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IONIC MECHANISMS MEDIATING 5-HYDROXYTRYPTAMINE- AND NORADRENALINE-EVOKED DEPOLARIZATION OF ADULT RAT FACIAL MOTONEURONES

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SUMMARY

1. The actions of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on the membrane properties of facial motoneurones in slices from the adult rat brainstem *in vitro* were examined using intracellular recording techniques.

2. In voltage clamp recording, hyperpolarizing voltage steps (> 20 mV), from holding potentials at or close to the resting potential, induced a slowly activating, voltage-dependent inward current possessing properties similar to the hyperpolarization-activated current (I_h) seen in other cell types. From tail current analysis two groups of facial motoneurones can be distinguished in terms of the activation range for I_h, one with a half-maximal activation at −81 mV and the other at −94 mV but with similar shapes.

3. 5-HT (120/126) and NA (21/21) depolarized facial motoneurones. The reversal potentials (E_m) obtained from peak voltage amplitude *I–V* plots in varying extracellular potassium concentrations suggested mechanisms involving a decrease in K^+ conductance.

4. Under voltage clamp, close to the resting potential, both 5-HT (39/41) and NA (13/13) evoked inward currents.

5. *I–V* plots and plots of 5-HT-sensitive current at different membrane potentials, obtained from currents evoked by voltage steps and measured before the development of I_h (instantaneous current), indicated that the 5-HT-evoked inward current was predominately associated with a decrease in conductance but with a range of reversal potentials for 5-HT ($E_{5\text{-HT}}$) from close to, to much more negative than the reversal potential for a potassium conductance (E_K). In some cases no change or increases in instantaneous conductance were observed.

6. Steady-state *I–V* relationships and plots of 5-HT-sensitive current, measured after development of I_h, indicated a 5-HT-associated conductance increase with a time and voltage dependence close to that of I_h, which could be abolished by extracellular caesium (2–5 mM).

7. The NA-evoked inward current was always associated with a decrease in

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conductance. Instantaneous and steady-state *I–V* relationships as well as plots of NA-sensitive current indicated a reversal potential at E_K.

8. The activation curve for I_h was shifted to more positive potentials in the presence of 5-HT. The time constant for activation of I_h showed a similar shift.

9. 5-Carboxamidotryptamine (5-CT), a 5-HT receptor agonist, was selective for the enhancement of I_h and only evoked an inward current when the holding potential was within the activation range of I_h.

10. It is concluded that 5-HT depolarizes facial motoneurones through a combination of mechanisms. One involves a decrease in a K^+ conductance active at all membrane potentials, the other an enhancement of a hyperpolarization-activated current, I_h. NA-evoked depolarization appears to involve only K^+ channel closure.

INTRODUCTION

Adult rat facial motoneurones *in vitro* have been shown to possess a characteristic electrophysiological profile (Larkman, Penington & Kelly, 1989). Membrane voltage responses are dominated by voltage-dependent 'sag' indicative of inward 'anomalous' rectification which has also been described in adult spinal motoneurones *in vivo* (Ito & Oshima, 1965; Barrett, Barrett & Crill, 1980) and neonatal spinal motoneurones *in vitro* (Takahashi, 1990). In facial motoneurones it is not clear whether this inward rectification derives from the potassium-mediated I_{IR} type (Hagiwara, Miyazaki & Rosenthal, 1976; Hestrin, 1981; Constanti & Galvan, 1983) or the mixed cation form, I_h (Yanagihara & Irisawa, 1980; DiFrancesco, 1981; Halliwell & Adams, 1982; Mayer & Westbrook, 1983) an issue which we have addressed at the start of this study.

Excitatory actions of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on central neurones have been reported to occur either alone or in combination with inhibitory actions in a biphasic manner (for reviews see Anwyl, 1990; Bobker & Williams, 1990). Recent reports have demonstrated that both forms of inward rectifier can be targets for neurotransmitter action in central neurones (North & Uchimura, 1989; McCormick & Pape, 1990*b*; Takahashi & Berger, 1990; Yamaguchi, Nakajima, Nakajima & Stanfield, 1990). Adult rat facial motoneurones in common with spinal motoneurones receive a dense 5-HT and NA innervation (McCall & Aghajanian, 1979; Steinbusch, 1981) though the source of this projection has not been characterized. Facial motoneurones *in vivo* (VanderMaelen & Aghajanian, 1980) and *in vitro* (Aghajanian & Rasmussen, 1989; Larkman *et al.* 1989) are depolarized by exogenously applied 5-HT through a mechanism assumed to involve K^+ channel closure. Given the prominent expression of inward rectification by facial motoneurones we have further investigated this action of 5-HT and compared it with the depolarizing action of NA on the same motoneurones. We report that the mechanism by which 5-HT, but apparently not NA, evokes a depolarization, involves more than just a simple K^+-mediated mechanism. Some of this work has been presented in abstract form (Larkman & Kelly, 1990, 1991*a*).

METHODS

Brainstem slices were prepared from adult Cob Wistar rats (160–250 g) as described previously (Larkman *et al.* 1989). The animals were decapitated with a small animal guillotine and the

brainstem with attached cerebellum was quickly removed and placed in artificial cerebrospinal fluid (ACSF) at 4 °C. A block of tissue containing the facial nucleus was prepared and slices (350 μm thick) were cut using a vibroslice (Camden Inst.). Slices were transferred to an interface-type chamber superfused at 0·5–1 ml/min with ACSF and incubated for at least 1 h before intracellular recording was commenced. The ACSF was of composition (mM): NaCl, 124; KCl, 5; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; HEPES, 1·25; D-glucose, 10 and pH was 7·4 after equilibration with a 95% oxygen–5% carbon dioxide gas mixture. When the external KCl concentration was changed, equimolar substitution with NaCl was performed.

In early experiments, to provide protection against anoxia, hydrogen peroxide (0·003% w/v) was added to the ACSF (Walton & Fulton, 1983) but this was later abandoned without prejudicing motoneurone viability. In later experiments we utilized the method of Aghajanian & Rasmussen (1989). The slice preparation and initial incubation was carried out using a modified ACSF in which the NaCl was replaced with equimolar sucrose (252 mM). Slices were maintained at 37 °C for current-clamp studies and 32 °C for voltage-clamp experiments. Some voltage-clamp studies were performed at 25 and 37 °C as indicated in the text. Results were qualitatively similar at each experimental temperature.

Recording microelectrodes were made from thin wall fibre, glass capillaries (1·2 mm o.d. Clark Electromedicals) using a Flaming-Brown horizontal puller (P80/PC, Sutter Instruments) and filled with 3 M KCl. DC resistances of the electrodes ranged between 10–50 MΩ. A single-electrode voltage-clamp amplifier (Axoclamp 2A) was used for both current- and voltage-clamp recording. During current-clamp recordings bridge balance was monitored continuously on a digital oscilloscope (Gould 1425) and capacitance was fully compensated. During voltage-clamp recordings head-stage output was continuously monitored to ensure adequate settling time. Sampling frequencies were routinely in the range 8–10 kHz and amplifier gain was 3–8 nA/mV. Output bandwidth was 1 kHz. Membrane potential and currents were recorded and stored on videotape using an analog interfaced digital audio processor (Sony PCM 701ES)–video cassette recorder (Sony SLF 30) system. In later experiments this system was replaced by a modified digital audio tape (DAT) system (Fentronix, Cambridge. UK). Data analysis was performed using a CED502 interfaced to a PDP 11 computer (Cambridge Electronic Design, Cambridge, UK) incorporating a range of 'in-house' written subroutines. Selection criteria required stable membrane potentials more negative than −55 mV, evoked action potentials of brief duration which overshot 0 mV and membrane voltage responses which displayed smooth charging curves and voltage-dependent 'sag'. Input resistance was determined from the slope of current–voltage (*I–V*) relationships constructed from averaged (four to six individual records) membrane voltage responses to injected current pulses measured at the peak of the voltage deflection. Activation and deactivation kinetics of I_h were determined using a CED software package and Boltzmann fits to activation curves were obtained using an 'in-house' written program operating on an IBM computer.

When the $[K^+]_o$ was altered, control 5-HT or NA responses were first obtained in 5 mM $[K^+]_o$ then further responses were obtained after the $[K^+]_o$ had been changed at least once to either 2·5, 6·25, 10 or 15 mM. Drugs and solutions of altered ionic composition were applied via a perfusion system incorporating 3-way stopcocks. CsCl (2–5 mM) and tetrodotoxin (TTX, 1 μM) were added to the perfusing ACSF. Drugs used were 5-carboxamidotryptamine (5-CT, Glaxo, Ware, UK), *m*-chlorophenylpiperazine (*m*-CPP, Sandoz, Basel, Switzerland), noradrenaline-HCl, 5-hydroxytryptamine creatinine sulphate and TTX (all Sigma, UK). Final concentrations were prepared immediately prior to use.

RESULTS

The results are based on intracellular recordings from 201 current-clamped and 65 voltage-clamped facial motoneurones. The mean values for resting potential and input resistance of a representative sample at 37 °C were $-70·6 \pm 6·2$ mV (mean ± S.D., $n = 201$; range, −56 to −88 mV) and $10·0 \pm 3·9$ MΩ ($n = 188$, range 3·1 to 25·7 MΩ), respectively.

Facial motoneurones exhibit I_h

Voltage responses from facial motoneurones evoked by hyperpolarizing current steps displayed a time-dependent depolarizing 'sag' (Larkman *et al.* 1989). When

facial motoneurones were voltage clamped at or near to their resting potential ($V_h = -63 \pm 4{\cdot}6$ mV, $n = 51$ at 32 °C), step hyperpolarizing voltage commands elicited an initial current peak, referred to as the instantaneous current, followed by the development of a slow inward current reaching a steady-state level at the end of

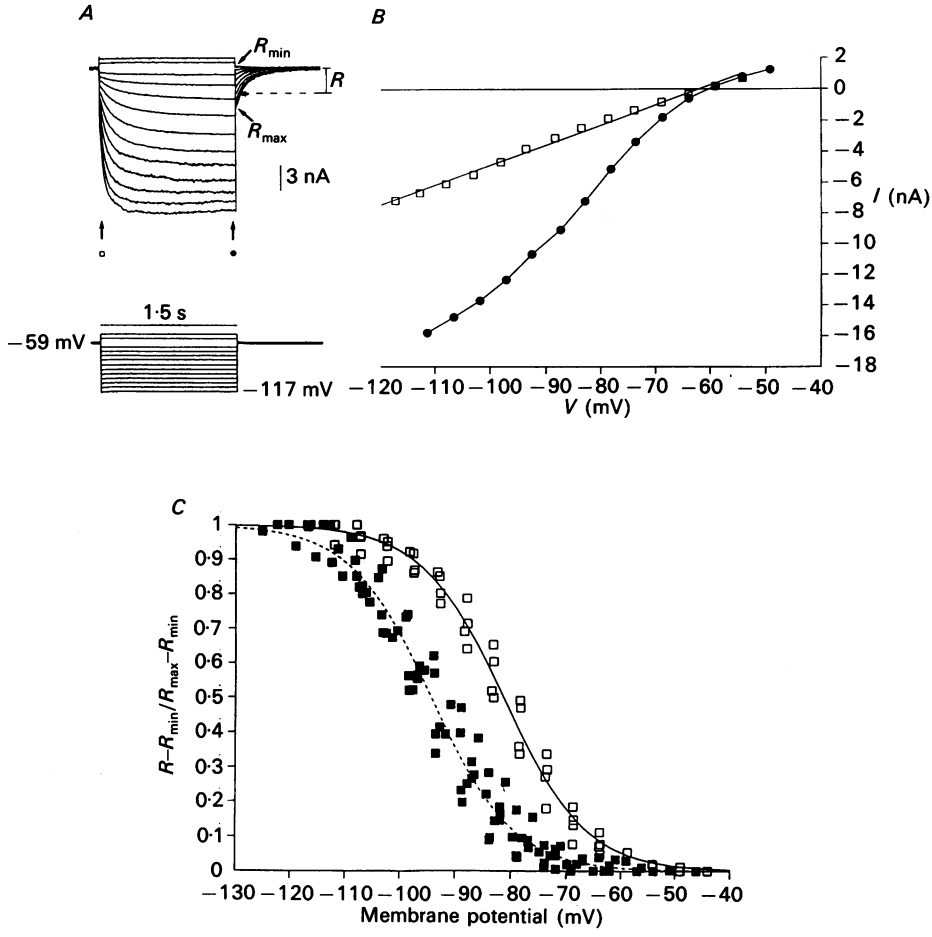

Fig. 1. Step currents evoking I_h in a motoneurone voltage clamped at −59 mV. *A*, voltage steps (1·5 s duration) elicit a slow voltage-dependent inward current which is followed by an inward relaxation tail current of amplitude, *R*. R_{min} and R_{max} indicate the threshold and maximum activation levels of the conductance and are used to construct I_h activation curves (see text for details). *B*, *I–V* relationship for instantaneous (□) and steady-state (●) current as indicated in *A*. Instantaneous current is linear, giving a slope conductance of 128 nS, while the steady-state plot shows inward rectification reaching a maximum conductive state around −112 mV. *C*, activation curves for I_h from thirteen different motoneurones indicate two cell populations with different activation ranges. Conductance at each potential was normalized according to eqn (1) and lines fitted using eqn (2). See text for details.

the voltage command (Fig. 1*A*). The amplitude of the inward relaxation increased with increasing hyperpolarization. Termination of the step voltage command resulted in an inward relaxing tail current. The linear ranges of instantaneous *I–V*

plots gave a mean value for input conductance of 88·9 ± 21·8 nS ($n = 49$). Plots of steady-state current displayed inward rectification throughout the range −60 to −115 mV (Fig. 1*B*). This time- and voltage-dependent inwardly rectifying current possessed properties similar to those characteristic of I_h described in many other cell types and will thus be referred to as I_h.

The voltage dependence of I_h was examined using conventional tail current analysis. An activation curve can be derived from the current relaxations evoked on stepping back to V_h after full activation of the conductance at varying membrane potentials. Normalization of the conductance according to the equation

$$R - R_{min}/R_{max} - R_{min}, \tag{1}$$

where R is the relaxation amplitude, R_{min} is the maximum amplitude relaxation in response to stepping from depolarized potentials and R_{max} is the maximum amplitude in response to stepping from hyperpolarized potentials (Fig. 1*A*), gave sigmoidal activation curves fitted by a Boltzmann equation of the form

$$1/1 + \exp[(V_h - V_{0.5})/k], \tag{2}$$

where V_h is the holding potential, $V_{0.5}$ the potential for half-maximal activation and k is a slope constant. This form of analysis in thirteen facial motoneurones produced evidence for two distinct activation ranges (Fig. 1*C*). The differences were judged to be significant according to a Mann–Whitney rank test ($P < 0.01$) and a Student's t test ($P < 0.001$). The two sets of data are fitted by eqn (2) with values for $V_{0.5}$ of −81·4 mV ($n = 4$) and −94·4 mV ($n = 9$) and k values of 7·5 and 7·4 respectively. Subsequent examination suggested related differences in resting potential and input conductance. The motoneurones with the more negative activation range had a mean resting potential of −67·6 ± 2·6 mV and an input conductance of 75·6 ± 15·1 nS compared to −59·8 ± 2·8 mV and 105·4 ± 6·3 nS for the other group. The suggestion from these data is that I_h may be active at the resting potential of only a proportion of facial motoneurones.

The actions of 5-HT and NA on facial motoneurones

As reported previously (VanderMaelen & Aghajanian, 1980; Larkman *et al.* 1989), addition of 5-HT (50–200 μM) or NA (10–100 μM) to the superfusing ACSF evoked a slow depolarization (amplitude range 1–16 mV) of facial motoneurones (120/126 and 21/21 for 5-HT and NA, respectively). Both depolarizations were accompanied by an increase in input resistance. *I–V* plots obtained in the presence and absence of either transmitter indicated reversal potentials ($E_{5\text{-HT}}$ and E_{NA}) of −94·2 ± 12·4 mV ($n = 70$) and −85 ± 9 mV ($n = 21$) in an extracellular K^+ concentration ($[K^+]_o$) of 5 mM, for 5-HT and NA respectively. Altering the $[K^+]_o$ showed that between 5 and 15 mM the values for $E_{5\text{-HT}}$, obtained in current-clamp experiments, closely fitted the values predicted by the Nernst equation assuming a $[K^+]_i$ of 150 mM (dashed line, Fig. 2). Deviation from this relationship at lower $[K^+]_o$ will be discussed later. In 10 mM $[K^+]_o$ the E_{NA} moved to −72·0 ± 0·9 mV ($n = 2$), while deviation from predicted values in 2·5 mM K^+ was similar to that for 5-HT (not shown). However, when 5-HT and NA were applied separately to the same facial motoneurones in 5 mM $[K^+]_o$ the

reversal potentials were $-95{\cdot}4 \pm 10{\cdot}6$ and $-86{\cdot}9 \pm 4{\cdot}8$ mV, respectively ($n = 9$). These values are significantly different ($P = 0{\cdot}05$, paired t test). Thus while the current-clamp evidence suggests that both 5-HT and NA act to reduce a K^+ conductance, this difference in the reversal potentials could result from differences

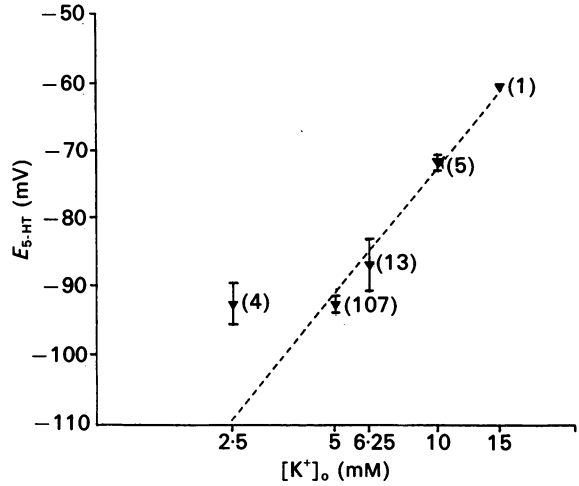

Fig. 2. 5-HT-evoked depolarization shows potassium dependence. The reversal potential for the depolarization ($E_{5\text{-HT}}$), taken from I–V data obtained in current-clamp experiments, plotted against the $\log_{10}[K^+]_o$. Vertical bars are S.E. of the mean for the number of observations in parentheses. The dashed line represents the predicted values obtained from the Nernst equation for a conductance solely mediated by K^+.

between their mechanisms of action or, alternatively, a differential localization of 5-HT and NA receptors.

5-HT, but not NA, enhances I_h

Under voltage clamp at or close to the resting potential, superfusion with 5-HT (50–200 μM) evoked a slowly developing inward current (39/41, amplitude range $-0{\cdot}1$ to $-1{\cdot}6$ nA, Fig. 3*A*). The inward current was unaffected by the presence of tetrodotoxin (1 μM) or cadmium (100 μM) suggesting a direct postsynaptic action independent of the external calcium concentration. When input conductance was monitored during the 5-HT application with a hyperpolarizing voltage step command of constant amplitude the inward current appeared to be associated with a conductance decrease ($n = 14$), a conductance increase ($n = 5$) or no change in conductance ($n = 4$).

Subsequent analysis of records suggests this is a consequence of the amplitude of test voltage step employed. Large amplitude hyperpolarizing commands evoked large amounts of I_h. In Fig. 3*A* closer inspection shows that while overall the conductance appears to decrease a small amount, the contribution of I_h to the total current actually increases in amplitude during 5-HT application (compare expanded responses). In contrast superfusion of facial motoneurones with NA (50–100 μM)

evoked a slow inward current (−0·1 to −0·6 nA) which was always associated with a decrease in conductance (13/13) irrespective of the amplitude of the test pulse (Fig. 3*B*).

Analysis of step currents evoked in the absence and presence of 5-HT allowed the two aspects of its action to be discerned (Fig. 4*A*). *I–V* relationships obtained by

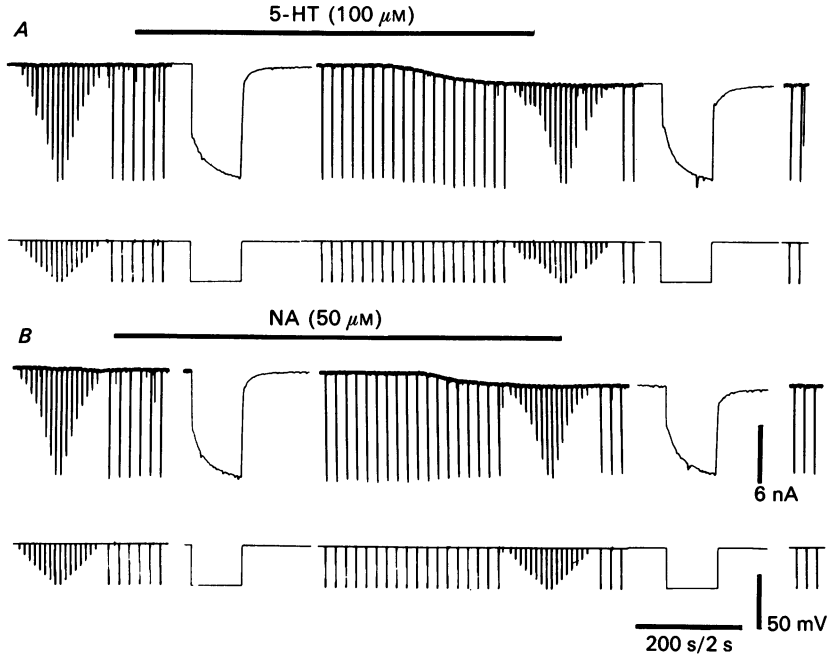

Fig. 3. 5-HT and NA evoke inward currents in a facial motoneurone voltage-clamped close to the resting potential. *A*, slow inward current (*I*) evoked by 5-HT (100 μM) at a V_h (*V*) of −60 mV at 25 °C. Conductance, tested with hyperpolarizing voltage steps −40 mV in amplitude, 1 s duration and at a frequency of 0·05 Hz, appears to decrease in 5-HT though the expanded records show the amplitude of I_h to increase. Families of step currents were generated before, during and after (not shown) the 5-HT-evoked inward current for *I–V* analysis. *B*, NA (50 μM) evokes a similar inward current in the same motoneurone clamped at −60 mV. Conductance can clearly be seen to decrease while I_h remains unchanged in the presence of NA. Dead space in the perfusion system accounts for a delay of about 3 min prior to drug entering the recording chamber. The horizontal scale bar values are 200 s for the unexpanded records and 2 s for the expanded records, in *A* and *B*.

plotting instantaneous current showed that the inward current was associated with an obvious decrease in slope conductance in 17/26 cases (32 °C) (Fig. 4*B*). A point of intersection obtained either by direct observation ($n = 7$) or by extrapolation of linear ranges ($n = 10$) gave a mean value for the $E_{5\text{-HT}}$ of $-90{\cdot}7 \pm 15{\cdot}0$ mV (range −76 to −128 mV). The remaining motoneurones showed either very small decreases in instantaneous conductance ($E_{5\text{-HT}} < -170$ mV, $n = 4$) or parallel or even positively converging *I–V* relationships ($n = 5$). Results obtained at 25 °C ($n = 9$) and 37 °C ($n = 5$) showed the same variation.

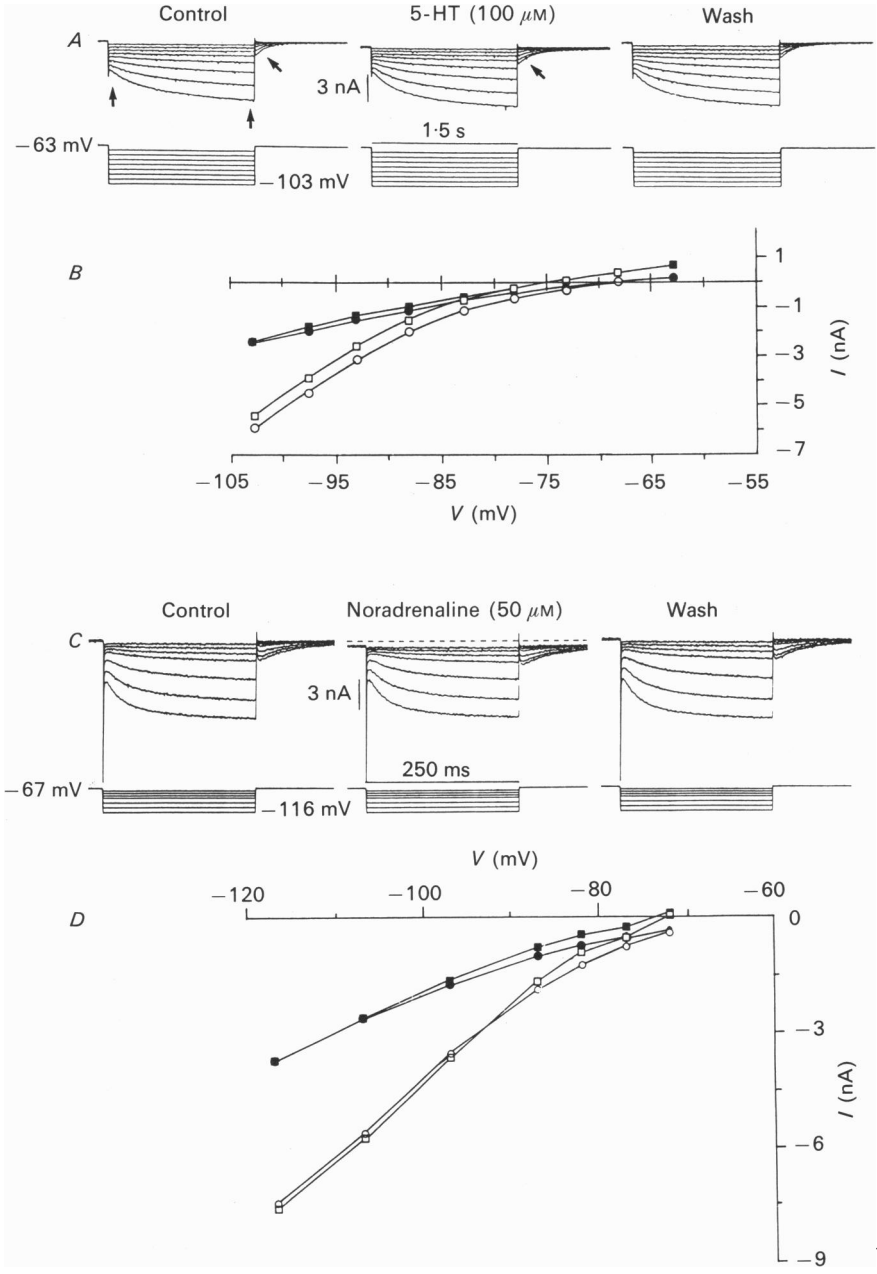

Fig. 4. 5-HT decreases instantaneous conductance but increases steady-state conductance while NA decreases both. *A* membrane currents displaying I_h (upper records) evoked by voltage steps (lower records) obtained before, during and after 5-HT application. Note the increased amplitude of inward relaxing tail currents in the presence of 5-HT (arrows). *B*, instantaneous (filled symbols) and steady-state (open symbols) *I–V* plots obtained in control (squares) and 5-HT (circles) from the records in *A*. 5-HT decreases the slope of the instantaneous *I–V* plot giving a point of intersection at −102 mV. In this motoneurone 5-HT evokes a parallel shift in the steady-state *I–V* relationship to more positive potentials. *C*, membrane currents (upper records) evoked by voltage steps (lower records) before, during and after NA application. *D*, instantaneous (filled symbols) and steady-

When plotted at the steady state after development of I_h, *I–V* relationships were usually non-intersecting in the tested range even when instantaneous relationships did intersect (Fig. 4*B*). Steady-state relationships were parallel or even showed divergence as hyperpolarization increased ($n = 40$). Tail currents at the offset of hyperpolarizing step voltage commands, which reflect I_h deactivation, are increased in amplitude in the presence of 5-HT (Fig. 4*A*, arrows). An explanation of this will be given later in the text.

For NA-evoked inward currents, *I–V* plots showed that in all cases both instantaneous and steady-state conductance decreased (Fig. 4*C* and *D*) giving points of intersection around the same potential ($-83{\cdot}3 \pm 7{\cdot}8$ mV, $n = 9$, 32 °C). No change in amplitude of tail currents was observed (Fig. 4*C*).

The current evoked by the 5-HT at different membrane potentials (5-HT-sensitive current) can be obtained by subtracting step currents evoked in the absence of 5-HT from those generated in its presence. The subtracted records indicate an instantaneous conductance decrease upon which is superimposed a time- and voltage-dependent conductance increase (Fig. 5*A*, inset). The time dependence of this conductance change closely reflects the time dependence of I_h at each potential. When plotted against voltage, at a time point equivalent to the instantaneous total current, the 5-HT-sensitive current was linear. A slope reflecting a conductance decrease was obtained on 16/25 occasions giving a mean reversal potential of $-92{\cdot}8 \pm 13{\cdot}9$ mV (Fig. 5*A*). No change or an increase in instantaneous conductance was seen on nine occasions. When measured at a time point equivalent to steady-state activation of I_h the 5-HT-sensitive current deviates greatly from the instantaneous current over the voltage range where I_h is active (Fig. 5*A*). Only two cells failed to exhibit this time- and voltage-dependent conductance increase in the presence of 5-HT.

Records of NA-sensitive current indicated a uniform conductance decrease throughout the duration of the pulse, irrespective of the activation of I_h. Plots of NA-sensitive current were linear giving values for E_{NA} of $-83{\cdot}0 \pm 9{\cdot}7$ and $-83{\cdot}3 \pm 10{\cdot}8$ mV, when measured at instantaneous or steady-state time points, respectively ($n = 9$; Fig. 5*B*). 5-HT-sensitive currents from the same cells showed steady-state currents that deviate from the instantaneous currents in all cases.

I_h can be abolished by addition of caesium ions (Cs^+) (2–5 mM) to the perfusing ACSF. Under these conditions 5-HT still evoked an inward current; however, the 5-HT-sensitive current was identical where measured at the instantaneous or steady-state time points reversing close to the predicted E_K ($n = 4$).

These lines of evidence suggest 5-HT has two actions on facial motoneurones. One involves an action to decrease a resting K^+ conductance, an action shared by NA, the second involves an enhancement of the inward rectifier, I_h.

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state (open symbols) *I–V* relationships in the absence (squares) and presence (circles) of NA from the records in *C*. Both instantaneous and steady-state relationships show a decrease in conductance in the presence of NA irrespective of steady-state inward rectification with points of intersection at -106 and -94 mV, respectively. *A* and *B* were performed at 32 °C, and *C* and *D* at 37 °C. $[K^+]_o$ was 5 mM in all cases.

5-HT evokes a positive shift in the I_h activation curve

While we earlier observed that an increase in tail current amplitude occurred in the presence of 5-HT it is clear that maximal I_h activation had not been achieved (Fig. 4*A*). If 5-HT is acting on I_h it could operate either by increasing the maximum level

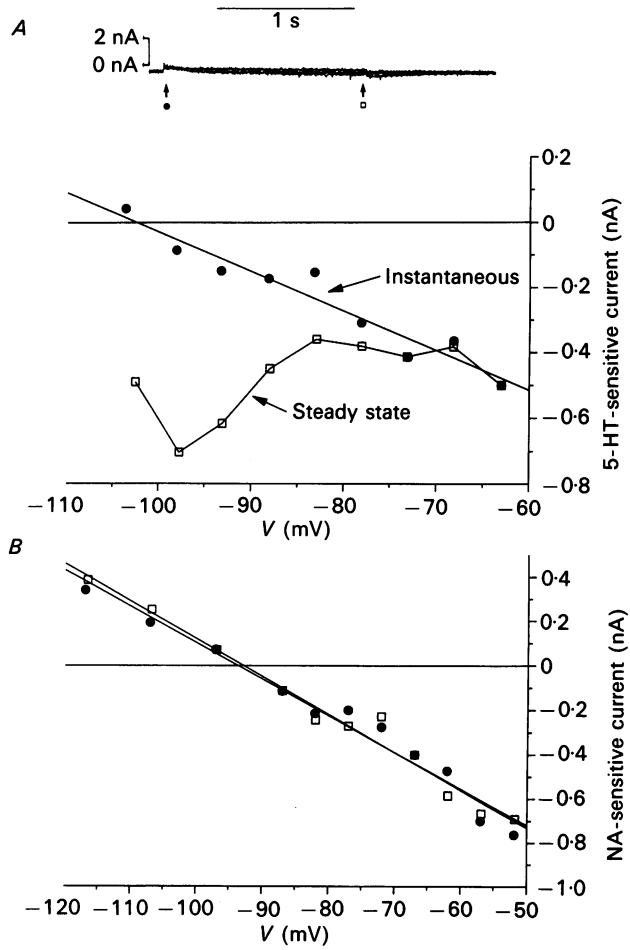

Fig. 5. 5-HT- and NA-sensitive currents at different membrane potentials. Currents were obtained by subtraction of step currents elicited in the absence and presence of 5-HT or NA and plotted against voltage achieved at instantaneous and steady-state equivalent time points. *A*, instantaneous 5-HT-sensitive current for the motoneurone in Fig. 4*A* and *B* was linear, reversing at −102 mV (line fitted by linear regression). The steady-state current showed hyperpolarization-sensitive deviation from the instantaneous current. *B*, NA-sensitive current for a second application of NA to the motoneurone shown in Fig. 4*C* and *D*, this time in the presence of TTX (1 μM). Instantaneous and steady-state currents were identical. Lines fitted by linear regression gave a reversal potential of −94 mV.

of current or by shifting the activation curve for the existing I_h to more positive potentials.

Maximum activation of I_h by large-amplitude hyperpolarizing voltage step

commands showed maximum tail current amplitude to be unaffected by 5-HT application (Fig. 6*A*). Sigmoidal I_h activation curves (generated by eqn (1) and fitted by eqn (2)) showed a shift of $3{\cdot}4 \pm 0{\cdot}4$ mV ($n = 3$) to more positive potentials in the presence of 5-HT (Fig. 6*B*).

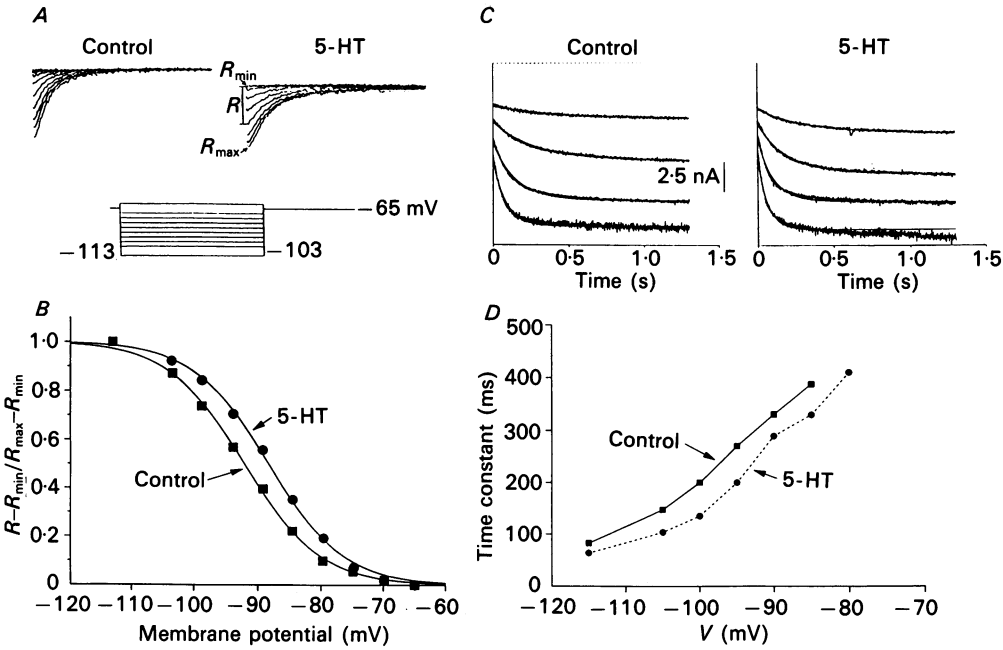

Fig. 6. Effects of 5-HT on the activation curve and kinetics of I_h. *A*, tail currents evoked at the offset of the illustrated voltage commands in control and in the presence of 5-HT from a facial motoneurone voltage clamped at −65 mV. Maximum amplitude was similar in both conditions. Note the slowing of I_h deactivation in the presence of 5-HT. *B*, normalization of the tail current amplitude (see main text) shows the activation curve for the conductance underlying I_h to be shifted to more positive potentials in 5-HT. Values of $V_{0\cdot5}$ and k for the fitted Boltzmann curves were −92 mV and 6 in control (■) and −88 mV and 5·9 in 5-HT (●). *C*, superimposed single exponential fits to I_h inward relaxations evoked by voltage commands to −85, −94, −103 and −113 mV from a V_h of −65 mV in the absence and presence of 5-HT. The fit at −113 mV in the presence of 5-HT was made over the first 500 ms prior to the observed current instability. *D*, plot of the time constant for I_h activation against potential from the data shown in *C* and additional currents not illustrated. 5-HT increases the rate of activation at any given potential.

Accompanying this shift in the activation curve was a reduction in the time constant of activation of I_h at any given potential (Fig. 6*C* and *D*). Activation of I_h was fitted by a single exponential function of the form

$$I_t = A + B e^{-t/\tau}, \tag{3}$$

where I_t is the current amplitude at time t, A and B are constants and τ is the time constant of activation. The time constant decreased with hyperpolarization. 5-HT further decreased the activation time constant shifting a plot of the time constant against potential to more positive levels (Fig. 6*D*). Thus at −85 mV in Fig. 6*C* the

activation time constant was 387 ms in control and 329 ms in 5-HT while at −113 mV the corresponding values were 83 and 64 ms, respectively. The rate of I_h deactivation was not studied in detail but from the decaying tail currents it can be seen that 5-HT lengthens the time constant of a single exponential fit from 78 ms in control conditions to 112 ms in the presence of 5-HT (Fig. 6*A*).

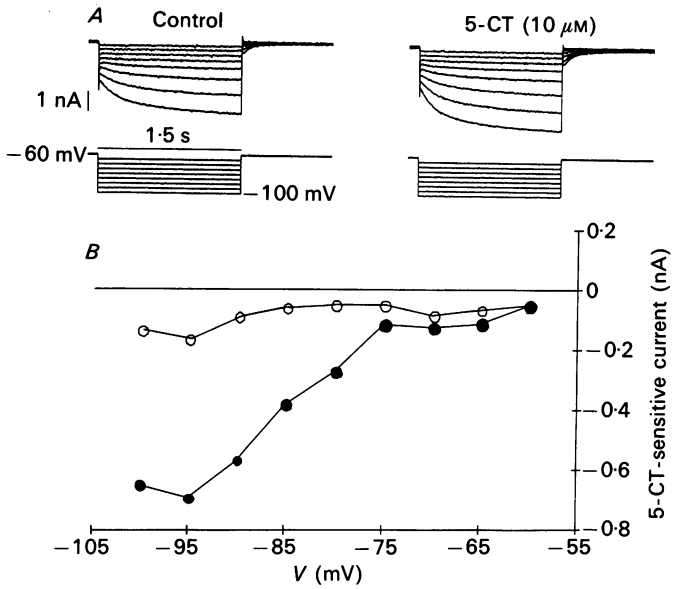

Fig. 7. 5-Carboxamidotryptamine (5-CT) selectively enhances I_h. *A*, evoked step currents before and during application of 5-CT (10 μM) to a motoneurone voltage clamped at −60 mV. No obvious inward current was evoked by 5-CT; however, I_h and associated tail currents were markedly enhanced. *B*, instantaneous (○) and steady-state (●) 5-CT-sensitive current from the records in *A*. No change in instantaneous current was evoked by 5-CT. The steady-state current showed marked inward deviation from the instantaneous current at potentials negative to −75 mV.

5-Carboxamidotryptamine selectively enhances I_h

Voltage-clamp experiments using 5-carboxamidotryptamine (5-CT) (10–25 μM), a general 5-HT_1 receptor agonist, showed that this ligand can selectively differentiate between the two actions of 5-HT described here.

Figure 7 illustrates that 5-CT (10 μM) fails to evoke an inward current in a facial motoneurone clamped at −60 mV, above the threshold for I_h activation. 5-HT previously evoked an inward current at this holding potential through a decrease in K^+ conductance (not shown). However, hyperpolarizing voltage commands into the activation range of I_h show an enhancement of this current in the presence of 5-CT (Fig. 7*A*). This is clearly indicated by the deviation of the 5-CT-sensitive current when measured at the steady state compared to the instantaneous time point (Fig. 7*B*). When applied to facial motoneurones held at potentials within the activation range of I_h, 5-CT evoked an inward current ($n = 4$). Co-application of 5-HT during the continued presence of 5-CT evoked an additional inward current due solely to a

decrease in K^+ conductance indicating that 5-CT can occlude the action of 5-HT on I_h (not shown). Changes in instantaneous current in the presence of 5-CT were difficult to detect though in one case a small increase was observed giving an extrapolated reversal level around -20 mV. In the same motoneurone the reversal potential for I_h was estimated to be -39 mV.

As for 5-HT the action of 5-CT on I_h involved a decrease in the time constant for activation at any given potential. At potentials between -80 and -100 mV the time constant decreased from 334 ± 124 to 292 ± 95 ms ($n = 4$).

DISCUSSION

The results indicate that the excitatory actions of 5-HT are mediated through two distinct depolarizing mechanisms occurring concurrently in the majority of facial motoneurones examined. The ionic basis of these mechanisms involves the reduction of a resting K^+ conductance and the enhancement of the hyperpolarization-activated mixed cation current, I_h, which we have characterized as being responsible for time-dependent inward rectification of facial motoneurones. Depolarization of the same motoneurones by NA appears to be mediated solely by K^+ channel closure. Voltage-clamp studies indicated that the difference in reversal potentials for 5-HT- and NA-evoked depolarizations seen in current-clamp studies is the result of an action of 5-HT on I_h; however, a contribution to the difference in reversal potentials due to a differential localization of the sites of action of 5-HT and NA, leading to greater electrotonic attenuation of the 5-HT-evoked resistance change when recorded intrasomatically, cannot be ruled out.

Hyperpolarization-activated I_h exhibited by facial motoneurones has been characterized in many other cell types (Yanagihara & Irisawa, 1980; DiFrancesco, 1981; Halliwell & Adams, 1982; Mayer & Westbrook, 1983). Interestingly two populations of motoneurones with different I_h activation ranges were identified, suggesting that I_h is active at the resting potential of some but possibly not all motoneurones. The evidence that 5-HT enhances I_h can be summarized thus: steady-state current–voltage plots obtained in voltage clamp show an increase in slope with hyperpolarization which is further increased by 5-HT indicating an increase in conductance at hyperpolarized potentials; subtracted 5-HT-sensitive current records show this conductance increase to have a time dependence similar to that of I_h activation; plots of steady-state 5-HT-sensitive current show a voltage dependence close to that of I_h; blockade of I_h with extracellular Cs^+ also abolishes the 5-HT-evoked conductance increase; 5-HT shifts the activation curve for I_h to more positive potentials.

Neurotransmitters, including 5-HT, modulate I_h in other central neurones and also the same current named I_f, in cardiac cells (DiFrancesco, 1985; Bobker & Williams, 1989; McCormick & Pape, 1990*b*; Takahashi & Berger, 1990). In these studies the inward current and conductance increase evoked by 5-HT was blocked by $[Cs^+]_o$ and had a reversal potential positive to the resting potential. In thalamic neurones and cardiac cells shifts in the I_h activation curve rather than changes in absolute current amplitude were observed, as indicated here for facial motoneurones.

As reported elsewhere (VanderMaelen & Aghajanian, 1980; Aghajanian &

Rasmussen, 1989; Larkman *et al.* 1989) 5-HT and NA evoke K^+-dependent depolarizations, confirmed here by the reversal potentials for each neurotransmitter being close to the values predicted by the Nernst equation especially at higher $[K^+]_o$. We speculate that deviation from the predicted values in 2·5 mM K^+ may be due to pronounced rectification at hyperpolarized potentials though another possibility may be that the actual $[K^+]_o$ never reaches the desired concentration due to leakage from damaged cells within the slice. In voltage-clamp experiments, 5-HT still evoked an inward current at potentials where I_h was not active or in conditions where I_h was blocked by $[Cs^+]_o$, both showing a reversal potential at the predicted E_K.

In nucleus accumbens neurones, 5-HT reduces an inwardly rectifying K^+ conductance which shows high sensitivity to blockade by Ba^{2+} (North & Uchimura, 1989) and presumably higher concentrations of Cs^+ (Uchimura, Cherubini & North, 1989). The K^+ conductance reduced in facial motoneurones showed no clear inward rectification and was insensitive to blockade by external Cs^+. As prominent rectification and Cs^+ blockade is greater at more hyperpolarized potentials (Uchimura *et al.* 1989; Yamaguchi *et al.* 1990) this may be due to the range of voltages tested which commonly did not exceed −100 mV in our experiments. However, the evidence suggests greater similarity to a 'leak' K^+ conductance reduced by 5-HT in hippocampal pyramidal neurones (Andrade & Nicoll, 1987; Colino & Halliwell, 1987).

The existence of two mechanisms acting at the same time on the same motoneurone explains the variation in $E_{5\text{-HT}}$ obtained from *I–V* measurements. At holding potentials within the activation range of I_h, instantaneous *I–V* relationships and plots of instantaneous 5-HT-sensitive current gave apparent reversal potentials for the 5-HT-evoked inward current more negative than the E_K. A component of the instantaneous current at the holding potential will be due to a steady-state level of I_h activation. In the presence of 5-HT the level of I_h activation is increased in addition to a decrease in K^+ conductance. The instantaneous conductance change is determined by the interaction between the two mechanisms resulting in the various forms of *I–V* relationships and range of apparent reversal potentials. Carbachol-evoked inward currents in hippocampal pyramidal neurones show similar properties where a model incorporating a dendritic conductance increase in addition to a decrease in a K^+ conductance was proposed (Benson, Blitzer & Landau, 1988).

The physiological evidence for two distinct depolarizing actions of 5-HT on facial motoneurones is supported by pharmacological data. Selective enhancement of I_h by 5-CT indicates that distinct receptor subtypes may be linked to the two ionic mechanisms of depolarization. The identity of the receptor subtype linked to enhancement of I_h is not clear. Similar actions in thalamic and prepositus hypoglossi neurones show a pharmacology which has been called 5-HT_1-like (Bobker & Williams, 1989; McCormick & Pape, 1990*b*). The action of 5-CT coupled with previous results showing 5-HT-, but not NA-, evoked depolarization of facial motoneurones to be completely blocked by methysergide and LY-53856, partly blocked by ketanserin but insensitive to spiperone and methiothepin may be consistent with this classification (Kelly, Larkman, Penington, Rainnie, McAllister-Williams & Hodgkiss, 1991; Larkman & Kelly, 1991*b*). Other 5-HT receptor agonists, 8-OH-DPAT, dipropyl-5-CT and 2-methyl-5-HT are without effect on

facial motoneurones. The high concentrations of 5-CT required to enhance I_h could suggest a 5-HT_4 receptor; however, ICS 205 930 was ineffective as an antagonist against 5-HT-evoked depolarization.

The conclusion that a 5-HT_{1C} or 5-HT_2 receptor is linked to K^+ channel closure remains valid (Rasmussen & Aghajanian, 1990; Kelly *et al.* 1991; Larkman & Kelly, 1991*b*). This is supported by the action of *m*-chlorophenylpiperazine (*m*-CPP) which we have seen to show agonist and antagonist actions on the 5-HT-evoked conductance decrease at equimolar concentrations with 5-HT (P. M. Larkman & J. S. Kelly, unpublished observations). This partial agonist activity is similar to the peripheral actions of this ligand at 5-HT_{1C} sites (Curzon & Kennett, 1990). Thus the receptor can be broadly classified in the 5-HT_2 receptor group along with 5-HT actions on neurones of the rat cortex and nucleus accumbens (North & Uchimura, 1989; Araneda & Andrade, 1991) but not the hippocampus (Chaput, Araneda & Andrade, 1990). Clearly though, given the possibility that I_h may not be active at the resting potential of all facial motoneurones, a re-examination of the pharmacology of the 5-HT-evoked inward current needs to be undertaken.

The involvement of multiple 5-HT receptor subtypes linked to distinct ionic mechanisms of depolarization may be reflected in spinal motoneurones. Ventral root depolarization by 5-HT shows a complex pharmacology (Connell & Wallis, 1989; Wallis, Connell & Kvaltinova, 1991). Intracellular recordings from motoneurones in the neonatal rat spinal cord *in vitro* show a 5-HT-evoked depolarization to be associated with a decrease in K^+ conductance linked to a receptor of the 5-HT_2 family (Wang & Dun, 1990). Whole-cell patch-clamp studies from motoneurones in thin slices, however, show depolarization associated with a conductance increase due to an action on I_h with a receptor pharmacology distinct from a 5-HT_2 type (Takahashi & Berger, 1990).

It is not known whether the two receptors/mechanisms co-exist at the same synapses or if 5-HT acts on both mechanisms at the same time under normal physiological conditions. In unclamped facial motoneurones the 5-HT-evoked depolarization was always associated with an increase in resistance making the cell more responsive to excitatory input. The action of 5-HT will depend on the resting potential and the activation range of I_h which appears to have a bimodal distribution. The range of apparent reversal potentials for the 5-HT effect may be because I_h is not activated at the resting potential in all cells or that the relative importance of each mechanism varies between cells. We saw no indications that the effects on I_h were specific to either of the cell groups.

The excitatory actions, while both being depolarizing at the apparent resting potential, have opposite effects on neuronal resistance and thus will have different modulatory effects on fast synaptic events such as those evoked by the excitatory amino acid transmitters (VanderMaelen & Aghajanian, 1982). It may be that it is not the depolarizing action of 5-HT on I_h *per se* that is of primary importance but more its modulatory effects on synaptic potentials and possibly firing properties. I_h has been suggested to play a role in pacemaker potentials and oscillatory burst firing in thalamic neurones and cardiac muscle cells (DiFrancesco, 1985; McCormick & Pape, 1990*a*, *b*), and that 5-HT and NA can modulate these rhythms through their augmenting actions. Facial motoneurones in common with other motoneurones

possess limited firing patterns such that similar modulatory actions of 5-HT appear unlikely. However, I_h has been proposed to play a role in the form of the spike after-hyperpolarization (AHP) (Gustafsson & Pinter, 1985; Schwindt, Spain & Crill, 1988) which could lead to modifications in response properties of the cell in the presence of 5-HT. Although we saw no obvious effect of 5-HT on facial motoneurone AHPs (P. M. Larkman & J. S. Kelly, unpublished observations) this may be a consequence of the additional action of increasing neuronal resistance through K^+ channel closure. We have not investigated the actions of 5-CT or Cs^+ (i.e. modulating I_h in isolation) on facial motoneurone firing properties.

The facial muscles of the rat control the orientation of the vibrissae and pinna and also the eye blink reflex, each performing important functions in the perception of sensory stimuli. We speculate that 5-HT and NA may play a role in sensory arousal and alertness by 'priming' motoneurones in readiness for excitatory synaptic input enabling rapid orientation of these organs in response to stimuli.

In conclusion this study indicates that 5-HT depolarizes facial motoneurones through a combination of mechanisms, one involving a decrease in K^+ conductance, the other an enhancement of the inward rectifier, I_h. These actions may be mediated through distinct receptors. Whether this is complicated by a differential somato-dendritic localization of the two receptor types is unknown. However, multiple depolarizing actions of 5-HT may be common to rat motoneurones (Takahashi & Berger, 1990; Wang & Dun, 1990; Wallis *et al.* 1991).

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