

tude determined. We estimated the half-activation \( (V_{0.5}) \) of Ih by fitting the data with the Boltzmann equation (see Materials and Methods). The average \( V_{0.5} \) of activation under control conditions was −81 ± 1.3 mV (Fig. 2, \( n = 12 \) cells). Furthermore, the time constant of Ih activation increased with increasing hyperpolarization and could be fit by a second-order exponential decay equation. These results are consistent with the properties of Ih seen in other studies (Banks et al., 1993; Solomon and Nerbonne, 1993).

We next examined the effect of bath application of 5-HT on the activation curve for Ih. Bath application of 5-HT (10 μM) induced an approximately 10 mV depolarizing shift in the voltage dependence of activation of this current (Figs. 1 and

![Fig. 1](image1.png)

**Fig. 1.** Ih in the ADn is regulated by serotonin. A, hyperpolarizing current pulses elicit a slowly activating inward current (Ih). Bath administration of serotonin (10 μM) increases the amplitude of this current (symbols). \( V_m = -40 \) mV, steps to −50 mV, −65 mV, and −80 mV. Holding current at −40 mV = 220 pA. The time constants at −65 mV were \( \tau^2 = 189 ± 20 \), \( \tau^2 = 1820 ± 170 \); the time constants at −80 mV were \( \tau^2 = 109 ± 1 \), \( \tau^2 = 1043 ± 10 \). B, voltage dependence of Ih determined in this same cell. Serotonin shifts the voltage dependence of this current in the depolarizing direction by approximately 8 mV. Data illustrated in this plot were normalized to the maximal Ih amplitude.

![Fig. 2](image2.png)

**Fig. 2.** Effects of 5-HT and 5-CT on the voltage dependence of Ih. Bath administration of 5-HT (10 μM) shifts the Ih activation curve in the depolarizing direction by approximately 10 mV. Bath administration of 5-CT (3 μM) similarly shifts the voltage dependence of Ih by approximately 7 mV (\( n = 4 \) cells). All of the 5-CT experiments and one of the 5-HT experiments were conducted in the presence of 10 μM cymenopindol. ■, controls (\( n = 7 \) cells for 5-HT and \( n = 6 \) cells for 5-CT); ●, 5-HT (\( n = 7 \) cells); and ▲, 5-CT (\( n = 6 \) cells). Error bars represent the S.E.M.
2). This shift in the voltage dependence of activation is evident in the raw traces as an increase in the amplitude of \( I_h \) at more depolarized potentials (Fig. 1A). The \( V_{0.5} \) for \( I_h \) in the presence of serotonin (10 \( \mu \)M) was \(-71 \pm 2.3\) mV \((n = 7\) cells\). Thus, 5-HT increases \( I_h \) at resting membrane potentials by shifting its voltage dependence.

**Pharmacology of the 5-HT-Induced Shift in the Voltage Dependence of \( I_h \) Activation.** To test a possible role for the 5-HT\(_7\) receptor subtype in mediating the serotonin-induced shift in the voltage dependence of \( I_h \), we used agonists and antagonists capable of distinguishing between serotonin receptor subtypes. One of the defining characteristics of the 5-HT\(_7\) receptor is its sensitivity to carboxamidomethylproline tetrahydrochloride (5-CT; Lovenberg et al., 1993; Ruat et al., 1993). Therefore, we first tested the effects of this compound on \( I_h \). As illustrated in Fig. 2, bath administration of 5-CT (3 \( \mu \)M) mimicked 5-HT and elicited a shift in the voltage dependence of \( I_h \) \((V_{0.5} = -74 \pm 1.5\) mV, \(n = 6\) cells; \(t\) test, \(p < 0.01\)). A second pharmacological characteristic of the 5-HT\(_7\) receptor is its sensitivity to hydroxydypropylaminotetralin (8-OHD-PAT). This ligand has a high affinity for the 5-HT\(_{1A}\) receptor (Hoyer et al., 1994) and a moderate affinity for the 5-HT\(_5\) and 5-HT\(_7\) receptors (Shen et al., 1993), yet has a negligible affinity for most other serotonin receptor subtypes. Bath application of 100 \( \mu \)M 8-OHD-PAT to neurons of the ADn had no detectable effect on the voltage dependence of \( I_h \), but effectively blocked the response to 5-HT \((n = 4\) cells, ANOVA \(F = 0.14, p > 0.5\) not shown). Finally, the 5-HT\(_7\) receptor also exhibits very high affinity for LSD, a compound that appears to function as a low intrinsic activity partial agonist when tested on 5-HT\(_7\) receptors expressed in heterologous systems (Ruat et al., 1993). Bath administration of LSD (1 \( \mu \)M) had no detectable effect on the voltage dependence of \( I_h \), but blocked the effect of 5-HT \((n = 2\) cells). These results exclude several 5-HT receptor subtypes, including most 5-HT\(_5\), receptors, as well as receptors of the 5-HT\(_{1A}\), 5-HT\(_{3}\), and 5-HT\(_{8}\) subtypes. As such, they are consistent with the possible involvement of a receptor of the 5-HT\(_7\) subtype, but they do not rule out the involvement of receptors of the 5-HT\(_{1A}\), 5-HT\(_{3}\), or 5-HT\(_{8}\) subtypes.

Mesulergine and cyanopindolol are useful agents for identifying 5-HT\(_7\) receptors in functional assays. Mesulergine exhibits low nanomolar affinity for the 5-HT\(_7\) receptor, but micromolar affinity for those of the 5-HT\(_5\) and 5-HT\(_{6}\) subtypes (Hoyer et al., 1994) and therefore can be used to distinguish 5-HT\(_7\) from 5-HT\(_5\) and 5-HT\(_{6}\) receptors. Cyanopindolol, in contrast, exhibits high affinity for the 5-HT\(_{1A}\) receptor, but negligible affinity for receptors of the 5-HT\(_7\) subtype. Therefore, we next tested both of these antagonists. Bath application of cyanopindolol (10 \( \mu \)M) by itself had no detectable effect on the voltage dependence of \( I_h \), but failed to antagonize the effects of 5-HT or 5-CT (Fig. 2, \(n = 4\) cells). This failure was unlikely to reflect inadequate penetration of cyanopindolol into the slice because when administered at this same concentration to hippocampal slices, under essentially identical recording conditions, it readily inhibits 5-HT\(_{1A}\) responses (Chapin and Andrade, 2001). In contrast to the lack of effect of cyanopindolol, bath administration of mesulergine (10 \( \mu \)M) completely blocked the 5-CT-induced shift in the voltage dependence of activation of \( I_h \) (Fig. 3A). These results are inconsistent with the involvement of 5-HT\(_{1A}\), 5-HT\(_{5}\), or 5-HT\(_{6}\) receptors and suggested the involvement of a receptor of the 5-HT\(_7\) subtype. More definitive characterization was performed using the selective 5-HT\(_7\) antagonist SB-269770. Bath administration of this compound (1–10 \( \mu \)M) blocked the shift in the voltage dependence of activation induced by 5-HT (Fig. 3B). This indicates that 5-HT modulates \( I_h \) through the 5-HT\(_7\) receptor subtype.

**Molecular Mechanism of the Shift in the Voltage Dependence of \( I_h \) Activation.** Previous studies have shown that 5-HT\(_7\) receptors couple to stimulation of adenylate cyclase and the cAMP signaling pathway (Lovenberg et al., 1993; Meyerhof et al., 1993; Shen et al., 1993; Heidmann et al., 1998). Hence, a 5-HT\(_7\)-mediated response may be signaled by increases in cAMP. Therefore, we examined the possible involvement of cAMP in signaling the effect of 5-HT in the ADn. To test this possibility, we added 1 mM cAMP directly into the intracellular solution used for whole-cell recording. Intracellular perfusion of cAMP produced a large shift in the voltage dependence of \( I_h \) (Fig. 4), such that the \( V_{0.5} \) of \( I_h \) under these conditions was shifted to \(-66.4\) mV \(\pm\) 1.8 mV \((n = 4\) cells tested\). This value was significantly...
different from the $V_{0.5}$ obtained without cAMP in the intracellular recording solution (ANOVA, $F = 11.06$, $p < 0.001$, control versus cAMP, Tukey’s post hoc test, $p < 0.01$). This shift in voltage dependence was associated with a significant increase in holding current at near-rest membrane potentials ($-60$ to $-70$ mV; Fig. 4). Thus, cAMP mimics the effects of serotonin on $I_h$ in the ADn. This is consistent with the well known regulation of $I_h$ by cAMP (Bobker and Williams, 1989; Pape and McCormick, 1989; DiFrancesco and Tortora, 1991).

If serotonin signaled its effect on $I_h$ in the ADn through cAMP, then the effect of serotonin should be occluded by inclusion of cAMP in the electrode. Therefore, we examined the ability of cAMP to block the 5-HT-induced shift in the voltage dependence of $I_h$ after intracellular perfusion with cAMP. As illustrated in Fig. 4, under these conditions, serotonin failed to produce any further shift in the voltage dependence of activation of $I_h$. In fact, the $V_{0.5}$ for $I_h$ recorded in the presence of cAMP in the electrode was unaltered by the application of 10 μM serotonin (cAMP = 66 ± 1.8 mV; serotonin in cAMP 66 ± 2.2 mV). Hence, cAMP occluded a further response to 5-HT. These results are consistent with 5-HT shifting the voltage activation of $I_h$ via cAMP in the ADn.

Intracellular cAMP can regulate $I_h$ channels directly (DiFrancesco and Tortora, 1991) by binding to the channel itself (Santoro et al., 2000), or indirectly via PKA through an unidentified mechanism (Accili et al., 1997). To distinguish between these two possibilities, we used the relatively broad-spectrum protein kinase inhibitors staurosporine (1 μM) and H-8 (200 μM). To ensure that the inhibitors would reach equilibrium, we preincubated the slices with the inhibitors for 1 to 6 h before recording. These experimental manipulations have been shown previously to be effective in blocking PKA-mediated responses in brain slices (Chang et al., 1991, 1995; Torres et al. 1995). We found that staurosporine and H-8 both failed to inhibit the ability of 5-HT to induced a shift in the voltage dependence of $I_h$ ($p > 0.5$, Fig. 5). These results suggest that the 5-HT shift in the voltage dependence of $I_h$ does not require activation of PKA in the ADn.
The Role of \( I_h \) in the 5-HT\(_7\)-Induced Inward Current.

In the last set of experiments, we wished to ascertain what role the modulation of \( I_h \) played in the 5-HT\(_7\) receptor-mediated inward current described in the previous paper (Chapin and Andrade, 2001). To determine how much of the 5-HT\(_7\)-induced inward current is due to modulation of \( I_h \), we took advantage of the selective \( I_h \) blocker ZD7288 (BoSmith et al., 1993). As illustrated in Fig. 6A, administration of ZD7288 (25–100 \( \mu M \)) greatly reduced, but did not completely block, the 5-HT\(_7\) receptor-induced inward current (Fig. 6). In a group of four cells tested using this protocol, the modulation of \( I_h \) accounted for 71% ± 6% of the 5-HT\(_7\)-induced inward current (Fig. 7). The residual 5-HT\(_7\)-induced inward current that remained after ZD7288 was unlikely to be an effect on \( I_h \) since ZD7288 completely blocked \( I_h \), measured using a hyperpolarizing pulse (Fig. 6B, inset). From these experiments, we conclude that an effect on \( I_h \) accounts for a large fraction, but not all, of the 5-HT\(_7\)-induced inward current.

**Fig. 6.** Effect of the \( I_h \) blocker ZD7288 on the 5-HT\(_7\) receptor-induced inward current. A, administration of 5-HT (10 \( \mu M \)) elicits an inward current in an ADn neuron. We have previously shown that this effect is mediated by the activation of receptors of the 5-HT\(_7\) subtype (Chapin and Andrade, 2001). \( V_h = -60 \) mV, \( I_{\text{inh}} = 20 \) pA. Bath administration of ZD7288 (100 \( \mu M \)) greatly reduced the inward current elicited by a second administration of serotonin to the same cell. This inhibition does not reflect desensitization, because this response to serotonin does not show detectable desensitization under our recording conditions (Chapin and Andrade, 2001). B, quantitative comparison of the 5-HT\(_7\) receptor-mediated inward current recorded under control conditions or in the presence of ZD7288 (25–100 \( \mu M \)). ZD7288 significantly reduced the effect of serotonin (\( n = 4 \) cells, \( p < 0.05 \)) and completely blocked \( I_h \) estimated using a hyperpolarizing step from -40 to -80 mV (inset, \( p < 0.001 \)).

Discussion

In the preceding study, we showed that 5-HT\(_7\) receptor stimulation induces a membrane depolarization and inward current in neurons of the ADn. In the present study, we examined the possibility that this inward current may result from a modulation of \( I_h \). We found that 5-HT in the ADn shifts the voltage dependence of activation of \( I_h \) and that the pharmacology of the receptor mediating this effect has the characteristics of a 5-HT\(_7\) receptor. In addition, we found that, consistent with the involvement of a 5-HT\(_7\) receptor in this response, the effect on \( I_h \) is likely mediated via cAMP. Finally, we found that the selective \( I_h \) blocker ZD7822 greatly reduced the 5-HT\(_7\) receptor-mediated inward current seen in ADn neurons. Combined, these results suggest that 5-HT\(_7\) receptors signal a depolarization in the ADn primarily by increasing \( I_h \) through a cAMP-dependent mechanism.

**Pharmacology of the 5-HT-Induced Modulation of \( I_h \).**

Previous studies in a variety of central neurons have described a serotonin-induced depolarization mediated by an increase in \( I_h \) (Bobker and Williams, 1989; Pape and McCormick, 1989; Takahashi and Berger, 1990; Larkman and Kelly, 1992; Spain, 1994). Many of these same studies have shown that this effect is mediated through cAMP. Therefore, since 5-HT\(_7\) receptors are known to couple to G proteincoupling, it seemed reasonable to hypothesize that \( I_h \) could mediate at least part of the 5-HT\(_7\)-induced depolarization in the ADn.

To begin examining this possibility, we first ascertained whether neurons of the ADn expressed \( I_h \). Hyperpolarizing pulses revealed the presence of a slowly developing inward current that was sensitive to ZD7288, thus identifying this current as \( I_h \). The expression of \( I_h \) in these cells is consistent with the expression of \( I_h \) channel subunits in the ADn (Santoro et al., 2000). Then, we examined whether serotonin regulated \( I_h \) in the ADn. Bath administration of serotonin increased \( I_h \) by shifting its voltage sensitivity in the depolarizing direction by approximately 10 mV. This effect is similar to, albeit considerably larger than, that seen in other thalamic nuclei (Pape and McCormick, 1989).

Next, we examined whether the pharmacology of this effect on \( I_h \) was consistent with the involvement of a 5-HT\(_7\) receptor. The ability of serotonin to shift the voltage dependence of \( I_h \) was mimicked by 5-CT and inhibited by 8-OHDPAT and LSD, whereas the effect of 5-CT was antagonized by mesulergine, but not by cyanopindolol. This is the pharmacological profile expected for a receptor of the 5-HT\(_7\) subtype. The only potential discrepancy is that 8-OHDPAT and LSD have been reported to be partial agonists at the 5-HT\(_7\) receptor (Lovenberg et al., 1993; Ruat et al., 1993), whereas they function as antagonists at the receptor regulating \( I_h \) in the ADn. This discrepancy could easily reflect differences in the strength of receptor G-protein coupling. LSD exhibits very low intrinsic activity at 5-HT\(_7\) receptors even when heterologously expressed in Chinese Hamster ovary cells (Ruat et al., 1993) and can be expected to function as an antagonist in a more weakly coupled system. Similarly, 8-OHDPAT has been reported to exhibit a reasonably high intrinsic activity when tested in heterologous expression systems (50–90%; Lovenberg et al., 1993; Plassat et al., 1993; Adham et al., 1998), but a lower intrinsic activity when tested on natively expressed 5-HT\(_7\) receptors (Hirst et al., 1997; Thomas et al., 1999; Chapin and Andrade, 2001). If the 8-OHDPAT-induced shift
in the $V_{o,5}$ of $I_h$ was similar in magnitude to its effect on the 5-HT$_7$-induced inward current (approximately 20% of the maximal 5-HT response), then the response would be below the ability of our assay to detect agonist activity. As such, these results support the involvement of a 5-HT$_7$ receptor in mediating the effects of serotonin on $I_h$ in the ADn.

The results above exclude some receptors, most notably those of the 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ subtypes. Furthermore, the effectiveness of mesulergine, which is more than 100-fold selective for the 5-HT$_7$ receptor versus 5-HT$_5$ and 5-HT$_6$ (Hoyer et al., 1994), strongly argues against the involvement of a receptor of the 5-HT$_6$ subtype. Additional evidence supporting the involvement of 5-HT$_7$ receptors in mediating the effect of 5-HT on $I_h$ was obtained with the selective 5-HT$_7$ antagonist SB-269770 (Bacon and Beck, 2000; Lovell et al., 2000), which blocked the 5-HT-induced response. Unfortunately, the shift in voltage dependence of $I_h$ does not lend itself to the kind of quantitative pharmacological analysis required for a definitive receptor subtype identification. Therefore, we explored additional avenues to test the possible involvement of 5-HT$_7$ receptors in the regulation of $I_h$ in the ADn.

**Signal Transduction Mechanism of the 5-HT$_7$-Induced Shift in the Voltage Dependence of Activation of $I_h$.** Because the 5-HT$_7$ receptor is coupled to $G_{o,5}$ and hence adenylate cyclase and CAMP production (Adham et al., 1998), it seemed reasonable to expect that responses signaled by a 5-HT$_7$ receptor be mediated via cAMP. In the present study, we found that cAMP can mimic and occlude the serotonin-induced shift of the voltage activation of $I_h$. This is consistent with a response that is mediated through the cAMP-signaling cascade. This is in accord with previous studies showing that cAMP can regulate $I_h$ (DiFrancesco and Tortora, 1991) and that serotonin regulates $I_h$ through cAMP in other brain areas (Bobker and Williams, 1989; Pape and McCormick, 1989).

Previous studies have shown that $I_h$ can be regulated by cAMP through PKA-independent, as well as PKA-dependent, mechanisms. In their now classic work, DiFrancesco and Tortora (1991) demonstrated that, in the heart, cAMP directly acts on the $I_h$ channel to shift the voltage dependence of activation. This conclusion has been substantiated by the cloning of four $I_h$ channel subunits (HCN1–4), each containing a cAMP binding domain in their sequence (Gauss et al., 1989). These subunits carry a large proportion of the 5-HT$_7$ receptor-mediated inward current by about three-fourths. These results indicated that $I_h$ channels carry a large proportion of the 5-HT$_7$ receptor-induced inward current. Interestingly, a small residual current remained even after the $I_h$ channels were blocked. This indicates that a second ionic mechanism may depolarize neurons of the ADn, but it is not clear whether this second component represents another receptor subtype or simply a second ionic mechanism by which the 5-HT$_7$ receptor can alter cell properties. This uncertainty notwithstanding, the results outlined above identify the receptor signaling the increase in $I_h$ in the ADn as belonging to the 5-HT$_7$ subtype, and suggest that this effect on $I_h$ accounts for most of the depolarization seen in the ADn in response to serotonin.

Serotonin has been shown previously to elicit a membrane depolarization by increasing $I_h$ in a variety of neurons (Bobker and Williams, 1989; Pape and McCormick, 1989; Takahashi and Berger, 1990; Larkman and Kelly, 1992; Spain, 1994; Cardenas et al., 1999). However, the serotonin receptor subtype responsible for these actions has remained controversial. In most of these studies, the receptor has been reported to resemble the 5-HT$_1$ subtype, although a more recent study on dorsal root ganglion cells has also suggested the possible involvement of a 5-HT$_7$ subtype (Cardenas et al., 1999). Such discrepancies are perhaps not unexpected, given the complex pharmacology of the 5-HT$_7$ receptor. In the present study, we have used several distinct approaches to identify the receptor regulating $I_h$ in the ADn. These approaches converge in identifying the serotonin receptor regulating $I_h$ in the ADn as belonging to the 5-HT$_7$ subtype. Admittedly, it is difficult to extrapolate with certainty from the ADn to other brain areas. However, these results nevertheless suggest that the “orphan” serotonin receptor regulating $I_h$ in the brain corresponds to the 5-HT$_7$ receptor.

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References


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