Journal of Physiology (1993), **461**, *pp.* 213–233
With 11 *figures*
Printed in Great Britain

MODULATION OF RESPIRATORY ACTIVITY OF NEONATAL RAT PHRENIC MOTONEURONES BY SEROTONIN

BY AMY D. LINDSAY AND JACK L. FELDMAN

From the Systems Neurobiology Laboratory, Department of Physiological Science, University of California Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90024-1527, USA

(*Received 8 January 1992*)

SUMMARY

1. The effects of serotonin on phrenic motoneurones were studied in an *in vitro* preparation of the isolated brainstem and spinal cord from neonatal rats.

2. Serotonin (5-HT; $\geqslant 5$–$10\ \mu$M) increased inspiratory-modulated phrenic nerve activity and produced a small amount of tonic activity during expiration. Inspiratory-modulated activity of the fourth cervical ventral root also increased, but was accompanied by robust tonic activity, which often obscured the rhythmic activity.

3. Serotonin, in both normal and tetrodotoxin-containing medium, depolarized phrenic motoneurones and increased cell input resistance. Serotonin also increased inspiratory-modulated firing as well as the response of phrenic motoneurones to injected current. The *y*-intercept of the relationship between firing frequency and injected current (*f–I*) was increased, but the slope was not affected. There was no bistable firing behaviour.

4. Under voltage clamp conditions, 5-HT produced a tonic inward current of 0·07–0·37 nA. This current increased with less negative holding potentials and decreased with more negative holding potentials (-75 to -90 mV) but did not reverse.

5. In addition, 5-HT decreased inspiratory-modulated synaptic current by $23 \pm 6\%$. The degree of attenuation was not affected by holding potential. The time course of the decrease in inspiratory-modulated synaptic current was similar to the changes seen in tonic inward current and input resistance.

6. Depolarization, tonic inward current, and shift in the *f–I* relationship produced by 5-HT were antagonized by the 5-$HT_{2/1C}$ receptor antagonist ketanserin and mimicked by the 5-$HT_{2/1C}$ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI). However, the 5-HT induced decrease in inspiratory-modulated synaptic current was not reduced by ketanserin nor mimicked by DOI.

7. We conclude that exogenously applied 5-HT simultaneously increases cell excitability and decreases inspiratory-modulated synaptic current in phrenic motoneurones via different types of receptors. When these responses occurred simultaneously, the increase in excitability predominated and the net effect was an augmentation of inspiratory-modulated phrenic motoneurone activity.

INTRODUCTION

Serotonin (5-HT) affects a variety of motor behaviours, and modulates the behaviour of many neurones involved in motor control (see Jacobs, 1991; Axmitia & Whitaker-Axmitia, 1991 for review). This includes Purkinje cells (Hicks, Krupa & Crepel, 1989), neocortical neurones (Lorenzon & Foehring, 1992) and spinal (Berger & Takahashi, 1990) and facial motoneurones (Larkman, Penington & Kelly, 1989). The behaviour of spinal motoneurones is modulated by 5-HT by mechanisms which include: direct excitation (Wang & Dun, 1990; Wallis, Connell & Kvaltinova 1991), inhibition (Wu, Wang & Dun, 1991; Zhang, 1991), and modulation of the response to synaptic inputs (Wu *et al.* 1991). In addition, 5-HT induces bistable firing behaviour in cat and turtle spinal motoneurones (Hounsgaard, Hultborn, Jespersen & Kiehn, 1988; Hounsgaard & Kiehn, 1989).

Phrenic motoneurones (which control the diaphragm) respond to exogenously applied 5-HT with increased inspiratory-modulated firing (Lalley, 1986*b*; Schmid, Bohmer & Merkelbach, 1990). However, stimulation of endogenous sources of 5-HT (raphe nuclei) can produce both an excitation and an inhibition of phrenic nerve activity (Lalley, 1986*a*). It is not clear if these opposing effects of raphe nuclei stimulation are mediated by 5-HT acting on phrenic motoneurones or on medullary respiratory control centres.

We studied the effects of 5-HT on phrenic motoneurone activity in the isolated brainstem and spinal cord preparation of neonatal rats (Liu, Feldman & Smith, 1990). The mechanisms underlying these effects were investigated with intracellular recordings from phrenic motoneurones under both current and voltage clamp conditions.

Our principal finding was that 5-HT, applied selectively to the spinal cord, increased phrenic motoneurone excitability and decreased inspiratory-modulated synaptic current. When 5-HT was added alone, the increase in excitability always dominated and the net effect was increased inspiratory-modulated phrenic nerve activity. Only when the increase in excitability was blocked was a reduction in phrenic motoneurone firing produced by 5-HT. The two effects of 5-HT appear to be mediated by distinctly different receptors.

A preliminary account of these data have been reported (Lindsay, Schwartz & Feldman, 1990).

METHODS

The brainstem and cervical spinal cord were isolated from 0- to 6-day-old ether-anaesthetized Sprague–Dawley rats ($n = 43$) and maintained under *in vitro* conditions (Smith & Feldman, 1987; Liu *et al.* 1990). Preparations were continuously superfused with physiological Krebs solution composed of (mM): NaCl, 128; KCl, 3·0; NaH_2PO_4, 0·5; $CaCl_2$, 1·5; $MgSO_4$, 1·0; $NaHCO_3$, 21; glucose, 30 and equilibrated with 95% O_2–5% CO_2; pH 7·45 at 27 °C.

For the preliminary pharmacological experiments, the phrenic nerve was left attached up to where it crossed the brachial plexus. The preparation was maintained in an *in vitro* chamber that was divided at the spinomedullary junction into separate brainstem and spinal cord compartments. The bathing solution was continuously aerated with a gas mixture of 95% O_2–5% CO_2 at 27 °C and exchanged every 15–25 min. For intracellular recording experiments, the preparation was maintained in an unpartitioned chamber (volume 1·3–1·5 ml) and continuously superfused at 65–85 ml h^{-1} with oxygenated Krebs solution at room temperature (24–25 °C). An environment of moist oxygen was maintained above the chamber. A patch made in the pial membrane overlying

the phrenic nucleus (C3–C5 segments) facilitated the penetration of microelectrodes into the spinal cord.

Electrophysiology

Respiratory activity was recorded from the fourth cervical ventral root, and in some experiments the phrenic nerve, using suction electrodes. Signals were amplified with a Grass amplifier (model P511K, USA).

Intracellular recordings from motoneurones were made with glass microelectrodes filled with either 2 M potassium acetate (DC resistance: 60–120 MΩ), or 2–3 M potassium chloride (KCl; DC resistance: 35–65 MΩ). All voltage clamp recordings employed KCl electrodes. Electrodes were coated with Sylgard™ to within 400 μm of the tip to reduce electrode capacitance, and attached to the headstage of an Axoclamp II amplifier (Axon Instruments, USA). Electrodes used in voltage clamp experiments met the following criteria: in discontinuous current clamp mode (extracellular) the electrode was stable at a sampling frequency $\geqslant 5$ kHz and a 1 nA current pulse resulted in a transient voltage deflection $\leqslant 2$ mV; in single electrode voltage clamp mode (intracellular) the electrode was stable at a gain $\geqslant 0{\cdot}3$ nA mV^{-1}. Such electrodes clamped 10–15 mV inspiratory drive potentials to within 2–3 mV of the command holding potential throughout the experimental protocol (Fig. 1; see also Liu *et al.* 1990).

Only those neurones that could be antidromically activated by stimulation of the C4 ventral root, had an antidromic action potential amplitude greater than 70 mV, a membrane potential during neural expiration more negative than −60 mV and an inspiratory drive potential greater than 10 mV were selected for analysis. Acceptable cells were located approximately half-way between the midline and the lateral edge of the cord and 130–300 μm from the ventral surface. These co-ordinates are consistent with the location of the phrenic motoneurone pool in neonatal rats (Lindsay, Greer & Feldman, 1991). Stable penetrations of such cells could be maintained for 2–4 h.

Changes in cell input resistance during drug application were measured under both current and voltage clamp conditions. During current clamp, −0·3 nA current pulses of 100 ms duration were applied at 0·5–1 Hz before and during the response of the cell to 5-HT; in voltage clamp, −6 mV command voltage pulses were used.

Changes in repetitive firing characteristics in response to 5-HT were investigated using a triangular current pulse which had a peak amplitude of 1 nA with a slope of ± 1 nA s^{-1} (e.g. Fig. 4). Instantaneous firing frequency was calculated as the reciprocal of the interspike interval.

Pharmacological substances

The following drugs were used: 5-HT (Sigma, USA), 1 mM dissolved in Ringer solution and adjusted to pH 7·4; 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI, Research Biochemicals, Inc., USA), 1 mM dissolved in Ringer solution and adjusted to pH 7·4; ketanserin tartrate, (Research Biochemicals, Inc.) 10–100 μM dissolved in deionized water; tetrodotoxin (TTX, Sigma), 200 μM in deionized water. All solutions were made in batches of 10 ml, divided into 1 ml aliquots and stored at −20 °C until use.

Drugs were administered either by addition to the bathing medium or local application via pressure ejection from a micropipette positioned over the ventral surface of the spinal cord close to the intracellular recording electrode. The amount of drug solution delivered by pressure ejection was chosen to be the minimum necessary to give a clear change in ventral root activity lasting between 3 and 5 min. The specific protocol used for each experiment is described in the Results. Pressure ejection pipettes had a tip diameter of ≈ 8 μm. Drugs were ejected with a 1–4 s train of 50 ms pulses at 50 ms intervals with pressures between 100 and 150 kPa.

To ensure that a response to a drug was due to an effect on spinal neurones, and not inadvertent diffusion of drug to brainstem neurones, control experiments employing selective drug application to the brainstem were conducted for each drug studied. The specific controls used for each drug are described in the Results. In addition, we observed that during pressure ejection of 5-HT, an increase of less than 100 μm in the distance of the pipette from the surface of the spinal cord significantly reduced the response recorded from ventral roots or single motoneurones.

Data acquisition and analysis

Data were recorded on video tape via pulse code modulation (Vetter model 3000; sampled at 10–40 kHz per channel). Selected portions of records were digitized at 6 kHz using an analog-to-digital converter and stored on a Vaxstation 3200 computer disk for subsequent analysis. The

frequency at which the data were digitized was sufficient for an accurate analysis of the events of interest (changes in baseline, and inspiratory-modulated membrane potential and current). Details of the analysis of specific results are given in the Results. Data are presented as mean ± standard deviation unless otherwise stated. In all figures the action potentials have been truncated.

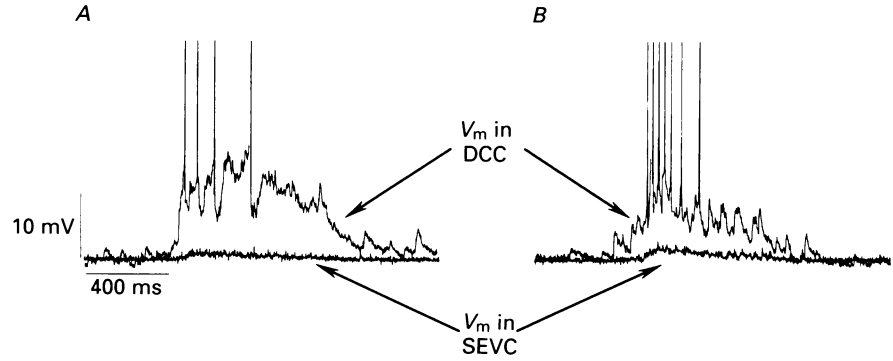

Fig. 1. Examples of the range of the voltage clamp efficacy in the present experiments. In each pair of traces, the upper trace is an inspiratory drive potential, with action potentials, recorded in discontinuous current clamp (DCC), while the lower trace is a consecutive inspiratory drive potential recorded during single electrode voltage clamp (SEVC). *A*, an example of one of the best voltage clamps obtained. *B*, an example of one of the worst voltage clamps obtained.

RESULTS

Effect of 5-HT on phrenic nerve activity

Application of 5-HT ($\geqslant 10\ \mu$M) to the spinal cord compartment of a partitioned chamber induced only a very small amount of tonic activity in the phrenic nerve ($n = 18$; Fig. 2*A*). In contrast, 5-HT induced robust tonic activity in the C4 ventral root, which includes axons from both phrenic and non-phrenic motoneurones ($n = 29$; Fig. 2*B*). The tonic activity was accompanied by increased inspiratory activity of the phrenic nerve ($n = 18$; Fig. 2*A*) which could also be seen on the C4 ventral root, when not obscured by tonic activity (Fig. 2*B*). There was no change in respiratory rate with selective application of 5-HT to the spinal cord.

The response to 5-HT administered to the bath began between 1 and 2 min following the addition of the drug, peaked at 2–4 min and then decreased with time after ≈ 10 min. Often some enhanced activity remained after 20–30 min, as long as the drug remained present. It proved difficult to completely wash out the effect of bath-applied 5-HT. After a 30 min wash, a second application of the same concentration of 5-HT often produced a significantly larger response than the first (i.e. tachyphylaxis). Consequently, a cumulative-dose protocol was used to determine the effect of increasing concentration; 5–7 min between drug applications.

The tonic and inspiratory-modulated activity produced by 5-HT increased with increasing concentration up to 30–40 μM, at which point the response saturated (Fig. 2). The increase in inspiratory activity with concentration was much more subtle than the increase in tonic activity.

To ensure that the observed changes in ventral root and phrenic nerve activity were due to 5-HT acting at sites within the spinal cord and not within the brainstem,

the effect of 5-HT selectively applied to the brainstem compartment of a partitioned chamber was studied in separate experiments ($n = 7$). (A complete investigation of the effect of 5-HT on medullary respiratory control centres was beyond the scope of this study, so only those results pertinent to the effect of 5-HT on spinal

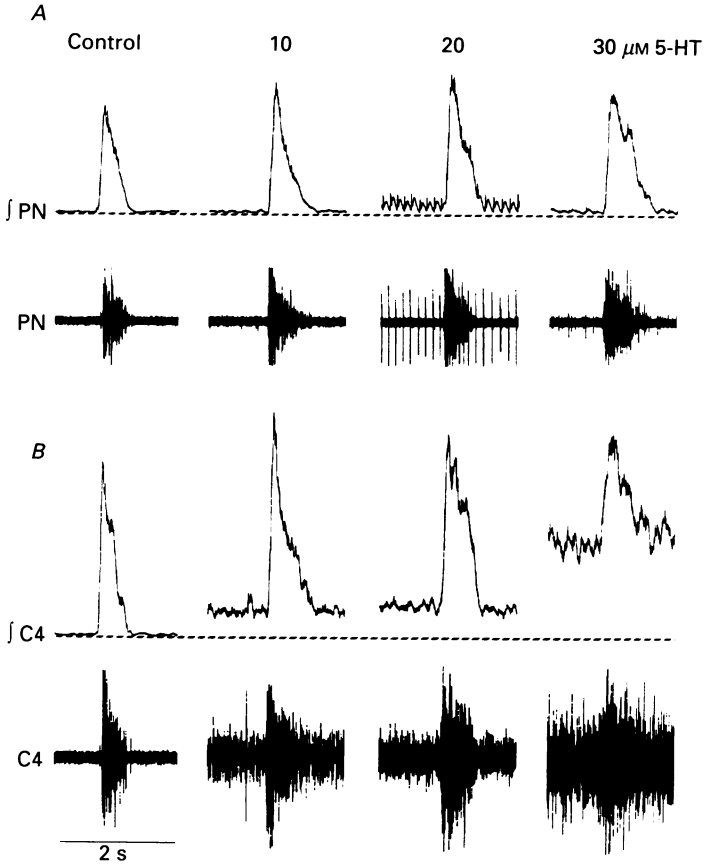

Fig. 2. Effect of increasing concentrations of 5-HT on simultaneously recorded activity from phrenic nerve and contralateral C4 ventral root. 5-HT added to the spinal cord compartment of a partitioned chamber. *A*, integrated and raw phrenic nerve activity (∫PN and PN, respectively). *B*, integrated and raw C4 ventral root activity (∫C4 and C4, respectively). Bursts are 2–3 min after drug application.

motoneurones are described here.) In contrast to 5-HT applied to the spinal cord, application of 5-HT to the brainstem produced time- and concentration-dependent changes in respiratory rate but no change in either tonic or inspiratory-modulated spinal motoneurone activity. With concentrations between 5 and 10 μM, 5-HT increased respiratory rate, while 5-HT concentrations greater than 30–40 μM decreased respiratory rate. However, application of 5-HT concentrations as high as 500 μM to the brainstem failed to produce any tonic spinal motoneurone activity or any changes in the amplitude of inspiratory-modulated spinal motoneurone activity.

Effect of 5-HT on membrane potential and spontaneous firing

The cellular events underlying the changes in phrenic nerve activity produced by 5-HT were investigated with intracellular recordings from phrenic motoneurones ($n = 18$). The partition used to divide the recording chamber into separate

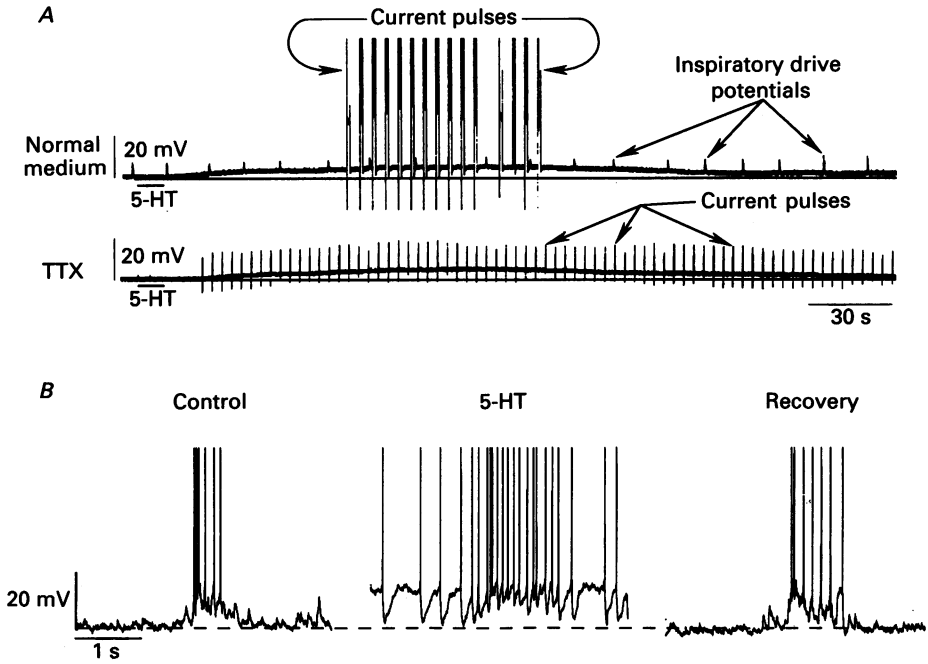

Fig. 3. Effect of local application of 5-HT on phrenic motoneurone membrane potential and spontaneous firing. *A*, changes in membrane potential produced by 5-HT applied in normal and TTX-containing medium. In normal medium, inspiratory-modulated activity is present and current pulses were given to test for changes in repetitive firing properties. In TTX-containing medium no inspiratory-modulated activity is present and brief current pulses were given to test for changes in input resistance (changes not visible at this gain). *B*, changes in inspiratory-modulated firing and membrane potential produced by 5-HT in a different cell. Resting membrane potential was ≈ -80 mV in both cells.

compartments interfered with the placement of electrodes for intracellular recording. Consequently, local application of 5-HT via pressure ejection from micropipettes was used in an unpartitioned chamber (see Methods). Local application of 5-HT at C4 never produced any changes in respiratory rate that would indicate that the drug had diffused to the brainstem.

Local application of 1 mM 5-HT depolarized phrenic motoneurones by $14{\cdot}56 \pm 7{\cdot}7$ mV ($n = 15$; Fig. 3). This depolarization lasted for 3–5 min and persisted in the presence of tetrodotoxin (TTX; 5 μM) sufficient to block antidromic action potentials and all spontaneous respiratory activity (Fig. 3*A*). The 5-HT-induced depolarization was accompanied by an apparent increase of 32–75% in input resistance (control input resistance ranged from 19–50 MΩ).

The primary effect of 5-HT on inspiratory-modulated phrenic motoneurone firing

was an increase in the number of impulses during the inspiratory burst (thirteen out of fifteen cells; Fig. 3*B*). The average number of impulses during the inspiratory burst was 5 ± 4 under control conditions and 11 ± 3 in the presence of 5-HT. The change in impulse frequency was more variable, due to the considerable range and

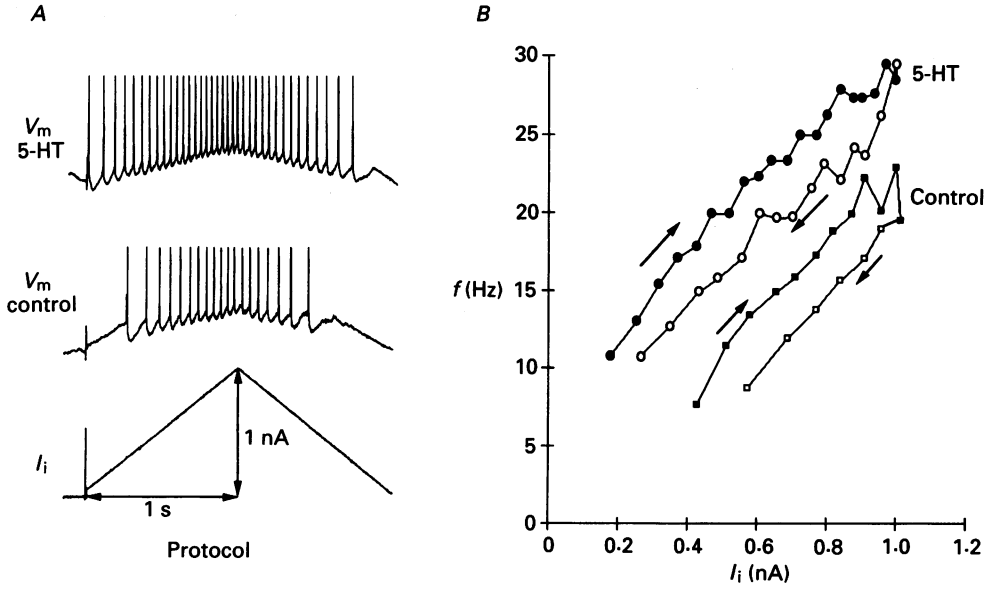

Fig. 4. Effect of local application of 1 mM 5-HT on repetitive firing produced by triangular current pulse injection. *A*, current injection protocol (see Methods). I_i, injected current amplitude. *B*, relationship between I_i and instantaneous firing frequency (f) under control conditions and following exposure to 5-HT. Current plotted was amplitude of injected current at time of occurrence of the spike. Filled symbols, values for rising phase of triangle; open symbols, values for falling phase of triangle. Slope of rising phase: 22·4 Hz nA^{-1} for control, 21·6 Hz nA^{-1} with 5-HT. Slope of falling phase: 26·2 Hz nA^{-1} for control, 22·2 Hz nA^{-1} with 5-HT.

variability in the mean frequency of inspiratory-modulated firing under control conditions (range, 0–24 Hz; mean, 13 ± 10 Hz). Only those cells which fired four or fewer impulses per inspiratory burst under control conditions increased impulse frequency with 5-HT (ten out of fifteen cells). Those cells that fired more than four impulses per burst under control conditions exhibited either a decrease ($n = 1$) or no change ($n = 4$) in peak or mean burst firing frequency following 5-HT. The inspiratory-modulated firing frequency in the presence of 5-HT ranged from 12–30 Hz (mean, 20 ± 5 Hz).

Half of the cells also exhibited some tonic impulse activity in response to 5-HT, but the rate was always well below the impulse frequency during the inspiratory burst.

Effect of 5-HT on repetitive firing properties

The increase in inspiratory-modulated phrenic motoneurone impulse activity was accompanied by an enhancement of cell excitability to injected current ($n = 6$, Fig. 4). This increase in excitability in the presence of 5-HT is evident in the change in

the relationship between firing frequency and injected current amplitude (f–I relationship; Fig. 4*B*). In the presence of 5-HT the f–I relationship was shifted upward and to the left. The y-intercept increased by 3–11 Hz and the x-intercept was reduced by 0·15–0·44 nA. Occasionally a small but statistically significant increase

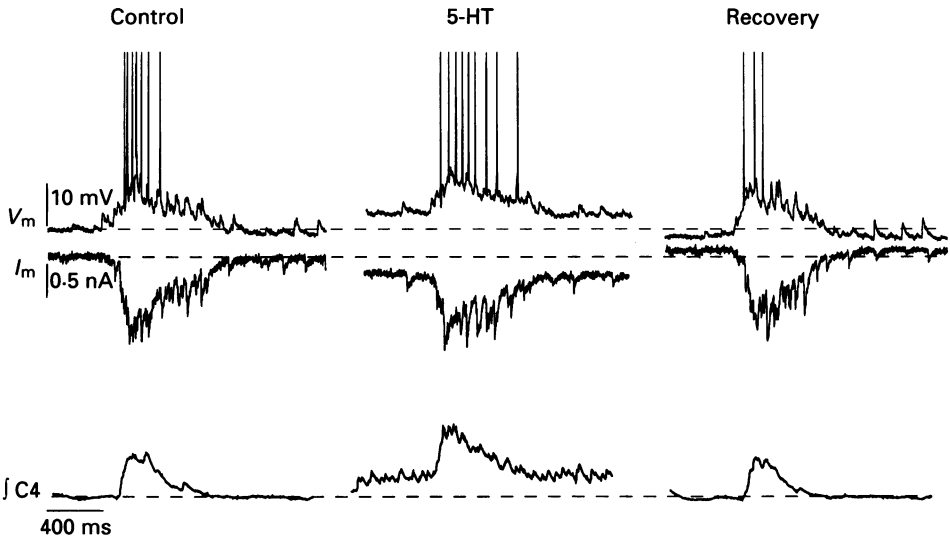

Fig. 5. Effect of local application of 5-HT on phrenic motoneurone inspiratory-modulated activity and membrane current and integrated C4 ventral root activity. V_m, membrane potential recorded during current clamp, resting potential ≈ -61 mV. I_m, membrane current recorded during voltage clamp, holding potential ≈ -61 mV. $\int$C4, integrated C4 ventral root activity. The paired V_m and I_m recordings for each condition were taken from consecutive inspiratory bursts ($\approx$ 10–20 s apart).

or decrease in the slope of the f–I relationship was seen in individual cells but, taken together, the changes were neither consistent nor significant.

In cat (Hounsgaard *et al.* 1988) and turtle (Hounsgaard & Kiehn, 1989) spinal motoneurones, 5-HT causes the cells to respond to an injected current pulse with repetitive firing that persists for seconds to minutes after pulse termination (Hounsgaard *et al.* 1988) and may continue until a hyperpolarizing current pulse is given. This type of repetitive firing is referred to as bistable firing behaviour (see Kiehn, 1991, for review), and can also be identified by changes in the response to a triangular current pulse (Hounsgaard *et al.* 1988; Hounsgaard & Kiehn, 1989). In normal medium, the f–I curves produced by a triangular current pulse are characterized by clockwise hysteresis (Fig. 4), i.e. the impulse frequency in response to the same current is less on the falling phase than the rising phase of the triangle. In cat and turtle spinal motoneurones, 5-HT produces a shift to counter-clockwise hysteresis (Hounsgaard *et al.*, 1988; Hounsgaard & Kiehn, 1989), indicating bistable behaviour. Serotonin never produced such a change in the direction of hysteresis in phrenic motoneurones (Fig. 4).

In addition, we also tested for the induction of bistable behaviour by 5-HT in phrenic motoneurones by applying 1–3 s pulses of up to 1·5 nA positive current to cells before, during and after exposure to 5-HT. All cells responded to positive

current pulses with repetitive firing that stopped when the current was turned off. In addition, in those cells which responded to 5-HT with tonic firing, 1–3 s pulses of 1–1·5 nA negative current were given in an attempt to abolish the tonic activity. The negative current pulse resulted in a cessation of firing for the duration of the current pulse only; impulse activity always resumed when the pulse was terminated.

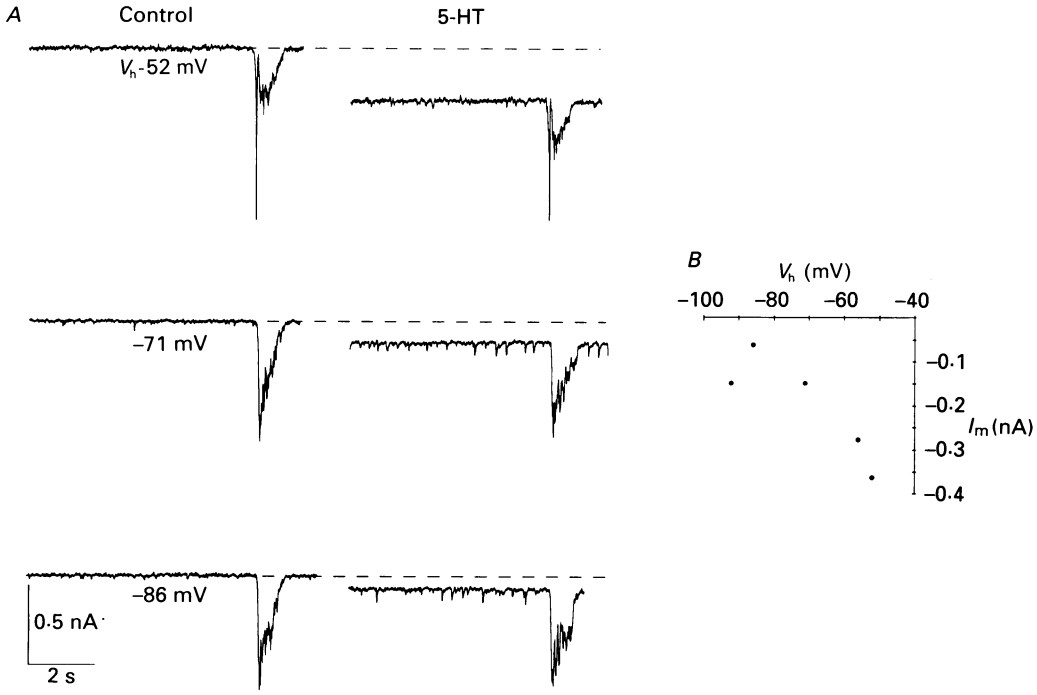

Fig. 6. Voltage dependence of tonic inward current induced by 5-HT. *A*, changes in inward currents produced by local application of 5-HT at three different holding potentials. Data filtered with an exponential filter ($t = 5$ ms). *B*, amplitude of tonic inward current produced by 5-HT as a function of holding potential. V_h, holding potential; I_m, membrane current.

Effect of 5-HT on membrane currents

To elucidate the mechanisms underlying the changes in membrane potential and repetitive firing produced by 5-HT, the response of phrenic motoneurones to 5-HT was examined under voltage clamp conditions ($n = 10$). In most trials, the amplifier was switched between current and voltage clamp modes every 2–3 respiratory cycles. This switching did not alter the responses to 5-HT.

In each cell, 5-HT induced a tonic inward current concurrent with the depolarization observed under current clamp conditions (Fig. 5). This tonic inward current ranged from 0·07 to 0·37 nA depending on holding potential; the amplitude increased with depolarizing holding potentials (Fig. 6). The minimum values for this current were obtained at holding potentials between -75 and -90 mV, but the current did not reverse with holding potentials as negative as -100 mV (Fig. 6*B*).

Concurrent with the tonic inward current, 5-HT decreased inspiratory-modulated synaptic current in phrenic motoneurones (Fig. 5). In the presence of 5-HT, the peak inspiratory current was reduced by $23 \pm 6\%$ (range, 10–30%) and the area under the inspiratory current curves decreased $17 \pm 11\%$ (range, 0–33%). Control values for

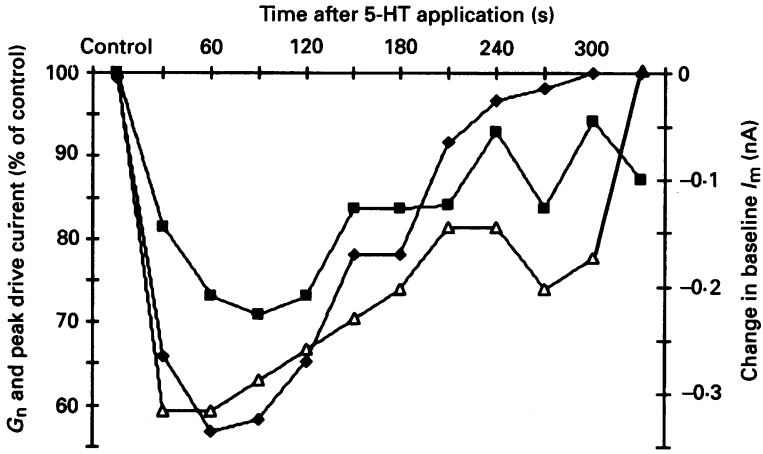

Fig. 7. Time course of the changes in baseline membrane current (I_m, ◆), input conductance (G_n, △) and peak inspiratory drive current (■) following local application of 5-HT.

peak inspiratory-modulated current ranged from −0·36 to −1·09 nA, depending on holding potential (see also Liu *et al.* 1990). However, the percentage decrease in inspiratory-modulated current, produced by 5-HT, did not vary systematically with holding potentials between −55 and −100 mV.

The changes in baseline membrane potential, tonic and inspiratory-modulated current, and input conductance followed a similar time course (Fig. 7).

In five cells, 5-HT induced small (0·05–0·2 nA) transient ($\approx$ 100–300 ms) inward currents during the expiratory phase (Fig. 6) that increased with hyperpolarizing holding potentials and decreased with depolarization. It is beyond the scope of the present study to examine these small currents in detail, but they provide anecdotal evidence that 5-HT may be activating many different currents, some of which may originate from synaptic activity (see Discussion).

Pharmacology of response to 5-HT

Several investigations have suggested that at least some of the excitatory effects of 5-HT on spinal motoneurone activity in the neonatal rat are mediated by 5-HT_2 or 5-HT_{1C} receptors (Morin, Hennequin, Monteau & Hilaire, 1990; Wang & Dun, 1990; Wallis *et al.* (1991). We investigated the possible involvement of these receptors in the response of phrenic motoneurones to 5-HT using the 5-$HT_{2/1C}$ receptor antagonist ketanserin, and the 5-$HT_{2/1C}$ receptor agonist DOI.

The 5-$HT_{2/1C}$ receptor antagonist, ketanserin, by itself, did not cause any change in spinal motoneurone activity but was a potent antagonist of the tonic activity

produced by a selective application of 5-HT to the spinal cord ($n = 13$; Fig. 8). In early experiments, a 10 min incubation with 30–40 μM ketanserin was used. However, we determined that a 15–20 min incubation in 0·5–1 μM ketanserin was sufficient to produce a profound and typically irreversible blockade of the excitatory activity

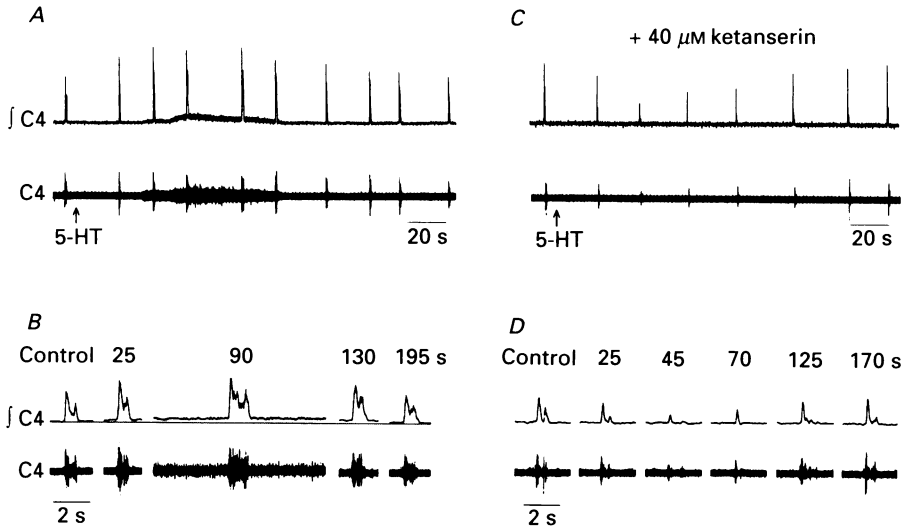

Fig. 8. Effects of local application of 5-HT on C4 ventral root activity, when given alone (*A* and *B*), and in the presence of 40 μM ketanserin (*C* and *D*). *B* and *D* illustrate expanded views of selected bursts from *A* and *C* respectively. Numbers indicate approximate time, in seconds, after 5-HT application. The difference in the amplitudes of control bursts in *B* and *D* is commonly seen in this preparation with time and cannot be attributed to a direct effect of ketanserin.

recorded from the phrenic nerve or ventral root following exogenous (bath or local) application of 5-HT.

In addition, ketanserin blocked the increase in inspiratory-modulated spinal motoneurone activity produced by 5-HT. Often, 5-HT applied in the presence of ketanserin not only failed to produce an excitatory response but instead produced varying degrees of inhibition of inspiratory-modulated ventral root or phrenic nerve activity ($n = 5$, Fig. 8*D*).

To ensure that the decrease in inspiratory-modulated spinal motoneurone activity was not due to an inadvertent action of 5-HT on the brainstem, the effect of the selective application of 5-HT with ketanserin to the brainstem was studied ($n = 6$). When 5-HT (10–20 μM) was applied to the brainstem in the presence of ketanserin it produced a decrease in respiratory rate but no change in the amplitude of inspiratory-modulated spinal motoneurone activity. Concentration of 5-HT greater than 10–20 μM mixed with ketanserin in the brainstem compartment produced a reversible cessation of all respiratory activity lasting several minutes.

To study the effect of ketanserin on the cellular response to 5-HT, the entire preparation was bathed with 1 μM ketanserin for 20 min and then 5-HT applied locally via pressure ejection from a micropipette. Ketanserin reduced or eliminated

the depolarization and tonic inward current produced by 5-HT (Fig. 9), and eliminated the shift in the *f–I* relationship. The completeness of the block by ketanserin was determined by the ratio of ketanserin to 5-HT concentration and the duration of exposure to ketanserin; a more complete blockade was achieved with

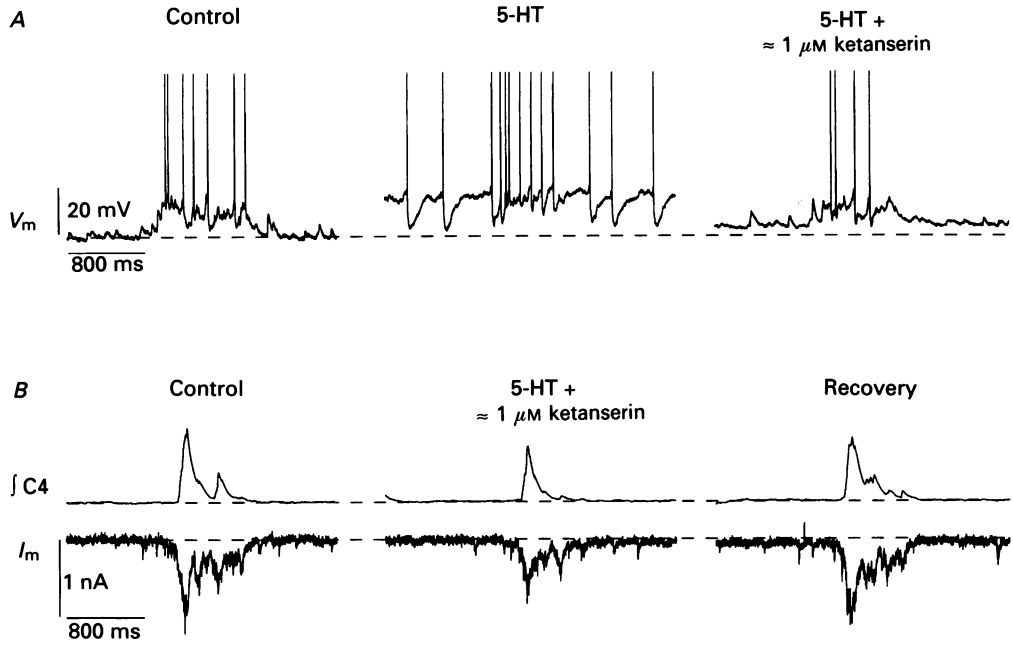

Fig. 9. Effect of local application of 5-HT on phrenic motoneurone activity and membrane current when given alone and after 20 min incubation in 1 μM ketanserin. *A*, current clamp recording from a phrenic motoneurone under control conditions, with 5-HT and with ketanserin plus 5-HT. Resting potential, −83 mV. *B*, integrated C4 ventral root activity and voltage clamp recording (same cell as in *A*) under control conditions, with ketanserin plus 5-HT, and following recovery from effects of 5-HT. Holding potential, −79 mV; V_m, membrane potential recorded during current clamp; ∫C4, integrated C4 ventral root activity; I_m, membrane current recorded during voltage clamp.

higher ratios or longer exposures. In addition, 5-HT applied in the presence of ketanserin either failed to increase inspiratory-modulated firing or caused it to decrease (Fig. 9*A*). However, the 5-HT-induced decrease in inspiratory modulated current was unaffected by ketanserin (Fig. 9*B*). Local application of 5-HT to the ventral surface of the spinal cord at C4 in an unpartitioned chamber and in the presence of ketanserin, did not produce any changes in respiratory rate.

Selective application of the $5\text{-HT}_{2/1C}$ agonist, DOI, to the spinal cord mimicked both the increase in tonic activity and the facilitation of inspiratory-modulated ventral root and phrenic nerve activity produced by 5-HT ($n = 5$; Fig. 10). As was true for 5-HT, DOI produced more tonic activity on the C4 ventral root than the phrenic nerve. The tonic activity produced by DOI was more persistent than that produced by 5-HT, with the effects often irreversible. None of these effects of DOI could be produced by selective application of DOI to the brainstem (up to 240 μM;

$n = 1$). Ketanserin was a potent antagonist of the effects of DOI ($n = 6$) but, unlike 5-HT, DOI never produced a decrease in inspiratory-modulated spinal motoneurone activity when applied in the presence of ketanserin.

At the cellular level, 20 μM DOI in the bath increased inspiratory-modulated firing, produced a depolarization accompanied by higher input resistance, and a tonic

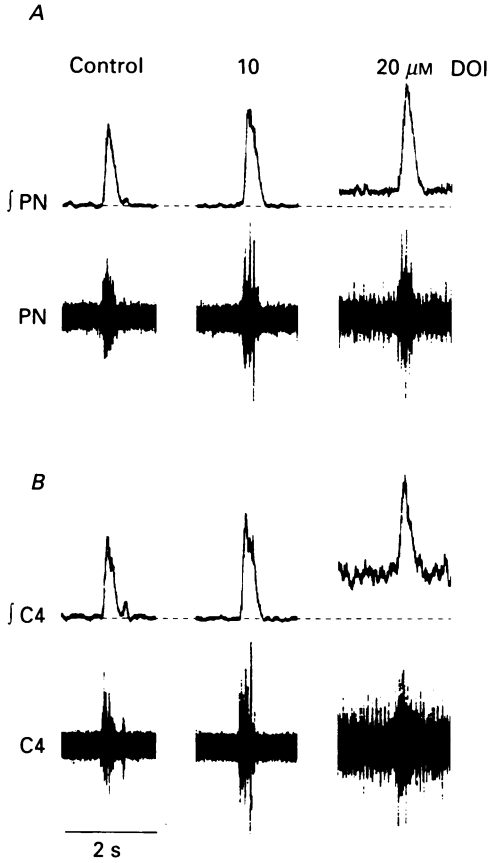

Fig. 10. Effect of increasing concentrations of DOI on simultaneously recorded activity from the phrenic nerve and contralateral C4 ventral root. DOI added to the spinal cord compartment of a partitioned chamber. *A*, integrated and raw phrenic nerve activity (∫ PN and PN, respectively); *B*, integrated and raw C4 ventral root activity (∫ C4 and C4, respectively). Bursts are 2–3 min after drug application.

inward current (Fig. 11*A*). DOI also caused a leftward shift in the *f–I* relationship, with no change in slope or the direction of hysteresis (Fig. 11*B*). However, DOI had no effect on inspiratory-modulated synaptic current and did not antagonize the decrease in inspiratory-modulated synaptic current produced by 5-HT when the two were applied together (Fig. 11*A*). The addition of 5-HT following DOI application always produced a decrease in inspiratory-modulated current. Occasionally, there was also a slight additional depolarization. The presence or absence of additional

depolarization was dependent on the concentration and duration of the previous exposure to DOI; being absent following high concentrations or long exposures.

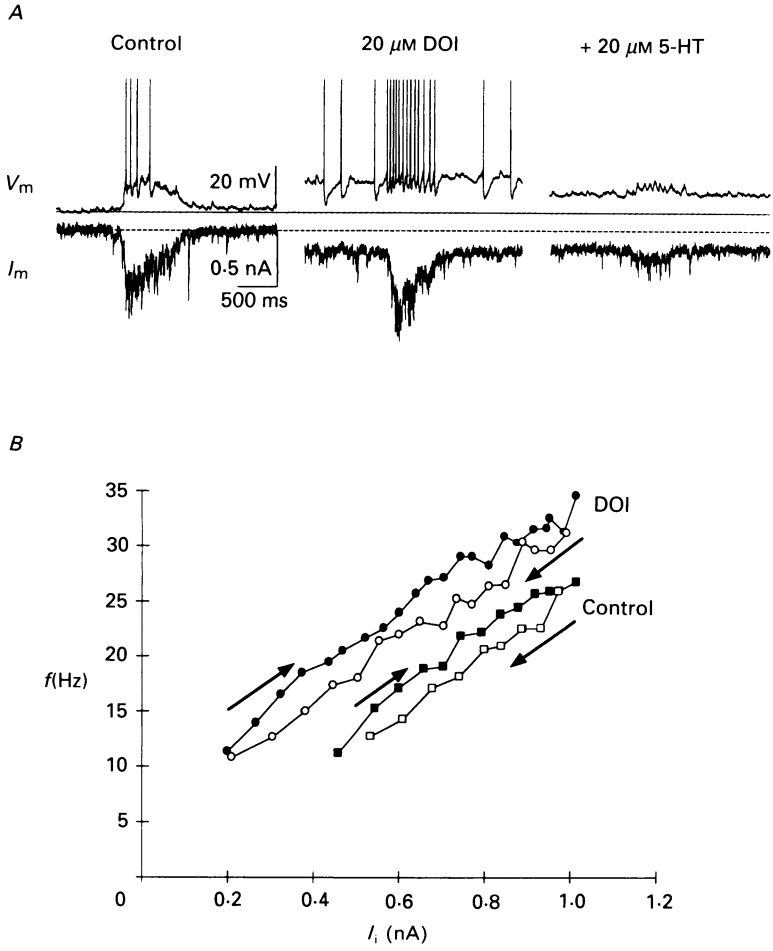

Fig. 11. Effect of DOI on phrenic motoneurone activity, membrane current and f–I relationship. *A*, current and voltage clamp recording of a phrenic motoneurone under control conditions, after exposure to 20 μM DOI, and after addition of 20 μM 5-HT (25 min after DOI). The difference in membrane potential between the DOI and 5-HT responses was due to the decay of the DOI response and not caused by the addition of 5-HT. V_m, membrane potential recorded during current clamp; I_m, membrane current recorded during voltage clamp; holding potential, −72 mV. *B*, relationship between injected current (I_i) and instantaneous firing frequency (f) under control conditions and following exposure to DOI. Filled symbols, values for rising phase of triangle; open symbols, values for falling phase of triangle.

DISCUSSION

The principal finding of our study was that exogenous application of 5-HT to phrenic motoneurones produced both an increase in excitability and a concurrent

reduction of inspiratory-modulated synaptic current. These two effects of 5-HT on phrenic motoneurones were mediated by distinctly different receptor subtypes. Drugs specific for the $5\text{-HT}_{2/1C}$ receptor selectively affected the increase in excitability, but neither mimicked nor blocked the decrease in inspiratory-modulated current produced by 5-HT. When the excitatory and inhibitory effects occurred simultaneously, the excitatory effect predominated, leading to an increase in motoneurone firing during the inspiratory burst. The reduction of synaptic current only affected the firing behaviour of phrenic motoneurones when the excitatory response to applied 5-HT was blocked by ketanserin.

Serotonin also has both excitatory and inhibitory effects on neurones in the hippocampus (Colino & Halliwell, 1987; Baskys, Niesen, Davies, & Carlen, 1989). The different effects are mediated by distinctly different 5-HT receptor subtypes acting on three separate potassium conductances (Andrade & Nicoll, 1987; Colino & Halliwell, 1987). In addition, 5-HT agonists can produce both facilitation (Jackson & White, 1990) and inhibition (Nagano, Ono & Fukuda, 1988) of the monosynaptic spinal reflex in rats. As is the case in the hippocampus, the inhibitory and excitatory effects of 5-HT on spinal reflexes appear to be mediated by distinctly different 5-HT receptor subtypes.

In immature rats, unidentified spinal lumbar motoneurones studied in slices also exhibit a range of responses to 5-HT which include depolarization, hyperpolarization and attenuation of dorsal root evoked excitatory and inhibitory postsynaptic potentials (Wu *et al.* 1991). In many cases, several of these responses appeared in the same cell (Wu *et al.* 1991). A heterogeneous response to applied 5-HT appears common throughout the nervous system.

Increase in phrenic motoneurone excitability

The increase in phrenic motoneurone excitability produced by 5-HT was characterized by enhanced firing in response to both synaptic (Fig. 3) and injected current (Fig. 4). The threshold for repetitive firing was reduced and the firing frequency for a given injected current was elevated by 5-HT. However, 5-HT did not produce any fundamental changes in the repetitive firing properties of phrenic motoneurones, i.e. there was no change in the slope of the *f–I* relationship and no indication of bistable firing behaviour. The increase in excitability was accompanied by a depolarization and an increase in input resistance, both of which persisted in the presence of TTX. Under voltage clamp conditions, 5-HT produced a tonic inward current that increased with depolarization.

A similar increase in excitability in the presence of 5-HT is seen in lamprey spinal motoneurones (Wallen, Buchanan, Grillner, Hill, Christenson & Hokfelt, 1989), human neocortical neurones (Lorenzon & Foehring, 1992), rat facial motoneurones (Larkman *et al.* 1989; Kelly, Larkman, Penington, Rainnie, McAllister-Williams & Hodgkiss, 1991) and neonatal rat cervical respiratory motoneurones (Morin, Monteau & Hilaire, 1991). In each case the response to synaptic or injected current is enhanced by 5-HT, without any indication of the bistable behaviour seen in cat (Hounsgaard *et al.* 1988) and turtle (Hounsgaard & Kiehn, 1989) spinal motoneurones. In rat facial and cervical respiratory motoneurones, the increase in excitability is also accompanied by a depolarization and an increase in input resistance. Serotonin also

produces a depolarization and an increase in input resistance in neonatal rat thoracolumbar motoneurones (Wang & Dun, 1990), hippocampal (Colino & Halliwell, 1987) and nucleus accumbens neurones (North & Uchimura, 1989).

In several cell types in which 5-HT produces a depolarization accompanied by an increase in input resistance, the phenomenon has been studied under voltage clamp conditions. In rat facial (Kelly *et al.* 1991) and thoracolumbar (Wang & Dun, 1990) motoneurones, 5-HT produces an inward current that increases with depolarizing holding potentials, as does the inward current seen in the present study. In these, and other studies (for example North & Uchimura, 1989), the inward current and its resultant depolarization are thought to be primarily due to a decrease in a resting potassium conductance. However, in rat lumbar (Wang & Dun, 1990) and facial (Larkman *et al.* 1989) motoneurones the depolarization produced by 5-HT is reduced at hyperpolarizing membrane potentials but does not reverse. This suggests that a change in another conductance with a more positive reversal potential is also involved.

We believe that the tonic inward current and resultant increase in excitability produced by 5-HT in phrenic motoneurones is also primarily due to a decrease in a resting potassium conductance. However, there is evidence that changes in other conductances are contributing to the inward current we observed. As in rat lumbar and facial motoneurones, the inward current in rat phrenic motoneurones decreased but did not reverse with hyperpolarizing membrane potentials. This suggests that an additional conductance(s) with a reversal potential positive from rest is contributing to the tonic inward current we observed.

In our experiments, the use of KCl electrodes would shift the chloride equilibrium potential to less negative values. Consequently, a reversed (outward) chloride current could contribute to the net inward current we observed. The mammalian 5-HT_{1C} receptor, when inserted into *Xenopus laevis* oocytes, mediates both an increase in a chloride conductance and a decrease in a potassium conductance (Paniker, Parker & Miledi, 1991). In addition, small transient inward currents that increased in amplitude with hyperpolarization were produced by 5-HT in some cells. The brief time course of these transient currents suggests that they may be synaptic in origin. Serotonin also enhances a calcium current in rat spinal motoneurones (Berger & Takahashi, 1990). Activation of any of these currents either directly, or through activation of transmitter release, could account for the failure to reverse the tonic inward current. Part of the response of rat phrenic motoneurones to 5-HT could also be mediated by an augmentation of a cationic current that activates slowly with hyperpolarization (I_h; Bobker & Williams, 1990). The presence of such a current (Takahashi, 1990) and its modulation by 5-HT (Takahashi & Berger, 1990) in neonatal rat lumbar spinal motoneurones has been described.

The increase in phrenic motoneurone excitability produced by 5-HT was mimicked by the 5-HT_2 receptor agonist DOI and blocked by the 5-HT_2 receptor antagonist ketanserin. This strongly implicates the 5-HT_2 receptor as the mediator of the increase in excitability. However, both of these drugs have significant affinity for the 5-HT_{1C} receptor, which is structurally and functionally very similar to the 5-HT_2 receptor (Pierce & Peroutka, 1989; Bobker & Williams, 1990). Activation of the 5-HT_2 receptor is associated with a decrease in a potassium conductance, while

activation of the 5-HT_{1C} receptor is associated with both a decrease in a potassium conductance and an increase in a chloride conductance (Paniker *et al.* 1991). Consequently, we consider it likely that the increase in excitability is produced by 5-HT acting at both 5-HT_2 and 5-HT_{1C} receptors. Several other investigators have suggested that similar increases in motoneurone excitability by 5-HT in other preparations may be at least partially produced by a 5-$HT_{2/1C}$-like receptor (Morin *et al.* 1990; Wang & Dun, 1990; Wallis *et al.* 1991).

Decrease in inspiratory-modulated current

There are two principal mechanisms by which 5-HT may cause a decrease in inspiratory-modulated synaptic current: (i) by decreasing the response of motoneurones to the endogenous neurotransmitter for inspiratory drive; or (ii) by decreasing the amount of neurotransmitter being released.

There are many examples of 5-HT-mediated postsynaptic inhibition in hippocampal (Colino & Halliwell, 1987), dorsal raphe (Rainnie, 1988) and lateral septal neurones (Joels, Shinnick-Gallagher & Gallagher, 1987) among others (for review, see Bobker & Williams, 1990). In these cells 5-HT produces a hyperpolarization, often accompanied by an increase in conductance. In rat phrenic motoneurones, 5-HT never produced a hyperpolarization or an increase in conductance, even when the excitatory response was blocked by ketanserin. Nonetheless, 5-HT could postsynaptically reduce the inspiratory drive current recorded at the soma in interfering with the propagation of current from distal excitatory synapses. This type of shunting of excitatory current could occur if a conductance increase is produced between the site of the excitatory synapse and the soma (Jack, Noble & Tsien, 1975; Vu & Krasne, 1992). This could be caused by either a direct action of 5-HT on phrenic motoneurones, or via the activation of inhibitory interneurones. It is possible that the small, transient inward currents produced by 5-HT in some cells may be reversed inhibitory synaptic currents. Consequently, despite the lack of any direct evidence of postsynaptic inhibition it would be premature to exclude it as a possible mechanism by which 5-HT decreases inspiratory-modulated current.

Serotonin inhibits the release of aspartate in rat cerebellar slices and synaptosomes (Maura, Barzizza, Folghera & Raiteeri, 1991) and inhibits both glutamate and GABA-evoked synaptic potentials in the rat locus ceruleus via presynaptic mechanisms (Bobker & Williams, 1989). It is also proposed that the inhibition by 5-HT of synaptic potentials in rat lumbar motoneurones is mediated by a presynaptic mechanism (Wu *et al.* 1991). Presynaptic modulation of transmission from bulbospinal inspiratory neurones to phrenic motoneurones has been suggested as the mechanism by which 2-amino-4-phosphonobutyric acid attenuates inspiratory-modulated synaptic current in phrenic motoneurones (Liu *et al.* 1990). However, at this time, the only reason to suspect a presynaptic mechanism for the attenuation of inspiratory-modulated current by 5-HT in phrenic motoneurones is the inability to establish a postsynaptic mechanism.

Both pre- and postsynaptic inhibition by 5-HT are associated with 5-HT_1 receptors. Generally, presynaptic inhibition by 5-HT is associated with activation of the 5-HT_{1B} receptor and postsynaptic inhibition is associated with the 5-HT_{1A} receptor (Colino & Halliwell, 1987; Bobker & Williams, 1989; Kelly *et al.* 1991; for

exception see Maura *et al.* 1991). However, in rat lumbar motoneurones, there is evidence that the 5-HT-induced attenuation of synaptic potentials is mediated by both 5-HT_{1A} and 5-HT_{1B} receptors acting presynaptically (Wu *et al.* 1991). In contrast, the 5-$HT_{2/1C}$ receptor has been implicated in the 5-HT-mediated attenuation of inspiratory-modulated activity in rat hypoglossal motoneurones (Morin, Monteau & Hilaire, 1992); the effect was mimicked by the 5-$HT_{2/1C}$ receptor agonist DOI and antagonized by the 5-$HT_{2/1C}$ receptor agonist ketanserin. The 5-HT receptor mediating the decrease in synaptic current in phrenic motoneurones cannot be identified at this time, but it is unlikely that it is a 5-HT_2 receptor as the effect was neither mimicked by DOI or blocked by ketanserin. Therefore, it appears that the response of phrenic motoneurones to 5-HT more closely resembles the response of other spinal motoneurones than the response of other respiratory motoneurones located outside of the spinal cord.

Functional implications

Stimulation of different nuclei within the raphe can produce either facilitation or inhibition of phrenic nerve activity that is at least partially mediated by 5-HT (Lalley, 1986*a*, *b*; Holtman, Dick & Berger, 1986, 1987). However, raphe nuclei stimulation produces similar changes in activity of medullary respiratory neurones concurrent with changes in phrenic nerve activity (Lalley, 1986a, b). Consequently, it is not clear if the changes in motoneurone activity are due to a direct effect on phrenic motoneurones or to an effect on respiratory neurones in the medulla. Studies of the effect of exogenously applied 5-HT on phrenic (Lalley, 1986*b*; Schmid *et al.* 1990) and high cervical respiratory motoneurones (Morin *et al.* 1991) have all reported an augmentation of inspiratory-modulated activity. Our observation that 5-HT can produce both an increase in excitability and a decrease in inspiratory-modulated synaptic current demonstrates that mechanisms exist by which both the excitatory and inhibitory effect of raphe nuclei stimulation on phrenic nerve activity could be mediated at the level of the spinal cord (Lalley, 1986*a*, *b*; Holtman *et al.* 1986, 1987).

In addition, 5-HT modulated the response of phrenic motoneurones to inspiratory-modulated inputs via mechanisms that are independent of those that would modulate phrenic motoneurone excitability to other inputs. Under our experimental conditions, exogenous application of 5-HT to the spinal cord always produced concurrent excitatory and inhibitory effects. This may not occur during the endogenous release of 5-HT. The central nervous system may employ more specific release mechanisms to activate these different effects in isolation, enabling precise control of ventilation under changing physiological conditions.

The release of 5-HT in the spinal cord may not be limited to conventional synapses; it may be released non-synaptically (Leger & Descarries, 1978). Serotonergic fibres form a dense plexus throughout the ventral horn of the cervical spinal cord of the rat, including within the dense dendritic bundles formed by phrenic motoneurones (Zhan, Ellenberger & Feldman, 1989; Lindsay *et al.* 1990). This could provide an anatomical substrate for non-localized release of 5-HT in the phrenic nucleus. If such a release occurred, a concurrent increase in phrenic motoneurone excitability and decrease in inspiratory current may result. The reduction of inspiratory-modulated current may serve as an important protective mechanism

against overstimulation of the diaphragm by the predominantly excitatory effects of 5-HT on spinal motoneurones. Such a mechanism could be critical following moderate trauma to the cervical spinal cord, which causes a dramatic elevation of extracellular 5-HT concentration (Salzman, Hirufuji, Llados-Eckman, MacEwen & Beckman, 1987). Without some mechanism to protect phrenic motoneurones from being overwhelmed by the increase in excitability that 5-HT can produce, the ability to maintain respiratory homeostasis would be compromised.

We thank Dr Aharon A. Lev-Tov for his critical review of an earlier version of this manuscript. This work was supported by NIH grant NS24742. A. D. L. was a recipient of a National Research Service Award (NS 08530) and a fellowship from the Muscular Dystrophy Association.

REFERENCES

ANDRADE, R. & NICOLL, R. A. (1987). Pharmacologically distinct actions of serotonin on single pyramidal neurones of the hippocampus recorded *in vitro*. *Journal of Physiology* **394**, 99–124.

AXMITIA, E. C. & WHITAKER-AXMITIA, P. M., (1991). Awakening the sleeping giant: anatomy and plasticity of the brain serotonergic system. *Journal of Clinical Psychiatry* **52**, suppl., 4–16.

BASKYS, A., NIESEN, C. E., DAVIES, M. F. & CARLEN, P. L. (1989). Modulatory actions of serotonin on ionic conductances of hippocampal dentate granule cells. *Neuroscience* **29**, 443–451.

BERGER, A. & TAKAHASHI, T. (1990). Serotonin enhances a low-voltage-activated calcium current in rat spinal motoneurones. *Journal of Neuroscience* **10**, 1922–1928.

BOBKER, D. H. & WILLIAMS, J. T. (1989). Serotonin agonists inhibit synaptic potentials in the rat locus ceruleus *in vitro* via 5-hydroxytryptamin$_{1A}$ and 5-hydroxytryptamin$_{1B}$ receptors. *Journal of Pharmacology and Experimental Therapeutics* **250**, 37–43.

BOBKER, D. H. & WILLIAMS, J. T. (1990). Ion conductances affected by 5-HT receptor substypes in mammalian neurons. *Trends in Neurosciences* **13**, 169–173.

COLINO, A. J. & HALLIWELL, J. V. (1987). Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature* **328**, 73–77.

HICKS, T. P., KRUPA, M. & CREPEL, F. (1989). Selective effects of serotonin upon excitatory amino acid-induced depolarizations of Purkinje cells in cerebellar slices from young rats. *Brain Research* **492**, 371–376.

HOLTMAN, J. R. JR, DICK, T. E. & BERGER, A. J. (1986). Involvement of serotonin in the excitation of phrenic motoneurones evoked by stimulation of the raphe obscurus. *Journal of Neuroscience* **6**, 1185–1193.

HOLTMAN, J. R. JR, DICK, T. E. & BERGER, A. J. (1987). Serotonin-mediated excitation of recurrent laryngeal and phrenic motoneurons evoked by stimulation of the raphe obscurus. *Brain Research* **417**, 12–20.

HOUNSGAARD, J., HULTBORN, H., JESPERSEN, B. & KIEHN, O. (1988). Bistability of α-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *Journal of Physiology* **405**, 345–367.

HOUNSGAARD, J. & KIEHN, O. (1989). Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. *Journal of Physiology* **414**, 265–282.

JACK, J. J. B., NOBLE, D. & TSIEN, R. W. (1975). *Electric Current Flow in Excitable Cells*, pp. 197–213. Oxford University Press, Oxford.

JACKSON, D. A. & WHITE, S. R. (1990). Receptor subtypes mediating facilitation by serotonin of excitability of spinal motoneurons. *Neuropharmacology* **29**, 787–797.

JACOBS, B. L. (1991). Serotonin and behaviour: emphasis on motor control. *Journal of Clinical Psychiatry* **52**, suppl., 17–23.

JOELS, M., SHINNICK-GALLAGHER, P. & GALLAGHER, J. P. (1987). Effect of serotonin and serotonin analogs on passive membrane properties of lateral septal neurons *in vitro*. *Brain Research* **417**, 99–107.

KELLY, J. S., LARKMAN, P., PENINGTON, N. J., RAINNIE, C. G., MCALLISTER-WILLIAMS, H. & HODGKISS, J. (1991). Serotonin receptor heterogeneity and the role of potassium channels in neuronal excitability. *Advances in Experimental Medicine and Biology* **287**, 177–191.

KIEHN, O. (1991). Plateau potentials and active integration in the 'final common pathway' for motor behaviour. *Trends in Neurosciences* **14**, 71–76.

LALLEY, P. M. (1986*a*). Responses of phrenic motoneurones of the cat to stimulation of medullary raphe nuclei. *Journal of Physiology* **380**, 349–371.

LALLEY, P. M. (1986*b*). Serotonergic and non-serotonergic responses of phrenic motoneurones to raphe stimulation in the cat. *Journal of Physiology* **3780**, 373–385.

LARKMAN, P. M., PENINGTON, N. J. & KELLY, J. S. (1989). Electrophysiology of adult rat facial motoneurones: the effects of serotonin (5-HT) in a novel *in vitro* brainstem slice. *Journal of Neuroscience Methods* **28**, 133–146.

LEGER, L. & DESCARRIES, L. (1978). Serotonin nerve terminals in the locus ceruleus of adult rat: a radioautographic study. *Brain Research* **145**, 1–13.

LINDSAY, A. D., GREER, J. J. & FELDMAN, J. L. (1991). Phrenic motoneuron morphology in the neonatal rat. *Journal of Comparative Neurology* **308**, 169–179.

LINDSAY, A. D., SCHWARTZ, N. & FELDMAN, J. L. (1990). Serotonin in the cervical spinal cord of neonatal rat. *Society for Neuroscience Abstracts* **16**, 73.

LIU, G., FELDMAN, J. L. & SMITH, J. C. (1990). Excitatory amino acid mediated transmission of inspiratory drive to phrenic motoneurons. *Journal of Neurophysiology* **64**, 423–436.

LORENZON, N. M. & FOEHRING, R. C. (1992). Relationship between repetitive firing and afterhyperpolarizations in human neocortical neurons. *Journal of Neurophysiology* **67**, 350–363.

MAURA, G., BARZIZZA, A., FOLGHERA, S. & RAITEERI, M. (1991). Release of endogenous aspartate from rat cerebellar slices and synaptosomes: inhibition mediated by a 5-HT_2 receptor and by a 5-HT_1 receptor of a possibly novel subtype. *Naunyn-Schmeideberg's Archives of Pharmacology* **343**, 229–236.

MORIN, D., HENNEQUIN, S., MONTAU, R. & HILAIRE, G. (1990). Serotonergic influences on central respiratory activity: an *in vitro* study in the newborn rat. *Brain Research* **535**, 281–287.

MORIN, D., MONTEAU, R. & HILAIRE, G. (1991). Serotonin and cervical respiratory motoneurones: intracellular study in the newborn rat brainstem–spinal cord preparation. *Experimental Brain Research* **84**, 229–232.

MORIN, D., MONTEAU, R. & HILAIRE, G. (1992). Compared effects of serotonin on cervical and hypoglossal inspiratory activities: an *in vitro* study of the newborn rat. *Journal of Physiology* **451**, 605–629.

NAGANO, N., ONO, H. & FUKUDA, H. (1988). Functional significance of subtypes of 5-HT receptors in the rat spinal reflex pathway. *General Pharmacology* **19**, 789–793.

NORTH, R. A. & UCHIMURA, N. (1989). 5-Hydroxytryptamine acts at 5-HT_2 receptors to decrease potassium conductance in rat nucleus accumbens neurones. *Journal of Physiology* **417**, 1–12.

PANIKER, M. M., PARKER, I. & MILEDI, R. (1991). Receptors of the serotonin 1C subtype expressed from cloned DNA mediate the closing of K^+ membrane channels encoded by brain mRNA. *Proceedings of the National Academy of Sciences of the USA*, **88**, 2560–2562.

PIERCE, P. A. & PEROUTKA, S. J. (1989). The 5-hydroxytryptamine receptor families. *Seminars in the Neurosciences* **1**, 145–153.

RAINNIE, D. G. (1988). The biophysical and pharmacological properties of presumptive serotinergic neurones recorded intracellularly from the dorsal raphe nucleus in the *in vitro* slice preparation. PhD Thesis, University of Edinburgh.

SALZMAN, S. K., HIROFUJI, E., LLADOS-ECKMAN, C., MACEWAN, G. D. & BECKMAN, A. L. (1987). Monoaminergic responses to spinal trauma. *Journal of Neurosurgery* **66**, 431–439.

SCHMID, K., BOHMER, G, & MERKELBACH, S. (1990). Serotonergic control of phrenic motoneuronal activity at the level of the spinal cord of the rabbit. *Neuroscience Letters* **116**, 204–209.

SMITH, J. C. & FELDMAN, J. L. (1987). *In vitro* brainstem–spinal cord preparations for study of motor systems for mammalian respiration and locomotion. *Journal of Neuroscience Methods* **21**, 321–333.

TAKAHASHI, T. (1990). Inward rectification in neonatal rat spinal motoneurones. *Journal of Physiology* **423**, 47–62.

TAKAHASHI, T. & BERGER, A. J. (1990). Direct excitation of rat spinal motoneurones by serotonin. *Journal of Physiology* **423**, 63–76.

VU, E. T. & KRASNE, F. B. (1992). Evidence for a computational distinction between proximal and distal neuronal inhibition. *Science* **255**, 1710–1772.

WALLEN, P., BUCHANAN, J. T., GRILLNER, S., HILL, R. H., CHRISTENSON, J. & HOKFELT, T. (1989). Effects of 5-hydroxytryptamine on the afterhyperpolarization, spike frequency regulation and oscillatory membrane properties in lamprey spinal cord neurons. *Journal of Neurophysiology* **61**, 759–768.

WALLIS, D. I., CONNELL, L. A. & KVALTINOVA, Z. (1991). Further studies on the action of 5-hydroxytryptamine on lumbar motoneurones in the rat isolated spinal cord. *Naunyn-Schmiedeberg's Archives of Pharmacology* **343**, 344–352.

WANG, M. Y. & DUN, N. J. (1990). 5-Hydroxytryptamine responses in neonate rat motoneurones *in vitro*. *Journal of Physiology* **430**, 87–103.

WU, S. Y., WANG, M. Y. & DUN, N. J. (1991). Serotonin via presynaptic 5-HT_1 receptors attenuates synaptic transmission to immature rat motoneurons *in vitro*. *Brain Research* **554**, 111–121.

ZHAN, W.-Z, ELLENBERGER, H. H. & FELDMAN, J. L. (1989). Monoaminergic and gabaergic terminations in phrenic nucleus of rat identified by immunohistochemical labelling. *Neuroscience* **31**, 105–113.

ZHANG, L. (1991). Effects of 5-hydroxytryptamine on cat spinal motoneurons. *Canadian Journal of Physiology and Pharmacology* **69**, 154–163.