Journal of Physiology (1992), **451**, *pp.* 605–629
With 8 *figures*
Printed in Great Britain

COMPARED EFFECTS OF SEROTONIN ON CERVICAL AND HYPOGLOSSAL INSPIRATORY ACTIVITIES: AN *IN VITRO* STUDY IN THE NEWBORN RAT

BY DIDIER MORIN, ROGER MONTEAU AND GÉRARD HILAIRE

From Equipe Biologie des Rythmes et du Développement, Département de Physiologie et Neurophysiologie, URA CNRS 0205, B.P. 332 Faculté des Sciences et Techniques St Jérôme, 13397 Marseille Cedex 13, France

(*Received 24 June 1991*)

SUMMARY

1. Experiments were performed on the brain stem–spinal cord preparation of newborn rats, in which the phrenic and hypoglossal nerves continue to show rhythmic respiratory activity *in vitro*, in order to compare the effects of serotonin (5-HT) on both activities and to analyse the mechanisms responsible for the depression by 5-HT of the hypoglossal activity.

2. Under control conditions, simultaneous recordings of the inspiratory discharges of hypoglossal and cervical roots showed that the two bursts did not start simultaneously and had different patterns (time-to-peak and peak values); this suggests that both pools of motoneurons did not share the same central drive(s).

3. Adding 5-HT and related agents to the bathing medium delayed and depressed the hypoglossal inspiratory discharge via activation of 5-HT_2 receptors since these effects were elicited by 5-HT_2 agonists (α-methyl-5-HT and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-HCl (DOI)) but not by 5-HT_1 agonists (RU 24969 and ($\pm$)-8-hydroxy-2-(di-*N*-propylamino)tetralin hydrobromide (8-OH-DPAT)). The 5-HT depression of the hypoglossal discharge was prevented by applying a pretreatment with a specific 5-HT_2 antagonist (ketanserin). Parallel to the hypoglossal discharge decrease, 5-HT elicited a permanent cervical root discharge along with a persistent inspiratory bursting. Adding the 5-HT precursor L-tryptophan to the bathing medium depressed the hypoglossal (XII) discharge without affecting the cervical one.

4. Local application of 5-HT within the hypoglossal motor nucleus decreased the hypoglossal output, revealing that the 5-HT depression of the hypoglossal discharge was at least partly mediated by the 5-HT effects at the level of the motoneurons. Local application of 5-HT within the cervical motor nucleus elicited a permanent firing in the cervical root with a persistent inspiratory bursting.

5. Intracellular analysis confirmed the existence of differences in central respiratory drive between cervical and hypoglossal motoneurons under control conditions, as well as differences in response to 5-HT. All the hypoglossal motoneurons became silent under 5-HT bathing, and showed no change in the input membrane resistance, a moderate depolarization, and a delayed central respiratory

MS 9503

drive with a decreased amplitude. The cervical motoneurons became more active during inspiration, despite a decrease in the amplitude of the central respiratory drive, which was compensated for by a large depolarization and an increased input membrane resistance. Some cervical motoneurons even fired at a low rate during expiration.

6. Taken as a whole, these results suggest that the hypoglossal depression induced by 5-HT was due to the activation of 5-HT_2 receptors located within the hypoglossal nucleus but the exact mechanisms whereby 5-HT modulates the hypoglossal outputs are still open to debate. The functional significance of the effects of 5-HT on hypoglossal inspiratory activity is discussed with reference to the obstructive apnoea disorders occurring in both adults and newborn infants.

INTRODUCTION

In the isolated newborn rat brain stem–spinal cord preparation, periodic discharges can be recorded from cranial and cervical ventral roots, and particularly from the phrenic nerve (Suzue, 1984). These rhythmic nervous discharges are consistent with the persistence of respiratory function, since (i) they elicit periodic inspiratory movements of the rib cage (Suzue, 1984), (ii) they originate from central respiratory neurons (Onimaru, Arata & Homma, 1988; Hilaire, Monteau, Gauthier, Rega & Morin, 1990; Smith, Greer, Liu & Feldman, 1990), (iii) they are sensitive to respiratory stimuli such as lung vagal afferents (Murakoshi & Otsuka, 1985) and pH and CO_2 levels (Harada, Kuno & Wang, 1985; Monteau, Morin & Hilaire, 1990*b*). This preparation has turned out to be a suitable method for pharmacological studies on central respiratory activity, since drugs added to the bathing medium diffuse within the brain stem, act on respiratory (and non-respiratory) neurons, and induce respiratory changes which are not affected by the periphery (Murakoshi, Suzue & Tamai, 1985; Errchidi, Hilaire & Monteau, 1990). For the purpose of pharmacological studies, this lack of peripheral effects is particularly advantageous in the case of serotonin (5-HT): previous *in vivo* experiments have yielded contradictory results as to the influence of 5-HT on central respiratory activity (for review see Eldridge & Millhorn, 1981) and no definite conclusions have been reached. Both excitatory and inhibitory influences have been thought to occur, mainly because it is difficult to separate the effects of 5-HT on the respiratory centres from those on either peripheral targets or other centres known to interact via the periphery with respiration (Moss, Denavit-Saubie, Eldridge, Gillis, Herkenham & Lahiri, 1986). It has been clearly established on the isolated brain stem–spinal cord preparation of newborn rats, however, that 5-HT exerts a facilitatory modulation on the activity of the respiratory rhythm generator through a permanent release of endogenous 5-HT acting via 5-HT_1 medullary receptors (Morin, Hennequin, Monteau & Hilaire, 1990*a*: Morin, Monteau & Hilaire, 1991*a*). Besides these facilitatory effects on respiratory frequency, 5-HT may also modulate the amplitude of the respiratory motor output: pharmacological (Monteau, Morin, Hennequin & Hilaire, 1990*a*) and electrophysiological studies (Morin, Hennequin, Monteau & Hilaire, 1990*b*) have shown, however, that 5-HT has depressing effects on the hypoglossal respiratory motor output, whereas the cervical output was not depressed. The hypoglossal (XII) nerves supply muscles in the upper airways, and a balance between the inspiratory

activities of the chest and upper airways has to be maintained in order to prevent obstruction of the upper airways (Remmers, de Groot, Sauerland & Anch, 1978). Many pharmacological agents such as diazepam, anaesthetics, and alcohol induce differential changes in the activity of the chest and upper airway respiratory muscles (for review see Iscoe, 1988) but no differential effect originating from an endogenous neurotransmitter as widely distributed as 5-HT, which might even be involved in sleep mechanisms (for review see Vogt, 1982), has ever been reported previously.

The aim of the present study was to investigate more closely the mechanisms through which 5-HT depresses the inspiratory activity of the XII motoneurons. First, the pattern of discharge of both XII and cervical motoneurons under normal conditions was analysed using extra- and intracellular approaches. Changes induced in both discharges by 5-HT and related compounds were then studied, leading to the conclusion that the effects on the XII discharge were due to the activation of 5-HT_2 receptors. Further experiments were performed to determine whether 5-HT acted directly or indirectly on the XII motoneurons. These investigations reveal that, at least in part, 5-HT acts directly at the level of the motor nucleus. Since the XII activity was also depressed by the 5-HT precursor L-tryptophan added to the bathing medium, it is suggested that a dysfunction of the 5-HT biosynthesis mechanisms leading to an abnormally high release of endogenous 5-HT may considerably depress the XII discharge, favouring the occurrence of obstructive apnoea. The latter point is discussed in relation to the genesis of sleep obstructive apnoea, both in adults, where it is a fairly common condition, and in the newborn, where it can lead to Sudden Infant Death Syndrome.

METHODS

Newborn rats (0–3 days old) were anaesthetized with ether and decerebrated just rostrally to the fifth cranial nerves. A section was performed at the level of the last cervical vertebra and the skin and muscles were rapidly removed. The dissection was then continued in a 1·5 ml chamber filled with artificial cerebrospinal fluid identical to that previously used (Murakoshi *et al.* 1985; Hilaire, Monteau & Errchidi, 1989) containing (mM): NaCl, 129; KCl, 3·35; $CaCl_2$, 1·16; $MgCl_2$, 1·15; $NaHCO_3$, 21; NaH_2PO_4, 0·58; glucose, 30, equilibrated with 95% of O_2 and 5% of CO_2, warmed to 27 ± 0·5 °C, and permanently drained by suction with a perfusion rate such that the total volume chamber was replaced within 30 s. After removal of the cranial and vertebral bones under binocular control, the brain stem was fixed with the dorsal surface upwards. For the intracellular recording of cervical motoneurons, the brain stem was fixed, however, with the ventral surface upwards.

The inspiratory electrical activities of one of the cervical ventral roots (generally C1 or C2) and the hypoglossal (XII) roots were recorded using suction electrodes. The signals were filtered (5–3000 Hz), amplified and fed to two identical leaky integrators (time constant, 100 ms), oscilloscopes and a paper recorder (Gould TA 2000). For the intracellular experiments, the right C2 segment or the right hypoglossal nucleus was explored with micropipettes (either 3 M-potassium acetate or KCl, impedance 60–100 MΩ) moved by a motorized microdrive (step 1 μm). The DC signal was fed to a laboratory-made microelectrode amplifier, oscilloscope, and paper recorder and stored on tape. The input membrane impedance was calculated from the voltage changes elicited by injection of positive and negative pulses of current (100 ms, 100–400 pA). For the antidromic identification of the impaled motoneurons, the right C2 ventral root or some of the twelfth nerve roots were sucked into an electrode and stimulated (200 μs, 25–100 μA) via an isolation unit and a digital stimulator (WPI 830).

The following drugs were used as pharmacological tools: serotonin (5-HT), α-methyl-5-HT and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-HCl (DOI) as 5-HT_2 agonists, RU24969 and (±)-8-hydroxy-2-(di-*N*-propylamino)tetralin hydrobromide (8-OH-DPAT) as 5-HT_1 agonists and ketanserin as 5-HT_2 antagonist (Bradley, Engels, Feniuk, Fozard, Humphrey, Middlemiss,

Mylecharane, Richardson & Saxena, 1986) and L-tryptophan as 5-HT precursor. The drugs were dissolved in the bathing medium and applied by superfusion. 5-HT and agonists were applied over a period of 6 min and 5-HT antagonist for 10 min. Apart from the experiments where antagonist and agonist were successively applied, only one drug was generally applied during each experiment and only one trial was performed per experiment. In some experiments, multi-barrelled micropipettes were used to apply 5-HT locally by pressure pulse ejection within the XII or cervical motor nuclei. Prior to the experiments, control ejections were performed under the microscope: the ejected volumes never exceeded 5 nl. The position of the electrode within the motor nucleus was determined by recording multi-unitary activities of the motoneuron pool via the central barrel filled with saline. In this location, 5 min before the ejection of 5-HT, saline was ejected in order to confirm the absence of any mechanical effects.

Prior to any application of drug (control period), the mean amplitude of the inspiratory bursts of XII and cervical roots were defined as the average of at least twenty integrated inspiratory discharges. Drug was then applied and the mean amplitudes of the two discharges were calculated every minute. Since 5-HT and related agents often elicited a tonic firing on cervical roots superimposed on the inspiratory bursts (see Results), two types of measurements were performed: (i) measuring the total height of the integrated inspiratory bursts, (ii) substracting to this value the deflection of the baseline due to the tonic discharge in order to eliminate the tonic component thought to contaminate the inspiratory burst. The minute average values were expressed as a percentage of the corresponding control value. All the experiments were repeated on several brain stem preparations with a standardized procedure to evaluate the mean effect. Results were expressed as the means $\pm$ S.E.M. and differences were taken to be significant at P values of less than 0·05 using paired and unpaired Student's t test.

RESULTS

Patterns of discharge of XII and cervical ventral roots under normal bathing medium

The brain stem–spinal cord preparation of newborn rat (Fig. 1*A*) continues *in vitro* to show rhythmic inspiratory discharges which were recorded for several hours in both the XII nerve and cervical roots (C1–C4). The two discharge patterns were similar on low-speed recordings (Fig. 1*B*), but differences were noted when they were analysed in detail, especially on high-speed recordings (Fig. 1*C*), taking into account (i) the onset of firing, (ii) the changes in the pattern of discharge, and (iii) the variability of the discharge.

The first difference concerned the onset of the discharges: the inspiratory burst did not start simultaneously on both nerves and the cervical root often fired earlier than the XII (12/19 cases); the lag ranged from 20 to 100 ms depending on the experiments (mean lag, 48 ± 11 ms). In four cases the two discharges started simultaneously, and in three cases, the XII discharge preceded the cervical one for around 40 ms. Whether or not they started simultaneously, the time between the two events remained constant throughout each experiment. When comparing the discharges from two different cervical ventral roots, no lags were observed between left and right roots from the same segment, and short lags occurred between ipsilateral roots from adjacent segments, with a 5–10 ms precession of the rostral (C1) *versus* the caudal (C2) root firing.

The second difference between the XII and cervical discharges concerned the shape of the discharge: the two discharge patterns were not identical and the time to peak of the cervical discharge (measured from the beginning of the discharge to its maximum) was significantly shorter (103 ± 12 ms) than that of the XII (164 ± 26 ms). No significant differences were observed between the durations of the

two discharges, which both lasted around 1 s under our experimental conditions (1080 ± 180 ms).

The third difference between the two discharges concerned the changes in amplitude which appeared spontaneously during the successive respiratory cycles.

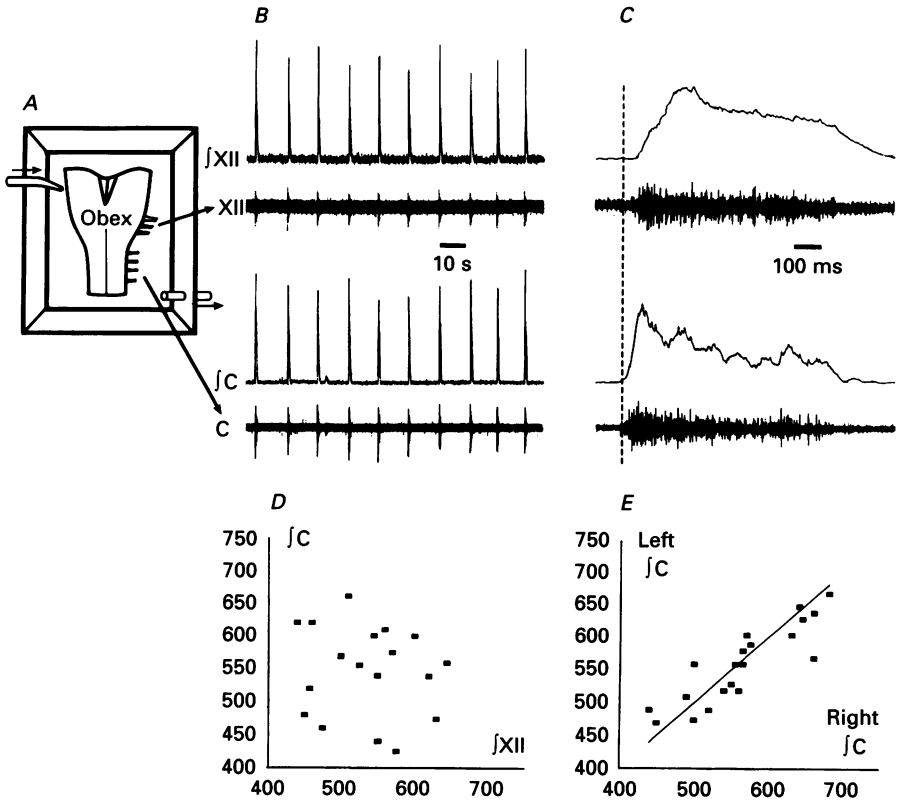

Fig. 1. Patterns of discharge of XII and cervical (C) ventral roots under normal bathing medium. *A*, schematic drawing of the brain stem–spinal cord preparation of the newborn rat, dorsal surface upwards, showing the XII and cervical ventral roots from which recordings were made. *B*, from top to bottom, integrated and raw discharges of the XII and cervical roots illustrating the inspiratory rhythmic discharges persisting in this *in vitro* preparation; note that the amplitude of the integrated activities displayed spontaneous changes which did not occur simultaneously on both roots. *C*, expansion of one inspiration from *B*, with same order of presentation as in *B*; note the difference in time onset and in time to peak between the two discharges. *D*, scatter plots showing the absence of relationships between the spontaneous changes in amplitude of the integrated inspiratory discharges recorded simultaneously in XII and cervical roots during eighteen successive respiratory cycles; surface area of the integrated XII (abscissa) and the cervical root (ordinate) discharges expressed in arbitrary units. *E*, same plots as in *D* but for left and right ventral roots; note the significant correlation between the two variables ($r = 0{\cdot}77$; twenty successive respiratory cycles).

As illustrated in Fig. 1*B*, in some respiratory cycles, one of the two discharges might increase or decrease whereas the other was not affected. These spontaneous changes in the peak amplitudes (and the total areas) of the two integrated signals were in the range of ±5–10 % of the mean value. In eight experiments, the peak amplitude and

total area of both activities were measured in twenty to thirty successive discharges, and linear regression calculations were performed. No significant coefficients of correlation were obtained between the changes observed on the XII and cervical recordings (Fig. 1*D*). In four other experiments, recordings were performed on either the left and right C1 roots or the ipsilateral C1 and C2 roots. Both signal amplitudes increased or decreased simultaneously and highly significant coefficients of correlation were obtained (Fig. 1*E*).

Under normal medium, the XII and cervical inspiratory bursts were therefore not identical: they (i) did not start simultaneously, (ii) did not increase with the same time course, and (iii) did not show the same changes in amplitude.

Effects of medium containing 5-HT and related compounds on the amplitude of the inspiratory discharge of XII nerve and cervical ventral roots

When the normal bathing medium was replaced by medium containing 5-HT or related agents, changes in respiratory frequency and changes in XII and cervical discharges were elicited. The respiratory frequency data were completely in agreement with those published previously (Monteau *et al.* 1990*a*; Morin *et al.* 1990*a*, 1991*a*) and will not be given in detail in the present study, which focused rather on the motor output amplitude changes.

Effects of 5-HT

In five experiments, 6 min application of a medium containing 5-HT (30 μM) led to an increase in the respiratory frequency and changes in discharge pattern of the inspiratory bursts (Fig. 2) as regards both the amplitude and the timing of the discharges.

After 2 min of 5-HT bathing, the amplitude of the XII discharge began to diminish, and after 6 min, it had decreased significantly by $59 \pm 13\%$ of the mean control amplitude. During the XII discharge decrease, a permanent firing with clearly distinguishable inspiratory bursts occurred in the cervical roots as shown in Fig. 2*A*, which illustrates one of these five experiments. The tonic cervical firing could be either continuous or interrupted by brief episodes with a different period from the respiratory one. Depending on whether or not the tonic firing level was taken into account to estimate the amplitude of the cervical motor output during inspiration, different conclusions may be put forward on the effect of 5-HT. Estimating the inspiratory motor output to be the difference between the expiratory and inspiratory levels (i.e. excluding the deflection of the trace due to the tonic firing) suggests that the cervical inspiratory discharge was depressed by 5-HT ($-23 \pm 9\%$) but the depression remains statistically weaker than that of the XII. Estimating the inspiratory motor output to be the overall amplitude of the cervical burst during inspiration (i.e. taking into account the deflection of the trace due to the tonic firing) reveals that the cervical inspiratory discharge was not depressed by 5-HT. This might be the actual effect (see Discussion).

Differences were also observed in the timing of the XII and cervical discharges evoked by 5-HT. In the first minute of 5-HT bathing, and regardless of the timing during control conditions (i.e. simultaneous onset, cervical activity occurring first, XII activity occurring first) the XII inspiratory bursts began to be delayed and in

all cases occurred later than the cervical ones (Fig. 2*C* and *D*). This lag increased throughout the 5-HT bathing and reached 124 ± 31 ms within 6 min. This effect was observed for all the bursts in all the preparations.

Shortly after the return to control medium, the permanent firing of the cervical discharge ceased and total recovery occurred within 5 min. The XII discharge,

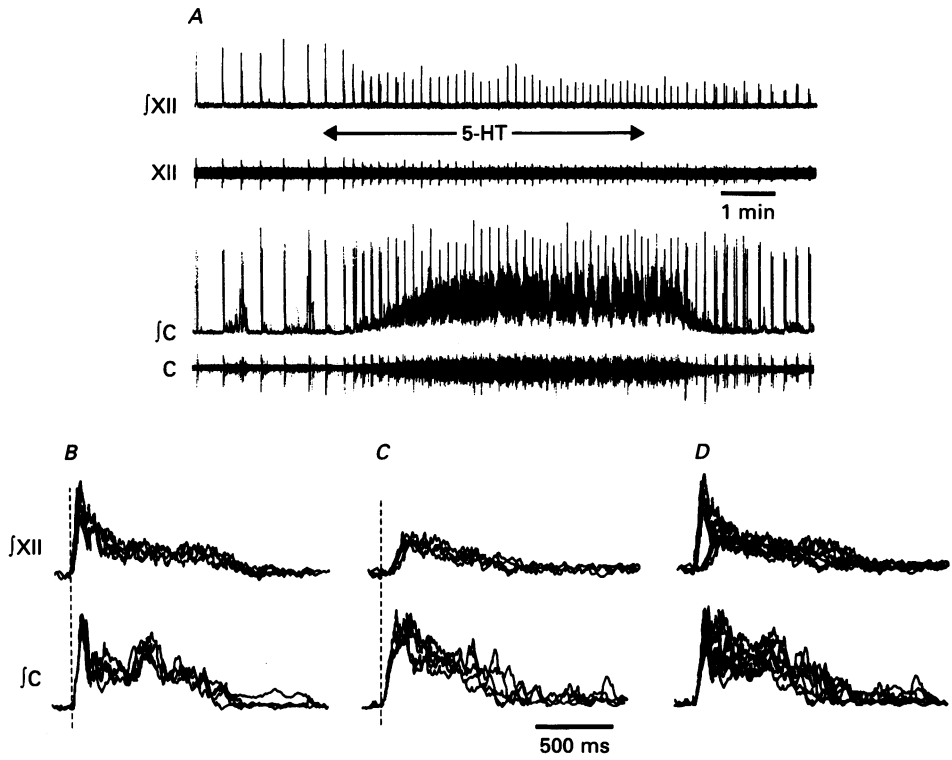

Fig. 2. Effects of medium containing 5-HT (30 μM) on the amplitude of the inspiratory discharge of XII nerve and cervical ventral roots. *A*, from top to bottom, integrated and raw discharges of the XII and cervical roots under normal medium and under 5-HT (between arrows); note (i) the drastic decrease in XII amplitude, (ii) the tonic discharge with the persistence of inspiratory burst in the cervical recording, and (iii) the increase in respiratory frequency (Morin *et al.* 1990*a*). *B* and *C*, high-speed recordings of the integrated XII (upper traces) and cervical (lower traces) discharges (five sweeps) before (*B*) and after (*C*) exposure to 5-HT medium. *D*, same traces as in *B* and *C* but superimposed; note that after 5-HT the XII decreased discharge was delayed.

however, was still low (−48·5 ± 19·5 % of the control value) and delayed after 5–10 min in control medium, and showed only partial recovery, even 30 min after the end of 5-HT bathing.

Effects of 5-HT-related agents

A medium containing the 5-HT_2 antagonist ketanserin (40–80 μM) was applied for 10 min before applying a second medium, containing both ketanserin and 5-HT (30 μM). After ketanserin pretreatment (40 μM), 5-HT still decreased the XII discharge amplitude in five experiments (−41 ± 18 %). Larger concentrations of

ketanserin were used (80 μM) in five other experiments; the ketanserin pretreatment then decreased the 5-HT efficiency: (i) 5-HT elicited only a weak decrease in the XII discharge ($-24{\cdot}4 \pm 19\%$), which was significantly less than that observed under 5-HT alone, with no ketanserin pretreatment, and (ii) no permanent firing was elicited in the cervical roots.

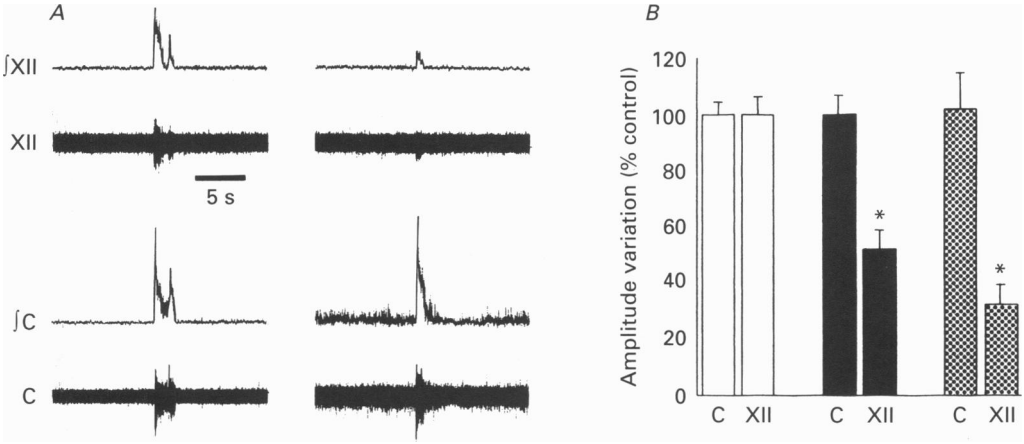

Fig. 3. Effects of medium containing the 5-HT_2 agonist DOI (30 μM) on the amplitude of the inspiratory discharge of XII nerve and cervical root. *A*, expanded recordings showing, from top to bottom, the integrated and raw inspiratory discharges of XII and cervical roots under normal medium (on the left) and under DOI (on the right); note that the 5-HT_2 agonist, like 5-HT, decreased the XII discharge and elicited tonic cervical firing. *B*, histograms showing the mean (vertical bar) and S.E.M. (vertical line) amplitude of the XII and cervical inspiratory discharges ($n = 5$ experiments) during DOI bathing; open bars, normal medium; filled bars, after 6 min of DOI bathing; stippled bars, 5 min after control conditions were resumed. Asterisks indicate significant changes. Note that the decrease in XII amplitude persisted for 5 min after the return to normal medium (stippled bar).

In order to define the type of receptors responsible for the decrease in the amplitude of the XII discharge under 5-HT, drugs known to be 5-HT_1 or 5-HT_2 agonists were added to the normal medium and applied for 6 min instead of the 5-HT medium.

In four experiments, the 5-HT_1 agonist 8-OH-DPAT (30 μM) was added to the bathing medium and did not elicit any significant changes in the XII discharge amplitude ($-16{\cdot}5 \pm 19{\cdot}2\%$) whereas the cervical discharge increased slightly ($10{\cdot}8 \pm 4{\cdot}3\%$). In six other experiments, the 5-HT_1 agonist RU24969 did not evoke any significant changes in the amplitude of either discharge ($5{\cdot}7 \pm 8{\cdot}6$ and $0{\cdot}6 \pm 20{\cdot}1\%$ for XII and cervical discharges, respectively). Both 5-HT_1 agonists increased the respiratory frequency as already reported (Morin *et al.* 1990*a*).

In five experiments, the 5-HT_2 agonist DOI (30 μM) was applied for 6 min (Fig. 3). After bathing, the XII discharge amplitude decreased significantly ($-48{\cdot}1 \pm 6{\cdot}2\%$), whereas the cervical level was not affected ($0{\cdot}3 \pm 6{\cdot}4\%$). After DOI bathing, the XII inspiratory discharge never recovered, even partially (Fig. 3*B*). In five other experiments, the 5-HT_2 agonist α-methyl-5-HT (30 μM) elicited significant decreases in the amplitude of the discharges, which were significantly larger at XII

(−38·4±13·1%) than at cervical (−16·8±5·9%) level. Five to ten minutes after α-methyl-5-HT bathing was resumed, a tendency to recover was observed as after 5-HT. In all the 5-HT_2 agonist experiments, the overall effects were identical to those obtained with 5-HT: a tonic discharge was evoked in the cervical roots but never in the XII nerve, the amplitude of which was decreased.

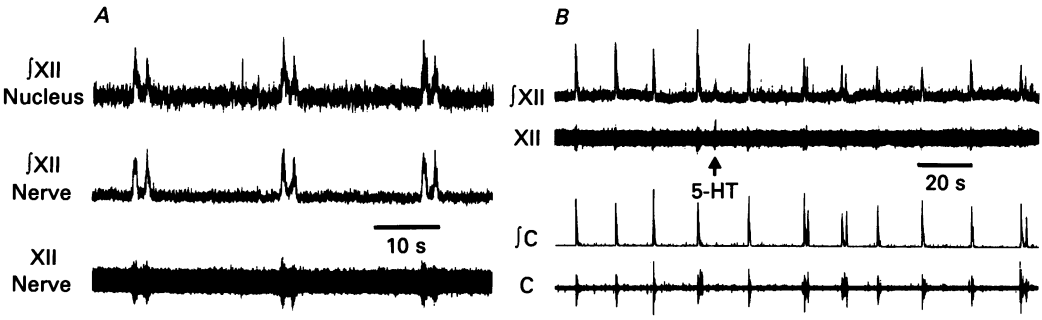

Fig. 4. Effects of local application of 5-HT within the hypoglossal nucleus on the XII inspiratory discharge. *A*, from top to bottom, integrated respiratory signal recorded via the central canal of a multi-barrelled micropipette placed within the XII nucleus, and integrated and raw XII inspiratory discharge. Horizontal bar, time scale. *B*, from top to bottom, integrated and raw discharges of the XII and cervical roots before and after ejection of 5 nl of 5-HT (0·1 M; at the arrow) within the hypoglossal nucleus at the corresponding site indicated in *A*. Note the decrease in XII discharge which occurred almost immediately after the ejection whereas the cervical discharge was not affected.

As with 5-HT (see above), the XII inspiratory burst occurred significantly later than the cervical one when the preparation was bathed with 5-HT_2 but not 5-HT_1 agonists. For example, α-methyl-5-HT bathing (30 μM) increased significantly the time lag between the two discharge onsets from 90±25·8 ms under normal medium to 240±46·2 ms.

It has already been demonstrated that 5-HT biosynthesis mechanisms remain functional in the brain stem–spinal cord preparation (Morin *et al.* 1991*a*). In five experiments, both cervical and hypoglossal discharges were recorded first under normal medium and then under medium containing L-tryptophan (50 μM). L-Tryptophan bathing significantly decreased within 15 min the discharge amplitude of the XII nerve (−43·9±26·4%) but not of the cervical ventral roots (3·9±19·7%). Permanent tonic firing was never observed on cervical roots.

Effects of local application of 5-HT within the motor nuclei on cervical and XII nerve discharges

Multi-barrelled micropipettes were used to apply 5-HT by pressure pulse ejection within the XII and cervical motor nuclei. In order to place the micropipette within the motor nuclei, extracellular recordings were performed via the central barrel filled with saline until a respiratory multi-unitary burst was recorded.

In ten experiments, a clearly identifiable inspiratory discharge was recorded within the hypoglossal nucleus (Fig. 4*A*). In this location, saline ejection did not elicit any changes in either the ipsilateral XII or the cervical activity. Five minutes later, ejection of 5-HT was performed, and the integrated XII discharges decreased

almost immediately within the first minute (Fig. 4*B*). Three minutes after the ejection, the mean integrated discharge dropped (by $-34 \pm 6\%$ of the control value) but this drop was statistically smaller than that observed during general 5-HT bathing. Simultaneous recordings of the cervical root activity did not reveal any

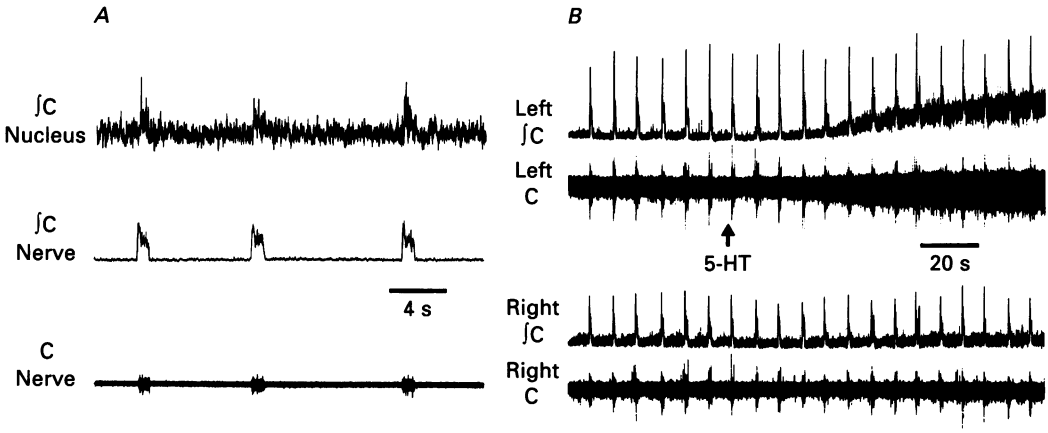

Fig. 5. Effects of local application of 5-HT within the cervical nucleus on the cervical inspiratory discharge. *A*, from top to bottom, example of integrated respiratory signal recorded via the central canal of a multi-barrelled micropipette in the left cervical C2 nucleus, integrated and raw C2 inspiratory discharges. *B*, from top to bottom, integrated and raw discharges of the left and right C2 roots before and after ejection of 5 nl of 5-HT (0·1 M; at the arrow) within the left C2 nucleus. Note the occurrence of a large tonic discharge on the left cervical root after the ejection and the persistence of inspiratory bursts; the right cervical discharge was not affected.

significant changes in either the frequency or in the amplitude ($-5{\cdot}0 \pm 6{\cdot}5\%$), which suggests that the drive from the medullary respiratory centres was not affected by the ejection. Five minutes after the 5-HT ejection, the XII discharge was still lower than the control value ($-27 \pm 8\%$) and had not fully recovered 10 min later. Unlike bath application, local application of 5-HT within the XII nucleus never increased the time lag between XII and cervical discharges.

In five further experiments, multi-barrelled micropipettes were placed within the cervical motor nuclei 200–300 μm below the ventral surface (Fig. 5*A*). Ejection of saline did not elicit any changes in the cervical motor output, whereas ejection of 5-HT elicited a large and continuous discharge on the ipsilateral C2 root but not on the contralateral root (Fig. 5*B*). This tonic discharge lasted for several minutes and recovery occurred 10–15 min after the ejection. During the C2 tonic discharge, inspiratory bursts remained clearly distinguishable, superimposed on the permanent firing, at the same frequency as before the 5-HT ejection. Depending on data compilation, the amplitude of the motor output during inspiration appeared either affected or not affected by ejection of 5-HT. On the one hand, a weak but significant decrease in amplitude appeared when the inspiratory motor output was estimated as the overall amplitude of the inspiratory burst minus the deflection of the trace due to tonic firing ($-7{\cdot}2 \pm 3{\cdot}5\%$). On the other hand, no significant decreases were observed when the inspiratory motor output was defined as the overall amplitude of the inspiratory burst.

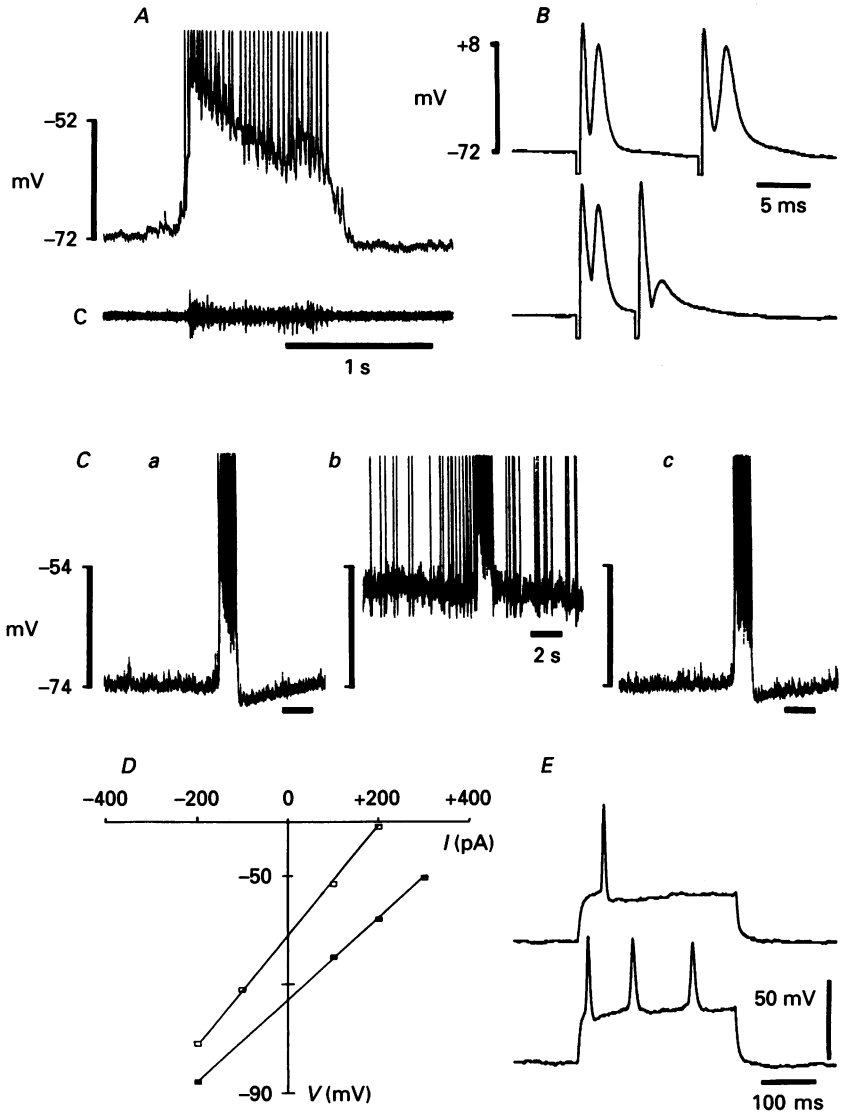

Fig. 6. Effects of 5-HT bathing on the intracellularly recorded activity of inspiratory cervical motoneurons. *A–E*, same C2 motoneuron. In *A* and *C*, the spikes were truncated. *A*, upper trace, intracellular recording from a C2 motoneuron showing the central respiratory drive potential and the firing during inspiration. *A*, lower trace, raw inspiratory discharge of the C2 nerve. *B*, antidromic identification by stimulating the C2 ventral root with two successive stimuli; upper trace, two stimuli 12 ms apart elicited two full antidromic spikes; lower trace, spike dissociation occurred when the second stimulus was applied less than 6 ms after the first. *C*, intracellular recording of the same motoneuron during bathing with normal medium (*Ca*), with 5-HT medium (*Cb*) and 5 min after 5-HT bathing (*Cc*); note in *Cb* that under 5-HT the motoneuron was strongly depolarized (17 mV) and showed an increased firing rate which overlapped onto expiratory phase; in *Cc*, after 5-HT, the depolarization disappeared. In *D*, the current–voltage relationships before (■) and during (□) 5-HT superfusion; note that the input membrane resistance of the cervical motoneuron increased by more than 42% under 5-HT superfusion. *E*, the depolarizing current pulse (0·35 nA, 300 ms), which elicited the firing of one spike under normal medium (upper trace), elicited sustained firing under 5-HT.

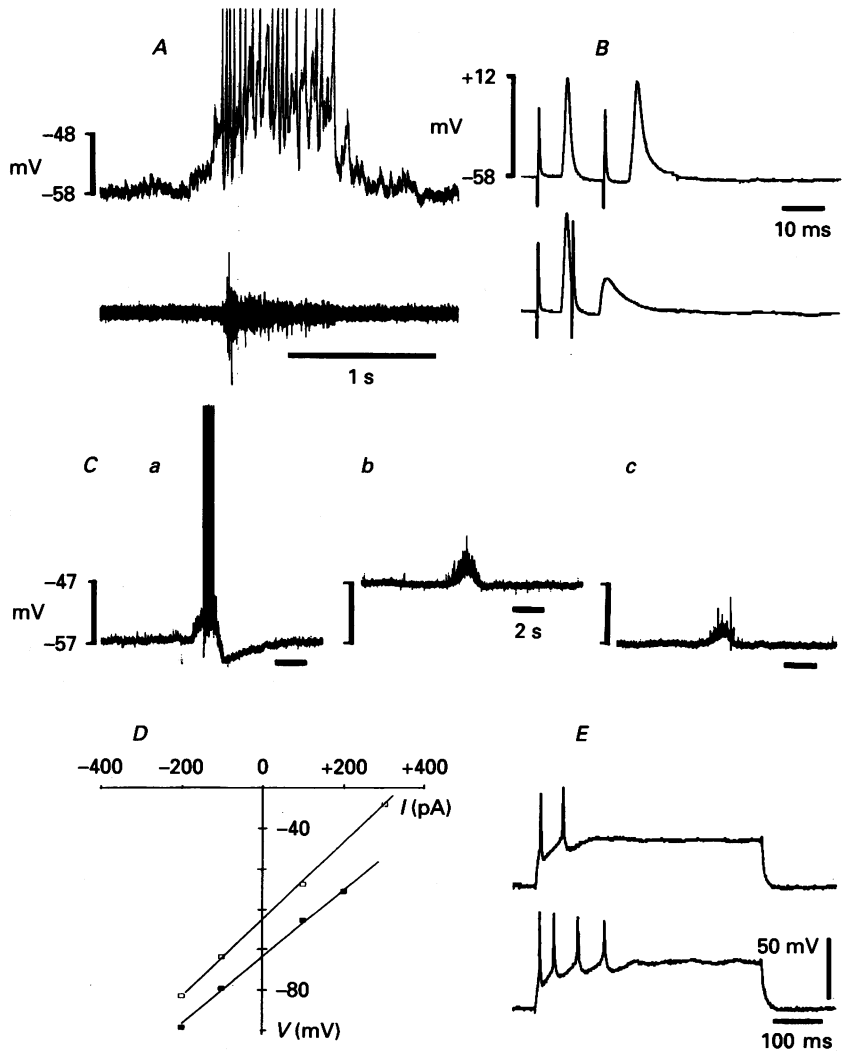

Fig. 7. Effects of 5-HT bathing on the intracellularly recorded activity of XII motoneurons. Three different XII motoneurons are illustrated in *A–B*, *C* and *D–E*, respectively. In *A* and *C*, the spikes were truncated. *A*, upper trace, intracellular recording from a XII motoneuron showing the central respiratory drive potential and the firing during inspiration; lower trace, raw inspiratory discharge of the C2 nerve. *B*, antidromic identification of the motoneuron by applying two successive stimuli to the XII roots; upper trace, two stimuli 16 ms apart elicited two full antidromic spikes; lower trace, spike dissociation occurred when the second stimulus occurred less than 8 ms after the first. *C*, intracellular recording during bathing with normal medium (*Ca*), with 5-HT medium (*Cb*) and 5 min after 5-HT bathing (*Cc*); note in *Cb* that under 5-HT the resting membrane potential of the motoneuron was slightly depolarized (9 mV) and that the motoneuron became inactive and showed a decreased central respiratory drive potential; in *Cc*, after control conditions were resumed, the central respiratory drive potential remained depressed, but the 5-HT depolarization disappeared and the motoneuron was still silent 5 min after 5-HT bathing. *D*, the current–voltage relationships before (■) and during (□) 5-HT superfusion; note that input membrane resistance of the XII motoneuron was not significantly affected by 5-HT superfusion (70 MΩ). *E*, depolarizing current pulse injected into this XII motoneuron (0·7 nA, 500 ms) elicited the firing of two

After recovery, the electrode was moved 100 μm to a more superficial or a deeper site; thereafter, no changes were elicited by 5-HT ejection except when the ejection was performed again at the same effective site.

Intracellular study of cervical and XII motoneurons

Intracellular recordings of twenty-one neurons (membrane polarization greater than 55 mV) were performed within the cervical and hypoglossal nuclei. On the basis of antidromic activation, eleven cervical (C2) and five XII motoneurons were unambiguously identified; five further neurons recorded within the hypoglossal nucleus, but without antidromic identification, were assumed to be XII motoneurons (see Discussion). Recording was easy to perform at the cervical level but extremely difficult at the hypoglossal level: one cervical motoneuron could be regularly recorded in every preparation, whereas at least sixty preparations were needed to record the ten XII motoneurons.

Under normal medium

During inspiration, both types of motoneurons showed depolarization (Figs 6*A* and 7*A*) named central respiratory drive potentials (CRDP, Sears 1964) which always occurred before the inspiratory burst in the C2 cervical root. Its onset sometimes occurred sooner in XII (135 ± 97 ms before the C2 discharge) than in cervical motoneurons (62 ± 27 ms before the C2 discharge), but comparisons between the means did not reveal any significant differences. The motoneurons then slowly depolarized during inspiration until reaching peak depolarization. A significant difference in the time to peak of the CRDP was observed between the two types of motoneurons; the cervical motoneurons reached the plateau sooner (139 ± 36 ms after the onset of the CRDP) than the XII motoneurons (418 ± 111 ms). The magnitude of the CRDP (measured from the resting potential during expiration to the peak depolarization during inspiration) appeared to be greater in the cervical ($16{\cdot}6 \pm 2{\cdot}2$ mV) than in the XII motoneurons ($11{\cdot}4 \pm 1{\cdot}3$ mV), but the difference was significant at $P < 0{\cdot}1$ and not at $P < 0{\cdot}05$. All the C2 and 8/10 XII motoneurons were active during inspiration in the same frequency range (26 ± 7 Hz), and fired only during inspiration. The mean input membrane resistances measured during expiration did not differ statistically between XII (62 ± 18 MΩ) and C2 motoneurons (47 ± 27 MΩ).

Under 5-HT medium

The normal medium was replaced by a medium containing 5-HT (30 μM) until a tonic discharge began to occur in the C2 root (2–3 min); the behaviour of XII and C2 motoneurons differed under 5-HT bathing, in complete agreement with the results described above: the C2 motoneurons remained active (or became more so), whereas all the XII motoneurons fell silent.

In the cervical motoneurons (Fig. 6*C*), the resting membrane potential slowly depolarized within 2–3 min of 5-HT bathing, whereas a permanent cervical root firing was recorded and the mean depolarization reached $19{\cdot}6 \pm 4{\cdot}4$ mV; the CRDP

spikes under normal medium (upper trace), and four spikes under 5-HT; this increased efficiency might be attributable to the rather strong depolarizing effects of 5-HT on this motoneuron (10 mV), which was inactive under 5-HT.

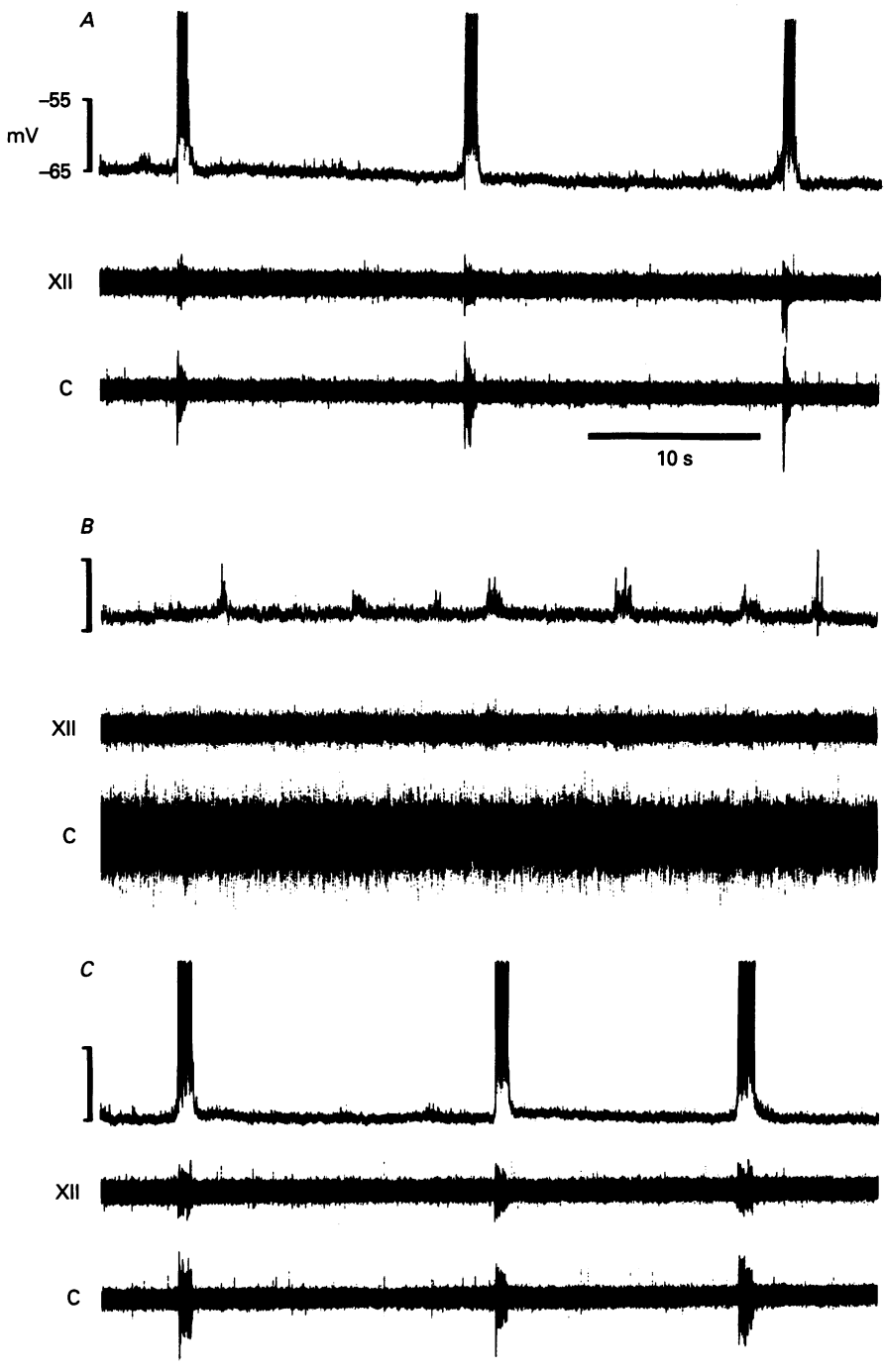

Fig. 8. Effects of 5-HT bathing on XII motoneuron. From top to bottom, intracellular recording of the activity of a XII motoneuron, XII and cervical C2 gross discharges. Recording on the same motoneuron under normal medium (*A*), under 5-HT medium (*B*) and again under normal medium (*C*). Note that under 5-HT (i) the XII motoneuron was depolarized (2 mV) but silenced, and showed a decreased central respiratory drive potential, (ii) the XII roots were also silent, and (iii) a large tonic discharge occurred from

superimposed on this depolarization was still clearly distinguishable. The amplitude of the CRDP, measured from the resting potential during expiration to the peak depolarization during inspiration, was significantly less (9 ± 3 mV) than under control conditions. Taking into account both the slow 19·6 mV depolarization of the resting potential and the 9 mV CRDP depolarization, the actual membrane resting potential during inspiration was 12 mV more depolarized under 5-HT than under control conditions. In keeping with this high level of depolarization, the discharge frequency of the cervical motoneurons increased significantly (paired *t* test) during inspiration (31 ± 5 Hz), and 4/11 were sometimes even recruited at a low frequency during expiration (around 10 Hz). In all cases, the input membrane resistance of the cervical motoneurons increased significantly (38 ± 14%; Fig. 6*D*). Pulses of depolarizing current set to elicit the firing of 1 spike under normal medium became able to elicit a sustained burst of 3–4 spikes under 5-HT (Fig. 6*E*). After the return to normal bathing medium, the slow depolarization disappeared within 2–4 min and the control firing level was rapidly recovered.

In one case, the effects of 5-HT on a cervical motoneuron could be tested prior to and after tetrodotoxin (TTX) treatment. Before TTX, 5-HT depolarized the motoneuron from 15 mV and increased the input membrane resistance by more than 60%. Bathing was then performed with medium containing TTX (10 μM) during 3 min; all spontaneous firing (in the impaled motoneuron and the respiratory nerves) was rapidly suppressed. After TTX treatment, 5-HT medium was again applied and again elicited a depolarization (8 mV) and an increase in the input membrane resistance (20%).

In XII motoneurons, 5-HT also elicited a depolarization of the resting membrane potential in 2–3 min of bathing (Fig. 7*C*), but this effect was significantly weaker ($8{\cdot}0 \pm 3{\cdot}5$ mV) than in the cervical motoneurons. The CRDP decreased significantly and its mean value reached only $5{\cdot}9 \pm 3{\cdot}7$ mV. Statistically, the CRDP under 5-HT was significantly lower in XII than in cervical motoneurons. Taking into account both the slow 8 mV depolarization and the 5·9 mV CRDP depolarization, the resting potential during inspiration of the XII motoneurons was therefore in the same range under both 5-HT and control conditions. All the XII motoneurons became inactive within 2–3 minutes of 5-HT bathing, but firing could still be initiated by delivering depolarizing pulses during the silent period. In 4/5 motoneurons, high-speed recordings revealed that the onset of the CRDP *versus* the beginning of the inspiratory cervical burst was delayed under 5-HT (mean, 180 ms; range, 80–400 ms). In all the XII motoneurons analysed, the input membrane resistance was not statistically affected by 5-HT (Fig. 7*D*). The effects of depolarizing pulses before and under 5-HT could be fully analysed in only one XII motoneuron: pulses set to elicit two spikes under normal medium were still efficient under 5-HT while the motoneuron was not spontaneously firing (Fig. 7*E*). This motoneuron showed the same input membrane resistance under 5-HT, although it was the most strongly depolarized (12 mV) by 5-HT bathing. After control conditions were resumed, only

---

the C2 cervical root (rhythmic inspiratory bursting could still be detected in the integrated signal, not shown). In this experiment, the XII inspiratory discharge recovered in both the motoneuron and XII roots after the return to normal medium (*C*). In *A* and *C*, the spikes were truncated.

one XII motoneuron recovered normal firing (Fig. 8), while the others remained inactive with a normal resting membrane potential but a reduced CRDP. The effects of 5-HT bathing on XII motoneurons after TTX treatment were not investigated for technical reasons (no impalement lasted long enough to allow 5-HT, recovery and 5-HT and TTX studies).

DISCUSSION

The present results obtained on the *in vitro* brain stem–spinal cord preparation of newborn rats shed some light on the mechanisms whereby serotonin (5-HT) modulates the activity of cervical and hypoglossal (XII) respiratory motoneurons. They are in complete agreement with previous data obtained on the same *in vitro* preparation indicating that although the activity of the spinal respiratory motoneurons was preserved, the activity of the XII motoneurons was drastically reduced when exogenous 5-HT was added to the bathing medium (Monteau *et al.* 1990*a*) and when 5-HT endogenous release was induced by electrical or pharmacological activation of the raphe nuclei (Morin *et al.* 1990*b*). This depressant effect of 5-HT appears to be fairly specific to XII motoneurons, since 5-HT is generally agreed to have excitatory effects on both cranial (McCall & Aghajanian, 1979) and spinal motoneurons (Holtman, Dick & Berger, 1986). The results obtained here furthermore showed that 5-HT delayed the XII inspiratory bursts as compared with the cervical ones. The data presented suggest that the 5-HT_2 receptors may be involved in several mechanisms acting either directly on the XII motoneurons or indirectly on their central drivers. The functional significance of this modulation will be discussed in connection with respiratory disorders to which both adults and newborn infants are subject.

The XII sample analysed intracellularly was limited to five unambiguously identified motoneurons and to five neurons located within the XII nucleus which were not identified antidromically. The latter were assumed to be XII motoneurons because (i) the hypoglossal nuclei are aggregates of motoneurons, (ii) the absence of antidromic activation may have been due to technical reasons (axons running in XII roots not sucked into the stimulating electrode, lesion of the axons within the electrode, etc.), and (iii) the unidentified motoneurons were identical to the five identified ones in their location, membrane properties, firing patterns under control conditions and response to 5-HT application. Furthermore, perihypoglossal neurons (which were not retrogradely labelled via rodamine injection into the XII nerve) have been identified in the area of the XII nucleus but have different electrophysiological properties from motoneurons (Viana, Gibbs & Berger, 1990). Intracellular recordings were very difficult to perform in XII motoneurons for unknown reasons; this cannot be attributable to the smallness of the soma at birth, since (i) the input membrane resistance of both XII and cervical motoneurons was in the same range under normal medium, and (ii) histological studies did not reveal any noteworthy differences between XII and phrenic motoneurons (Cameron, Fang, Brozanski & Guthrie, 1989). A previous intracellular study on XII motoneurons performed on newborn rat brain stem slices yielded lower input membrane resistance values (around 25 MΩ for Haddad, Donnelly & Getting, 1990); this may be due to technical differences.

Differences between XII and cervical patterns of discharge under control conditions

Both extracellular and intracellular analysis showed the existence of differences between the XII and cervical activities under control conditions. In this isolated preparation, from which any peripheral inputs which might interact with the central respiratory drive have been eliminated, the XII and cervical motoneurons might have been expected to display identical discharges. In fact, they differed in both the firing onset and the discharge pattern. The XII discharge has been reported to occur before the phrenic one in adults (for review see Iscoe, 1988) and was assumed to be necessary for efficient ventilation: to avoid a collapsus, the upper airways must be opened before the air is pumped by the chest muscles (Lowe, 1990). The reverse relationship with the cervical discharge preceding the XII one was frequently observed in this study and has already been described both *in vivo* during hypoxia (Hwang, St John & Bartlett, 1983) and *in vitro* (Smith *et al.* 1990). These differences in the onset of XII firing between the adult and newborn suggest that the control of the upper airways may be immature at birth, as already hypothesized in the newborn pig (Sica, Steele, Gandhi & Prasad, 1988), unless the lack of afferents from the periphery in this isolated preparation may explain the differences. Under normal medium, both at the extracellular level in nerves and at the intracellular level in motoneurons, comparisons between the discharge patterns and the CRDPs showed the existence of differences in behaviour between XII and cervical motoneurons. On the one hand, it can be assumed that the two pools of motoneurons both received the same central drive and that differences in their intrinsic properties explain the differences in the discharge patterns. This was not confirmed by our intracellular study, which did not show the existence of any noteworthy differences between the intrinsic properties. On the other hand, the two types of motoneurons probably did not share the same central drive. Although the drivers of the spinal respiratory motoneurons have been fairly thoroughly documented (for review see Monteau & Hilaire, 1991), the respiratory drives of the XII motoneurons still remain to be identified and the possibility that there may exist a specific drive to the upper airway motoneurons cannot be ruled out. Furthermore, the XII motoneurons are involved in other functions than breathing, such as swallowing and mastication, and receive other central drives (Amri & Car, 1988; Amri, Car & Roman, 1990). This heterogeneity among the inputs may explain the lack of significant correlation observed between the discharges of XII and cervical roots and the differences in the time course of the CRDP observed intracellularly.

Differences between XII and cervical patterns of discharge during drug superfusion

Already published studies of 5-HT effects on the respiratory activity of the brain stem–spinal cord preparation reported that adding exogenous 5-HT to the bathing medium (Monteau *et al.* 1990*a*) and eliciting release of endogenous 5-HT by raphe activation (Morin *et al.* 1990*b*) increased the respiratory frequency and depressed the XII inspiratory motor output. The experiments reported herein fully agree with these results since the depression of the XII activity by 5-HT is unambiguous but the remaining question is to know whether or not the 5-HT depressing effect is indeed specific to the XII motoneurons as suggested in previous studies (Monteau *et al.* 1990*a*; Morin *et al.* 1990*b*). The integrated recordings of the whole nerve discharges

revealed that the decrease in respiratory motor output was drastic in the XII (50 and 34% for general and local applications, respectively) but questionable in the cervical nerves. In the latter case, interpretation of the results was hardened in relation to the occurrence of a 5-HT-induced permanent firing. On the one hand, when the tonic firing level is subtracted from the overall amplitude of the inspiratory bursts, a weak but significant decrease in cervical discharge was observed (23 and 7% for general and local 5-HT applications, respectively). Although the mean decrease is statistically less in cervical than in XII motoneurons, specificity should be disregarded since 5-HT depresses both activities. On the other hand, when taking into account the tonic discharge (measurements of the overall amplitude of the inspiratory bursts without excluding the tonic level), no significant changes in the amplitude of the cervical discharge were observed and specificity of 5-HT inhibition of XII activity should be put forward. The 5-HT-induced tonic activity has been shown to involve both previously silent non-respiratory motoneurons which became recruited under 5-HT and inspiratory motoneurons, the firing of which became prolonged during expiration (Morin, Monteau & Hilaire, 1991*b*). Therefore measurements performed with exclusion of the tonic level eliminated an inspiratory component of the total inspiratory activity and constitute an erroneous data compilation leading to a false conclusion of decreased inspiratory motor output. This was again confirmed by intracellular recordings reported here which demonstrated that under 5-HT the firing of the cervical inspiratory motoneurons was slightly (but significantly) increased during inspiration (mean rate 31 Hz against 25 Hz, paired *t* test) and prolonged during expiration (mean rate 10 Hz) while all the XII motoneurons were silenced. Thus, the actual change elicited by 5-HT bathing on inspiratory activity is an increase for cervical motoneurons and a decrease for XII motoneurons.

When the preparation was bathed with a medium containing the 5-HT precursor L-tryptophan, the XII discharge was drastically depressed whereas the cervical discharge was not statistically affected. The XII depression has to be related to the release of endogenous 5-HT newly synthesised from L-tryptophan since 5-HT biosynthesis mechanisms are still functioning under the *in vitro* conditions (Morin *et al.* 1991*a*). In these experimental conditions where no tonic firing disturbed the analysis of the cervical discharge, the amplitude of the cervical discharge was not affected and the 5-HT effect on XII and cervical motoneurons was clearly different.

Involvement of 5-HT$_2$ receptors in the 5-HT-induced depression of the XII discharge

Serotonergic inputs to the XII motoneurons have been described (Takeuchi, Kojima, Matsuura & Sano, 1983) and 5-HT profiles detected throughout all the regions of the XII motor nucleus (Aldes, Marco & Chronister, 1989). These 5-HT inputs may originate from the medial reticular formation (Borke, Nau & Ringler, 1983), particularly the raphe nuclei which give rise to processes entering the XII motor nucleus (Aldes, Chronister, Marco, Haycock & Thibault, 1988). This was also demonstrated by injecting into the tongue Pseudorabies virus which first labelled hypoglossal motoneurons, and then, after a transneuronal passage, raphe nuclei (Card, Rinaman, Schwaber, Miselis, Whealy, Robbins & Enquist, 1990). The raphe 5-HT inputs may be responsible for the decrease in XII discharge elicited by raphe electrical and pharmacological stimulations (Morin *et al.* 1990*b*).

It is evident from electrophysiological, behavioural and radioligand binding studies that there exist multiple 5-HT receptor subtypes in the central nervous systems (for review see Frazer, Maayani & Wolfe, 1990). One of the aims of this study was therefore to define what 5-HT receptor subtypes are responsible for the depressant effects of 5-HT on the XII discharge. The pharmacological tools used in the present study are known to be specific agonists and antagonists of 5-HT receptor subtypes: 8-OH-DPAT and RU24969 are 5-HT_1 agonists, while DOI and α-methyl-5-HT specifically activate 5-HT_2 sites (Bradley *et al.* 1986).

A decrease in the XII discharge and an increase in the time lag between cervical and XII discharges were elicited by bathing the brain stem with 5-HT_2 agonists, but not 5-HT_1 agonists. Accordingly, ketanserin, which is a specific 5-HT_2 antagonist (Leysen, Niemegeers, Van Nueten & Laduron, 1981), protected the XII discharge from the depressant effects of 5-HT. These pharmacological data therefore point to the conclusion that 5-HT_2 receptors are responsible for the effects of 5-HT on the XII inspiratory discharge. Evidence exists that 5-HT_2 receptors are to be found on rat brain stem neurons (Davies, Wilkinson & Roberts, 1988), and more specifically within the XII nucleus of the rat (Pazos, Cortés & Palacios, 1985); at this site, the m-RNA for the 5-HT_2 receptors (Mengod, Pompeiano, Martinez-Mir & Palacios, 1990) have been detected.

In this study using the same pharmacological tools and analysing the cervical respiratory motoneurons, the conclusion is reached that activation of 5-HT_2 spinal receptors excited cervical respiratory motoneurons. This is in agreement with previous studies in which it was reported that 5-HT increases the input membrane resistance and depolarizes cervical (Morin *et al.* 1991*b*) and thoracic (Wang & Dun, 1990) motoneurons in neonatal rats via 5-HT_2 receptors. The question therefore arises as to what mechanisms govern the effects of 5-HT_2 on XII and spinal motoneurons.

Sites and mechanisms of action of 5-HT in XII depression

The serotonergic inactivation of XII motoneurons may be due to 5-HT acting directly on the motoneurons or to indirect actions on the network(s) controlling the motoneurons. As discussed below, none of these hypothetical mechanisms can be safely ruled out so far.

Direct effects

Local and general applications of 5-HT led to the same qualitative results in a given pool of motoneurons, which suggests that the changes in motor output observed under 5-HT may be at least partly due to the direct action of 5-HT within the nuclei. This action might be mediated either postsynaptically by the membrane of the motoneurons or presynaptically by the axon terminals of the central drivers impinging on the motoneurons.

In the case of the cervical motoneurons, both local and general applications elicited a tonic discharge with persistent inspiratory bursts. The intracellular studies showed that general application of 5-HT depolarized the motoneurons (20 mV), increased their input membrane resistance (40%), and hence their firing; these *in vitro* results, which complete those presented in a preliminary report (Morin *et al.* 1991*b*), are in agreement with results demonstrating the excitatory effects of 5-HT

on spinal respiratory (Holtman *et al.* 1986) and non-respiratory motoneurons (Myslinski & Anderson, 1978; Takahashi & Berger, 1990). In slices, 5-HT depolarizes motoneurons and increases the input membrane resistance by activating the 5-HT_2 receptors (Wang & Dun, 1990). The 5-HT changes in input membrane resistance and polarization, and more particularly their persistence after TTX, argue in favour of the idea that the 5-HT has direct excitatory effects. The existence of 5-HT receptors on the membrane of the cervical respiratory motoneurons has even been confirmed in ultrastructural studies which described 5-HT synaptic terminals in direct contact with the soma and dendrites of phrenic motoneurons (Holtman, Vascik & Maley, 1990).

In the case of the XII motoneurons, both local and general applications of 5-HT depressed the XII activity. Intracellular analysis demonstrated that 5-HT did not affect the input membrane resistance and evoked only a slight depolarization (8 mV). This suggests that 5-HT has weak postsynaptic effects on XII motoneurons, which is in agreement with ultrastructural studies in which it was reported that most of the 5-HT profiles were non-synaptic (68%, Aldes *et al.* 1989). The possibility cannot be totally ruled out, however, that the observed effects of 5-HT on XII motoneurons were mediated postsynaptically, since (i) some (however few) 5-HT synaptic profiles do exist on dendrites or soma of XII motoneurons (Aldes *et al.* 1989), and (ii) m-RNA for the 5-HT_2 receptor is present within the XII nucleus (Mengod *et al.* 1990).

The 5-HT-induced decrease in CRDP observed in XII motoneurons might be at least partly due to presynaptic effects of 5-HT on the axon terminals from the respiratory centres impinging on the motoneurons, since local application of 5-HT within the XII nucleus elicited a decrease in the XII discharge which was not attributable either to changes in the motoneuron intrinsic properties (the weak depolarization without any changes in input membrane resistance observed in the intracellular study) or to indirect effects on other central structures. Possible presynaptic depressant actions of 5-HT have been reported (Klein, Camardo & Kandel, 1982; Maura, Fedele & Raiteri, 1989; Buchanan & Grillner, 1991). In histological studies on the 5-HT innervation of the XII nucleus, no 5-HT axo-axonic synapses were observed which might have mediated 5-HT presynaptic inhibition, but occasionally some 5-HT preterminal axons were detected running among several unlabelled axons (see Fig. 3, from Aldes *et al.* 1989), which might have been some of the axons of the inspiratory central drivers of the XII motoneurons. This suggests the idea that a modulation of the transmission of the respiratory drive to the XII motoneurons might take place. In the case of the XII motoneurons, where 5-HT elicited only a weak depolarization, the CRDP might have decreased sufficiently to silence the motoneurons whereas in the case of the cervical motoneurons, the large depolarization easily counterbalanced the decrease in central respiratory drive. It cannot be excluded that quantitative differences (number of synapses, receptor density, etc.) are responsible for differences in behaviour of XII and cervical motoneurons under 5-HT excess.

Indirect effects

Upon comparing the effects of general and local applications of 5-HT on the XII discharge, quantitative differences were found to exist: the decrease in XII discharge amplitude was significantly greater during general 5-HT application (−59%) than

during local 5-HT application within the XII nucleus (−34%). On the one hand, this quantitative difference may be attributable to the diffusion of 5-HT being wider within the whole XII nucleus during general *versus* local applications. On the other hand, this suggests the possibility that in the case of general bathing, 5-HT may have acted on other central targets (respiratory centres, swallowing centres, etc.). The XII motoneurons receive numerous brain stem afferents (Borke *et al.* 1983) and, as stated above, besides the respiratory drive, other drives such as those originating from the swallowing and mastication centres (Amri & Car, 1988; Amri *et al.* 1990) which are sensitive to 5-HT (Kessler & Jean, 1987). The hypothesis that 5-HT may act indirectly on the various inputs to the XII motoneurons (respiratory and others) cannot be excluded during general bathing experiments and might account for the XII behaviour under 5-HT.

Local applications of 5-HT within the XII nucleus did not affect the onset time of the XII discharge *versus* the cervical one as did general applications of 5-HT_2 agonist. This suggests that the increase in the time lag resulting from general application may not involve a local effect of the amine on the XII nucleus but rather an action on other central targets such as the central drivers of the XII motoneurons. This is in agreement with intracellular data which revealed that the onset of the CRDP was delayed by 5-HT: this effect of 5-HT cannot be explained in terms of local action.

Functional significance of the 5-HT-induced modulation of the XII discharge

The results described above may help to throw considerable light on obstructive apnoeas, these respiratory disorders which occur in both adults and newborn infants. In the adult, the sleep apnoea syndrome has been described as transient respiratory arrests occurring as soon as the patient falls asleep or as soon as sleep deepens (Kurtz, Krieger & Stierle, 1978). These transient arrests may be due to either central apnoea (a complete cessation of the activity of all the respiratory muscles), obstructive apnoea (cessation of the activity of the respiratory muscles of the upper airways but persistence of the activity of the chest respiratory muscles) or mixed apnoea (a combination of both types). In the case of obstructive apnoea, a partial or total invagination of the posterolateral pharyngeal walls was described, along with a significant decrease in, or the complete disappearance of, EMG activity of several muscles in the upper airways including those innervated by the XII, particularly the genioglossus (Guilleminault, Hill, Simmons & Dement, 1978). Despite the fact that the precise locus of upper airway closure has not yet been systematically identified and may vary from one patient to another (Guilleminault *et al.* 1978; Remmers, 1984), any inadequacy of the inspiratory upper airway muscles to overcome the negative pressure generated in the airway by the inspiratory pump muscles will favour the occurrence of obstructive apnoea (Sauerland & Harper, 1976; Remmers *et al.* 1978; Iscoe, 1988). These respiratory disorders may be serious in obese adults, who can suffer from recurrent obstructive apnoea leading to hypoxaemia and sleep deprivation due to frequent arousals during airway collapses. The results reported here demonstrate that a drastic decrease in the inspiratory activity of the XII nerve and an increased time lag between the onsets of the cervical and hypoglossal discharges can result from 5-HT excess. Convincingly, adding L-tryptophan to the bathing medium to increase endogenous 5-HT release depressed XII discharge. The

depression of the XII motor output may favour the occurrence of obstructive apnoea: in normal subjects, the activity of the genioglossus muscle, which tends to dilate the upper airways, starts prior to inspiration, but in the case of obstructive sleep apnoea syndrome, its activity is delayed and this time lag may predispose patients to upper airway collapse and obstruction (Lowe, 1990).

In patients suffering from sleep obstructive apnoea, increases in the levels of the inactive catabolite (5-hydroxyindoleacetic acid) were reported to occur (Cramer, Warter, Renaud, Krieger, Marescaux & Hammers, 1981) and might be attributable to an abnormally high level of 5-HT release. Furthermore, 5-HT has been assumed to play a role in sleep regulation, since tryptophan may restore the capacity to sleep in animals with 5-HT-depleted raphe neurons (for review see Vogt, 1982) and 5-HT_2 antagonist modifies human sleep (Idzikowski, Mills & Glennard, 1986). Taken as a whole, these results may suggest that a dysfunction of the mechanisms governing 5-HT synthesis and release may lead to sleep and respiratory disorders. In the newborn, developmental changes in the 5-HT biosynthesis mechanisms occur during the first few weeks of life (Hamon & Bourgoin, 1982). A dysfunction in the maturation of these mechanisms may lead to abnormal levels of 5-HT release and therefore to obstructive apnoea, ventilatory disorders and acute hypoxia. Hypoxia is known to elicit, after a transient ventilatory response, a depression of ventilation in the newborn (for review see Jansen & Chernick, 1983). These two respiratory disorders may be coupled via a positive feedback (obstructive apnoea leading to hypoxia leading to a decrease in central respiratory drive which worsens the obstructive apnoea condition) and have drastic consequences in the newborn and might be responsible for some cases of Sudden Infant Death Syndrome.

The present study on the brain stem–spinal cord preparation of newborn rats confirms previous data (Monteau *et al.* 1990*a*; Morin *et al.* 1990*b*) demonstrating that 5-HT specifically depresses XII inspiratory activity, and suggests the involvement of 5-HT_2 receptors presumably located at the presynaptic level on the terminal of the inspiratory axons impinging on the XII motoneurons. The functional significance of this depression may be highly relevant to the obstructive apnoea occurring both in adults and newborn infants. Nevertheless, all these results were obtained under *in vitro* conditions and need first to be confirmed under physiological *in vivo* conditions before any final conclusions can be made.

The authors would like to acknowledge the highly useful assistance of Mrs A. M. Lajard with the photography and that of Mr M. Manneville with the electronic equipment. This research was supported by CNRS (URA 0205), the INSERM (Grant 886006) and the foundation 'Naître et Vivre'. They also wish to thank Dr J. Blanc for revising the English.

REFERENCES

ALDES, L. D., CHRONISTER, R. C., MARCO, L. A., HAYCOCK, J. W. & THIBAULT, J. (1988). Differential distribution of biogenic amines in the hypoglossal nucleus of the rat. *Experimental Brain Research* **73**, 305–314.

ALDES, L. D., MARCO, L. A. & CHRONISTER, R. B. (1989). Serotonin-containing axon terminals in the hypoglossal nucleus of the rat. An immuno-electronmicroscopic study. *Brain Research Bulletin* **23**, 249–256.

AMRI, M. & CAR, A. (1988). Projections from the medullary swallowing center to the hypoglossal motor nucleus: a neuroanatomical and electrophysiological study in the sheep. *Brain Research* **441**, 119–126.

AMRI, M., CAR, A. & ROMAN, C. (1990). Axonal branching of medullary swallowing neurons projecting on the trigeminal and hypoglossal motor nuclei: demonstration by electrophysiological and fluorescent double labelling techniques. *Experimental Brain Research* **81**, 384–390.

BORKE, R. C., NAU, M. E. & RINGLER, R. L. (1983). Brain stem afferents of hypoglossal neurons in the rat. *Brain Research* **269**, 47–55.

BRADLEY, P. B., ENGELS, G., FENIUK, W., FOZARD, J. R., HUMPHREY, P. A., MIDDLEMISS, D. N., MYLECHARANE, E. J., RICHARDSON, B. P. & SAXENA, P. R. (1986). Proposal for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology* **25**, 563–576.

BUCHANAN, J. T. & GRILLNER, S. (1991). 5-Hydroxytryptamine depresses reticulospinal excitatory postsynaptic potentials in motoneurons of the lamprey. *Neuroscience Letters* **112**, 71–74.

CAMERON, W. E., FANG, H., BROZANSKI, B. S. & GUTHRIE, R. D. (1989). The postnatal growth of motorneurons at three levels of the cat neuraxis. *Neuroscience Letters* **104**, 274–280.

CARD, J. P., RINAMAN, L., SCHWABER, J. S., MISELIS, R. R., WHEALY, M. E., ROBBINS, A. K. & ENQUIST, L. W. (1990). Neurotropic properties of Pseudorabies virus: uptake and transneuronal passage in the rat central nervous system. *Journal of Neuroscience* **10**, 1974–1994.

CRAMER, H., WARTER, J.-M., RENAUD, B., KRIEGER, J., MARESCAUX, CHR. & HAMMERS, R. (1981). Cerebrospinal fluid adenosine 3′,5′-monophosphate, 5-hydroxyindoleacetic acid and homovanillic acid in patients with sleep apnoea syndrome. *Journal of Neurology, Neurosurgery and Psychiatry* **44**, 1165–1167.

DAVIES, M., WILKINSON, L. S. & ROBERTS, M. H. (1988). Evidence for excitatory 5-HT_2 receptors on rat brainstem neurones. *British Journal of Pharmacology* **94**, 483–491.

ELDRIDGE, F. L. & MILLHORN, D. E. (1981). Central regulation of respiration by endogenous neurotransmitters and neuromodulators. *Annual Review of Physiology* **43**, 121–135.

ERRCHIDI, S., HILAIRE, G. & MONTEAU, R. (1990). Permanent release of noradrenaline modulates respiratory frequency in the newborn rat: an *in vitro* study. *Journal of Physiology* **429**, 497–510.

FRAZER, A., MAAYANI, S. & WOLFE, B. B. (1990). Subtypes of receptors for serotonin. *Annual Review of Pharmacology and Toxicology* **30**, 307–348.

GUILLEMINAULT, C., HILL, M. W., SIMMONS, F. B. & DEMENT, W. C. (1978). Obstructive sleep apnea: electromyographic and fibroptic studies. *Experimental Neurology* **62**, 48–67.

HADDAD, G. G., DONNELLY, D. F. & GETTING, P. A. (1990). Biophysical properties of hypoglossal neurons *in vitro*: intracellular studies in adult and neonatal rats. *Journal of Applied Physiology* **69**, 1509–1517.

HAMON, M. & BOURGOIN, S. (1982). Characteristics of 5-HT metabolism and function in developing brain. In *Biology of Serotonergic Transmission*, ed. OSBORNE, N. N., pp. 197–220. J. Wiley, New York.

HARADA, Y., KUNO, M. & WANG, Y. Z. (1985). Differential effects of carbon dioxide and pH on central chemoreceptors in the rat *in vitro*. *Journal of Physiology* **368**, 679–693.

HILAIRE, G., MONTEAU, R. & ERRCHIDI, S. (1989). Possible modulation of the medullary respiratory rhythm generator by the noradrenergic A5 area: an *in vitro* study in the newborn rat. *Brain Research* **485**, 325–332.

HILAIRE, G., MONTEAU, R., GAUTHIER, P., REGA, P. & MORIN, D. (1990). Functional significance of the dorsal respiratory group in adult and newborn rats: *in vivo* and *in vitro* studies. *Neuroscience Letters* **111**, 127–132.

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

HOLTMAN, J. R., VASCIK, D. S. & MALEY, B. E. (1990). Ultrastructural evidence for serotonin-immunoreactive terminals contacting phrenic motoneurons in the cat. *Experimental Neurology* **109**, 269–272.

HWANG, J.-C., ST JOHN, W. M. & BARTLETT, D. (1983). Respiratory-related hypoglossal nerve activity: influence of anesthetics. *Journal of Applied Physiology* **55**, 785–792.

IDZIKOWSKI, C., MILLS, F. J. & GLENNARD, R. (1986). 5-Hydroxytryptamine-2 antagonist increases human slow wave sleep. *Brain Research* **378**, 164–168.

ISCOE, S. D. (1988). Central control of the upper airway. In *Respiratory Function of the Upper Airway*, ed. MATHEW, O. P. & SANT' AMBROGIO, G., pp. 125–186. Marcel Dekker, Inc., New York.

JANSEN, A. H. & CHERNICK, V. (1983). Development of respiratory control. *Physiological Reviews* **63**, 437–483.

KESSLER, J. P. & JEAN, A. (1987). Effects of catecholamines on the swallowing reflex after pressure microinjections into the lateral solitary complex of the medullary oblongata. *Brain Research* **386**, 69–77.

KLEIN, M., CAMARDO, J. & KANDEL, E. R. (1982). Serotonin modulates a specific potassium current in the sensory neurons that show presynaptic facilitation in *Aplysia*. *Proceedings of the National Academy of Sciences of the USA* **79**, 5713–5717.

KURTZ, D., KRIEGER, J. & STIERLE, J. C. (1978). EMG activity of cricothyroid and chin muscles during wakefulness and sleeping in the sleep apnea syndrome. *Electroencephalography and Clinical Neurophysiology* **45**, 777–784.

LEYSEN, J. E., NIEMEGEERS, J. E., VAN NUETEN, J. M. & LADURON, P. M. (1981). ^{3}H ketanserin (R 41 468), a selective ^{3}H ligand for serotonin 2 receptors binding sites. Binding properties, brain distribution and functional role. *Molecular Biology* **21**, 301–314.

LOWE, A. A. (1990). The tongue and airway. *Otolaryngologic Clinics of North America* **23**, 677–698.

MCCALL, R. B. & AGHAJANIAN, G. K. (1979). Serotonergic facilitation of facial motoneuron excitation. *Brain Research* **169**, 11–27.

MAURA, G., FEDELE, E. & RAITERI, M. (1989). Acetylcholine release from rat hippocampal slices is modulated by 5-hydroxytryptamine. *European Journal of Pharmacology* **165**, 173–179.

MENGOD, G., POMPEIANO, M., MARTINEZ-MIR, M. I. & PALACIOS, J. M. (1990). Localization of the m-RNA for the 5-HT_2 receptor by *in situ* hybridization histochemistry. Correlation with the distribution of receptor sites. *Brain Research* **524**, 139–143.

MONTEAU, R. & HILAIRE, G. (1991). The spinal respiratory motoneurons. *Progress in Neurobiology* **37**, 83–144.

MONTEAU, R., MORIN, D., HENNEQUIN, S. & HILAIRE, G. (1990*a*). Differential effects of serotonin on respiratory activity of hypoglossal and cervical motoneurons: an in vitro study on the newborn rat. *Neuroscience Letters* **111**, 127–132.

MONTEAU, R., MORIN, D. & HILAIRE, G. (1990*b*). Acetylcholine and central respiratory chemosensitivity: in vitro study in the newborn rat. *Respiration Physiology* **81**, 241–254.

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

MORIN, D., HENNEQUIN, S., MONTEAU, R. & HILAIRE, G. (1990*b*). Depressant effect of raphe stimulation on inspiratory activity of the hypoglossal nerve: *in vitro* study in the newborn rat. *Neuroscience Letters* **116**, 299–303.

MORIN, D., MONTEAU, R. & HILAIRE, G. (1991*a*). 5-Hydroxytryptamine modulates central respiratory activity in the newborn rat: an *in vitro* study. *European Journal of Pharmacology* **192**, 89–95.

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

MOSS, I. R., DENAVIT-SAUBIE, M., ELDRIDGE, F. L., GILLIS, R. A., HERKENHAM, M. & LAHIRI, S. (1986). Neuromodulators and transmitters in respiratory control. *Federation Proceedings* **45**, 2133–2147.

MURAKOSHI, T. & OTSUKA, M. (1985). Respiratory reflexes in an isolated brainstem–lung preparation of the newborn rat: possible involvement of gamma-aminobutyric acid and glycine. *Neuroscience Letters* **62**, 63–68.

MURAKOSHI, T., SUZUE, T. & TAMAI, S. (1985). A pharmacological study on respiratory rhythm in the isolated brainstem–spinal cord preparation of the newborn rat. *British Journal of Pharmacology* **86**, 95–104.

MYSLINSKI, N. R. & ANDERSON, E. G. (1978). The effects of serotonin precursors on α- and γ-motoneuron activity. *Journal of Pharmacology and Experimental Therapeutics* **204**, 19–26.

ONIMARU, H., ARATA, A. & HOMMA, I. (1988). Primary respiratory rhythm generator in the medulla of brainstem–spinal cord preparation from newborn rat. *Brain Research* **445**, 314–324.

PAZOS, A., CORTES, R. & PALACIOS, J. M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Research* **346**, 231–249.

REMMERS, J. E. (1984). Obstructive sleep apnea. *American Review of Respiratory Diseases* **130**, 153–155.

REMMERS, J. E., DE GROOT, W. J., SAUERLAND, E. K. & ANCH, A. M. (1978). Pathogenesis of upper airway occlusion during sleep. *Journal of Applied Physiology* **44**, 931–938.

SAUERLAND, E. K. & HARPER, R. M. (1976). The human tongue during sleep: electromyographic activity of the genioglossus muscle. *Experimental Neurology* **51**, 160–170.

SEARS, T. A. (1964). The slow potential of thoracic respiratory motoneurones and their relation to breathing. *Journal of Physiology* **175**, 404–424.

SICA, A. L., STEELE, A. M., GANDHI, M. R. & PRASAD, N. (1988). Factors affecting central inspiratory modulation of hypoglossal motoneuron activity in newborn pigs. *Journal of Developmental Physiology* **10**, 285–295.

SMITH, J. C., GREER, J. J., LIU, G. & FELDMAN, J. (1990). Neural mechanisms generating respiratory pattern in Mammalian brain stem–spinal cord *in vitro*. I. Spatiotemporal patterns of motor and medullary neuron activity. *Journal of Neurophysiology* **64**, 1149–1169.

SUZUE, T. (1984). Respiratory rhythm generation in the *in vivo* brainstem–spinal cord preparation of neonatal rat. *Journal of Physiology* **93**, 173–183.

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

TAKEUCHI, Y., KOJIMA, M., MATSUURA, T. & SANO, Y. (1983). Serotonergic innervation of the motoneurons in the mammalian brainstem. Light and electron microscopic immunohistochemistry. *Anatomy and Embryology* **167**, 321–333.

VIANA, F., GIBBS, L. & BERGER, A. J. (1990). Double- and triple-labeling of functionally characterized central neurons projecting to peripheral targets studied *in vitro*. *Neuroscience* **38**, 829–841.

VOGT, M. (1982). Some functional aspects of central serotoninergic neurons. In *Biology of Serotonergic Transmission*, ed. OSBORNE, N. N., pp. 299–315. J. Wiley, New York.

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