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WHOLE-CELL RECORDINGS OF INWARDLY RECTIFYING K^+ CURRENTS ACTIVATED BY 5-HT_{1A} RECEPTORS ON DORSAL RAPHE NEURONES OF THE ADULT RAT

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SUMMARY

1. An inwardly rectifying K^+ current activated by serotonin (5-HT) was recorded from acutely isolated adult dorsal raphe (DR) neurones using the whole-cell recording mode of the patch clamp technique.

2. The 5-HT-induced K^+ current ($I_{5\text{-HT}}$) was only visible at an $[K^+]_o > 5$ mM and it was observed in 69% of the cells.

3. The reversal potential for $I_{5\text{-HT}}$ was close to the potassium equilibrium potential and was shifted by 51 mV per 10-fold change in $[K^+]_o$ indicating that $I_{5\text{-HT}}$ was carried predominantly by K^+. The chord conductance of $I_{5\text{-HT}}$ at -90 mV was proportional to the external $[K^+]$ raised to a fractional power.

4. A dose–response relationship revealed that $I_{5\text{-HT}}$ was activated with an ED_{50} of 30 nM. Ba^{2+} (0·1 mM) blocked $I_{5\text{-HT}}$ completely. Spiperone reversibly antagonized the response to 5-HT and 8-OHDPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) mimicked the response indicating that the receptor activated was of the 5-HT_{1A} subtype.

5. The response to 5-HT was largely prevented by *in vitro* pretreatment of the cells with pertussis toxin (PTX) indicating the involvement of a PTX-sensitive G-protein in the transduction mechanism.

6. cAMP and lipoxygenase metabolites, both implicated in the modulation of similar currents in other preparations, were found not to alter the effectiveness of 5-HT.

7. Glibenclamide and tolbutamide, blockers of the ATP-regulated K^+ channel, did not reduce the effect of 5-HT in DR neurones.

8. These results show that in acutely isolated adult DR neurones 5-HT activates an inwardly rectifying K^+ current and this involves a PTX-sensitive G-protein in the transduction pathway which may interact with the K^+ channel directly.

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INTRODUCTION

The serotonergic neurones of the dorsal raphe (DR) nucleus, located in the upper brainstem, innervate many of the higher centres of the brain. Regulation of the activity of these cells has a wide impact on brain function. Normally, DR neurones fire spontaneously in a slow pacemaker type fashion (Vandermaelen & Aghajanian, 1983), but this activity can be modified by external influences such as sensory input (Trulson & Jacobs, 1979).

Serotonin (5-HT) itself can modify the electrical properties of DR neurones. 5-HT is thought to contribute to recurrent inhibition following electrical stimulation of the nucleus or a major synaptic input (Wang & Aghajanian, 1977; Yoshimura, Higashi & Nishi, 1985; Pan & Williams, 1989). 5-HT may be released by neighbouring cells to generate a serotonergic IPSP at the soma of DR neurones which are densely endowed with 5-HT_{1A} receptors (Sotelo, Cholley, El Mestikawy, Gozlan & Hamon, 1991). Recently we have reported that 5-HT inhibits DR neurone calcium current and this effect is mediated by 5-HT_{1A} receptor activation (Penington & Kelly, 1990; Penington, Kelly & Fox, 1991*a*). 5-HT has been suggested to decrease the spontaneous firing rate of DR neurones by increasing a potassium conductance (Vandermaelen & Aghajanian, 1983; Lakoski & Aghajanian, 1984). This conductance has been investigated using intracellular recording in the brain slice preparation, where it has been shown to be inwardly rectifying (Williams, Colmers & Pan, 1988).

In this study and the accompanying paper (Penington, Kelly & Fox, 1993) we have attempted to investigate these conductances for the first time using whole-cell and single channel patch clamp techniques. We have been able to identify a component of K^+ current at the whole-cell level and the underlying single channels that are modulated by 5-HT when suitable conditions are employed. These channels are highly K^+ selective and inwardly rectifying. A brief communication of some of these results has been made to The Physiological Society (Penington, Kelly & Fox, 1991*b*).

METHODS

Three coronal slices (500 μm) through the brain stem at the level of the dorsal raphe nucleus were prepared from young adult rats (200–250 g) in a conventional manner using a 'vibroslice'. The slices were placed in cold Ringer solution containing (mM): NaCl, 119; KCl, 5; $MgCl_2$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; NaH_2PO_4, 1·2; glucose, 11; pH 7·3–7·4 when bubbled with 95% O_2–5% CO_2. The slices were placed on an Agar base and a piece of grey matter 2 × 2 mm was cut from immediately below the cerebral aqueduct containing the dorsal raphe nucleus. The pieces of tissue were then incubated in a Pipes buffer (piperazine-*N*-*N*′-bis(2-ethanesulphonic acid) solution containing 0·07% trypsin (Sigma Type XI) under pure oxygen for 90 min according to the method of Kay & Wong (1987). The pieces of tissue were then triturated in Dulbecco's modified Eagle's medium and the isolated cells allowed to settle on a glass coverslip coated with concanavalin A. Within 5 min of plating the cells were firmly anchored to the coated coverslip. Recording was carried out within 30–60 min after cell attachment at room temperature 22–25 °C.

Whole-cell currents consisting mainly of potassium currents were carried out using a potassium gluconate pipette solution containing (mM): potassium gluconate, 84; Hepes, 10; MgATP, 2; KCl, 38; EGTA, 11; KOH, 33; $CaCl_2$, 1; GTP, 300 μM, pH 7·3 with NaOH. Some experiments were done using NaATP in place of MgATP. The default bathing solution contained (mM): NaCl, 147; KCl, 2·5; $CaCl_2$, 2; $MgCl_2$, 2; glucose, 10; Hepes, 20; pH 7·3 with NaOH.

The extracellular solution was continually perfused at a rate of about 2 ml/min into a bath containing about 1 ml of recording solution. In order to eliminate the contribution of Na^+ ions to

the inward current, 0·1 μM (TTX, citrate salt; Sigma Chemical Co., St Louis, Mo. USA) was added to all bathing solutions. The osmolarity of the pipette solution was adjusted with sucrose to be 20 mosmol hyposomotic to the bathing solution (300 mosmol/l). Drugs were usually dissolved in the extracellular solution and added to the perfusate or occasionally they were added directly to the bath thus achieving a known final concentration. Spiperone HCl and 8-OHDPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) were obtained from Research Biochemicals Incorporated, Natick, MA, USA. Nordihydroguaiaretic acid (NDGA) and 5-HT creatine sulphate were obtained from Sigma. NDGA was dissolved at 200 mM in dimethylsulphoxide (DMSO) and diluted to 10 μM in the bathing solution. Similarly tolbutamide and glibenclamide (gift of Dr Deborah Nelson) were prepared at 100 and 10 mM and dissolved to the final concentration in control experiments. DMSO at final bath concentrations of < 0·001 % had no effect on K^+ channel currents. In two experiments 100 μM tolbutamide was tested (DMSO concentration, 0·1 %). No block of 5-HT sensitive current was observed, and so no controls were done for this concentration of DMSO. The pertussis toxin was obtained from Calbiochem (La Jolla, CA, USA) and 8-CPTcAMP 8-(4-chlorophenyl thio-cAMP) from Boehringer Mannheim (Germany). Liquid junction potentials were measured by first placing the pipette into the internal pipette solution (potassium gluconate) as a zero reference and then exchanging the bath solution. A liquid junction potential of 4–5 mV (relative to ground) was measured between potassium gluconate and bath solutions containing NaCl or KCl. The data in this paper have not been corrected for this small potential.

Neurones with truncated dendrites and a cell soma with one dimension of at least 20 μm were voltage clamped using an Axopatch 1c patch clamp amplifier in the whole-cell configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Electrodes ranging in resistance from 1·8–2·5 MΩ were pulled from soda-lime glass capillary tubes (Scientific Products, McGaw Park, IL, USA) and coated with Sylgard. The series resistance circuit of the amplifier was used to compensate ≈ 80 % of the apparent series resistance. Clamp settling time was always less than 1 ms. The seal resistance was often greater than 5 GΩ. The voltage clamp data were filtered at 3 kHz and then digitized at 100 or 200 μs per point. Voltage protocols were generated and analysed by an IBM PC/AT clone using Axobasic 1 software.

RESULTS

Activation of K^+ channels by 5-HT: requirement for elevated $[K^+]_o$

Solutions were continuously perfused over isolated neurones attached to the bottom of a chamber. Whole-cell currents were recorded in the presence of TTX to block Na^+ currents. Since most of the test voltage steps were hyperpolarizations contamination by other currents was not evident. The theoretical reversal potential for K^+ (E_K) was first set at −104 mV by the use of an internal $[K^+] = 155$ mM and an external $[K^+] = 2{\cdot}5$ mM. Under these conditions, no change in the holding current was detected at −60 mV ($n = 10$) when 5-HT was added to the bath. Current–voltage relationships showed no change in the membrane current even at voltages more negative to E_K. Figure 1*A* illustrates the effect of raising $[K^+]_o$ to 6 mM (which is close to the physiological range see Discussion). 5-HT (10 nM to 10 μM) produced a small outward shift in the holding current when held at −60 mV. 5-HT induced a small inward current (below the experimentally determined reversal potential of $-72 \pm 3{\cdot}2$ mV, mean ± S.E.M., $n = 5$). Figure 1*B* shows that 5-HT activated no measurable current at a holding potential of −60 mV in 10 mM $[K^+]_o$ (theoretical $E_K = -69$ mV). A large increase in inward current was observed at −90 mV (note the change in gain between panels *A*, *B* and *C* of Fig. 1). In elevated $[K^+]_o$ a large inward current was elicited by 5-HT in forty-three of sixty-two cells. In other experiments 20, 50, and 136 mM $[K^+]_o$ were also investigated. In all cases when $[K^+]_o$ was increased the $[Na^+]_o$ was decreased by the same amount.

Figure 1*C* shows that changing the $[K^+]_o$ to 136 mM (theoretical $E_K = -3{\cdot}3$ mV)

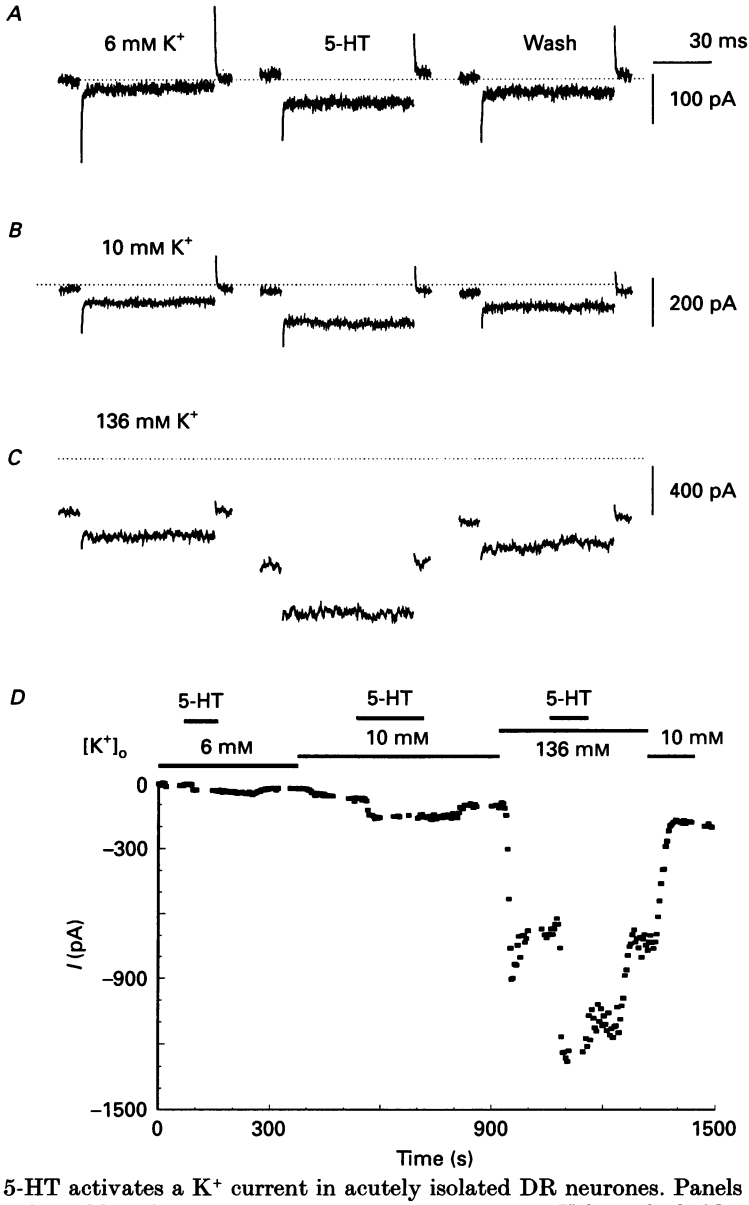

Fig. 1. 5-HT activates a K^+ current in acutely isolated DR neurones. Panels *A–C* show currents elicited by a hyperpolarizing current step to −90 mV from the holding potential of −60 mV. E_K was set at −83 mV in *A*, (6 mM $[K^+]_o$) at −70 mV in *B* (10 mM $[K^+]_o$) and at −3 mV in *C* (136 mM $[K^+]_o$). Left panel shows currents recorded in absence of 5-HT (control), middle panel currents in the presence of 5-HT (10 μM), and the right panel currents after 5-HT was washed from the bath. *D* shows the plot of current, measured at −90 mV, as a function of time. $[K^+]_o$ was changed as indicated (lower horizontal bars). Top horizontal bars indicate application of 10 μM 5-HT. Solutions are described in Methods.

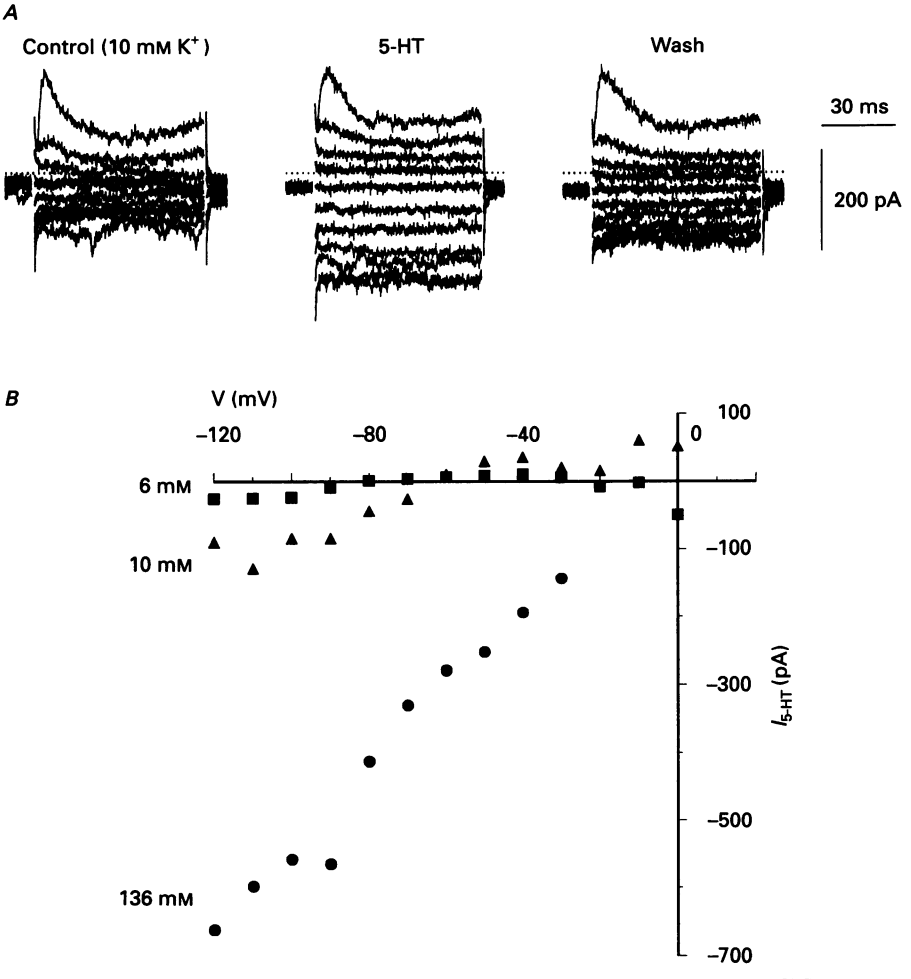

Fig. 2. Current–voltage relationships of the 5-HT-induced current ($I_{5\text{-HT}}$) in a DR neurone. *A*, current traces were elicited by voltage steps in the range from −120 to −20 mV were made from a holding potential (V_h) of −60 mV every 4 s. 5-HT (10 μM) was added as indicated. Currents for the panel on the right were obtained after 5-HT was washed from the bath. *B*, graph of $I_{5\text{-HT}}$ plotted as a function of potential. Thirty data points taken from the end of the current trace were averaged for each potential. Currents in 5-HT were subtracted from control to give $I_{5\text{-HT}}$ for three different concentrations of $[K^+]_o$ in this cell. $I_{5\text{-HT}}$ was clearly inwardly rectifying; it could not be studied at more depolarized potentials due to the activation of other conductances. The data shown in this figure are from the same cell as that illustrated in Fig. 1. The linear portions of the *I–V* plots were fitted using a simplex least-squares algorithm to find the reversal potentials for the 5-HT activated current ($E_{5\text{-HT}}$).

greatly increased both the holding current and the increase in K^+ conductance evoked by 5-HT. Figure 1*D* illustrates the steady-state current at the end of each step to −90 mV plotted against time for the entire experiment. This figure shows the inward current at −90 mV activated in three different $[K^+]_o$ and the effects of 5-HT. Note that all effects were reversible.

Figure 2 shows that the currents activated by 5-HT were inwardly rectifying. In Fig. 2*A* the currents activated by hyperpolarizing steps from −60 mV, in 10 mM $[K^+]_o$, were usually small and rectified weakly in the inward direction. At more negative voltages the current elicited by hyperpolarizing steps diminished or

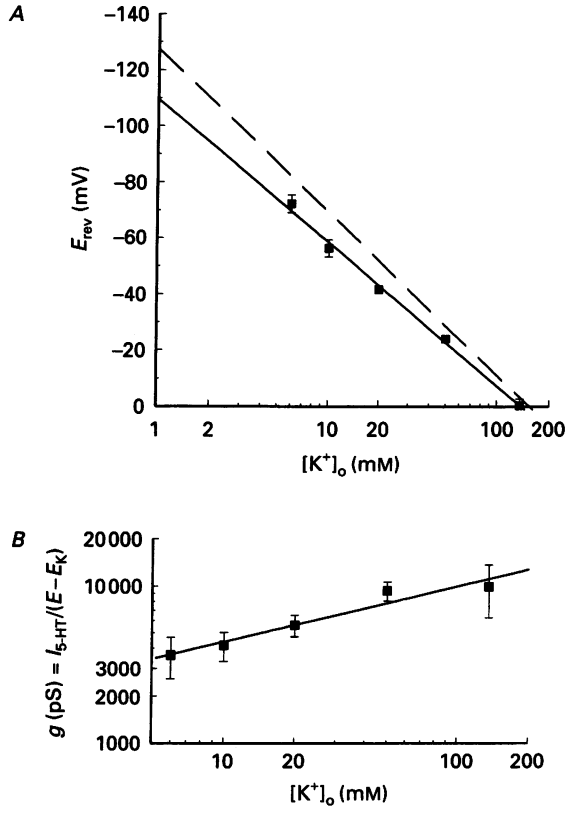

Fig. 3. $I_{5\text{-HT}}$ is a K^+-selective current. *A*, reversal potentials for $I_{5\text{-HT}}$ are plotted as a function of $[K^+]_o$. The data points were fitted using a simplex algorithm. The best fit was obtained by:

$$E_{rev} = -51{\cdot}1 \log [K^+]_o/139.$$

The dashed line shows the relationship predicted by the Nernst equation assuming an internal $[K^+]$ of 155 mM (pipette concentration):

$$E_{rev} = -58{\cdot}1 \log [K^+]_o/155.$$

Thus it can be seen that $I_{5\text{-HT}}$ was K^+ selective, but deviates from perfect selectivity. *B* plots the log of the 5-HT-induced conductance at −90 mV against $\log [K^+]_o$. The data was fitted with a function of the form:

$$g = c[K^+]_o^x$$

where g is conductance, c is a constant, and x is a fraction. For this data $x = 0{\cdot}35$ and $c = 1946$. Thus the 5-HT-induced conductance is proportional to $[K^+]_o^{0{\cdot}35}$.

saturated. In Fig. 2*B* replacement of the $[Na^+]_o$ with K^+ straightened out the *I–V* relationship and allowed larger currents to flow, as shown earlier in muscle cells (Matsuda & Stanfield, 1989). In Fig. 2*A* depolarizing steps from −60 to −20 mV

activated an I_A type K^+ current followed by a current with the characteristics of a delayed rectifier (I_{DR}) K^+ conductance. However, the currents activated in the presence of 5-HT by hyperpolarizing steps of less than 60 mV were larger in the inward as opposed to the outward direction. The voltage-dependent currents, I_A and I_{DR}-like were unaffected by 5-HT and the increase in K^+ conductance recovered when the 5-HT was washed out.

TABLE 1. Relation between $[K^+]_o$ and reversal potential

$[K^+]_o$ (mM)	$E_{5\text{-HT}}$ (mV)
6 ($n = 5$)	$-72{\cdot}4 \pm 3{\cdot}2$
10 ($n = 6$)	$-56{\cdot}3 \pm 3{\cdot}0$
20 ($n = 26$)	$-41{\cdot}7 \pm 0{\cdot}9$
50 ($n = 4$)	-24
136 ($n = 6$)	$-0{\cdot}4 \pm 2{\cdot}4$

Figure 2*B* illustrates the steady-state current plotted against voltage obtained by subtracting the current in control conditions from the current in 5-HT to yield 5-HT activated current ($I_{5\text{-HT}}$) for three different $[K^+]_o$. In 6 mM $[K^+]_o$ (■) a small inward current was activated by 5-HT below −80 mV, the apparent reversal potential for this cell. In the same cell, when the $[K^+]_o$ was increased to 10 mM, the 5-HT-activated inward current, $I_{5\text{-HT}}$, increased below −65 mV and saturated at −120 mV. Although values are plotted in the range from −30 to 0 mV they should be interpreted carefully, as other 5-HT-sensitive ionic conductances are active in this range (Penington & Kelly, 1990). In 136 mM $[K^+]_o$ $I_{5\text{-HT}}$ increased in an essentially linear fashion (Fig. 2*B*) between −30 and −120 mV (in low $[Na^+]_o$); the limiting chord conductance was ~ 7 nS. The linear section of the *I–V* plot was therefore extrapolated to obtain a reversal potential ($E_{5\text{-HT}}$) value of −8 mV, close to the predicted Nernst value of −3·3 mV. In some cell types internal Mg^{2+} causes mild inward rectification (Vandenberg, 1987; Matsuda, Matsuura & Noma, 1989). A preliminary attempt to examine the role of Mg^{2+} was undertaken by replacing 2 mM Mg^{2+} in the patch pipette with Na^+. In three cells NaATP was used instead of MgATP; no change in the inward rectification was observed.

Figure 3*A* plots the measured reversal potentials in 5-HT as a function of $[K^+]_o$. The dashed line represents the expected Nernstian relationship between $[K^+]_o$ and the reversal potential. Values for the reversal potential are given in Table 1. A simplex algorithm was used to fit the data; this yielded an $[K^+]_i$ of 139 mM and the slope was −51·1 mV per tenfold change in $[K^+]_o$. The E_{rev} for 5-HT was linearly related to the log of the $[K^+]_o$ with a slope which was 12% lower than that predicted. The small discrepancy between measured and predicted values for reversal potential may indicate that the 5-HT-induced current is carried predominantly but not solely by K^+ or that other conductances can be activated by 5-HT. Interestingly, the results predict that the $[K^+]_i$ measured at a reversal potential of 0 mV was 139 mM. This value is 16 mM lower than the concentration in the pipette solution and probably reflects the true $[K^+]_i$.

Figures 1 and 2 show that the 5-HT induced current is much larger in high $[K^+]_o$. Figure 3*B* illustrates the relationship between the size of the 5-HT-induced

conductance at −90 mV and $[K^+]_o$. The log of the 5-HT-induced conductance is linearly related to the log of $[K^+]_o$. Thus to a close approximation, the 5-HT-induced conductance is directly related to the $[K^+]_o$ raised to the power of 0·35.

$I_{5\text{-HT}}$ was sensitive to blockade by Ba^{2+} which helps to identify the type of K^+ channel activated as an inward rectifier. Figure 4*A* shows a current–voltage

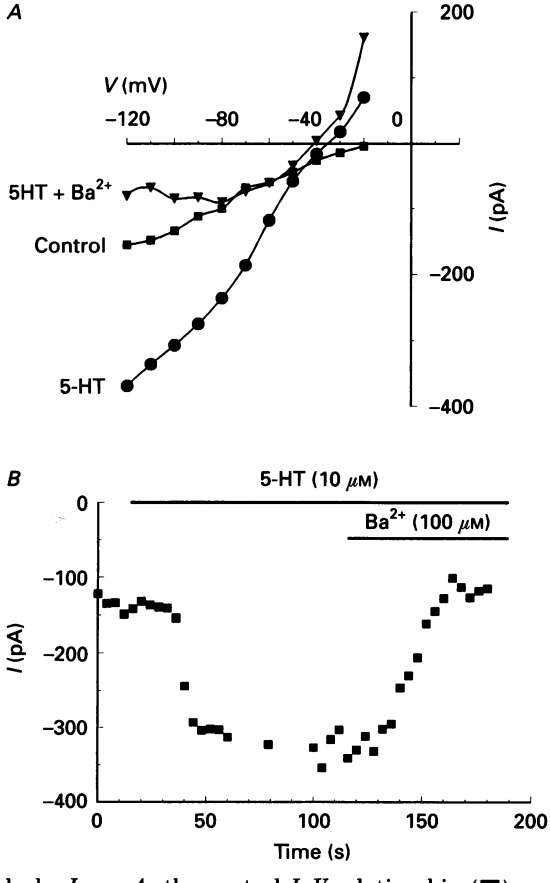

Fig. 4. Ba^{2+} blocks $I_{5\text{-HT}}$. *A*, the control *I–V* relationship (■) was quite linear between −120 and −20 mV. The steady-state *I–V* relationship in the presence of 5-HT (●) was inwardly rectifying down to −80 mV and then showed some saturation. Addition of 0·1 mM Ba^{2+} produced an *I–V* plot (▼) that was similar to the control *I–V* plot between −50 and −80 mV but was significantly smaller at potentials negative to −80 mV. All three *I–V* plots were measured with $[K^+]_o$ = 20 mM and V_h = −60 mV. *B*, graph of current as a function of time plots the K^+ current increase produced by the application of 5-HT (10 μM) and the subsequent blockade by Ba^{2+} (0·1 mM). Currents were measured at −90 mV; this cell is the same one as illustrated in *A*.

relationship in 20 mM $[K^+]_o$ where the membrane potential was stepped from −120 to −20 mV and the conductance in the absence of 5-HT appeared to be linear. Application of 5-HT clearly increased the inward current at potentials negative to −50 mV. However, currents evoked by hyperpolarizing steps in the range −80 to −120 mV showed some saturation. Application of 100 μM Ba^{2+} blocked the effect of

5-HT ($n = 5$) so that in the voltage range -80 to -50 mV the membrane current in 5-HT and Ba^{2+} was identical to control. At all other potentials, where it was measured, Ba^{2+} reduced the membrane current further than the control level and the membrane current showed a negative slope conductance region below -80 mV in all

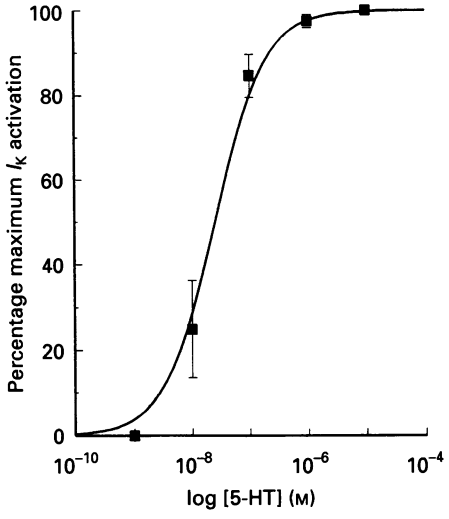

Fig. 5. 5-HT dose–response relationship indicates a high affinity for the receptor. Each data point is an average from five cells and represents the amount of inward current elicited at -90 mV by different concentrations of 5-HT. The data were fitted by eye with the binding curve

$$R = R_{max}/((1+ED_{50})/[5\text{-HT}]^n),$$

where n is the number of 5-HT molecules required to bind to the receptor for activation ($n = 1$). The ED_{50} was 30 nM.

the cells tested ($n = 4$ for $[K^+]_o = 20$ mM). In five cells 5-HT elicited $-90{\cdot}9 \pm 39{\cdot}2$ pA of inward K^+ current at -60 mV in 20 mM K^+, but in the presence of 100 μM Ba^{2+} $-14{\cdot}4 \pm 9{\cdot}0$ pA was observed. Figure 4*B* shows a plot of membrane current at -90 mV plotted against time. 5-HT activated -188 pA of inward K^+ current. Addition of 100 μM Ba^{2+} blocked 212 pA of inward 5-HT-induced current.

The main pharmacological characteristics of the 5-HT_{1A} receptor subtype are the high affinity for 5-HT, antagonism by spiperone and activation by low doses of 8-OHDPAT (Gozlan, El Mestikawy, Pichat, Glowinsky & Hamon, 1983). A dose–response curve to 5-HT was constructed by assigning the maximum 5-HT-induced current (always elicited by 10 μM 5-HT) the value 100%. The size of the response was then measured using concentrations of 1, 10, 100 nM and 1 μM 5-HT. The averaged data from five experiments are illustrated in Fig. 5. 5-HT (1 μM) produced a near maximal response ($97{\cdot}4 \pm 1{\cdot}6$%) and the data were fitted with the binding curve:

$$R = R_{max}/((1+ED_{50})/[5\text{-HT}]^n).$$

The ED_{50}, which in this case was 30 nM, is the concentration required to evoke half the maximum response and n is the number of molecules of 5-HT which have to bind to the receptor to cause activation. In this case n was assumed to be one.

Figure 6*A–D* shows how both the increased inward current and conductance evoked by 5-HT were readily antagonized by 1 μM spiperone. Figure 6*A* shows the control current in response to a voltage step from −60 to −90 mV. The centre panel illustrates the inward current induced by 5-HT and the right panels show that when

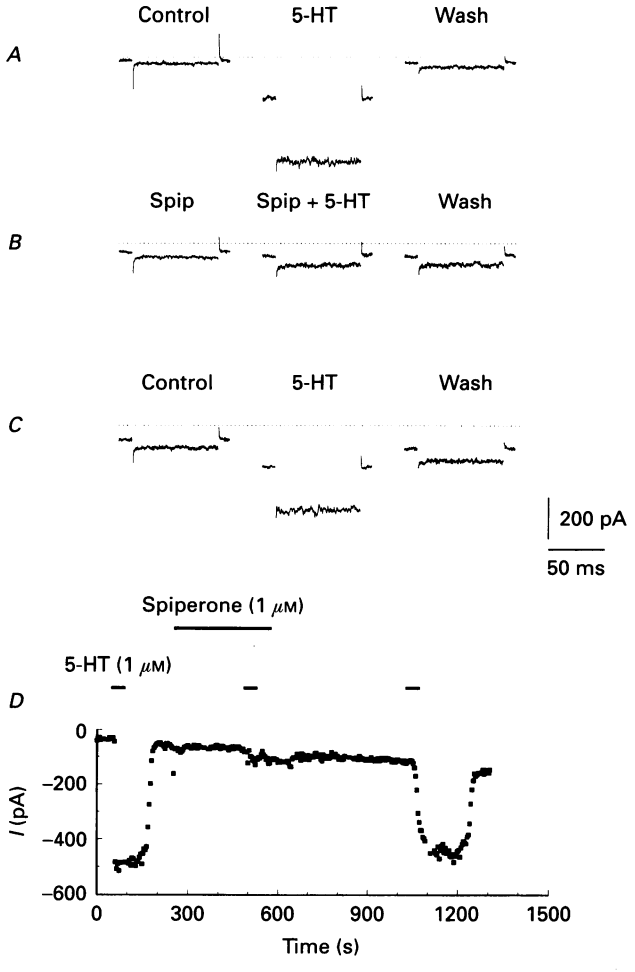

Fig. 6. The current produced by 5-HT was antagonized by the 5-HT_{1A} receptor antagonist, spiperone. *A* shows a typical response to 5-HT in 20 mM $[K^+]_o$, produced by a 100 ms hyperpolarization to −90 mV from V_h = −60 mV. *B* shows that 5-HT has little effect after the cell was exposed to spiperone (1 μM) for 5 min. *C*, the response to 5-HT could be recovered after spiperone was washed from the bath. *D*, currents produced by a 100 ms hyperpolarization to −90 mV were measured at the end of the step. Graph plots steady-state current as a function of time. 5-HT and spiperone were applied as indicated.

5-HT was washed from the bath near complete recovery followed. Panel *B* shows the large reduction in the size of the 5-HT-induced inward current 5 min after spiperone was added to the bath. When spiperone was washed out of the bath the response to 5-HT recovered as illustrated in Fig. 6*C*. The current measured at −90 mV is plotted against time in Fig. 6*D*. This shows that spiperone could antagonize the response to

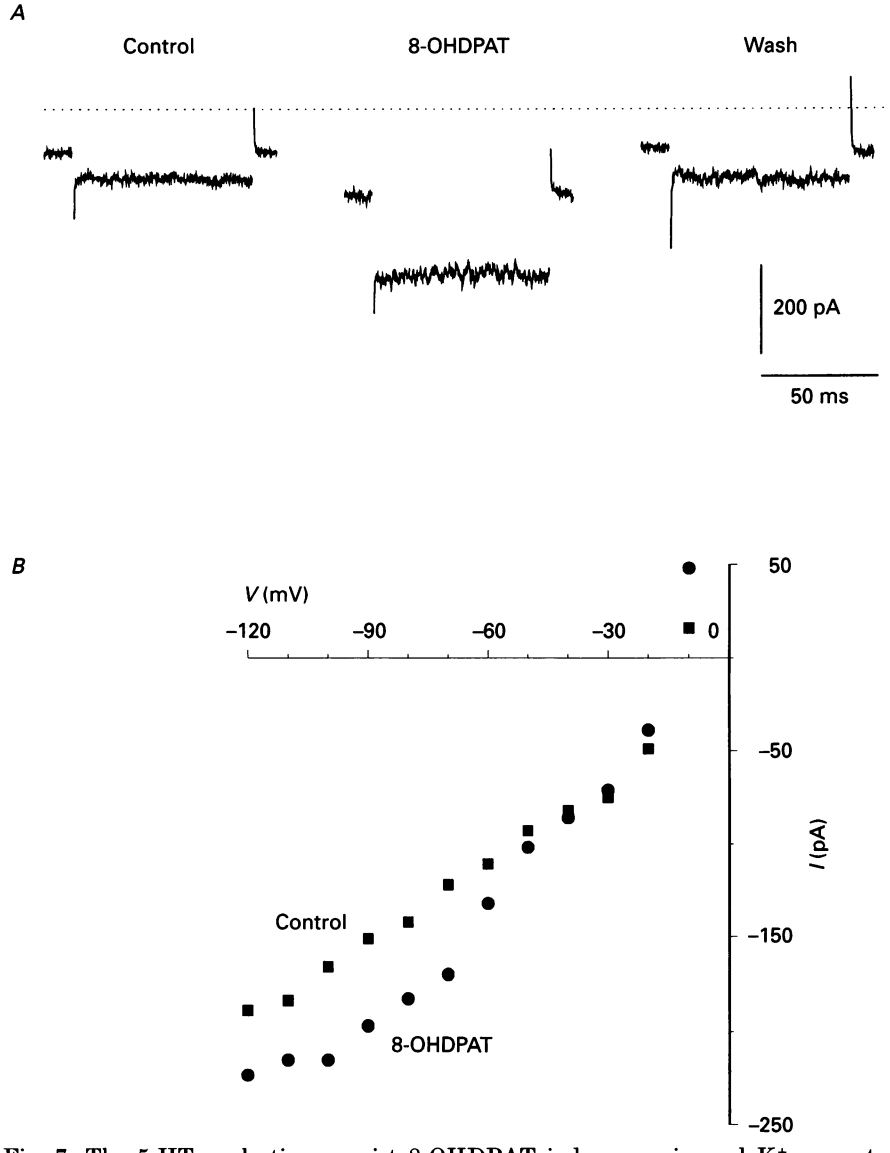

Fig. 7. The 5-HT_{1A}-selective agonist 8-OHDPAT induces an inward K^+ current with characteristics identical to those produced by 5-HT. *A*, current records obtained by holding the membrane potential at −60 mV and stepping the membrane to −90 mV. 8-OHDPAT increased the holding current at −60 mV, as well as the current during the hyperpolarizing step to −90 mV. *B*, *I–V* relationship for this cell in control conditions (■) and during 8-OHDPAT (●). In 20 mM $[K^+]_o$ the reversal potential was close to that expected of a current carried by K^+ (equilibrium potential, −40 mV).

5-HT. The effects of spiperone were reversible. In four cells (20 mM $[K^+]_o$) the 5-HT-induced current averaged 259·8 ± 82·0 pA at −90 mV and was reversibly reduced by 77% to 57·5 ± 23·0 pA by the application of 100 nM spiperone.

Figure 7*A* shows that 100 nM 8-OHDPAT mimicked the action of 5-HT by evoking a reversible inward current and increase in conductance. Figure 7*B* shows a

current–voltage relationship before (■) and after 8-OHDPAT (●) to illustrate the voltage dependence of the 5-HT_{1A}-selective agonist. This compound elicited an inward current below −40 mV which was similar to that evoked by 5-HT. At 100 nM, 8-OHDPAT increased the K^+ conductance in seven cells (in 20 mM $[K^+]_o$)

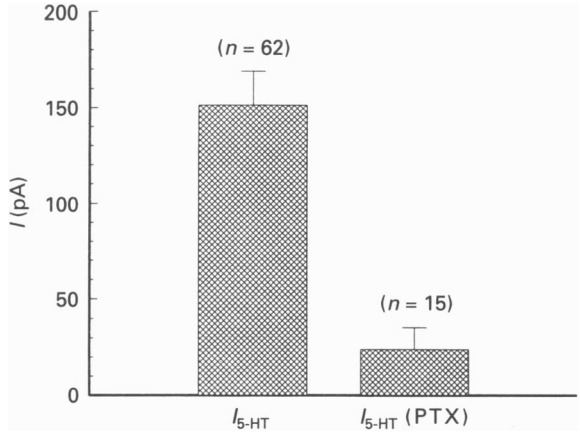

Fig. 8. The K^+ current produced by the application of 5-HT current was dramatically reduced by pertussis toxin (PTX) treatment. Slices were incubated in 1 μg/ml PTX for 12–16 h. Cells were then dissociated by trituration. In sixty-two cells not treated with PTX, 5-HT (1 μM) produced on average 151 pA of inward current (at −90 mV in 20 mM $[K^+]_o$). In a group of fifteen cells pretreated with PTX, 5-HT elicited only 24 pA of inward current, indicating that a PTX-sensitive G-protein is involved in the response.

and caused a reversible inward current of 89·8 ± 27·0 pA ($n = 6$) at −90 mV. In one cell 50 nM 8-OHDPAT caused an inward current of 103·4 pA and in the same cell 25 nM had no effect. In two cells 10 μM 8-OHDPAT failed to elicit an inward current in a cell which was responsive to 5-HT but instead strongly decreased the holding current and conductance of the cell, suggesting that high concentrations of this drug effectively block K^+ channels.

Coupling of the 5-HT_{1A} receptor to the K^+ channel

It is quite well established that several neurotransmitters including 5-HT interact with ion channels by means of a GTP-binding protein (Andrade, Malenka & Nicoll, 1986; Gilman, 1987; Ewald, Sternweis & Miller, 1988; Innis, Nestler & Aghajanian, 1988; Williams *et al.* 1988). In previous experiments using DR neurones, the involvement of a G-protein in responses to 5-HT was established by injecting pertussis toxin (PTX) into the lateral ventricle 2–3 days before the experiment to inactivate the G-protein responsible. The effectiveness of this treatment may vary depending on the access of the toxin to the DR cells. In the present study PTX (1 μg/ml) was added *in vitro* to the isolated nucleus before cell isolation and incubated for 13 h at 35 °C. Figure 8 illustrates the effect that incubation with PTX had on inward K^+ current elicited by 5-HT. PTX largely prevented the action of 5-HT on DR neurones; PTX pretreatment reduced the effectiveness of 5-HT by a factor of 84%. In a group of sixty-two cells recorded in 20 mM $[K^+]_o$ forty-three cells

(69%) responded to 5-HT (1 μM), with an average K^+ current increase of $151{\cdot}3 \pm 17{\cdot}6$ pA (mean $\pm$ S.E.M.); after PTX only four of the experimental group of fifteen cells (27%) responded to 5-HT. After PTX the average inward current was $23{\cdot}8 \pm 11{\cdot}5$ pA. Cells treated in exactly the same way with the exception that the PTX was not included in the incubation responded to 5-HT normally with an average current of $140{\cdot}7 \pm 30{\cdot}9$ pA ($n = 11$) and 73% of the cells responded. The mean of the control group was statistically different from the PTX-treated group (t test, $P < 0{\cdot}01$). These results confirm that the 5-HT receptor-induced activation is coupled to the opening of K^+ channels by means of a PTX-sensitive G-protein.

Ligand-gated opening of K^+ channels and the effect of cAMP

It has been suggested that a reduction in the level of cAMP by neurotransmitters may be an important step in the mechanism of K^+ channel activation in neurones (Andrade & Aghajanian, 1985; but see Zidichouski, Kehoe, Wong & Smith, 1989). Selyanko, Smith & Zidichouski (1990) reported that the responses of K^+ channels to agonists were best recorded when cAMP was included in the patch pipette during recordings from frog sympathetic ganglia. 5-HT_{1A} receptor activation has been reported to decrease levels of cyclic adenosine monophosphate (cAMP) in several tissues (Dumis, Sebben & Bockaert, 1988). To determine whether cAMP was required for the response to 5-HT observed in DR neurones, cAMP (100 μM) was included in the patch pipette. With $[K^+]_o$ of 2·5 mM, 5-HT had no effect on the current–voltage relationship in three cells. When the $[K^+]_o$ was increased to 20 mM, 5-HT elicited the same response as that observed with no cAMP in the pipette. In all subsequent experiments cAMP was removed from the patch pipette and responses to 5-HT occurred as usual. In order to evaluate the response before and after cAMP was present, a recording of the K^+ channel opening effect of 5-HT was made in 20 mM $[K^+]_o$ and the current measured at -90 mV (Fig. 9*A*). After one response to 5-HT, the membrane-permeable cAMP derivative, 8-CPTcAMP, was added to the bath. The response to 5-HT was unaltered ($n = 3$). Addition of 8-CPTcAMP to one cell which was unresponsive to 5-HT in 20 mM $[K^+]_o$ failed to convert the cell to a responsive one.

Roeper, Hainsworth & Ashcroft (1990) have observed that the ATP-regulated K^+ channel blocker tolbutamide could reverse the membrane hyperpolarization induced by D_2 dopamine and $GABA_B$ agonists in substantia nigra neurones, suggesting that an ATP-regulated K^+ channel was activated by dopamine and GABA. This possibility was tested in DR neurones (Fig. 9*B*) by recording the inward K^+ current induced by 5-HT at -90 mV in 20 mM $[K^+]_o$ and then incubating the cells with 1 μM glibenclamide or tolbutamide for varying periods before re-applying 5-HT. In four cells the control response to 5-HT was $-145{\cdot}2 \pm 33$ pA and in the presence of 1 μM glibenclamide the response was -153 ± 19 pA, which is not significantly different from control. In two cells 100 μM tolbutamide did not affect the response to 5-HT. However, addition of glibenclamide or tolbutamide did produce a small reduction in the holding current at -60 mV. This presumably indicates the presence of a small number of ATP-sensitive K^+ channels which are not influenced by 5-HT.

The lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA, 10 μM), was found to completely block transmitter activation of K^+ channels in *Aplysia* and heart cells

(Piomelli *et al.* 1987; Kurachi, Ito, Sugimoto, Shimizu, Miki & Ui (1989). NDGA was tested in three DR neurones. In these cells 1 μM 5-HT activated an average inward current at -90 mV of -262 pA. After 5 min preincubation with NDGA, which had no effect by itself, the average 5-HT-induced current was 239·6 pA, a decrement of

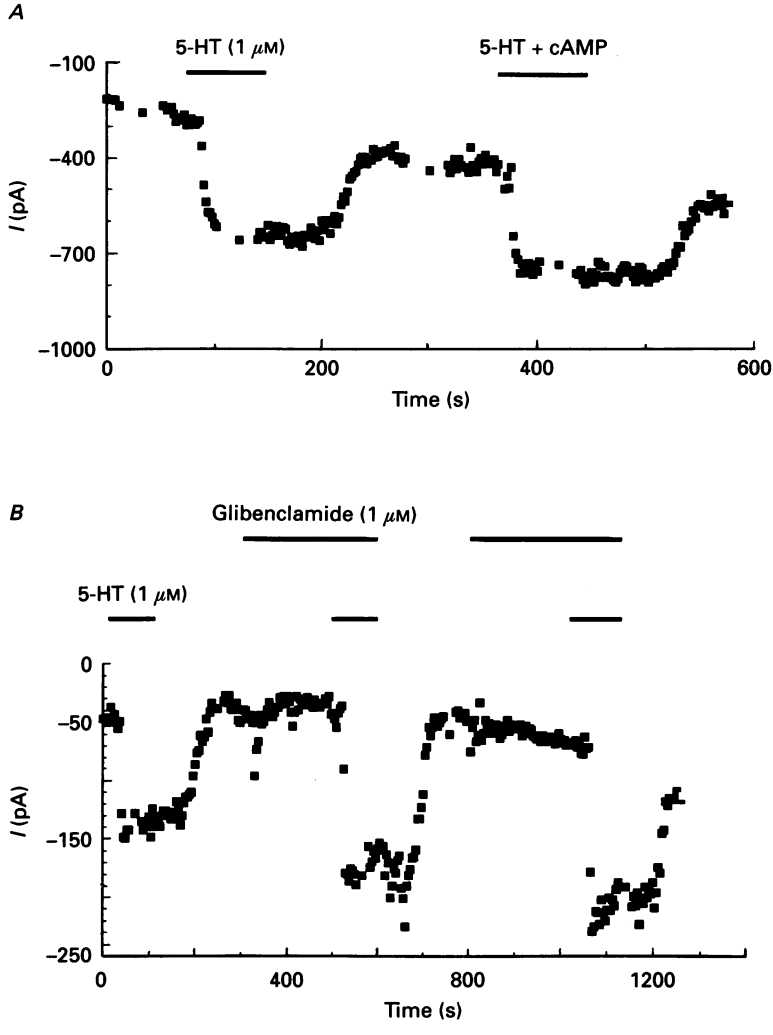

Fig. 9. Neither cAMP analogues nor blockers of ATP-gated K^+ channels (K^+_{ATP}) were able to alter the effect of 5-HT in DR neurones. *A*, hyperpolarizing steps to -90 mV were applied every 4 s from $V_h = -60$ mV, and membrane currents were recorded. 5-HT was applied as indicated. 8-CTPcAMP was added to the bath with the 5-HT during the second application. The response to 5-HT was virtually identical. *B*, glibenclamide (1 μM), a blocker of K^+_{ATP}, appeared to increase the size of the response to 5-HT in this cell but on average in four cells there was no change in the response to 5-HT. 5-HT and glibenclamide were added as indicated.

8%. This concentration of NDGA was 2–10 times that required to completely block transmitter activation of K^+ channels in *Aplysia* and heart cells.

DISCUSSION

In DR neurones the 5-HT-induced conductance was found to be proportional to the $[K^+]_o$ raised to the power of 0·35. In starfish eggs the inwardly rectifying K^+ channel conductance has been found to be proportional to $\sqrt{K_o^+}$ (Hagiwara, Ozawa & Sand, 1975) and Sakmann & Trube (1984) obtained a value of 0·62 in heart ventricular cells. Williams *et al.* (1988), who examined DR neurones in the slice preparation, found that the slope conductance activated by 5-HT_1 agonists doubled between −60 and −120 mV and increased upon raising the $[K^+]_o$.

In our study $E_{5\text{-HT}}$ changed by 51·1 mV instead of 58 mV per decade change in $[K^+]_o$, a value which was 12% less than expected. This finding suggests that the 5-HT induced current is carried predominantly but not exclusively by K^+. However, in high $[K^+]_o$ and low $[Na^+]_o$, $E_{5\text{-HT}}$ is close to the value predicted by the Nernst equation. If we assume that the contaminating ion is Na^+ then from the Nernst equation the selectivity ratio of the channel is approximately 30:1, suggesting some Na^+ permeability. These results are somewhat different from those obtained by Williams *et al.* (1988) which showed an almost perfect Nernstian relationship between 5-HT-induced current and E_K. However, our results are similar to those obtained in the heart where the reversal potential of the inwardly rectifying K^+ channel shifted by 49 mV per decade change in $[K^+]_o$ (Sakmann & Trube, 1984). Another possibility is that 5-HT activates a separate non-selective conductance, in DR neurones.

Another unexpected result was that the K^+ current evoked by 5-HT was only observed at $[K^+]_o$ of 6 mM and above in isolated cells. This finding raises the question of whether the response would be seen in the cells under physiological conditions? Williams *et al.* (1988) observed a clear effect of 5-HT in the slice preparation at $[K^+]_o$ ranging from 2·5–10 mM, as did Penington, Rainnie & Kelly (1987). Measurements of resting external $[K^+]_o$ using K^+-sensitive microelectrodes yielded values ranging from 2 to 5 mM in guinea-pig neocortex and cat spinal cord (Morris & Krnjevic, 1974) but Zeuthen, Hiam & Silver (1974) obtained a value of 3 mM $[K^+]_o$ in rat neocortex. The definitive answer to this question has not been resolved because the supporting glial cells may regulate $[K^+]_o$ locally. Another possibility is that acutely isolated neurones exhibit an altered response to $[K^+]_o$ or that dialysis with the whole-cell patch clamp somehow alters ion channel sensitivity. Recordings using the perforated patch technique (Levitan & Kramer, 1990) would help to rule out a cause residing with the isolated cell, as opposed to the dialysis resulting from the whole-cell technique.

The presence of two other types of inwardly rectifying channels was inferred in previous studies (Williams *et al.* 1988), both activated by hyperpolarization from rest; one is the slowly activating inward current called I_Q or I_H (Mayer & Westbrook, 1983) and the other a fast activating current similar to that seen in skeletal muscle (Katz, 1949; Constanti & Galvan, 1983). These two inwardly rectifying currents have not been observed in acutely isolated adult cells studied with the whole-cell patch

clamp. In the current study, time-dependent inwardly rectifying currents were not detected. This agrees with previous sharp electrode recordings in the DR slice preparation (Penington *et al.* 1987) but is in contrast to the work of Williams *et al.* 1988. The only inward rectification observed in this preparation occurred in the current that was activated by 5-HT, observed at the value of E_K with linear *I–V* relations negative to E_K.

In slice preparations of the DR nucleus high micromolar concentrations of 5-HT were required to produce a maximal K^+ current response (Williams *et al.* 1988). In the slice mechanisms responsible for the destruction or uptake of 5-HT normally keep the level of 5-HT near the cell bodies low. These mechanisms are greatly reduced or absent in acutely isolated cells perfused externally at a fast rate. Thus the dose–response curve, in acutely isolated neurones relates the magnitude of the K^+ current response to the concentration of 5-HT near the cell surface, giving an ED_{50} value of 30 nM may be the first accurate functional determination of 5-HT_{1A} receptor affinity using 5-HT as the agonist. The high sensitivity of the receptor to 5-HT, the block by spiperone (a 5-HT_{1A} antagonist) and sensitivity to 8-OHDPAT (a 5-HT_{1A}-selective agonist) all confirm that the receptor is of the 5-HT_{1A} subtype. Furthermore, the finding that pertussis toxin acting intracellularly on a G-protein subunit helps to confirm that the response is the same one that has been studied in the slice preparation by others (Lakoski & Aghajanian, 1984; Innis *et al.* 1988; Williams *et al.* 1988).

Inwardly rectifying K^+ currents may be activated by a direct interaction with G-proteins, although the exact mechanism of K^+ channel activation is not yet resolved. Two groups have reported that the lipoxygenase products of arachidonic acid (AA) metabolism in cardiac myocytes are capable of activating the pertussis toxin-sensitive G-protein-gated muscarinic K^+ channel (Kurachi *et al.* 1989; Kim, Lewis, Graziadei, Neer, Bar-Sagi & Clapham, 1989) possibly mediated by the $\beta\gamma$-subunit of a G-protein. This effect was prevented by the lipoxygenase inhibitor, NDGA. In *Aplysia*, lipoxygenase metabolites act as second messengers to activate S-type K^+ channels, a situation somewhat different from that found in mammalian neurones (Piomelli *et al.* 1987). Recently Koyano, Grigg, Nakajima & Nakajima (1990) investigated the role of AA metabolites on the cultured locus coeruleus neuronal response to somatostatin and substance-P. The activation of K^+ channels by somatostatin and inhibition by substance-P was reduced by a range of lipoxygenase inhibitors including NDGA, but 5′-lipoxygenase metabolites enhanced the response to somatostatin. The authors concluded that the 5′-lipoxygenase products were not directly involved in the responses but may act to maintain or enhance the function of the K^+ channel. The activation of K^+ channels by 5-HT in DR neurones is not reduced by NDGA which suggests that AA metabolites are not involved in this response. In addition, the state of the channels also did not appear to be altered by inclusion of cAMP in the patch pipette or by the addition of membrane-permeable cAMP analogues prior to and during the observation of K^+ channel activation by 5-HT. Thus cAMP did not seem to be involved in the response to 5-HT.

In the absence of 5-HT only a small component of membrane current was blocked by 0·1 mM Ba^{2+}. All the 5-HT-induced K^+ current was completely blocked by 0·1 mM Ba^{2+}, which helps to identify this as an inwardly rectifying K^+ channel similar to the

observations of Williams *et al.* (1988). The K^+ currents activated by 5-HT do not appear to be of the ATP-regulated K^+ channel family as the response to 5-HT was not reduced by tolbutamide or glibenclamide (ATP-regulated K^+ channel blockers). In conclusion $I_{5\text{-HT}}$ is carried by channels which are probably not regulated by ATP, and their mild inward rectification may not stem exclusively from the presence of internal Mg^{2+}, although further work is required to define the role of Mg^{2+}. The accompanying paper (Penington *et al.* 1993) examines the unitary properties of the K^+-selective channels activated by 5-HT.

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