5-HT-1A receptor-mediated modulation of medullary expiratory neurones in the cat

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1. The involvement of the 5-HT-1A receptor in serotoninergic responses of stage 2 expiratory (E-2) neurones was investigated in pentobarbitone-anaesthetized, mechanically ventilated cats.

2. The specific agonist of the 5-HT-1A receptor, 8-hydroxy-diproplaminotetralin (8-OH-DPAT), administered systemically or by ionophoresis directly on to the neurones, had a clear depressant effect.

3. Administration of 8-OH-DPAT at doses of 10–50 $\mu g\ kg^{-1}$ (I.V.) increased the membrane hyperpolarizations of E-2 neurones during the inspiratory and postinspiratory phases, and shortened their duration of activity in association with shortening of phrenic nerve activity. Discharges of E-2 neurones were also less intense. At doses of 50–90 $\mu g\ kg^{-1}$, 8-OH-DPAT reduced or abolished inspiratory hyperpolarizations, and reduced expiratory depolarizations of membrane potential and discharge in parallel with inhibition of phrenic nerve discharges. The effects of the larger doses were reversed by I.V. injection of NAN-190, an antagonist at the 5-HT-1A receptor.

4. Dose-dependent effects on the membrane potential and discharge of E-2 neurones, but not on phrenic nerve activity, were also seen by ionophoretic administration of 8-OH-DPAT on to E-2 neurones. At low currents, ejection of 8-OH-DPAT hyperpolarized the neurones without affecting the duration of inspiratory hyperpolarization and expiratory depolarization. This hyperpolarization depressed the intensity and the duration of expiratory discharges. Ejection with larger currents hyperpolarized the E-2 neurones further, and depressed expiratory depolarization leading to blockade of expiratory discharges.

5. The effects on membrane potential were accompanied by decreased neuronal input resistance. This depressed the excitability of E-2 neurones as tested by discharges evoked by intracellular current injection. The amplitudes of action potentials decreased in parallel with the changes in input resistance. The effects were attributed to a postsynaptic effect of 8-OH-DPAT leading to a gradually developing inhibition by activation of 5-HT-1A receptors.

6. Hyperventilatory apnoea depressed on-going synaptic activity and unmasked the effect of ionophoretically applied 8-OH-DPAT. The responses of the E-2 neurone were enhanced, as evidenced by increased membrane hyperpolarization and greater reduction of input resistance. Both responses faded appreciably, indicating receptor desensitization. The degree and rate of apparent desensitization depended on the dose/ejecting current. The greater sensitivity and faster desensitization to 8-OH-DPAT were attributed to the hyperventilatory alkalinization of the extracellular fluid, which might influence agonist binding to 5HT-1A receptors and/or receptor properties.

There is a growing body of evidence that serotonin (5-hydroxytryptamine, 5-HT) is involved in the central nervous control of respiration. Serotoninergic neurones originating in the medullary raphe complex and rostroventrolateral medulla send axons to many regions of the medulla and spinal cord to terminate directly at or as close appositions to neurones which subserve various physiological functions (Vandermaelin, 1985; Millhorn, Hökfelt, Seroogy & Verhofstad, 1988; Connelly, Ellenberger & Feldman, 1989). Serotoninergic neurones of

the raphe complex show on-going respiratory-modulated discharge activity (Lindsey, Hernandez, Morris & Shannon, 1992). There is feedback within the raphe complex itself and anatomical studies indicate that there is serotoninergic control of the respiratory network within the medulla (Maley & Eide, 1982; Jiang & Shen, 1985; Holtman, 1988; Voss, DeCastro, Lipsky, Pilowsky & Jiang, 1990).

The described effects of 5-HT are complex and the underlying mechanisms are as yet poorly understood. Electrical or chemical stimulation of the medullary raphe complex produces either excitation or inhibition of respiratory neurones, the effect being dependent on the site of stimulation within the raphe complex (Holtman, Anastasi, Norman & Dretchen, 1986; Holtman, Dick & Berger, 1986; Lalley, 1986*a*; Millhorn, 1986). When 5-HT analogues are applied systemically, intraventricularly or extracellularly, the discharge of bulbar respiratory neurones is mostly depressed in a dose-dependent fashion (Armijo, Mediavilla & Florez, 1979; Lalley, 1982; McCrimmon & Lalley, 1982), whereas synaptically coupled cranial motoneurones are generally activated (Berger, Bayliss & Viana, 1992; Kubin, Tojima, Davies & Pack, 1992). In addition, ionophoresis of 5-HT near to respiratory neurones has similarly diverse effects (Böhmer, Dinse, Fallert & Sommer, 1979; Champagnat, Denavit-Saubie, Henry & Leviel, 1979; Arita & Ochiishi, 1991). The data indicate that the 5-HT effects are most probably mediated by more than one type of 5-HT regulatory mechanism and point to the functional significance of various subtypes of 5-HT receptors.

Further investigations are therefore required to understand the mechanisms activated by 5-HT. Such studies of 5-HT influences on respiratory neurones have to distinguish between presynaptic and postsynaptic actions, the former influence probably being widespread to bring about a generalized depression or excitation of the respiratory network, the latter postsynaptic responses to 5-HT being diverse and dependent on the set of 5-HT receptors being expressed in individual neurones. Different subtypes of 5-HT receptor have been identified in the central nervous system using agonists and antagonists with high selectivity for specific receptor subtypes which differentially influence neuronal excitability (Anwyl, 1990). Similar techniques can be applied to identify the subtypes of 5-HT receptors, and to determine their relative numbers and distribution in respiratory neurones. The effect of 5-HT receptor activation on on-going changes of ionic conductances and fluctuations of the membrane potential which finally establish the rhythm of respiration (Richter, 1982; Richter, Ballantyne & Remmers, 1986) can therefore be investigated in order to understand the functional significance of the serotoninergic innervation of respiratory neurones.

In the present study, we have analysed, at the cellular and network level, the effects of 8-hydroxy-dipropyl-aminotetralin (8-OH-DPAT), a specific agonist, and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine (NAN-190), an antagonist of the 1A subtype of 5-HT receptor. 8-OH-DPAT was applied directly on to identified neurones to determine the postsynaptic effects of 5-HT-1A receptor activation, and also administered intravenously to observe the general effects of the modified network function on single respiratory neurone behaviour as well as on the respiratory output in phrenic nerves.

METHODS

Anaesthesia and preparation

Adult cats of either sex (2·5–5·0 kg) were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} I.P., initial dose) followed by supplementary I.V. doses of 4–12 mg as required if blood pressure, phrenic nerve activity and/or respiratory frequency increased spontaneously or in response to nociceptive test stimuli. Atropine sulphate (0·1–0·2 mg kg^{-1} I.V.) was given to reduce salivation, and dexamethasone (0·2 mg kg^{-1} I.M.) was administered to prevent brain oedema. Prior to electrode recordings, intravenous doses of gallamine triethiodide (4–8 mg kg^{-1} initially, followed by 4–8 mg h^{-1}) were administered for muscular paralysis. Animals were then mechanically ventilated with oxygen-enriched air through a tracheal cannula. A pneumothorax was established bilaterally to prevent respiratory movements and, therefore, improve stability of central recordings. Atelectasis was prevented by applying 1–2 cmH_2O end-expiratory pressure to the expiratory outflow. Ventilatory volume and rate were adjusted to maintain an end-tidal CO_2 level at 3–5%. Cannulae were placed in one femoral artery and both femoral veins for monitoring arterial blood pressure and for drug delivery, respectively. Body temperature was maintained between 36 and 38 °C by external heating devices. Experiments were terminated by intravenous injection of sodium pentobarbitone sufficient to produce irreversible cardiac arrest.

The cat's head was mounted rigidly in a head holder and the spinous processes of T1 and L5 were clamped in a spinal frame to suspend the animal. The head was ventroflexed to allow optimal exposure of the dorsal surface of the medulla by occipital craniotomy. Phrenic (C5 branches) and cervical vagus nerves were exposed bilaterally through a dorsal approach and were sectioned. The central ends of the phrenic nerves were desheathed and mounted on bipolar silver hook electrodes for recording. They were then covered with a mixture of vaseline and paraffin oil. The spinal cord was exposed by laminectomy from C2 to C4 for bilateral stimulation of the reticulospinal tracts with an array of four bipolar concentric steel electrodes. After these electrodes were placed, the spinal cord was covered with agar dissolved in Ringer solution. Occipital craniotomy and cutting of the dura exposed the medulla. The arachnoid membrane was removed from the medulla and the pia was opened at the site of recordings to allow insertion of fine-tipped microelectrodes. Up to three pressure feet were placed gently on the surface of the medulla, one over the site of microelectrode insertion, the others on the rostral and caudal parts of the medulla to increase stability of intracellular recordings.

Stimulation and recording procedures

The reticulospinal tracts at C2–C3 were stimulated with single shocks (30–70 V, 0·1 ms) in order to identify bulbospinal expiratory neurones by antidromic excitation and collision of

action potentials.

Phrenic nerve activity was recorded with bipolar silver hook electrodes, amplified (2000–10000×), band-pass filtered (80–3000 Hz), displayed on an oscilloscope and registered on magnetic tape (Racal; frequency response, DC–5 kHz) and chart recorder (Gould TA 2000) as direct discharges and moving averages of the rectified discharge (0·01–0·1 s time constant).

Intracellular recordings were obtained from thirty-one expiratory neurones (E-2 neurones) that revealed an augmenting discharge pattern during the stage 2 expiratory interval, which was identified by the silent period in phrenic nerve activity (for identification see: Ballantyne & Richter, 1986). E-2 neurones of the ventral respiratory group (VRG) were found at a level extending from obex to approximately 2 mm caudal to the obex. Five of seven E-2 neurones tested had reticulospinal projections. Recordings were made with micropipettes filled with 3 M KCl and had a DC resistance 40–70 MΩ when measured in brain tissue. Membrane potentials were recorded with an intracellular amplifier (Axoclamp 2A; frequency response, DC–5 kHz) equipped with bridge balance and capacity compensation circuits. Membrane potentials were recorded in DC mode, amplified (10×), displayed on an oscilloscope and registered on a chart recorder.

Arterial blood pressure, tracheal pressure and logic pulses signalling ejecting currents passed through ionophoresis barrels were also registered on the chart recorder and stored on magnetic tape.

Ionophoresis

8-OH-DPAT was applied ionophoretically from extracellular micropipette assemblies to E-2 neurones during intracellular recording. The assemblies were constructed using a modified version of the method described by Oliver (1971). Three-barrel pipettes (3B120F-6, World Precision Instruments, USA) were pulled and broken back to a total diameter of about 3 μm. Single micropipettes for intracellular recording were pulled and then bent over a heating coil to an angle of 20–30 deg at a point 3–5 mm from the tip. The single- and three-barrel pipettes were positioned in parallel under microscopic observation so that the shaft of the intracellular electrode fitted into a groove formed by two of the ionophoresis barrels. The single and three-barrel electrodes were cemented together with light-cured dental adhesive and the upper shafts of the single- and three-barrel pipettes were encased in cranioplast cement. The recording barrel was filled with 3 M KCl, ionophoresis barrels were filled with 8-OH-DPAT (40 mM) and γ-aminobutyric acid (GABA, 500 mM) in bidistilled water (pH 4). Drugs were ejected with cationic currents applied from a programmable ionophoresis unit (Polder, FRG). Anionic retaining currents (20 nA) were applied to prevent leakage between ejection periods.

Experimental protocol

E-2 neurones in the caudal VRG were first located with single micropipettes for intracellular recording. Several neurones were recorded before ionophoresis or I.V. administration of 8-OH-DPAT in order: (i) to locate a relatively dense pool of neurones; (ii) to assess and, when necessary, to improve conditions for stable intracellular recording; and (iii) to determine control patterns of on-going synaptic activity. Single pipettes were then replaced by compound micropipette assemblies for intracellular recording and extracellular ionophoresis. Before drug testing, seven E-2 neurones were tested for evidence of bulbospinal projection by stimulating the reticulospinal tracts with single shocks. E-2 neurones were accepted for testing of 8-OH-DPAT if they exhibited stable membrane potentials of at least −50 mV during the inspiratory phase. Results were considered unacceptable and pipette assemblies were replaced if: (i) membrane potential changed in the depolarizing direction directly with the switching on of cationic ejecting currents; (ii) voltage noise increased during ionophoretic current flow; and (iii) neurones failed to hyperpolarize during ionophoresis of GABA, since this inhibitory amino acid is known consistently to inhibit all types of respiratory neurones (Lalley, 1986*b*, *c*; Haji, Remmers, Connelly & Takeda, 1990). In cases in which E-2 neurones failed to respond to GABA as well as 8-OH-DPAT, microscopic examination of the electrodes revealed that there was a separation greater than 100 μm of the three-barrel pipettes from single pipettes.

Responses to I.V. administration of 8-OH-DPAT were recorded either with single micropipettes or with compound phoresis micropipette assemblies after the responses to ionophoretic administration had been tested. Intravenous injection of 8-OH-DPAT produced arterial hypotension, which occasionally made it necessary to maintain a steady level of blood pressure with slow intravenous infusion of noradrenaline (40 μg ml^{-1}) in glucose–Ringer solution. We assumed that this did not affect the neuronal effects of 8-OH-DPAT ionophoresis, as it is generally accepted that noradrenaline administered systemically does not pass the blood–brain barrier. This was confirmed by our observation that 8-OH-DPAT effects remained unchanged when tested before and after noradrenaline injections. NAN-190 was administered I.V. in concentrations of 100 μg ml^{-1} dissolved in Ringer solution.

Results are expressed as means ± S.E.M.

RESULTS

Findings reported here were obtained from twenty experiments. The effects of systemic application of 8-OH-DPAT on the membrane potential and membrane properties of E-2 neurones ($n = 11$), phrenic nerve activity and blood pressure were recorded in eleven experiments. In nine experiments, the cellular responses to ionophoresis of 8-OH-DPAT were measured in twenty E-2 neurones, two of which were additionally tested for their response to I.V. application of 8-OH-DPAT.

Effects of systemic administration of 8-OH-DPAT on phrenic nerve activity and E-2 neurones

Intravenous administration of 8-OH-DPAT produced effects on phrenic nerve activity which were dose dependent. Doses ranging from 10 to 50 μg kg^{-1} increased the frequency and shortened the duration of the augmenting inspiratory burst discharges in the phrenic nerve (Figs 1*B* and 2*B*). The initial rate of augmentation of the ramp-like inspiratory patterns was not significantly altered, but the earlier termination of the inspiratory discharge prevented compound action potential frequency of phrenic nerves from reaching the peak late-inspiratory

levels recorded under control conditions. Higher doses of 8-OH-DPAT (50–90 μg kg^{-1}) led to either a disappearance of all phrenic nerve activity or to a continuous low level discharge. Complete recovery from the low dose effects occurred after 20–45 min, whereas 60–120 min were required for recovery from the higher range of doses. Mean arterial blood pressure fell rapidly (42 $\pm$ 8·8 mmHg) following intravenous injection of 8-OH-DPAT. The effects of 8-OH-DPAT on respiratory neural discharges, however, were not attributable to hypotension, since elevation of arterial blood pressure to control levels by noradrenaline infusion did not alter the effect of 8-OH-DPAT on respiratory activity.

In this study, E-2 neurones were analysed whose membrane potential fluctuations revealed the typical synaptic behaviour described in detail in earlier studies (Mitchell & Herbert, 1974; Ballantyne & Richter, 1986). Periodic synaptic inhibition brought the membrane potential to maximal hyperpolarized levels at the beginning of phrenic nerve activity (inspiration) and then the membrane potential depolarized again as phrenic nerve discharge progressed (Fig. 1*A*). At the termination of inspiration, the membrane potential started to depolarize more rapidly, but then depolarization was slowed appreciably during the postinspiratory after-discharge of the phrenic nerve (postinspiration) by a second declining pattern of synaptic inhibition until the threshold for action potential discharge was reached during the stage 2 expiratory phase, when phrenic nerve activity was absent.

The membrane potential behaviour (Figs 1*A* and 2*A*) was altered by intravenous injection of 8-OH-DPAT. Doses which increased the frequency and shortened the duration of phrenic nerve discharges (10–50 μg kg^{-1}) increased the maximal membrane hyperpolarization by 3–10 mV (4·9 $\pm$ 2·3 mV; Fig. 2*B*), and slowed the rate of depolarization during the postinspiratory phase (Fig. 1*B*). As a consequence, firing level was not reached (Fig. 2*B*) or passed only late during expiration, resulting in a relative shortening of the burst discharge of action potentials (Fig. 1*B*).

Larger doses of 8-OH-DPAT (50–90 μg kg^{-1}, $n = 3$) eliminated respiratory-related fluctuations of membrane potential as well as phrenic nerve activity. With the loss of phrenic nerve activity, the membrane potential of the E-2 neurone stabilized at an intermediate level between the maxima of inspiratory hyperpolarization and expiratory depolarization as determined under control conditions.

The effects of 8-OH-DPAT could be antagonized in five cases by intravenous injection of NAN-190 (20 μg kg^{-1}), a 5-HT-1A receptor blocking agent (Glennon, Naiman, Pierson, Titlek, Lyon & Weisberg, 1988). An example of its antagonistic action is shown in Fig. 2*C–E*. Rhythmic phrenic nerve activity reappeared and the membrane potential of E-2 neurones started to fluctuate rhythmically,

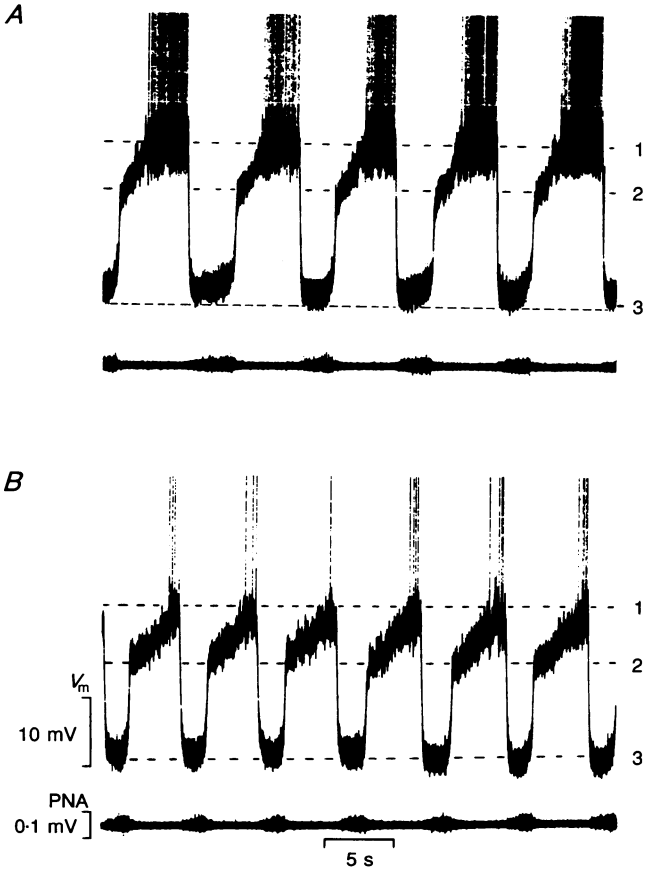

Figure 1. Effects of I.V. injection of 8-OH-DPAT on membrane potential (V_m) of a late expiratory (E-2) neurone and phrenic nerve activity (PNA)
A, control records. *B*, 3 min after I.V. injection of 8-OH-DPAT, 40 μg kg^{-1}. Reference (dashed) lines 1 and 2 are visual aids to show the slowed rate of depolarization during the postinspiratory period. Line 3 is a reference for maximum (inspiratory) membrane potential during control recordings and shows further inspiratory hyperpolarization produced by 8-OH-DPAT.

revealing all typical patterns of postsynaptic activity, i.e. declining early-inspiratory inhibition and declining postinspiratory inhibition (Fig. 2*C*).

Responses of E-2 neurones to ionophoretic administration of 8-OH-DPAT

The behaviour of E-2 neurones was influenced in several ways by ionophoretically administered 8-OH-DPAT, although phrenic nerve activity remained constant. These effects were dose dependent, i.e. they varied with the amount of 8-OH-DPAT ejected by various current intensities.

During ejection of 8-OH-DPAT with lower current intensities (20–50 nA cationic current; see Fig. 3*B*), the membrane potential of E-2 neurones was only slightly hyperpolarized. This became evident in a stronger hyperpolarization during inspiration and a longer delay until firing levels were reached. Consequently, the duration of expiratory discharge was shortened and maximal expiratory firing frequency was reduced. However, the discharge still had an augmenting pattern. Ejection of 8-OH-DPAT with larger current intensities, ranging between 70 and 100 nA (Fig. 3*C*), produced a steady membrane hyperpolarization of E-2 neurones and a significant decrease of the central respiratory drive potentials, consisting of inspiratory hyperpolarization and expiratory depolarization. This steady membrane hyperpolarization ($8{\cdot}6 \pm 0{\cdot}92$ mV; $n = 20$) and decrease of the periodic respiratory drive potentials ($4{\cdot}1 \pm 1{\cdot}9$ mV; $n = 20$) were responsible for the failure of the membrane

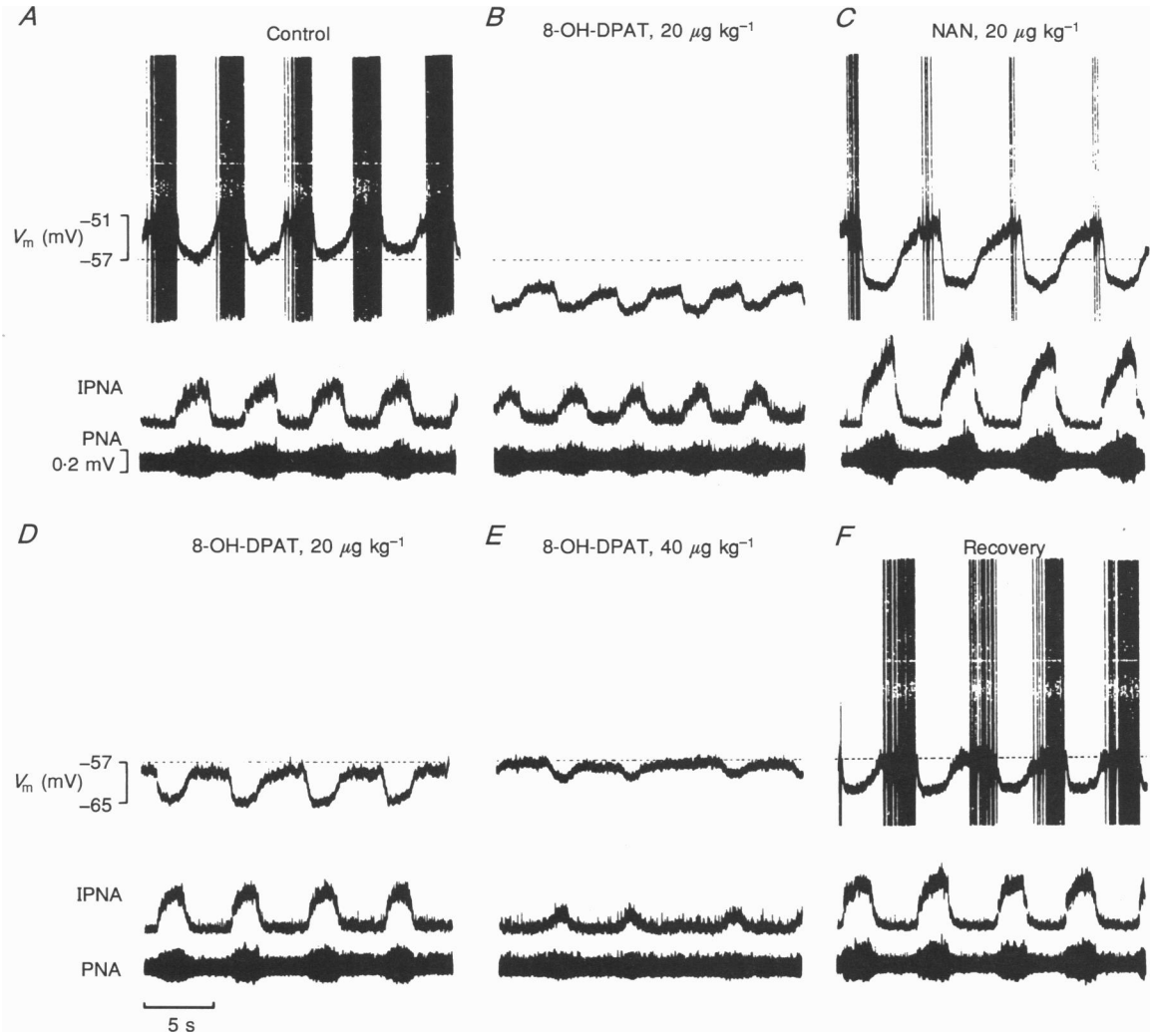

Figure 2. Effects of 8-OH-DPAT I.V. on phrenic nerve discharge and the activity of an E-2 neurone which were antagonized by NAN-190

A–F show membrane potential (V_m) of the E-2 neurone and phrenic nerve activity shown as AC recordings (PNA) and in integrated form (IPNA). Control records are shown in *A*. Records in *B* were obtained after injection of 8-OH-DPAT, 20 μg kg^{-1}. Injection of NAN-190 (20 μg kg^{-1}, I.V.) antagonized the effect of 8-OH-DPAT. The antagonistic effect of NAN-190 persisted and significantly depressed the effects of a second dose of 20 μg kg^{-1} 8-OH-DPAT applied 5 min later (*D*). An additional dose of 20 μg kg^{-1} 8-OH-DPAT given after 6 min (*E*) was necessary to provoke a significant depression of respiratory activities.

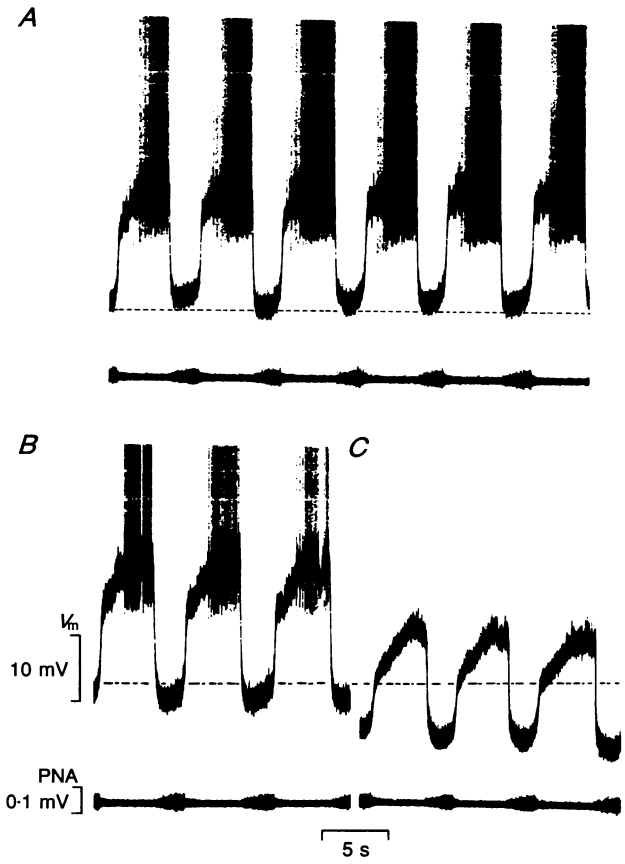

Figure 3. Effects of ionophoretic administration of 8-OH-DPAT on membrane potential and discharge properties of an E-2 neurone
Control records are shown in *A*. *B* and *C* show effects during ionophoresis of 8-OH-DPAT with two different ejecting currents: 50 nA in *B*, 100 nA in *C*. Both records are taken 30 s after the start of ionophoresis.

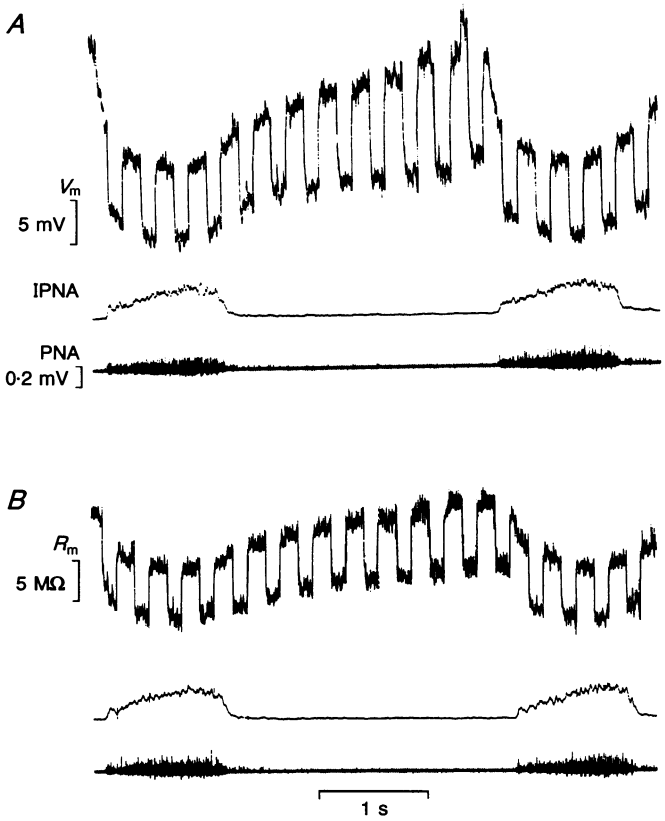

Figure 4. Effects of ionophoretic administration of 8-OH-DPAT on membrane potential (V_m) and neuronal input resistance (R_m) of an E-2 neurone
Hyperpolarizing current pulses (1 nA, 160 ms) were injected from the intracellular recording electrode to provide measures of R_m. Control records of V_m, phrenic nerve activity (PNA) and integrated phrenic nerve activity (IPNA) are shown in *A*. *B* shows records obtained during ionophoresis of 8-OH-DPAT (35 nA ejecting current). A calibration scale for R_m is shown in *B*.

potential to reach firing threshold. The results of Fig. 3 are representative of the effects of 8-OH-DPAT on the twenty E-2 neurones tested, although several neurones were not as strongly depressed as shown in Fig. 3*C* with currents up to 100 nA. Steady membrane hyperpolarization and depression of discharges began 15–60 s after the onset of ejecting current (28 ± 5·8 s), depending on current intensity. Recovery to control behaviour occurred gradually, requiring 30–150 s after terminating ejection (60 ± 10·8 s).

Tests performed on five neurones revealed that 8-OH-DPAT also significantly reduced neuronal input resistance in association with hyperpolarization and depression of the central respiratory drive potentials.

The records of Fig. 4 were obtained from an E-2 neurone which did not discharge, as the membrane potential was very negative (−75 mV) even under control conditions (Fig. 4*A*). Electrotonic potentials evoked by injection of current pulses (−1 nA) provided an estimate of input resistance. As seen in all E-2 neurones measured, input resistance was lowest during inspiration and highest during stage 2 of expiration. Ionophoretic administration of 8-OH-DPAT (35–100 nA) reduced the maximal input resistance during expiration by 27·6 ± 7 % and minimal input resistance during inspiration by 28·3 ± 9 % ($n = 5$; Fig. 4*B*). The other E-2 neurones exhibited a similar decrease of input resistance in association with the DC membrane hyperpolarization. The relative temporal variation of input restance became smaller as the central respiratory drive potentials of the E-2 neurones were depressed (Fig. 4*B*).

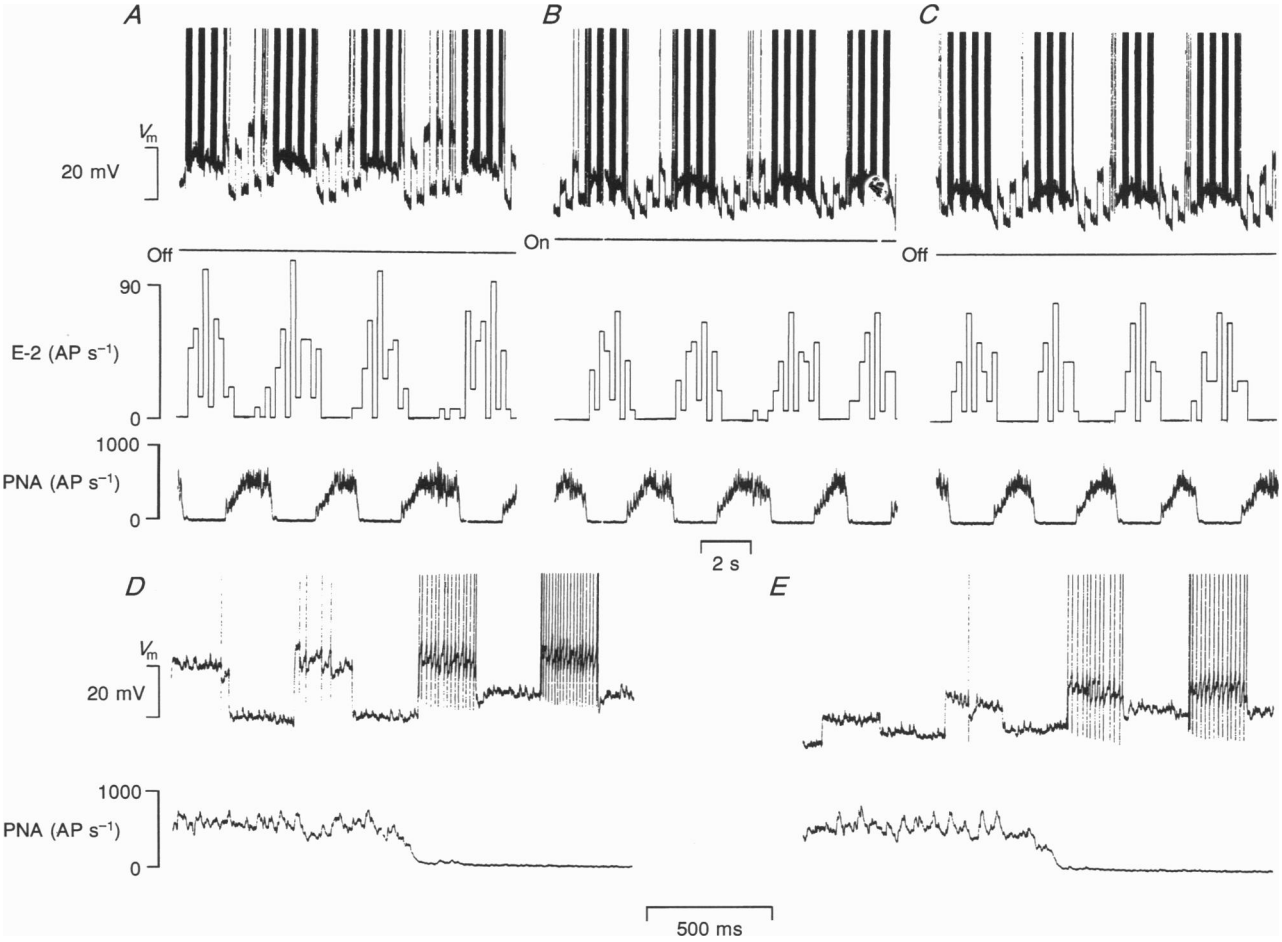

Figure 5. Depression of pulse-evoked E-2 discharges and neuronal input resistance produced during ionophoresis of 8-OH-DPAT

Steady hyperpolarizing current (−3 nA) abolished spontaneous discharges, but not respiratory-related slow V_m oscillations. Depolarizing current pulses (2 nA, 220 ms) were passed from the recording electrode to evoke discharges from the E-2 neurone. Records in *A–C* show membrane potential (V_m) of the E-2 neurone, ejection marker for 8-OH-ionophoresis (on–off line), histograms of neuronal action potential frequency (E-2, AP s^{-1}; sampling time 20 ms per bin) and integrated phrenic nerve activity (PNA, AP s^{-1}). Records *A–C* were obtained before (*A*), during ionophoresis of 8-OH-DPAT (*B*; 90 nA ejecting current) and 2 min after turning off ejection current (*C*). *D* and *E* are expanded time records of membrane potential and integrated phrenic nerve activity before (*D*) and during ionophoresis (*E*) of 8-OH-DPAT to further show hyperpolarization, decreased neuronal input resistance (note depression of depolarizing potential) and depression of evoked discharges.

Ionophoretically administered 8-OH-DPAT depressed not only spontaneous discharges of E-2 neurones, but also the repetitive discharge evoked by positive current pulses passed through the intracellular pipette.

Spontaneous discharges of the E-2 neurone shown in Fig. 5 were prevented by passage of constant hyperpolarizing current, during which depolarizing current pulses were applied to provoke repetitive discharges of action potentials (Fig. 5*A* and *D*), which varied throughout the respiratory cycle due to spontaneous changes in membrane potential and neurone input resistance. During administration of 8-OH-DPAT (Fig. 5*B* and *E*), the current-evoked discharges were less intense in all phases of the respiratory cycle. The changes were due to the decrease in neuronal input resistance. The amplitudes of action potentials varied in parallel with the changes in input resistance (Fig. 6), but there were no changes in threshold for discharge of action potentials, nor did the repetitive firing properties change significantly (Fig. 5).

Hence the reduction of discharge observed during 8-OH-DPAT application was due to a drug-dependent membrane hyperpolarization and reduction of neurone input resistance. The findings suggest that, within the range of ejecting current intensities used (20–100 nA), the effects of 8-OH-DPAT are predominantly postsynaptic and not presynaptic.

Within the dose range which produces significant depression of firing, the effect of ionophoresed 8-OH-DPAT seemed to be limited to the neurones tested and not related to its action on adjacent neurones. Evidence for this statement was derived from an experiment in which the activity of two E-2 neurones was recorded by the same microelectrode after impalement of an E-2 neurone.

Puncturing the E-2 neurone resulted in a poor sealing of the pipette and the measured membrane potential was relatively lower (−56 mV) compared to average inspiratory membrane potential (−64 mV; mean of 20 neurones). However, the high leak conductance allowed a simultaneous extracellular recording of the initial segment–soma dendriticic (IS–SD) spike discharge of a neighbouring E-2 neurone. The impaled neurone revealed a typical pattern of membrane potential fluctuations and

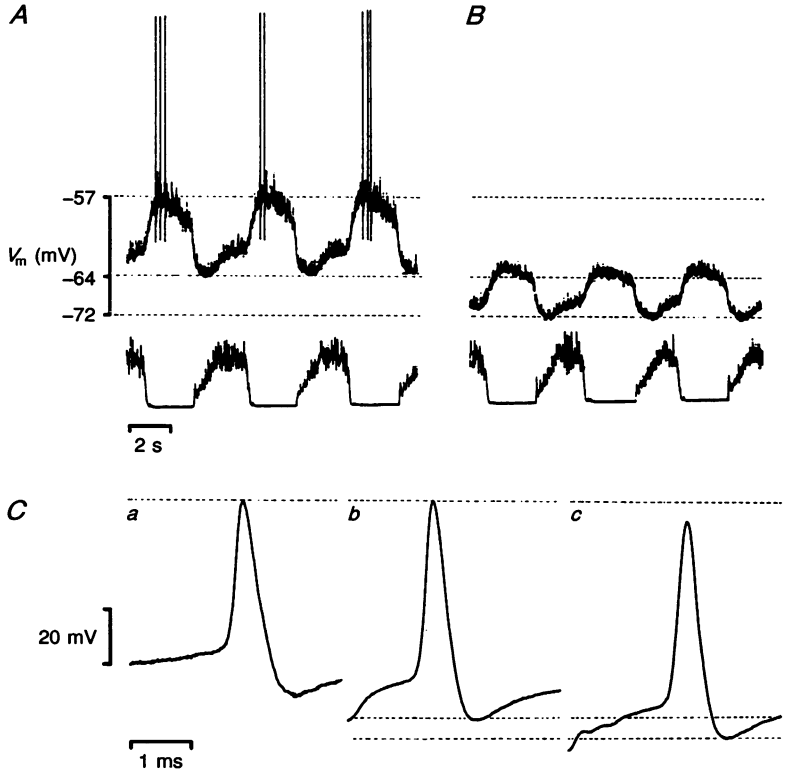

Figure 6. Effects of 8-OH-DPAT ionophoresis on spontaneous and evoked discharge properties of the E-2 neurone illustrated in Fig. 5

A, spontaneous discharge of the E-2 neurone and moving average of phrenic nerve activity. *B*, hyperpolarization and depression of spontaneous E-2 discharge during 90 nA ionophoresis of 8-OH-DPAT. *Ca*, average of 20 spontaneously discharged action potentials. *Cb*, averages of 20 action potentials evoked by 2 nA, 220 ms depolarizing current pulses injected on top of a 3 nA hyperpolarizing DC current to stop spontaneous discharge. *Cc*, averages obtained during 90 nA ionophoresis of 8-OH-DPAT. Averages show reduction of action potential amplitudes and depolarizing electrotonic potentials.

ejection of 8-OH-DPAT by current intensities of 20–70 nA resulted in appreciable membrane hyperpolarization and reduction of the amplitude of the respiratory drive potential as seen usually (Fig. 7*B* and *C*). At the same time, there were no noticeable effects of 8-OH-DPAT on the discharge of the neighbouring E-2 neurone (Fig. 7*B*, *C* and *E*). Ejection of 8-OH-DPAT by 90 nA current resulted in further depression of the impaled neurone without changing the discharge of the neighbouring E-2 neurone.

Responses of an E-2 neurone to 8-OH-DPAT ionophoresis during hyperventilation-induced apnoea

Synaptically induced changes of the membrane potential (central respiratory drive potentials) and neuronal input resistance were abolished when the animal was hyperventilated with oxygen-enriched air. Under these conditions, P_{CO_2} fell from 32 torr (ventilation rate 20 min^{-1}) to 22 torr (ventilation rate 52 min^{-1}). At the same time,

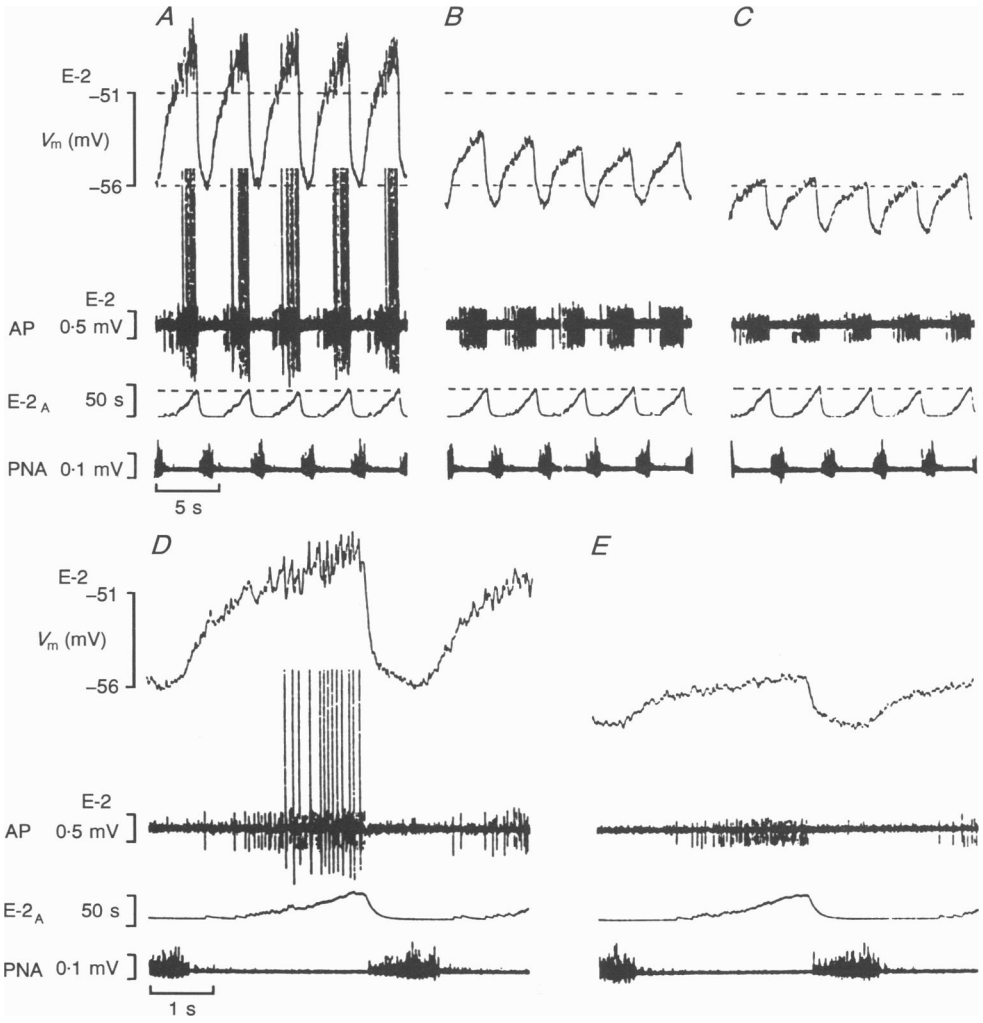

Figure 7. Responses of two E-2 neurones to ionophoresis of 8-OH-DPAT by different ejecting currents

Activities of an impaled neurone and a nearby E-2 neurone were picked up by the intracellular recording electrode. The figure illustrates low-pass filtered (15 Hz) records of the membrane potential (V_m) of the impaled neurone, its action potential discharge (large spikes; obtained by passing the output of the intracellular amplifier to a follower AC amplifier with band-pass filter, 100–5000 Hz) and the spike discharge (small spikes) as well as its integrated frequency (E-2$_A$) of the nearby E-2 neurone. Control records are shown in *A*. *B* and *C* show effects on the E-2 neurones produced by 8-OH-DPAT during ionophoresis with ejecting currents of 40 nA (*B*) and 70 nA (*C*). *D* and *E* are records at expanded time resolution taken before (*D*) and during ionophoresis of 8-OH-DPAT with 70 nA (*E*). Note that 8-OH-DPAT, 40–70 nA, produces marked dose/current-dependent depression of the impaled E-2 neurone without altering discharge properties of the nearby E-2 neurone.

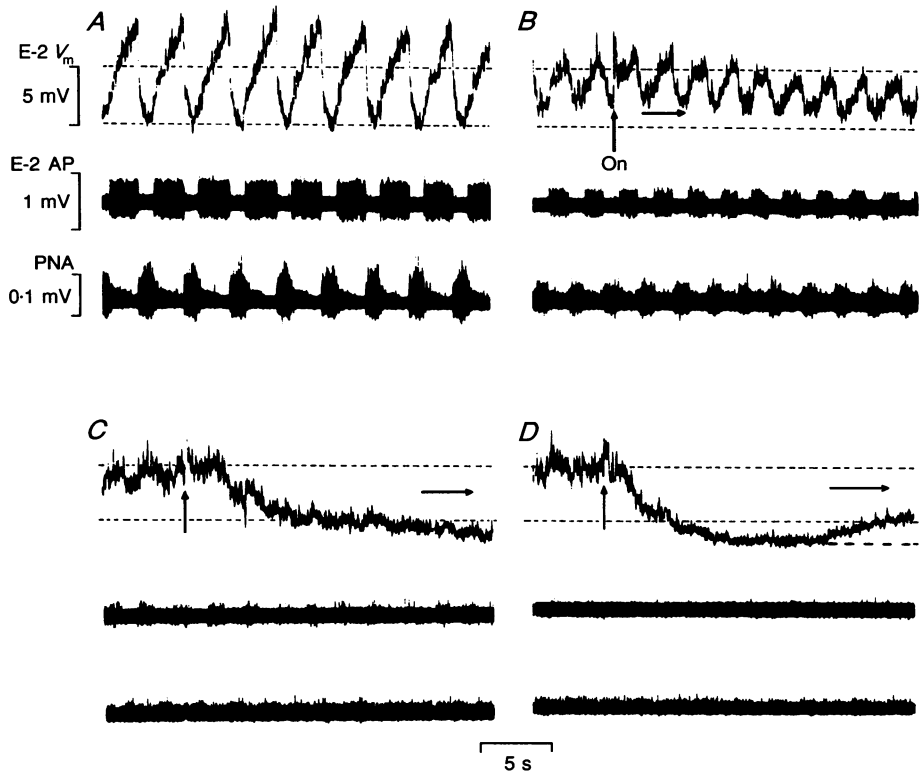

Figure 8. Change in response of an E-2 neurone to 8-OH-DPAT ionophoresis during hyperventilation apnoea

Records obtained from the E-2 neurones also shown in Fig. 7. Top records are low-pass filtered (15 Hz) records of the membrane potential (V_m) of the impaled neurone. Middle traces are the action potential discharges of a nearby E-2 neurone obtained by passing the output of the intracellular amplifier to a follower amplifier (AC; band-pass filter, 100–5000 Hz). Records shown in *A* were obtained under control conditions during mechanical ventilation at a rate of 20 min^{-1}, end-tidal P_{CO_2}, 32 torr. The maximal membrane potential was −67 mV. *B–D*, responses of the impaled E-2 neurone to 8-OH-DPAT, 60 nA ejecting current during hyperventilation (52 min^{-1}) for 4·5 min (*B*), 6 min (*C*) and 8 min (*D*). End-tidal P_{CO_2} had fallen to 22 torr when records in *D* were obtained. Turning on of ejecting current is signalled by vertical arrows. Currents were left on beyond the time course of each recorded segment in *B–D*, as indicated by horizontal arrows.

mean membrane potential stabilized at −62 mV, i.e. at a level between the two peaks of control membrane potential fluctuations (60 ± 2·3 mV during stage 2 expiration and 70 ± 1·2 mV during inspiration) and maximal neuronal input resistance increased more than twofold (Fig. 10).

The responses of an E-2 neurone to 8-OH-DPAT ionophoresis (60 nA ejecting current) were considerably enhanced during such hyperventilatory apnoea (Fig. 8). The hyperpolarizing responses to repetitive phoresis of 8-OH-DPAT with constant ejecting current intensity increased in parallel with the decrease of central respiratory drive potentials to reach a maximum when apnoea was complete (Fig. 8*D*). During complete apnoea, membrane hyperpolarization and reduction in neuronal input resistance and synaptic noise increased with current intensity until maxima were achieved with ejection of 8-OH-DPAT by 60 nA. A graphical summary of the effects produced before and after apnoea is seen in Fig. 10.

Under such apnoeic conditions, however, peak membrane hyperpolarization faded after 15 s although 8-OH-DPAT phoresis (60 nA) continued and attained a steady level which was 2·5 mV less than the maximum (Fig. 8*D*). Greater sensitivity and fading of the voltage response were also seen in the effects of 8-OH-DPAT on input resistance. In Fig. 9 it can be seen that the rates of adaptation of the changes in membrane potential, input resistance and synaptic noise increased with ejecting current intensity. Very rapid fading explained why the steady-state responses to 8-OH-DPAT during apnoea are less in response to 70 nA current than to 60 nA.

DISCUSSION

The findings of this study demonstrate the importance of the 5-HT-1A subtype of the serotonin receptor family in mediating serotoninergic inhibitory control of stage 2 expiratory neurones within the lower brainstem. They also

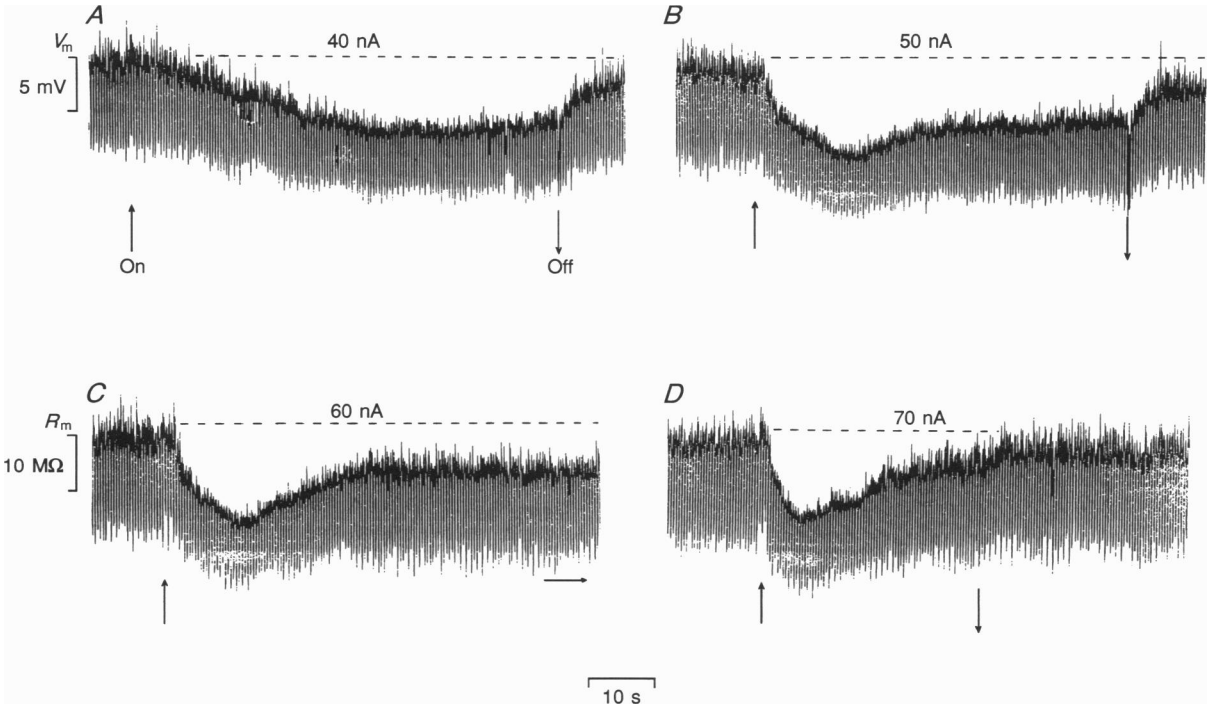

Figure 9. Effects of ionophoresis of 8-OH-DPAT on an E-2 neurone during complete hyperventilation apnoea (end-tidal P_{CO_2}, 22 torr)
Records show responses to ionophoresis of 8-OH-DPAT with ejecting currents of 40 nA (*A*), 50 nA (*B*), 60 nA (*C*) and 70 nA (*D*). Vertical arrows signal the turning on and off of ejecting currents. Hyperpolarizing current pulses (0·5 nA, 160 ms, 3 Hz) were injected to provide estimates of neuronal input resistance (R_m). Note the increasing hyperpolarization, decreasing R_m and synaptic noise (high frequency fluctuation of membrane potential) with ejecting currents until maximum effects were reached during ejection of 8-OH-DPAT by 60 nA. Later fading of responses is evident with all ejecting currents, but is more prominent with larger currents.

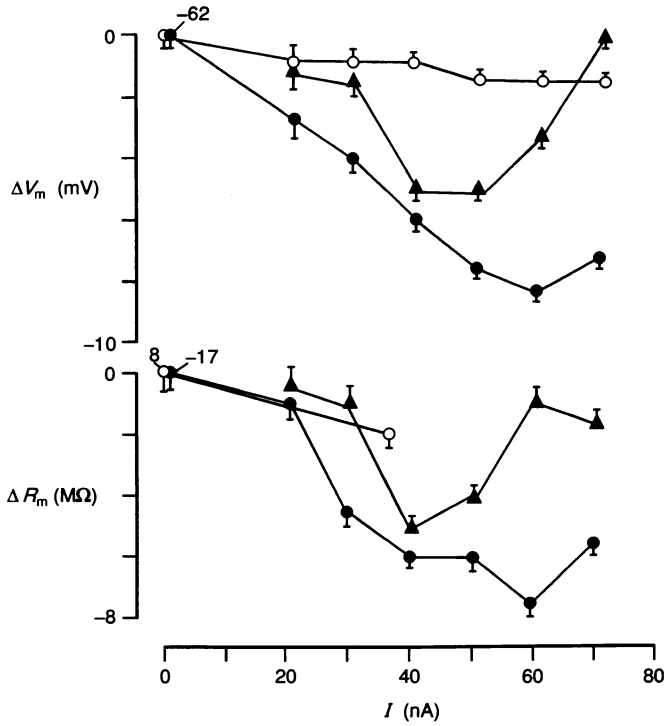

Figure 10. Summary of effects of ionophoresed 8-OH-DPAT on an E-2 neurone during normal ventilation (○) and during complete hyperventilation apnoea (●,▲)
Results obtained from the E-2 neurone shown in Fig. 9. Ordinates, changes in membrane potential (V_m) and neuronal input resistance (R_m). Abscissa, ejecting currents (*I*, nA). During control ventilation (end-tidal P_{CO_2}, 32 torr), maximum (inspiratory) membrane potential was −62 mV and R_m was 8 MΩ during inspiration when no 8-OH-DPAT was administered. During complete apnoea (end-tidal P_{CO_2}, 22 torr), membrane potential and input resistance were steady at −62 mV and 17 MΩ in the absence of 8-OH-DPAT ejection. ●, maximum changes in V_m and R_m; ▲, changes recorded after fade of maximum response to steady levels. Vertical bars indicate standard error of the means.

support the idea that 5-HT modulates the excitability of expiratory neurones not only directly on the postsynaptic neurone, but also indirectly through the actions on other types of neurones in the respiratory neural network.

Interactive influences of 5-HT receptor subtypes

8-OH-DPAT is a well-established specific agonist for the 5-HT-1A receptor (Anwyl, 1990). Activation of the receptor by 8-OH-DPAT leads to a G-protein-dependent activation of an inwardly rectifying potassium conductance, which leads to hyperpolarization and depression of neuronal excitability (Andrade & Nicoll, 1987; McCormick & Williamson, 1989). In E-2 neurones, the predominant effect of 5-HT is also inhibitory (P. M. Lalley, unpublished observations) and this suggests that the soma of these neurones have 5-HT-1A receptors with a high affinity. Other subtypes of 5-HT receptors are also expressed in E-2 neurones (Böhmer *et al.* 1979), but not analysed in the present study.

In neurones of the medullary respiratory network and in synaptically coupled motoneurones various other ligand-activated membrane conductances are activated during the respiratory cycle and these produce the periodic fluctuations of membrane potential. Activation of these conductances might interact or mask the potential excitatory influences of 5-HT-2 and 5-HT-3 receptors (Morin, Hennequinn, Monteau & Hilaire, 1990; Berger *et al.* 1992; Kubin *et al.* 1992) more effectively than the predominant inhibitory influence of 5-HT through binding to 5-HT-1A receptors.

Network effects of 5-HT-1A receptor activation

Certain effects of intravenously administered 8-OH-DPAT differed from those seen during ionophoresis and since they occurred in conjunction with changes in phrenic nerve activity, they seem to be related to indirect effects acting through the respiratory network. These effects include shortening of inspiration and concomittent waves of membrane hyperpolarization as well as activation of postinspiratory activity resulting in a decrease of central respiratory drive potentials in E-2 neurones. An increase in frequency of inspiratory discharges was also observed in an *in vitro* preparation of the newborn rat and was attributed to activation of bulbar 5-HT-1A receptors (Morin *et al.* 1990). Therefore, a brainstem site of action seems likely in the adult cat. Shortening of the inspiratory hyperpolarization of caudal and rostral E-2 neurones might reflect shortening of the discharge duration of early-inspiratory neurones, allowing late-inspiratory neurones to switch off inspiration earlier and more efficiently (Richter *et al.* 1986).

Systemic application of larger doses of 8-OH-DPAT abolished phrenic nerve activity and membrane potential fluctuations of E-2 neurones. These effects were partly reversed by NAN-190 and therefore reflect an action through 5-HT-1A receptors. The observed postsynaptic inhibition of E-2 neurones may be only one of the complex effects of systemically applied 8-OH-DPAT on cell bodies and dendrites of various neurones of the respiratory network. A detailed analysis of the 5-HT mechanisms, therefore, requires studies at the cellular level.

Postsynaptic effects of 8-OH-DPAT on membrane potential and discharge patterns of E-2 neurones

Ionophoretic administration of 8-OH-DPAT increased neuronal conductance of E-2 neurones leading to membrane hyperpolarization, inhibition of spontaneous discharges and depression of discharges evoked by intracellular injection of depolarizing current pulses. These results indicate that the effects are due to a postsynaptic action of 8-OH-DPAT on somatic or proximal dendritic 5-HT-1A receptors. Increasing membrane hyper-polarization prolonged the time to threshold and decreased the frequency of the subsequent action potential discharge without affecting the firing pattern, which was still augmenting when ionophoretic currents were small. This indicates that 8-OH-DPAT does not interfere with other ligand-activated conductances. We assume that 8-OH-DPAT-induced shunting of respiratory drive potentials (Fig. 3*B*) was due to activation of potassium conductances (Anwyl, 1990).

Extracellular ionophoretic application of 8-OH-DPAT acting on 5-HT-1A receptors could inhibit neighbouring neurones. The hyperpolarizing response of the neurones analysed in intracellular recordings could therefore also be due to inhibition of a tonic input from adjacent excitatory interneurones. This would be accompanied by a decrease of excitatory synaptic inputs and increase of neuronal input resistance. On the contrary, the observation of a steady membrane hyperpolarization, combined with a decrease of synaptic noise and decline of neurone input resistance, shows that the 8-OH-DPAT effect was due to an influence on the postsynaptic membrane of the E-2 neurones studied.

The discharge pattern of neighbouring neurones remained unchanged when 8-OH-DPAT was applied onto the intracellularly recorded E-2 neurone, as seen in Figs 7 and 8. The decrease in amplitude of extracellular action potentials picked up by the intracellular electrode seemed to result from the diminished input resistance of the impaled neurone rather than from an effect of 8-OH-DPAT on the neighbouring neurone.

Modulation of postsynaptic actions of 8-OH-DPAT during hyperventilatory apnoea

Decreased chemoreceptive drive of the respiratory network that results from hyperventilation reduces the discharge of medullary respiratory neurones (Bainton & Kirkwood, 1979). The depression seems to be primarily linked to removal of the P_{CO_2}-induced (H^+) stimulus to central

nervous chemoreceptors, which changes in parallel with the changes in end-tidal P_{CO_2} (Loeschcke, 1982). In the present study, hyperventilation depressed rhythmic respiratory drive potentials and discharges. When apnoea was complete, the responses of E-2 neurones to 8-OH-DPAT were enhanced, but also faded more rapidly. The higher sensitivity to 8-OH-DPAT during apnoea might be the consequence of reduced shunting of the 5-HT-1A receptor-activated potassium conductance by other ligand-activated, mostly GABAergic conductances, which are activated during the inspiratory and postinspiratory phase of the respiratory cycle (Haji, Remmers, Connelly & Takeda, 1990).

However, changes of extracellular pH might also affect the 5-HT-1A receptor-mediated activation of potassium conductances (Jordan, Frederickson, Phillis & Lake, 1972). Similar pH-dependent effects are also described for other ligand-activated conductances (Chesler & Kaila, 1992; Pasternak, Bountra, Voipio & Kaila, 1992). Fading of the changes in conductances as well as membrane potential of E-2 neurones might indicate down-regulation or desensitization of 5-HT-1A receptors on the postsynaptic membrane. There is no evidence that presynaptic mechanisms are involved in this loss of sensitivity.

Physiological implications

Serotoninergic neurones originating in the medullary raphe complex and rostroventrolateral medulla send axons to many regions of the medulla and spinal cord to terminate directly at or as close appositions to respiratory neurones (Connelly *et al.* 1989). Therefore, serotonin appears to be an important neuromodulator in the central nervous system which modifies the responses of neurones to the fast-acting neurotransmitters (Vandermaelin, 1985). Neuromodulation of respiratory control mechanisms by 5-HT explains why diverse effects are observed on various sorts of respiratory neurones. By activating different subtypes of receptor, 5-HT may act synergistically with or act antagonistically against the neuronal effects of excitatory and inhibitory amino acids, which seem to provide the primary mechanism for the respiratory rhythm generation (Champagnat, Denavit-Saubie, Moyanova & Rondouin, 1982; Haji *et al.* 1992). Acting in concert with such neurotransmitters at the postsynaptic membrane of respiratory neurones, 5-HT might fine-tune the discharge pattern of respiratory neurones and modulate respiratory rhythm in response to state-related changes in behaviour. Serotonin might also contribute to the changes occurring during energy deprivation of the respiratory network, such as hypoxia or ischaemia. This could explain why systemic application of 8-OH-DPAT effectively suppresses apneustic breathing patterns (P. M. Lalley, A.-M. Bischoff & D. W. Richter, unpublished observations), which seem to result from a hypoxia or ischaemia-induced weakening of late-inspiratory inhibition of the inspiratory network (M. C. Bellingham, C. Schmidt & D. W. Richter, unpublished observations).

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