Serotonin Reduces the Hyperpolarization-Activated Current (I_h) in Ventral Tegmental Area Dopamine Neurons: Involvement of 5-HT_2 Receptors and Protein Kinase C

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Liu, Zhaoping, E. Bradshaw Bunney, Sarah B. Appel, and Mark S. Brodie. Serotonin reduces the hyperpolarization-activated current (I_h) in ventral tegmental area dopamine neurons: involvement of 5-HT_2 receptors and protein kinase C. J Neurophysiol 90: 3201–3212, 2003. First published July 30, 2003; 10.1152/jn.00281.2003. Dopaminergic neurons of the ventral tegmental area (VTA) have been implicated in the rewarding properties of drugs of abuse and in the etiology of schizophrenia; serotonin modulation of these neurons may play a role in these phenomena. Whole cell patch-in-the-slice recording in rat brain slices was used to investigate modulation of the hyperpolarization-activated cationic current I_h by serotonin in these neurons. Serotonin (50–500 μM) reduced the amplitude of I_h in a concentration-dependent manner; this effect was reversible after prolonged washout of serotonin. This effect was mimicked by the 5-HT_1 agonist α-methylserotonin (25 μM) and reversed by the 5-HT_2 antagonist ketanserin (25 μM). Serotonin reduced the maximal I_h current and conductance (measured at −130 mV) and caused a negative shift in the voltage dependence of I_h activation. The serotonin-induced reduction in I_h amplitude was antagonized by intracellular administration of the nonspecific protein kinase inhibitor H-7 (75 μM) and the selective protein kinase C inhibitor chelerythrine (25 μM). The protein kinase C activator phorbol 12, 13 diacetate (PDA, 2 μM) reduced I_h amplitude; when PDA and serotonin were applied together, the effect on I_h was less than additive. These data support the conclusion that serotonin reduces I_h in dopaminergic VTA neurons by acting at serotonin 5-HT_2 receptors, which activate protein kinase C. This reduction of I_h may be physiologically important, as the selective inhibitor of I_h, ZD7288, significantly increased dopamine inhibition of firing rate of dopaminergic VTA neurons, an effect that we previously demonstrated with serotonin.

INTRODUCTION

Dopaminergic neurons of the ventral tegmental area (VTA) have been implicated in the rewarding effects of drugs of abuse (Wise 1987) and in the etiology of schizophrenia (White and Wang 1983). Dopaminergic neurons in the VTA are the cells of origin of the mesolimbic/mesocortical dopamine pathways and provide dopaminergic innervation of the nucleus accumbens (Oades and Halliday 1987). The mesolimbic/mesocortical dopamine pathway is important for self-administration of many drugs of abuse including, cocaine, amphetamine, nicotine, and ethanol (Wise 1987). The rewarding properties of some drugs of abuse, for example ethanol and nicotine, are related to their ability to excite dopaminergic cell bodies in the VTA (Brodie 1991; Brodie et al. 1999b; Di Chiara and Imperato 1988).

The VTA receives serotonergic innervation from the raphe nuclei (Oades and Halliday 1987). Serotonin has many effects in the VTA. Serotonin acts on 5-HT_2 receptors to reduce GABA release from GABAergic terminals onto GABA B receptors on dopaminergic neurons (Cameron and Williams 1994). Serotonin potentiates ethanol excitation (Brodie et al. 1995; Trifunovic and Brodie 1996) and dopamine inhibition (Brodie and Bunney 1996) of dopaminergic VTA neurons, and both of these effects are mediated by 5-HT_2 receptors. Cocaine also potentiates ethanol excitation of these neurons, an effect also mediated by 5-HT_2 receptors (Bunney et al. 2000).

Dopaminergic VTA neurons have a hyperpolarization-activated cationic current (I_h) (Lacey et al. 1989; Mueller and Brodie 1989). I_h has been shown to be modulated by serotonin in a number of different types of neurons. Serotonin increases I_h in neurons in the thalamus (McCormick and Pape 1990), nucleus prepositus hypoglossi (Bobker and Williams 1989) and dorsal root ganglion (Cardenas et al. 1999), and these effects of serotonin are mediated by an increase in cAMP. Serotonin also increases I_h in motoneurons of the facial motor nucleus but through an action at 5-HT_3 receptors (Garratt et al. 1993). In contrast to the enhancement seen in the preceding studies, serotonin decreases I_h in cerebellar Purkinje neurons, an effect that may be mediated by 5-HT_3 receptors (Li et al. 1993).

In earlier reports on the action of serotonin on dopaminergic neurons, serotonin was observed to increase I_h in substantia nigra neurons (Nedergaard et al. 1991) and in 25% of VTA neurons tested (Pessia et al. 1994). In our studies, serotonin potentiation of the effects of ethanol (Brodie et al. 1995) and dopamine (Brodie and Bunney 1996) was mediated through 5-HT_2 receptors and generally required higher serotonin concentrations or longer administrations of serotonin than were applied by others (Pessia et al. 1994). For this reason, we assessed the effects of higher serotonin concentrations on I_h. The present study demonstrates that serotonin reduces the amplitude of I_h in dopaminergic VTA neurons in a concentration-dependent manner and causes a negative shift in the voltage dependence of I_h activation. Furthermore, this effect of serotonin appears to be mediated by 5-HT_2 receptors and

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activation of protein kinase C. Some of these results have been previously reported in abstract form (Liu et al. 1999).

METHODS

Preparation of brain slices

Brain slices containing the VTA were prepared from male Fischer 344 rats (90–150 g) as previously described (Brodie et al. 1999a). Animals used in this study were treated in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Briefly, after rapid removal of the brain, the tissue was blocked coronally to contain the VTA and substantia nigra; the cerebral cortices and a portion of the dorsal mesencephalon were removed from the block. The tissue block was glued to the vibratome chuck and submerged in chilled artificial cerebrospinal fluid (ACSF). Coronal sections (400 µm thick) were cut, and the tissue was placed directly in the recording chamber (500 µl). The slice was totally submerged in ACSF maintained at a flow rate of 2 ml/min; the temperature in the recording chamber was kept at 35°C. The composition of the ACSF in these experiments was (in mM) 126 NaCl, 2.5 KCl, 1.24 NaH₂PO₄, 2.4 CaCl₂, 1.3 MgSO₄, 26 NaHCO₃, and 11 glucose. The ACSF was saturated with 95% O₂-5% CO₂ (pH = 7.4).

Extracellular recording electrodes were made from 1.5-mm-diam stainless steel wire (Dagan, Minneapolis, MN) and the tips were fire-polished. Electrodes had resistances of 3–5 MΩ when filled with a solution containing (in mM) 125 potassium gluconate, 15 NaCl, 1 CaCl₂, 2 MgCl₂, 10 EGTA, 3 ATP, and 0.3 GTP, adjusted to pH 7.3 with TRIZMA base. The electrode was advanced into the brain slice, and the extracellular spontaneous action potentials of dopaminergic VTA neurons were monitored in current clamp mode. Recordings were made with conventional whole-cell patch-in-the-slice methodology. Voltage- and current-clamp recordings were accomplished with an Axopatch-1B or Axoclamp-2A amplifier (Axon Instruments, San Rafael, CA). Current and voltage were monitored on a storage oscilloscope and on a rectilinear pen recorder and recorded on an on-line PC computer. Current and voltage steps, data acquisition and data analysis were controlled with pClamp 8 software (Axon Instruments, Foster City, CA).

Protocols and data analysis

In current-clamp experiments, Iᵩ was evoked by a family of hyperpolarizing current pulses (duration: 800 ms); the largest current pulse was adjusted to cause a peak voltage deflection to about −120 mV. Voltage-current (V-I) curves were constructed for peak and steady-state voltage responses. The voltage change due to Iᵩ was measured as the difference between the peak voltage and the steady-state voltage at the end of the current pulse. In voltage-clamp experiments, Iᵩ was evoked with a family of hyperpolarizing voltage steps (duration: 1 s) from a holding potential of −60 to −130 mV (in 10-mV increments). Current-voltage (I-V) curves were constructed for instantaneous and steady-state currents. The amplitude of Iᵩ was measured as the difference between the instantaneous current measured just after the decay of the capacitive transient and the steady-state current at the end of the voltage step; Iᵩ-V curves were constructed from these data.

The reversal potential of Iᵩ was determined from tail currents measured with the following voltage protocol. The membrane potential was stepped to −110 mV from a holding potential of −60 mV to activate Iᵩ; the step duration was 1 s that was sufficient time for full activation. The membrane potential was then stepped to test voltages between −120 and −10 mV (in 10-mV increments); step duration was 300 ms. The maximum amplitude of the tail current was measured just after the capacitive transient at each test voltage. Tail current amplitude was plotted as a function of the test voltage step, a curve fitted to the data points and the reversal potential was determined from the point on the curve at which the tail current reversed from outward to inward. To reduce contamination of Iᵩ tail currents by other currents, tail current experiments were performed in the presence of tetrodotoxin (TTX, 1 µM), tetraethylammonium chloride (TEA, 2 mM), 4-aminopyridine (2 mM), and MgCl₂ (20 mM) in the ACSF.

Drug-induced changes in Iᵩ were evaluated by examination of full I-V and Iᵩ-V curves measured in control and in the presence of drug in the same VTA neuron. For the purposes of averaging responses from different neurons and comparisons between drug conditions, drug-induced changes in Iᵩ were summarized as the percent decrease in Iᵩ amplitude measured at −120 mV. Percent decrease in Iᵩ was calculated as |Iᵩ(D) − Iᵩ(C)|/Iᵩ(C) × 100, where Iᵩ(D) and Iᵩ(C) are the amplitudes of Iᵩ measured in the presence of the drug and control, respectively. Percent decrease in Iᵩ was measured in control medium in the same cell prior to drug administration. Averaged data are expressed as means ± SE. Statistical significance of data from different drug conditions was assessed with a Student’s t-test or one- or two-way ANOVA as appropriate. When needed, the Student-Newman-Keuls post hoc test was used to test all pairwise multiple comparisons, and the Dunnett’s post hoc test was used for multiple comparisons versus the control group.

Extracellular recording

Extracellular recording electrodes were made from 1.5-mm-diam glass tubing with filament and were filled with 0.9% NaCl. Tip resistance of the microelectrodes ranged from 4 to 8 MΩ. The Fintronics amplifier used in these recordings includes a window discriminator, the output of which was fed to both a rectilinear pen recorder, and a computer-based data-acquisition system that was used for on- and off-line analysis of the data. The multiplexed output of the Fintronics amplifier was displayed on an analog storage oscilloscope for accurate adjustment of the window levels used to monitor single units. An IBM-PC-based data-acquisition system was used to calculate, display, and store the frequency of firing over 5-s and 1-min intervals. Firing rate was determined before and during drug application. Firing rate was calculated over a 1-min interval immediately prior to drug administration and a 1-min interval during the peak drug effect; drug-induced changes in firing rate were expressed as the percentage change from the control firing rate according to the formula [(FRᵩ − FRᵩ₋ᵩ)/FRᵩ₋ᵩ] × 100, where FRᵩ is the firing rate during the peak drug effect and FRᵩ₋ᵩ is the control firing rate. The change in firing rate thus is expressed as a percentage of the initial firing rate, which controls for small changes in firing rate which may occur over time.
**Drugs**

The flow rate of fluid to the recording chamber was continuously monitored with a flowmeter, and adjustable valves were used to keep the rate constant. The small volume chamber used in this study permitted the rapid application and washout of drug solutions. Because the slice was submerged in ACSF in the recording chamber, applied agents reach equilibrium in the chamber quickly (2–3 min). Drugs were added to the ACSF in the fluid delivery tubing by means of a calibrated infusion pump from stock solutions 100–1,000 times the desired final concentrations. The addition of drug solutions to the ACSF was performed in such a way as to permit the drug solution to mix completely with ACSF before this mixture reached the recording chamber. Final concentration was calculated from ACSF flow rate, pump infusion rate, and concentration of drug stock solution. Effects on $I_{h}$ were measured at 5 and 10 min after bath application of serotonin. In general, the effect on $I_{h}$ was greater at 10 min, and therefore the data shown in this paper were collected at this time point. Dopamine was given for 6 min, or less if firing rate was completely blocked. ZD7288 was given for 20 min before dopamine was retested. Most other drugs were administered by bath application as described in the preceding text and their effects on $I_{h}$ were quantitated after 10 min of application. The exceptions to this were H-7, chelerythrine, and cAMP, which were added to the internal solution and were applied intracellularly from the recording pipette. In this case, a period of 10–20 min was allowed after the rupture of the patch to permit the drug to enter the cell.

Serotonin creatinine sulfate complex, α-methylserotonin maleate, ketanserin tartrate, dopamine hydrochloride, and all other drugs used in this study were obtained from Sigma/RBI (St. Louis, MO) except for H-7 and dibutyryl-cAMP, which were obtained from Tocris Cookson, (Ballwin, MO); tetrodotoxin (TTX), which was used only in the tail-current experiments, was obtained from Calbiochem (La Jolla, CA).

**RESULTS**

**Serotonin reduces $I_{h}$: current-clamp studies**

Initially, $I_{h}$ was studied in whole cell clamp mode in a total of 21 VTA neurons with whole cell patch-in-the-slice recording. All cells met the criteria for dopaminergic VTA neurons described in Methods. At the resting membrane potential, these cells fired overshooting spontaneous action potentials. When tested with a series of hyperpolarizing current pulses 800 ms in duration (Fig. 1A, top), all of these neurons exhibited a robust time-dependent depolarizing “sag” in voltage responses more negative than about −70 mV (Fig. 1A, bottom), due to activation of $I_{h}$. The difference between the peak and steady-state voltage response indicates the magnitude of $I_{h}$. In the presence of 300 μM serotonin, the difference between the peak and steady-state voltage response was reduced, showing that $I_{h}$ is reduced by serotonin in these dopaminergic VTA neurons (Fig. 1B). Figure 1C is a voltage-current ($V$-$I$) curve in which the voltage responses shown in Fig. 1, A and B, are plotted as a function of the negative current pulses used to evoke them. This graph shows that serotonin produced a relatively small change in the peak voltage response but produced a large reduction in the steady-state response. Figure 1D plots the difference between the peak and steady-state voltage responses (magnitude of $I_{h}$) as a function of the peak voltage attained. This graph clearly shows the reduction in $I_{h}$ over the voltage range from about −70 to −120 mV.

The serotonin-induced reduction of $I_{h}$ in dopaminergic VTA neurons was concentration dependent ($n$ = 21). Figure 2 illustrates the effect of four different concentrations of serotonin tested on a dopaminergic VTA neuron. Figure 2A is a $V$-$I$ curve showing the effect of serotonin on the peak and steady-state components of the voltage responses in this neuron, and B...
Tritsch 1997; Jiang et al. 1993), the cell exhibited a serotonin-induced increase in 100 

Cesium (2.5 mM) caused complete block of I_h in five of nine dopaminergic VTA neurons and partial block in the remaining four neurons (data not shown). I_h was completely blocked by 5 mM cesium (Appel et al. 2003).

Voltage-clamp characterization of the serotonin-induced reduction of I_h

Following the current-clamp studies described in the preceding text, the effect of serotonin on I_h was further characterized with whole cell voltage-clamp recording in a total of 40 dopaminergic VTA neurons in brain slices. Voltage-clamp recording allowed the effect of serotonin on I_h to be studied at constant voltage and allowed determination of the effect of serotonin on the voltage dependence of activation of I_h (see Fig. 7, following text). The mean membrane potential of these neurons (measured in current-clamp mode) was $-44.4 \pm 0.5$ mV, and the mean input resistance was 108.6 $\pm 4.5$ M$\Omega$ ($n = 40$). Figure 3A illustrates the currents recorded in response to a series of negative voltage steps (duration: 1 s) from a holding potential $-60$ to $-130$ mV (in 10 mV increments). Following the capacitive artifact, there was a time-dependent increase in inward current reflecting the activation of I_h (bottom). The instantaneous current (ins.; ↑) was measured just after the decay of the capacitive transient and the steady-state current (ss.; ↑) was measured near the end of the voltage command. Figure 3B shows that in the presence of 500 $\mu$M serotonin, the steady-state inward current was substantially reduced in comparison to control (Fig. 3A), reflecting a serotonin-induced decrease in I_h. Figure 3C is a current-voltage (I-V) curve of the instantaneous and steady-state currents plotted as a function of the step voltage, in control (data from Fig. 3A) and in the presence of 500 $\mu$M serotonin (data from Fig. 3B). Note that serotonin decreased the steady-state current at all voltage steps more negative than $-70$ mV but had little or no effect on the instantaneous current. In Fig. 3D, the difference between the instantaneous and steady-state current (amplitude of I_h) is plotted versus the step voltage in the absence and presence of 500 $\mu$M serotonin. Note that I_h exhibited inward rectification and was activated at potentials more negative than $-70$ mV. The large reduction in I_h caused by the addition of 500 $\mu$M serotonin can be clearly seen.

![FIG. 2. Reduction of I_h by serotonin was concentration-dependent. A: V-I curves similar to Fig. 1C show the peak and steady-state voltage responses evoked by a series of hyperpolarizing current pulses in control and in the presence of 4 different concentrations of serotonin in a single dopaminergic VTA neuron. Serotonin (5-HT, 50–500 $\mu$M) caused a concentration-dependent reduction in the steady-state voltage responses (steady-state: symbols in legend) but had no effect on the peak responses (peak: symbols in legend). B: the magnitude of I_h in this neuron was determined from the difference between the peak and steady-state current responses and is plotted as in Fig. 1D. Serotonin (50–500 $\mu$M) caused a concentration-dependent decrease in the magnitude of I_h at all potentials negative to $-70$ mV.](http://jn.physiology.org/)

shows the voltage difference between peak and steady-state as a measure of the magnitude of I_h in the same neuron. Serotonin (50–500 $\mu$M) caused a concentration-dependent reduction in the steady-state current, but little change in the peak voltage (Fig. 2A). Figure 2B shows that this corresponds to a concentration-dependent reduction in the magnitude of I_h. In all nine cells tested with more than one serotonin concentration (100–500 $\mu$M) in current-clamp experiments, higher concentrations of serotonin produced a larger decrease in I_h than the lower concentrations. Table 1 shows the effects of serotonin (50–500 $\mu$M) on 21 dopaminergic VTA neurons; with concentrations of $\geq 100$ $\mu$M, >90% of the neurons showed a reduction in I_h. No cell exhibited a serotonin-induced increase in I_h, but a few showed no change in I_h, including four cells tested with 25 $\mu$M serotonin.

For the population of cells tested with 300 $\mu$M serotonin ($n = 15$), serotonin did not significantly change the membrane potential (paired t-test, $P > 0.05$) or the input resistance (paired t-test, $P > 0.05$). Specifically, prior to serotonin administration, the mean membrane potential was $-51.3 \pm 1.1$ mV and the mean input resistance was 291 $\pm 52$ M$\Omega$; in the presence of 300 $\mu$M serotonin, the mean membrane potential was $-50.2 \pm 1.2$ mV and the mean input resistance was 319 $\pm 46$ M$\Omega$.

As has been previously shown (Cathala and Paupardin-Tritsch 1997; Jiang et al. 1993), the I_h measured in this study was blocked by addition of cesium to the external medium.

![TABLE 1. Current-clamp data showing the effect of different concentrations of serotonin on I_h measured in 21 dopaminergic VTA neurons](http://jn.physiology.org/)

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Each neuron was tested with from one to four different concentrations of serotonin. When multiple concentrations were tested on the same neuron, the order of administration was from lowest to highest and each concentration was applied for 10–15 min before increasing the concentration. The number of cells showing each type of response is listed with the percent response of all cells tested with the same concentration given in parentheses. VTA: ventral tegmental area.
Figure 4 shows the concentration dependence of serotonin reduction of $I_h$ under voltage-clamp conditions. Figure 4A is an $I-V$ curve showing the instantaneous and steady-state currents plotted as a function of the hyperpolarizing voltage step; the currents were measured in control and after bath applications of three different concentrations of serotonin (100, 300, and 500 μM) to the same neuron. Serotonin (100–500 μM) decreased the steady-state current in a concentration-dependent manner but did not affect the instantaneous current. The difference between the instantaneous and steady-state current, $I_{h,ss}$, is plotted as a function of voltage in Fig. 4B; the concentration dependence of the reduction of $I_{h}$ by serotonin can be seen clearly in this graph. In all five cells tested with more than one serotonin concentration (≥100 μM) in voltage-clamp experiments, higher concentrations of serotonin produced a larger decrease in $I_{h}$ than the lower concentrations. A concentration-response curve for the mean serotonin-induced reduction of $I_h$ in voltage-clamp experiments is illustrated in Fig. 4C. The effect of serotonin was concentration-dependent (1-way ANOVA, $F = 12.47$, df = 2, 36, $P < 0.001$).

Table 2 shows that serotonin (30–500 μM) reduced $I_h$ in most of the 40 cells tested under voltage-clamp conditions. With serotonin concentrations of ≥50 μM, >90% of the neurons showed a reduction in $I_h$; no neurons exhibited an increase in $I_h$ and two neurons showed no change. By contrast, 30 μM serotonin (tested on 16 neurons) produced an increase in $I_h$ in 37.5% of the cells, a decrease in ~37.5%, and no change in the remaining 25% of the neurons.

When the recording could be held for sufficient time, the effect of serotonin was completely reversible. Figure 5 illustrates such an experiment. In this neuron, 300 μM serotonin clearly reduced the amplitude of $I_h$, and after a washout period of 1 h, $I_h$ returned to the pre-serotonin levels. Partial or total recovery on washout was observed in six neurons. For the six neurons tested, before serotonin administration $I_h$ at −130 mV was −288 ± 0.05 pA, in the presence of serotonin, $I_h$ at −130 mV was −202 ± 0.05 pA, and on washout of from 20 to 60 min, $I_h$ at −130 mV was −279 ± 0.05 pA. Serotonin significantly reduced $I_h$ amplitude (1-way repeated measures ANOVA, $F = 5.60$, df = 2, 10; $P < 0.05$), but there was no significant difference in $I_h$ at −130 mV between control and washout values (Student-Newman-Keuls, $P > 0.05$).

Effect of serotonin is mimicked by α-methylserotonin

The effect of serotonin to reduce $I_h$ was seen primarily at concentrations >50 μM. One reason for this could be efficient serotonin reuptake in the brain slice preparation, which would reduce the effective concentration of serotonin at the receptor. We have previously demonstrated serotonergic effects on ethanol potency with low concentrations of the reuptake inhibitors cocaine and cocaethylene in the absence of exogenous serotonin (Bunney et al. 2000, 2001), indicating that both release of serotonin and active reuptake of endogenous serotonin occur in our slice preparation. Because the results of experiments using reuptake blockers in combination with exogenous serotonin would be difficult to interpret due to unknown levels of endogenous serotonin, we used a serotonin analog that is not a substrate for reuptake, α-methylserotonin (α-Me5-HT). Figure 6 illustrates the results of voltage-clamp experiments to assess the effects of α-Me5-HT on $I_h$. In the I-V curve shown in Fig. 6A, α-Me5-HT (25 μM) caused a clear decrease in $I_h$ compared with control; α-Me5-HT (25 μM) decreased $I_h$ in all eight dopaminergic VTA neurons tested. Specifically, α-Me5-HT (25 μM) significantly reduced the mean $I_h$ amplitude measured at −120 mV from −281 ± 26 pA in control to −229 ± 29 pA in α-Me5-HT (paired t-test, $t =$
Concentration dependence of serotonin reduction of $I_h$ under voltage-clamp conditions: $A$: $I-V$ curve showing the instantaneous (open symbols) and steady-state (filled symbols) currents plotted as a function of the hyperpolarizing voltage step for a typical dopaminergic VTA neuron. Current responses were measured in control and after bath applications of 3 different concentrations of serotonin (5-HT, 100, 300, and 500 μM) to the same neuron (symbols on legend). Serotonin (100–500 μM) decreased the steady-state currents in a concentration-dependent manner but did not affect the instantaneous currents.

$B$: $I_h$, the difference between the instantaneous and steady-state currents, was inhibited in a concentration-dependent manner by serotonin (100–500 μM); same neuron as in $A$. $C$: pooled concentration-response curve for the effect of serotonin on $I_h$ amplitude in voltage-clamp experiments. The amplitude of $I_h$ at a command voltage of −120 mV was measured by subtracting the instantaneous current from the peak current and expressed as percent change from control $I_h$ amplitude (see METHODS). One or more concentrations of serotonin (30, 100, or 300 μM) was applied to 18 dopaminergic VTA neurons and the mean change in $I_h$ was plotted as a function of serotonin concentration (±SE).

$\Delta I_h$ by serotonin was reversible on washout. The mean percent decrease in $I_h$ amplitude caused by 25 μM α-Me5-HT was $-19.7 \pm 2.8\%$ ($n=8$), which was similar in magnitude to the mean percent decrease caused by 100 μM serotonin $-22.1 \pm 4.3\%$ ($n=5$).

This effect of α-methylserotonin was reversible after 30–40 min of washout (Fig. 6B) in all three dopaminergic VTA neurons tested. Specifically, α-Me5-HT (25 μM) significantly decreased the mean $I_h$ amplitude measured at −120 mV from $-258 \pm 5$ pA in control to $-197 \pm 6$ pA in α-Me5-HT and then fully reversed with washout to $-251 \pm 16$ pA (1-way repeated-measures ANOVA, $F=14.64, df=2, 4, P=0.014$, Student-Newman-Keuls post hoc tests, $P<0.05$).

**Effect of α-methylserotonin is reversed by ketanserin**

Because α-methylserotonin is a selective agonist for the 5-HT$_2$ receptor, the preceding results suggest that the 5-HT$_2$ serotonin receptor mediates the inhibitory effect of serotonin on $I_h$. To further establish this, we tested whether ketanserin, a selective 5-HT$_2$ antagonist, could reverse the reduction of $I_h$ by α-methylserotonin; this was found to be the case in all three dopaminergic VTA neurons tested. Specifically, α-Me5-HT (25 μM) significantly decreased the mean $I_h$ amplitude measured at −120 mV from $-354 \pm 39$ pA in control to $-302 \pm 55$ pA in α-Me5-HT. When ketanserin (25 μM) was added to the superfusate in the continued presence of α-Me5-HT, the effect of α-Me5-HT was fully reversed. In the presence of both ketanserin and α-Me5-HT, the mean $I_h$ amplitude was $-393 \pm 37$ pA, which was significantly larger than in the presence of α-Me5-HT alone (1-way repeated-measures ANOVA, $F=21.44, df=2, 4, P=0.007$, Student-Newman-Keuls post hoc tests $P<0.05$).

![FIG. 5. The reduction in $I_h$ by serotonin was reversible on washout. Current-voltage curve of $I_h$ amplitude (similar to Fig. 3D) in a dopaminergic VTA neuron. Serotonin (300 μM, 5-HT, ▲) caused a large decrease in $I_h$ over control (○), which was fully reversible after ~1 h of washout (wash, ■).](http://jn.physiology.org/)

**TABLE 2.** Voltage-clamp data showing the effect of different concentrations of serotonin on $I_h$ measured in 40 dopaminergic VTA neurons

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Serotonin causes a negative shift in the voltage-dependence of \( I_h \) activation

\( I_h \) was measured in 10 dopaminergic VTA neurons in the absence and presence of 300 \( \mu \)M serotonin. The voltage-clamp protocol and data analysis were the same as described for Fig. 3 in the preceding text. \( I_h \) currents were converted to conductance (\( G_h \)) with the equation \( G_h = I_h/(E - E_{rev}) \), where \( E \) is the voltage step used to evoke the current and \( E_{rev} \) is the reversal potential of \( I_h \). The values for \( E_{rev} \) were \(-39 \) mV for control and \(-42 \) mV for serotonin as determined by tail current analysis in three cells (see METHODS). The mean maximal \( G_h \) conductance (\( G_h \) measured at \(-130 \) mV) was \( 3.3 \pm 0.4 \) nS in control and \( 1.7 \pm 0.2 \) nS in 300 \( \mu \)M serotonin (\( n = 10 \)) or a mean percent reduction in maximal conductance of \(-47\% \) (with each cell serving as its own control). This serotonin-induced reduction in maximal \( G_h \) was significant (paired \( t \)-test, \( t = 4.75, df = 9, P = 0.001 \)).

The conductance (\( G_h \)) for each cell was normalized (\( G_h/G_{max} \)), where \( G_{max} \) was taken to be the value of \( G_h \) at \(-130 \) mV; this was done to permit the pooling of responses from the 10 neurons, as shown in Fig. 7. The mean normalized conductance (\( G_h/G_{max} \)) in the absence and presence of serotonin (300 \( \mu \)M) is plotted as a function of voltage. Serotonin shifted this conductance-voltage curve in the negative direction, indicating a negative shift in the voltage dependence for activation of \( G_h \).

The serotonin-induced shift was statistically significant (2-way ANOVA, \( F = 10.69, df = 1, 126, P = 0.001 \)). The effect of voltage was also significant (\( F = 167.57, df = 6, 126, P < 0.001 \)). The smooth curves on Fig. 7 were fit to the data points with the Boltzmann equation of the form

\[
\frac{G_h}{G_{max}} = \frac{1}{1 + e^{V - V_{1/2}}}
\]

The half-activation voltage (\( V_{1/2} \)) determined from the Boltzmann equation was \(-88 \pm 0.6 \) mV in control and \(-95 \pm 1.6 \) mV in 300 \( \mu \)M serotonin, a negative shift of \(-7 \) mV (Fig. 7). The slope factor (\( k \)) was similar in control (12 \pm 0.6) and in serotonin (11 \pm 1.5).

Effect of cAMP agonists and protein kinase inhibitors on the serotonin-induced reduction of \( I_h \)

Drug-induced changes in \( I_h \) were evaluated by examination of full \( I-V \) and \( I_h-V \) curves measured in control and in the presence of drug in the same VTA neuron. For the purposes of averaging responses from different neurons and comparisons between drug conditions, drug-induced changes in \( I_h \) were summarized as the percent decrease in \( I_h \) amplitude measured at \(-120 \) mV (see METHODS for formula). We first examined whether increasing intracellular cAMP would alter the serotonin-induced reduction of \( I_h \). Bath application of serotonin alone (300 \( \mu \)M) caused a mean percent decrease in \( I_h \) amplitude of \(-39.1 \pm 5.6\% \) in 12 dopaminergic VTA neurons (Fig. 8, far left bar). In another group of dopaminergic VTA neurons, 1 mM cAMP was included in the recording pipette. A small increase in the amplitude of \( I_h \) was seen after 10–15 min of recording in all five of these neurons, indicating that cAMP had diffused into the cell and was exerting a positive modulatory effect on \( I_h \). Figure 8 shows that after 10–15 min of intracel-

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**Figure 6.** The 5-HT\(_3\) agonist, \( \alpha \)-methyserotonin (\( \alpha \)-Me5-HT), reduces \( I_h \). A: current-voltage curve of \( I_h \) amplitude in a dopaminergic VTA neuron. \( \alpha \)-Me5-HT (25 \( \mu \)M, ■) caused a clear decrease in \( I_h \) as compared with control (○). In 8 dopaminergic VTA neurons tested, \( \alpha \)-Me5-HT (25 \( \mu \)M) significantly reduced the mean \( I_h \) amplitude measured at \(-120 \) mV from \(-281 \pm 26 \) pA in control, to \(-229 \pm 29 \) pA in \( \alpha \)-Me5-HT (paired \( t \)-test, \( P < 0.001 \)). B: the reduction of \( I_h \) by \( \alpha \)-Me5-HT was reversible on washout in all 3 dopaminergic VTA neurons tested. Specifically, \( \alpha \)-Me5-HT significantly decreased the mean \( I_h \) amplitude measured at \(-120 \) mV from \(-258 \pm 5 \) pA in control to \(-197 \pm 6 \) pA in \( \alpha \)-Me5-HT and then fully reversed with washout to \(-251 \pm 16 \) pA (1-way repeated-measures ANOVA, \( P = 0.014 \), Student-Newman-Keuls post hoc tests, \( P < 0.05 \)). *: a significant difference both from control and from wash. There was no difference between control and wash (\( P > 0.05 \)).

**Figure 7.** Serotonin causes a negative shift in the voltage dependence of activation of \( I_h \). \( I_h \) currents measured in 10 dopaminergic VTA neurons were converted to conductance (\( G_h \)) with the equation \( G_h = I_h/(E - E_{rev}) \), where \( E \) is the voltage step used to evoke the current and \( E_{rev} \) is the reversal potential of \( I_h \). The values for \( E_{rev} \) were \(-39 \) mV for control and \(-42 \) mV for serotonin as determined by tail current analysis in three cells (see METHODS). The mean maximal \( G_h \) conductance (\( G_h \) measured at \(-130 \) mV) was \( 3.3 \pm 0.4 \) nS in control and \( 1.7 \pm 0.2 \) nS in 300 \( \mu \)M serotonin (\( n = 10 \)) or a mean percent reduction in maximal conductance of \(-47\% \) (with each cell serving as its own control). This serotonin-induced reduction in maximal \( G_h \) was significant (paired \( t \)-test, \( t = 4.75, df = 9, P = 0.001 \)).

The conductance (\( G_h \)) for each cell was normalized (\( G_h/G_{max} \)), where \( G_{max} \) was taken to be the value of \( G_h \) at \(-130 \) mV; this was done to permit the pooling of responses from the 10 neurons, as shown in Fig. 7. The mean normalized conductance (\( G_h/G_{max} \)) in the absence and presence of serotonin (300 \( \mu \)M) is plotted as a function of voltage. Serotonin shifted this conductance-voltage curve in the negative direction, indicating a negative shift in the voltage dependence for activation of \( G_h \). The serotonin-induced shift was statistically significant (2-way ANOVA, \( F = 10.69, df = 1, 126, P = 0.001 \)). The effect of voltage was also significant (\( F = 167.57, df = 6, 126, P < 0.001 \)). The smooth curves on Fig. 7 were fit to the data points with the Boltzmann equation of the form

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The half-activation voltage (\( V_{1/2} \)) determined from the Boltzmann equation was \(-88 \pm 0.6 \) mV in control and \(-95 \pm 1.6 \) mV in 300 \( \mu \)M serotonin, a negative shift of \(-7 \) mV (Fig. 7). The slope factor (\( k \)) was similar in control (12 \pm 0.6) and in serotonin (11 \pm 1.5).
comparisons demonstrated that the effect of serotonin in the presence of H-7 or chelerythrine (25 μM) was included in the recording pipette, there was no consistent change in $I_h$ measured at -120 mV. Serotonin alone (5-HT, 300 μM) reduced the amplitude of $I_h$ ($-39.1 \pm 5.6\%$, $n = 12$). Serotonin (300 μM) also reduced $I_h$ amplitude in experiments in which 1 mM cAMP was included in the recording pipette (+cAMP, $-32.5 \pm 7.1\%$, $n = 5$). $I_h$ was also reduced when 300 μM serotonin was applied in the presence of 500 μM dibutyryl-cAMP (+dBcAMP, $-27.5 \pm 4.7\%$, $n = 3$), a membrane-permeable cAMP analogue. 

When the protein kinase inhibitor H-7 (75 μM) was included in the recording pipette, 300 μM serotonin caused only a very small mean percent decrease in $I_h$ amplitude ($+H-7, -4.4 \pm 11.0\%, n = 4$). Likewise, when the protein kinase C inhibitor chelerythrine (25 μM) was included in the recording pipette, the reduction in $I_h$ amplitude by 300 μM serotonin (+Chelery, $-16.2 \pm 3.8\%$, $n = 7$) was much smaller than the effect of serotonin alone. There was a significant difference between the drug conditions (1-way ANOVA, $P = 0.009$) and Dunnett’s post hoc comparisons demonstrated a significant difference between the control effect of serotonin alone and the effect of serotonin in the presence of H-7 and chelerythrine ($P < 0.05$), but the effect of serotonin in the presence of internal cAMP or external dibutyryl-cAMP was not significantly different from serotonin alone ($P > 0.05$).

Subsequent bath application of 300 μM dibutyryl-cAMP caused a further mean percent decrease in $I_h$ amplitude measured at -120 mV ($-4.7\%$, $n = 7$), a much smaller reduction than the effect of serotonin shown in Fig. 3D. Serotonin (300 μM) also reduced $I_h$ amplitude by 300 μM serotonin (+Chelery, $-16.2 \pm 3.8\%$, $n = 7$) was much smaller than the effect of serotonin alone. There was a significant difference between the drug conditions (1-way ANOVA, $P = 0.009$) and Dunnett’s post hoc comparisons demonstrated a significant difference between the control effect of serotonin alone and the effect of serotonin in the presence of H-7 and chelerythrine ($P < 0.05$). Because both H-7 and chelerythrine significantly attenuated the serotonin-induced reduction of $I_h$ and both are potent inhibitors of protein kinase C, the following experiments with the phorbol ester, PDA, were undertaken to further examine the possible involvement of protein kinase C.

Protein kinase C activator PDA mimics and occludes the serotonin-induced reduction in $I_h$

Figure 9A shows a current-voltage ($I-V$) curve of instantaneous and steady-state currents as a function of the step voltage in control and in the presence of 2 μM PDA. DMSO (0.1%) was used as a vehicle for the PDA and was present both in control and in the PDA-containing media. Like the effect of serotonin shown in Fig. 3C, PDA decreased the steady-state current but had little or no effect on the instantaneous current. In Fig. 9B, the difference between the instantaneous and the steady-state currents ($I_h$ amplitude) is plotted versus the step voltage in the absence and presence of 2 μM PDA. Note the large reduction in $I_h$ amplitude in the presence of PDA that closely resembles the effect of serotonin shown in Fig. 3D. Similar effects on $I_h$ current-voltage ($I-V$) curves were observed in all three dopaminergic VTA neurons tested with 2 μM PDA.

Figure 10 compares the effect on $I_h$ in dopaminergic VTA neurons of DMSO vehicle alone, serotonin (5-HT) in DMSO, PDA in DMSO, and 5-HT and PDA applied together in...
DMSO. Bars indicate the mean percent decrease in \( I_h \) measured at \(-120 \text{ mV}\) (see methods for formula). The far left bar shows that addition of 0.1% DMSO to the ACSF did not change \( I_h \) amplitude (\(-1.7 \pm 5.0\%\), \( n = 7 \)). DMSO (0.1%) was present in the ACSF for the other three experimental groups shown (remaining 3 bars). When tested in the presence of DMSO, 300 \( \mu \text{M} \) serotonin caused a mean percent decrease in \( I_h \) of \(-35.3 \pm 9.1\%\) (\( n = 4 \)). Bath application of PDA (2 \( \mu \text{M} \)) in DMSO vehicle also reduced \( I_h \) amplitude (\(-44.9 \pm 7.8\%\), \( n = 3 \)). When PDA (2 \( \mu \text{M} \)) and serotonin (300 \( \mu \text{M} \)) were applied together (in DMSO), the percent decrease in \( I_h \) amplitude was \(-51.5 \pm 20.0\%\) (\( n = 3 \)). Note that this effect was much less than the sum of the effects of serotonin and PDA when tested alone. Asterisks indicate a significant difference from the effect of DMSO vehicle alone. (1-way ANOVA, \( P = 0.006 \); Student-Newman Keuls post hoc tests \( P < 0.02 \)). The decreases in \( I_h \) measured in 5-HT, PDA, and 5-HT plus PDA were not significantly different from each other (\( P > 0.05 \)).

Reduction of \( I_h \) with ZD7288 potentiates dopamine inhibition

Because \( I_h \) is activated at membrane potentials negative to \(-70 \text{ mV}\) and the resting membrane potential of dopaminergic neurons is between \(-55\) and \(-40 \text{ mV}\) (Grace and Bunney 1983; Lacey et al. 1987), the physiological relevance of reduction of \( I_h \) by serotonin was examined. We previously have demonstrated that serotonin potentiates dopamine inhibition of the spontaneous firing rate of dopaminergic VTA neurons through an action at 5-HT\(_2\) receptors (Brodie and Bunney 1996). If serotonin potentiates dopamine inhibition through a reduction of \( I_h \), then selective blockade of \( I_h \) also would be expected to potentiate dopamine inhibition. ZD7288 is a selective blocker of \( I_h \) (Seutin et al. 2001). The spontaneous firing rate of dopaminergic VTA neurons was measured with extracellular single unit recording (see METHODS). Figure 11, A and B, illustrates the effect of 30 \( \mu \text{M} \) ZD7288 on dopamine inhibition of a single dopaminergic VTA neuron. For the population of cells tested before and in the presence of 30 \( \mu \text{M} \) ZD7288 (\( n = 14 \)), dopamine produced concentration-dependent decreases in firing rate (2-way ANOVA, \( F = 12.42, \text{df} = 4.76; P < 0.001 \)). ZD7288 alone increased the basal firing rate by 11.0 \( \pm 5.2\%\). The responses to dopamine (1–10 \( \mu \text{M} \)) were much larger in the presence of 30 \( \mu \text{M} \) ZD7288 than prior to ZD7288 addition in these cells (Fig. 11C); dopamine inhibition was significantly increased in the presence of ZD7288 compared with the control condition (2-way ANOVA, \( F = 31.591, \text{df} = 1.76; P < 0.001 \)).

Discussion

The present current- and voltage-clamp study demonstrates that serotonin (50–500 \( \mu \text{M} \)) reduces the amplitude of the hyperpolarization-activated cationic current (\( I_h \)) in dopaminergic VTA neurons. This reduction was concentration-dependent and reversible with washout of serotonin. \( I_h \) was also reduced by the 5-HT\(_2\) agonist \( \alpha\)-Me5-HT, and this effect was reversed by the 5-HT\(_2\) antagonist ketanserin. The maximal \( I_h \) current (measured at \(-130 \text{ mV}\)) was reduced by serotonin, as was the maximal conductance (\( G_{\text{max}} \)). The normalized conductance (\( G_{\text{h}}/G_{\text{max}} \)) curve showed that serotonin caused a negative shift in the voltage dependence of activation of \( I_h \). The serotonin-induced reduction in \( I_h \) amplitude was blocked by the nonspecific protein kinase inhibitor H-7 and the selective protein kinase C inhibitor chelerythrine, which suggests that it was mediated by protein kinase C. Consistent with this interpretation, the protein kinase C activator PDA mimicked and occluded the serotonin-induced reduction in \( I_h \) amplitude. Specifically, PDA applied alone reduced \( I_h \) amplitude. When serotonin and PDA were applied together, the effect on \( I_h \) was less than additive, suggesting that they were reducing \( I_h \) by a common mechanism. Taken together, these data demonstrate that serotonin reduces \( I_h \) in dopaminergic VTA neurons and support the conclusion that this effect is mediated by 5-HT\(_2\) receptors and protein kinase C.

The present study demonstrates that serotonin concentrations of 50–500 \( \mu \text{M} \) consistently decrease \( I_h \) in dopaminergic neurons of the VTA, whereas serotonin concentrations <50 \( \mu \text{M} \), produced mixed effects. For example, our current-clamp experiments with 25 \( \mu \text{M} \) serotonin showed no change in \( I_h \). Our voltage-clamp experiments testing 30 \( \mu \text{M} \) serotonin in 16 VTA neurons showed that 37.5% of cells showed a decrease in \( I_h \), but an equal proportion showed an increase in \( I_h \) with this lower serotonin concentration; the remaining 25% of neurons showed no change. The observation that a lower concentration of serotonin can increase \( I_h \) in some dopaminergic VTA neurons is consistent with earlier reports that 40 \( \mu \text{M} \) serotonin increased \( I_h \) in dopaminergic neurons in the substantia nigra (Nedergaard et al. 1991) and that serotonin (30–100 \( \mu \text{M} \)) increased \( I_h \) in 25% of dopaminergic VTA neurons tested (Pessia et al. 1994). It should be noted, however, that in the present study, increases in \( I_h \) were never observed with serotonin concentrations of \( \geq 50 \) \( \mu \text{M} \), whereas the decrease in \( I_h \) was clearly concentration dependent.

In view of estimates that the concentration of serotonin released at the synapse may reach the mM range (Bunin and Wightman 1998), it is likely that the effects of the relatively high concentrations of serotonin (50–500 \( \mu \text{M} \)) observed in the
present study are physiologically relevant. Furthermore, high concentrations of serotonin, such as were used in these studies, should have a faster onset of action than lower concentrations. In addition, serotonin reuptake blockers were not added to the superfusion medium, and, given active reuptake of serotonin present in brain slices, it is likely that the effective concentration of 5-HT at the receptor was lower than the concentration applied in the bath in our experiments. This is supported by the fact that 25 μM α-Me5-HT, a 5-HT2 agonist that is not a substrate for reuptake, was sufficient to significantly reduce \( I_h \) amplitude. The size of the reduction in \( I_h \) by 25 μM α-Me5-HT was very similar to the reduction by 100 μM serotonin, indicating the same effect could be produced by a fourfold lower concentration of α-Me5-HT.

The effectiveness of the 5-HT2 agonist α-Me5-HT to reduce \( I_h \) and the reversal of this effect by the 5-HT2 antagonist ketanserin, suggest that serotoninergic reduction of \( I_h \) is mediated by serotonin 5-HT2 receptors. An ultrastructural study has shown that 5-HT2A receptors are present on the dendrites and somata of dopaminergic VTA neurons (Doherty and Pickel 2000). Because the action of serotonin at 5-HT2 receptors is transduced by the phosphatidylinositol signal transduction system (Conn and Sanders-Bush 1984), the identity of the receptor as 5-HT2 is consistent with the antagonism of the effect of serotonin by protein kinase C inhibitor chelerythrine and the less selective protein kinase inhibitor H-7, and by the mimicking of the inhibitory effect of serotonin on \( I_h \) by PDA. Taken together, these results support the hypothesis that serotonin, by acting at a 5-HT2 receptor, causes the activation of protein kinase C, which then reduces \( I_h \) amplitude. Additional studies will be necessary to determine how activation of protein kinase C reduces \( I_h \) amplitude in dopaminergic VTA neurons.

The serotonin-induced inhibition of \( I_h \), observed in the present study in dopaminergic VTA neurons, has also been reported in cerebellar Purkinje cells (Li et al. 1993). The effect of serotonin in Purkinje cells closely resembles the effect in dopaminergic VTA neurons described in the present paper. In both studies, serotonin induced a similar negative shift in the voltage dependence of activation of \( I_h \). Interestingly, the serotonin-induced inhibition of \( I_h \) in Purkinje neurons was mimicked by the 5-HT2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (Li et al. 1993).

In contrast, in most studies in the literature, serotonin-induced increase of \( I_h \) has been observed. For example, serotonin increases \( I_h \) in inferior olivary neurons, and this increase is mediated by 5-HT2 receptors, as it is blocked by 5-HT2 antagonist ketanserin and is mimicked by 5-HT2 agonist DOI (Placantonakis et al. 2000). In addition, serotonin increases \( I_h \) in neurons in CA1 hippocampal pyramidal neurons (Gasparini and DiFrancesco 1999) and in neurons in the thalamus (McCormick and Pape 1990), nucleus prepositus hypoglossi (Bobker and Williams 1989), and dorsal root ganglion (Car-
denas et al. 1999); these effects of serotonin are mediated by an increase in cAMP. Conversely, receptors that inhibit adenyl cyclase can reduce \( I_h \) as demonstrated for opioids in nodose ganglion cells (Ingram and Williams 1994) and adenosine A1 receptor-mediated effects in thalamic (Pape 1992) and laterodorsal tegmental neurons (Rainnie et al. 1994). Because 5-HT\(_3\) receptors inhibit adenyl cyclase, we considered the possibility that the serotonin-induced reduction in \( I_h \) that we observed in dopaminergic VTA neurons might be mediated by a reduction in cAMP.

In the present study, intracellular application of cAMP from the recording pipette caused a small increase in the amplitude of \( I_h \) in all dopaminergic VTA neurons tested, indicating that cAMP had diffused into the cell and was exerting a positive modulatory effect on \( I_h \). This is consistent with previous reports that increasing intracellular cAMP can increase \( I_h \) in dopaminergic neurons in the ventral tegmental area (Jiang et al. 1993) and substantia nigra (Cathala and Paupardin-Tritsch 1997). Four genes encoding \( I_h \) channels (HCN1–4) recently have been cloned; all have a conserved cyclic nucleotide-binding domain, but the isoforms differ in the strength of their modulation by cAMP (Santoro and Tibbs 1999; Santoro et al. 2000). All four HCN genes are expressed in brain but show different regional localization (Moosmang et al. 1999; Robinson and Siegelbaum 2003). It remains to be determined which HCN isoforms are present in dopaminergic VTA neurons and their sensitivity to modulation by cAMP relative to neurons in other brain areas.

If the serotonin-induced reduction in \( I_h \) that we observed in dopaminergic VTA neurons was due to a decrease in cAMP, then increasing intracellular cAMP would be expected to prevent or significantly attenuate this inhibitory effect. Serotonin still reduced \( I_h \) amplitude after intracellular administration of cAMP from the recording pipette and after bath application with the membrane-permeable cAMP analogue dibutyryl-cAMP. The effect of serotonin on \( I_h \) in the presence of internal cAMP or external dibutyryl-cAMP was not significantly different from the effect of serotonin alone. These data suggest that the reduction in \( I_h \) amplitude in dopaminergic VTA neurons is not consistent with inhibition of adenylyl cyclase.

The results of the present study suggest that the serotonergic reduction of \( I_h \) in dopaminergic VTA neurons is mediated by protein kinase C. Specifically, serotonergic inhibition of \( I_h \) was reduced by the nonspecific kinase inhibitor H-7 and the selective protein kinase C inhibitor chelerythrine. Furthermore, the phorbol ester PDA mimicked and occluded the serotonininduced reduction in \( I_h \). In addition to the reduction in \( I_h \) amplitude, serotonin also caused a negative shift in the voltage dependence of activation of \( I_h \) in dopaminergic VTA neurons. Interestingly, protein kinase C also has been implicated in the reduction of \( I_h \) by neurotensin in dopamine neurons in the substantia nigra pars compacta (Cathala and Paupardin-Tritsch 1997). While the size of the decrease in \( I_h \) amplitude by neurotensin was similar to the serotonin-induced reduction in our study, neurotensin did not significantly change the voltage dependence of activation of \( I_h \) (Cathala and Paupardin-Tritsch 1997). In view of the fact that there are numerous isoforms of PKC (Way et al. 2000), it is possible that neurotensin activates a different PKC isoform than serotonin. This might result in phosphorylation of different sites on the \( h \)-channel, or phosphorylation of a different subset of \( h \)-channels by the two neurotransmitters, leading to differences in their modulation of \( I_h \).

Because \( I_h \) activates at membrane potentials negative to \(-70\) mV in dopaminergic VTA neurons, it may not contribute much to their spontaneous firing rate (but see Seutin et al. 2001). Interestingly, we have presented data in the present paper that reduction of \( I_h \) can increase the inhibitory effect of dopamine on the spontaneous firing rate of dopaminergic VTA neurons. We have previously reported that serotonin, acting through 5-HT\(_3\) receptors, also potentiates the inhibitory action of dopamine (Brodie and Bunney 1996). As even low concentrations of dopamine (e.g., 1 \( \mu \)M) were potentiated by ZD7288, the effect of \( I_h \) blockade on dopamine inhibition probably did not require reducing the whole cell membrane potential to \(-70\) mV because the cells were still spontaneously active. While further experiments will be necessary to uncover the precise mechanism of this action, it may be that the opening of the G-protein-coupled inwardly rectifying potassium channels (GIRKs) by the action of dopamine on D\(_2\) receptors (Kim et al. 1995; Lacey et al. 1987) produces local membrane potentials negative to \(-70\) mV, which causes the opening of \( h \)-channels in the vicinity of those GIRK channels. The inhibitory dopamine current would be shunted by the reduced membrane resistance due to the open \( h \)-channels, and the effect of the inhibitory dopamine current on the whole cell would be reduced. When \( I_h \) is reduced by serotonin or ZD7288, the action of dopamine would not be attenuated by the opening of nearby \( h \)-channels and there would be relatively greater reduction of firing rate by dopamine.

The neuromodulatory role of serotonin may also extend to excitatory burst firing of dopaminergic neurons. Selective blockade of \( I_h \) with ZD7288 has been shown to decrease spontaneous firing of midbrain dopamine neurons (Seutin et al. 2001) and, as hypothesized by those authors, \( I_h \) may be more important when the membrane achieves more negative potentials, for example, during the afterhyperpolarization after action potential burst firing. After a burst, the afterhyperpolarization can achieve potentials negative to \(-70\) mV, and the duration of this afterhyperpolarization may be reduced by the opening of \( h \)-channels. Reduction of \( I_h \) by serotonin may prolong the postburst afterhyperpolarization and reduce excitability. This more sustained decrease in neuronal excitability might reduce the response to subsequent excitatory stimulation and limit the frequency of bursting in these neurons. As burst firing may be induced by excitatory amino acid neurotransmission (Johnson et al. 1992), this action of serotonin could have a neuroprotective effect on mesencephalic dopamine neurons.

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DISCLOSURES

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