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PERMANENT RELEASE OF NORADRENALINE MODULATES RESPIRATORY FREQUENCY IN THE NEWBORN RAT: AN *IN VITRO* STUDY

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SUMMARY

1. Respiratory activity was recorded on ventral cervical roots during *in vitro* experiments performed on superfused newborn rat brain stem–cervical cord preparations.

2. Eliminating the pontine structures by performing a transection at the level of the ponto-medullary junction resulted in a sustained increase in respiratory frequency, which suggests the existence of a pontine inhibitory drive impinging on the medullary rhythm generator.

3. Noradrenaline (NA) and drugs affecting NA efficiency were added to the bathing medium and the resulting changes in respiratory frequency were analysed. NA decreased the respiratory frequency, and this effect was potentiated by pargyline (an inhibitor of the NA degradation by monoamine oxydases) and blocked by yohimbine (an α_2-antagonist).

4. Yohimbine or piperoxane (which blocks the α_2-adrenoceptors) increased the resting respiratory frequency to the level reached after ponto-medullary transection, whereas pargyline or desipramine (which potentiates NA efficiency) decreased the respiratory rate. Since these effects were no longer observed after elimination of the pons, it is suggested that a permanent release of endogenous NA by pontine areas may modulate the activity of the medullary respiratory rhythm generator.

5. When α-methyltyrosine (an inhibitor of NA biosynthesis) was applied to the pons, the respiratory frequency was increased, whereas when tyrosine (a precursor of NA) was applied, the respiratory frequency decreased. This decrease was enhanced by pargyline, suppressed by α-methyltyrosine and blocked by piperoxane.

6. To conclude, it is suggested that the mechanisms underlying NA biosynthesis (i) continue to function under these *in vitro* experimental conditions and (ii) are responsible for a permanent release of endogenous NA, which slows down the respiratory frequency. These results are discussed as regards the possibility that the medullary respiratory rhythm generator may be modulated via the noradrenergic area A5 in the newborn rat.

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INTRODUCTION

Isolated newborn rat brain stem–spinal cord preparation have been found to generate a rhythmic activity *in vitro* synchronized with upward movements of the thorax (Suzue, 1984). Periodic discharges may be recorded on cranial nerves, cervical ventral roots, and especially on the phrenic nerve (Suzue, 1984; Harada, Kuno & Wang, 1985*a*; Harada, Wang & Kuno, 1985*b*; Murakoshi & Otsuka, 1985; Smith & Feldman, 1987) and neurons of the medullary respiratory centres (Hilaire, Monteau, Gauthier, Rega & Morin, 1990). This preparation has turned out to be a suitable model for pharmacological studies on central respiratory activity (Murakoshi, Suzue & Tamai, 1985) since drugs added to the bathing medium diffuse within the whole brain stem and induce respiratory changes not affected by the periphery. In previous studies (Errchidi, Hilaire & Monteau, 1988; Hilaire, Monteau & Errchidi, 1989), it has been suggested on the basis of section, coagulation and stimulation experiments that the medullary respiratory rhythm generator may be tonically inhibited by a structure located in the caudal ventrolateral pons, more specifically in the vicinity of area A5. Adding noradrenaline (NA) to the bathing medium decreased the respiratory frequency whereas adding yohimbine or idazoxan (α_2-antagonists) had the opposite effect. Moreover, electrical stimulations performed in the vicinity of area A5 slowed down the respiratory rate, and this effect was blocked by α_2-antagonists. From these data, it was assumed that the activity of the medullary respiratory generator might be permanently modulated by the A5 pontine nucleus (Hilaire *et al.* 1989). This proposal was based on the assumptions that (i) the mechanisms underlying noradrenergic biosynthesis continue to function under the *in vitro* experimental conditions and (ii) the pontine A5 structures continuously release endogenous NA. The aim of the present study was to test these assumptions. Drugs known for affecting NA efficiency and NA biosynthesis were added to the medium used for bathing the brain stem. The changes in respiratory frequency elicited were consistent with the idea that the medullary respiratory generator is under the permanent modulating influence of a region in the pons.

METHODS

Newborn rats (0–3 days old) were anaesthetized with ether and decerebrated. A section was performed at the level of the last cervical vertebra and the skin and muscles were rapidly removed. The preparation was then placed in a 2 ml chamber filled with artificial cerebrospinal fluid. Cranial and vertebral bones were removed with thin forceps under binocular control. The brain stem was fixed with the ventral surface upwards. The artificial cerebrospinal fluid medium was identical to that reported by Murakoshi *et al.* (1985) (in mM: NaCl, 129; KCl, 3·35; $CaCl_2$, 1·16; $MgCl_2$, 1·15; $NaHCO_3$, 21·0; NaH_2PO_4, 0·58; glucose, 30·0), warmed to 27 ± 0·5 °C and equilibrated with 95% O_2 and 5% CO_2 (pH 7·3). The bathing medium was permanently drained by suction and the perfusion rate was such that the total chamber volume was replaced within 30 s.

Inspiratory electrical activities of the cervical ventral roots were recorded using suction electrodes. The respiratory frequency was defined as the frequency of these activities. Signals were filtered (5–3000 Hz), amplified (Neurolog System, Digitimer) and fed to a leaky integrator, oscilloscopes and a paper recorder (Gould TA 2000).

Drugs were dissolved in the bathing medium and applied by superfusion for 6–12 min. Occasionally, the *in vitro* bath was partitioned with a barrier (improved with Vaseline) at the ponto-medullary junction; each part of the bath contained a lateral inlet–outlet for bathing media, so that the pons and medulla could each be superfused with a different medium. In each

experiment, a control period of 3–5 min was run in order to define a mean respiratory frequency per minute under normal medium. The brain stem was then superfused with medium containing drugs and the changes in respiratory frequency were expressed each minute as a percentage of the mean control value. Only one trial was performed at each experiment, and experiments were repeated on several brain stem preparation with a standardized procedure in order to determine the mean effect. Results were expressed as the means ± S.E.M. Differences were taken to be significant at P values smaller than 0·05, using Student's t test to make paired and unpaired comparisons.

RESULTS

Types of preparation

In the present experiments, the following two types of preparation were used, in which the brain stem was transected at different levels: ponto-medullary preparations (transection at the intercollicular level, $n = 58$) and medullary preparations (transection at the level of the VI cranial nerves, $n = 42$). The elimination of the pontine structures by transection immediately elicited a sustained increase in respiratory frequency. Thereafter, the respiratory frequency remained high and the amplitude of the integrated inspiratory discharge decreased. Figure 1 shows the pattern of respiratory activity recorded on cervical ventral roots and illustrates the increase in respiratory frequency evoked by a ponto-medullary transection in one experiment. In all these experiments, the resting respiratory frequency in medullary preparations was more than twice that observed in ponto-medullary preparations ($10·5 \pm 0·5$ cycles min^{-1}, $n = 42$ *versus* $4·2 \pm 0·4$ cycles min^{-1}, $n = 58$, respectively; $P < 0·001$).

Effects of noradrenaline (NA) on respiratory frequency

In medullary preparations, adding NA to the bathing medium (25 μM) elicited decreases in respiratory frequency as shown in Fig. 2 in the case of a single experiment. With normal medium, the control resting respiratory frequency was around 12 cycles min^{-1}. Two minutes after applying the medium containing NA, the respiratory rate began to decrease and dropped to 8 cycles min^{-1} within 4–6 min while the amplitude of the integrated inspiratory discharge increased. This effect was reproducible from one experiment to another and the mean affect of NA medium on respiratory frequency is also shown in Fig. 2. In twelve experiments performed under standardized conditions, the mean respiratory frequency per minute decreased significantly 2 min after applying the NA medium (25 μM) and stabilized at around 80% of the control values 4–6 min after NA bathing began. When returned to normal medium, the control respiratory frequency was restored within 3–4 min. During the decrease in respiratory frequency, the amplitude of the inspiratory discharge increased. In five experiments, these NA effects were blocked by applying medium containing the α_2-antagonist yohimbine (200 μM) for 7 min prior to NA application.

In five experiments in which medium with a low NA content (5 μM) was used, the respiratory frequency either increased (1/5), decreased (1/5) or was not affected (3/5) and the mean changes observed were not statistically significant. In five further experiments, a pre-treatment was performed for 9 min with medium containing pargyline (100 μM). Pargyline medium elicited a decrease in respiratory frequency within 3–6 min and then stabilized (see below). The control respiratory frequency

values (100%) were then defined after stabilization under pargyline. Thereafter, whenever medium containing 5 μM–NA was applied, it efficiently decreased the respiratory frequency in all the experiments (Fig. 3). The mean effect was a significant decrease of 25 ± 12% in the control value occurring within 4–9 min of exposure to the medium.

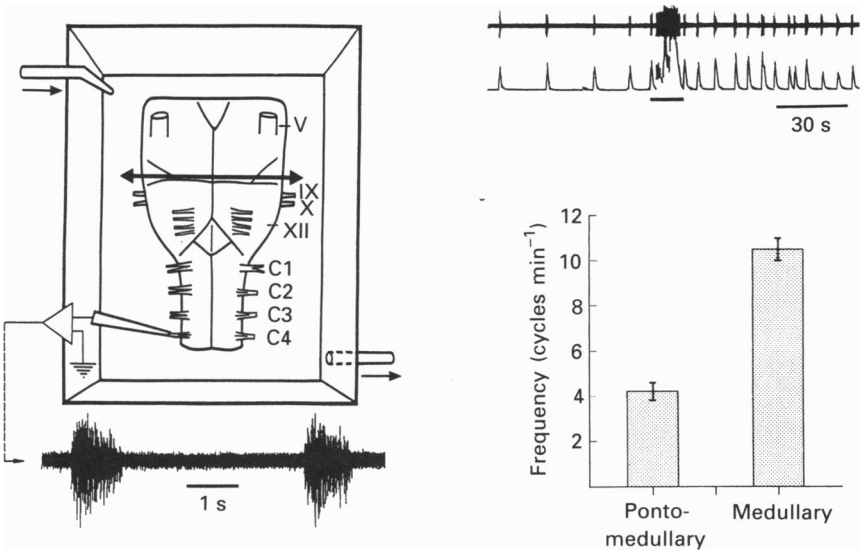

Fig. 1. Increases in respiratory frequency elicited by transection at the ponto-medullary level. On the left: schematic drawing of the newborn rat brain stem–spinal cord preparation showing the pattern of discharge recorded on cervical ventral roots; the horizontal double-headed arrow indicates the level of transection. Top right: inspiratory discharge recorded on cervical ventral roots (upper trace) and integrated activity (lower trace) at a different time base; prior to transection (on the left), during transection (horizontal bar) and after transection (on the right); note the immediate increase in respiratory frequency that occurred on removal of the pons. Bottom right: mean respiratory frequency (cycles min^{-1}) and S.E.M. plotted with the ponto-medullary ($n = 58$) and medullary preparations ($n = 42$) used in the present study.

Effects of drugs affecting NA efficiency

In ponto-medullary preparations, when the normal medium was replaced by a medium containing the α_2-antagonists (yohimbine, $n = 6$ or piperoxane, $n = 5$), the respiratory frequency increased slowly for 2–3 min and the increase was statistically significant within 3–6 min. Figure 4 illustrates one experiment performed with yohimbine (200 μM), where the respiratory frequency increased for 3–4 cycles min^{-1} in normal medium to 7 cycles min^{-1} under yohimbine, and gives the mean effect observed in six experiments. After 6 min of yohimbine bathing, the respiratory frequency was more than twice the control level. When returning to the normal medium, the control respiratory frequency was restored within 6–9 min. Increases in respiratory frequency were also observed when the brain stem was bathed with medium containing piperoxane (50–100 μM, $n = 5$). In four of five experiments, the

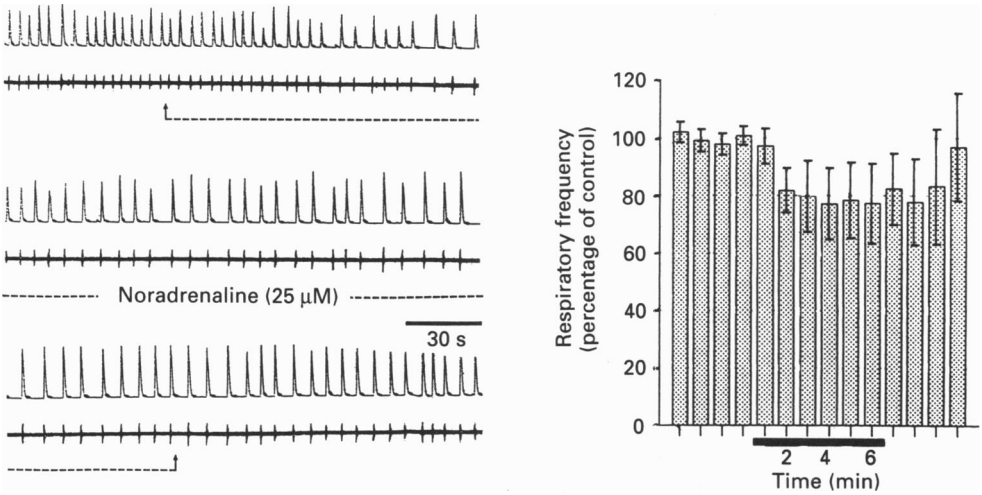

Fig. 2. Decreases in respiratory frequency elicited in medullary preparations by medium containing NA. On the left: inspiratory activity recorded on a cervical ventral root (lower beam) and its integrated activity (upper beam); the three superimposed traces (continuous recording) illustrate the decrease in respiratory frequency induced by NA during a single experiment; note the decrease in respiratory frequency when the normal medium was replaced by medium containing NA (25 μM, dashed horizontal line between arrows). On the right: histogram plotted on the basis of twelve experiments; ordinate, mean respiratory frequency and S.E.M. expressed each minute as a percentage of control frequency (100%); control frequency was the mean respiratory frequency during a 4 min period under normal medium; abscissa, time in minutes. Standardized protocol used in each experiment: control period of at least 4 min in normal medium, 6 min in medium containing 25 μM-NA (horizontal black bar), then return to normal medium. Note the mean decrease in respiratory frequency.

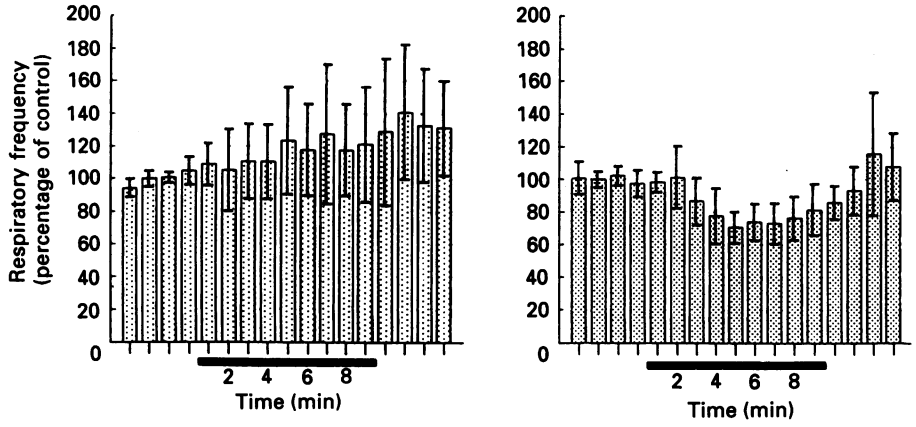

Fig. 3. Pargyline reveals respiratory effects of medium containing a low concentration of NA (5 μM). Same arrangement as for the histogram in Fig. 2; mean effect on respiratory frequency of medium containing NA (5 μM) applied for 9 min (black horizontal bar) plotted from five experiments performed on medullary preparations without (on the left) and with (on the right) pargyline pre-treatment. A weak concentration of NA (5 μM) significantly decreased the respiratory frequency only after pargyline pre-treatment.

respiratory frequency increased significantly, reaching 135±20% of the control values after 9 min of piperoxane bathing. With both yohimbine and piperoxane, the increase in respiratory frequency was accompanied by a decrease in the amplitude of the integrated inspiratory discharge.

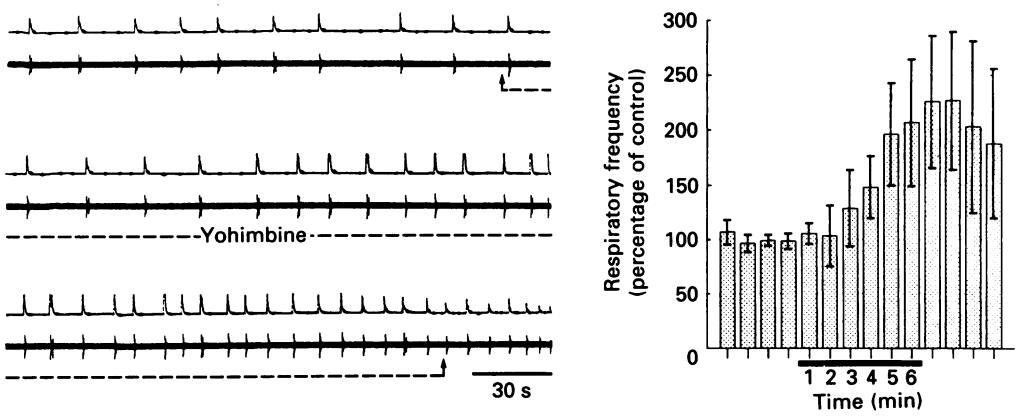

Fig. 4. Increases in respiratory frequency elicited by α_2 antagonist in ponto-medullary preparations. On the left: single experiment showing increase in respiratory frequency when the brain stem was superfused with a medium containing yohimbine; lower beam, respiratory discharge, upper beam, integrated activity; note the decrease in amplitude of the inspiratory discharge with high respiratory frequency. On the right: histogram showing the mean effect plotted on the basis of six experiments; same arrangement as in Figs 2 and 3; the mean respiratory frequency under medium containing yohimbine (black horizontal bar) was more than twice that of the control.

When the pontine structures were eliminated by ponto-medullary transection, exposure to medium containing either yohimbine ($n = 5$) or piperoxane ($n = 8$) no longer elicited any significant increases in the respiratory frequency.

Assuming that a permanent release of endogenous NA from the pons may modulate the respiratory frequency, drugs known to affect NA efficacy such as pargyline (an inhibitor of monoamine oxydases) and desipramine (a NA uptake inhibitor) should be expected to elicit changes in respiratory frequency.

Adding pargyline to the normal medium (200 μM, $n = 11$) elicited a significant decrease in the respiratory frequency which occurred after a 3 min period of weak, non-significant increase. After 6 and 9 min, the mean respiratory frequency dropped significantly to 74±5 and 72±7% of the control value, respectively (Fig. 5). In nine further experiments, the pontine structures were eliminated by transection. Medium containing pargyline (200 μM) then induced only minor changes in respiratory frequency, amounting to less than 9% of the control values. Statistically, the changes induced by pargyline were significantly weaker in the medullary preparations than in the ponto-medullary preparations ($P < 0{\cdot}001$).

Adding desipramine to normal medium (100 μM) elicited a weak but significant decrease in the respiratory frequency after a 3 min period of latency in nine experiments (Fig. 5). The frequency was decreased to 82±7% of the control value after 6–9 min. Here again, desipramine was tested in six further experiments after

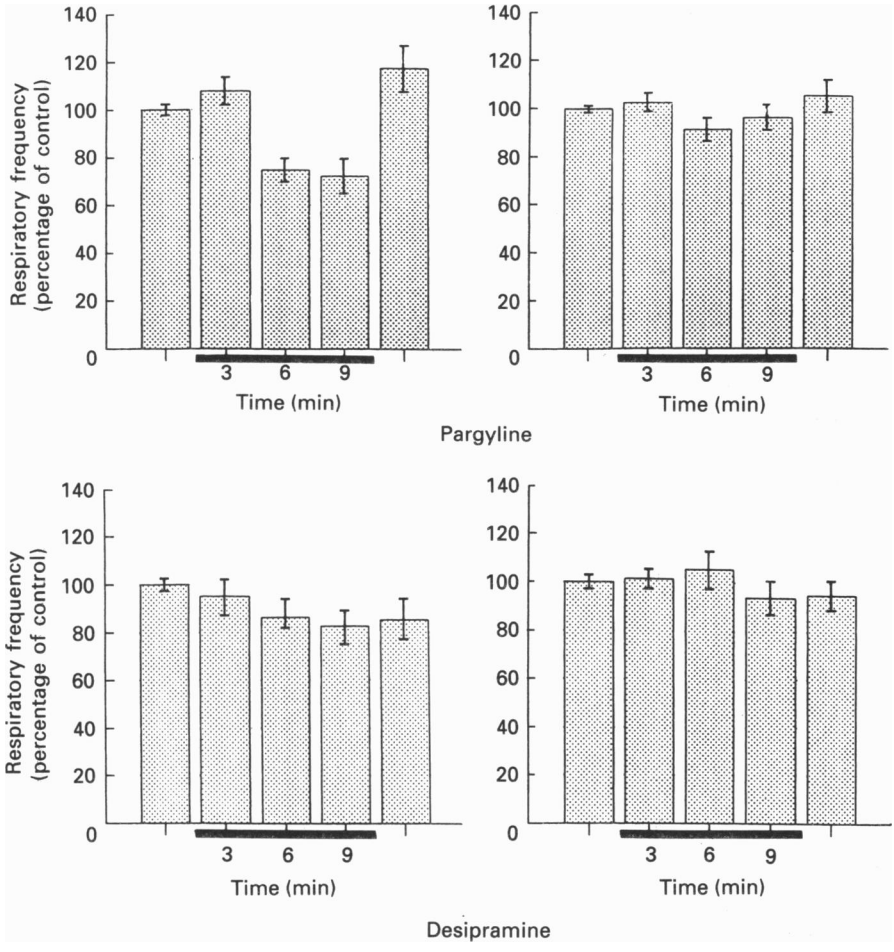

Fig. 5. Depressant effects of pargyline and desipramine on the respiratory frequency in ponto-medullary preparations. In these histograms, the same arrangement was used as before but the mean frequency was plotted every 3 min: the control frequency (100%) was defined during a 3 min period, then the drug was applied for 9 min (horizontal black bar) and finally the preparations were returned to normal medium. Upper histograms: effect of pargyline (200 μM) on respiratory frequency in ponto-medullary (on the left, $n = 11$) and medullary (on the right, $n = 9$) preparations. Lower histograms: effect of desipramine (100 μM) on respiratory frequency in ponto-medullary (on the left, $n = 9$) and medullary (on the right, $n = 6$) preparations. Note that both drugs significantly decreased the respiratory frequency in ponto-medullary but not in medullary preparations.

ponto-medullary transections. In medullary preparations, desipramine did not elicit any significant decreases in the respiratory frequency.

Effects of drugs affecting NA biosynthesis

In order to block NA biosynthesis, α-methyltyrosine, a tyrosine hydroxylase inhibitor, was added to the bathing medium (100 μM). In ponto-medullary preparations bathed with α-methyltyrosine ($n = 5$), complex changes in respiratory

frequency occurred. First, a decrease in the respiratory frequency was noted within the first 3 min and when α-methyltyrosine exposure continued, the respiratory frequency began to increase and reached the control values. Assuming that these dual effects (a decrease and a subsequent increase in frequency) might be due to α-

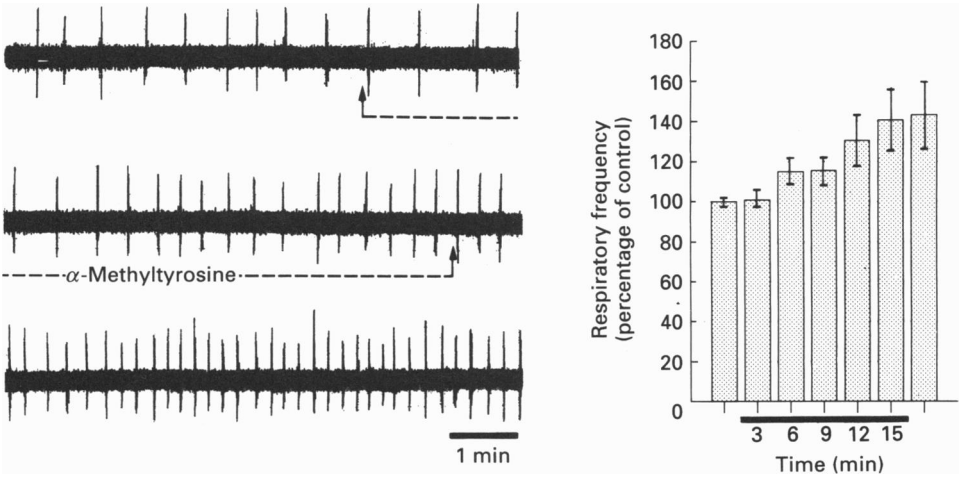

Fig. 6. Block of NA synthesis and respiratory frequency changes induced by α-methyltyrosine. On the left: single experiment showing an increase in respiratory frequency during superfusion of the pons with α-methyltyrosine. On the right: histogram showing the mean effect plotted on the basis of twelve experiments; the respiratory frequency increased significantly when the pons was superfused with α-methyltyrosine (100 μM; horizontal black bar). Time as indicated.

methyltyrosine acting on several sites, we attempted to simplify the problem by applying α-methyltyrosine at the pontine level only. In twelve further experiments, a barrier was then placed at the level of the ponto-medullary junction in order to bath the medulla with normal medium and the pons with medium containing α-methyltyrosine. Figure 6 illustrates an experiment where the effects of α-methyltyrosine were particularly obvious. After the pons had been bathed with α-methyltyrosine for 3 min, the respiratory frequency increased slowly and reached twice the control values after 12 min. The mean histogram plotted on the basis of twelve experiments is also illustrated in Fig. 6. The mean maximal effect was a significant 43% increase in respiratory frequency. No recovery was noted during the first 6 min after return to normal medium.

In order to activate NA biosynthesis, the normal medium was replaced by a medium containing tyrosine (25 μM), the precursor of NA. Figure 7 illustrates a single experiment where the respiratory frequency decreased from 6 cycles min^{-1} (control) to 4 cycles min^{-1} after 10 min in tyrosine medium. After the return to normal bathing medium, the respiratory frequency recovered within 3–4 min. In ten ponto-medullary preparations, exposure to tyrosine medium decreased respiratory frequency in six experiments with a 3–6 min latency but had no effect in four cases. In the sample as a whole, the mean effect was a significant decrease in the respiratory frequency (24 ± 13% of the control value) occurring within 8–12 min (Fig. 7).

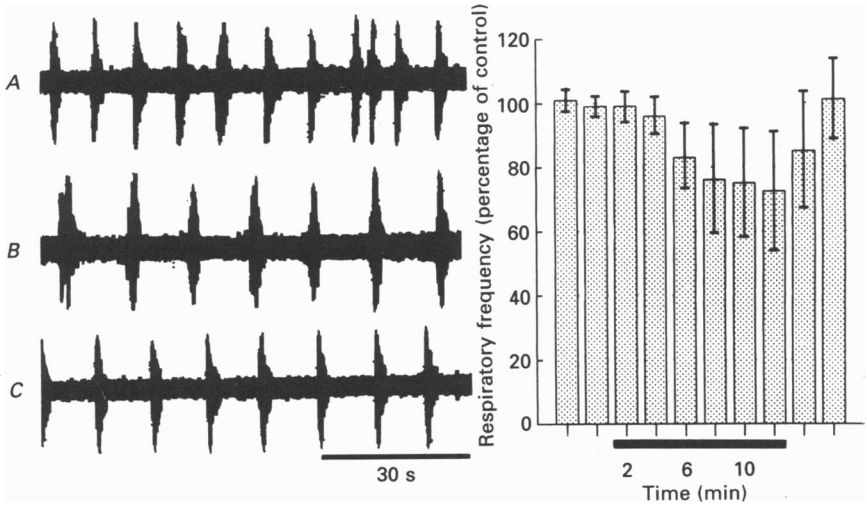

Fig. 7. Decreases in respiratory frequency elicited by tyrosine, the NA precursor. On the left: single experiment showing a decrease in respiratory frequency during superfusion with tyrosine (25 μM). In *A* and *C*, in normal medium; in *B* in medium containing tyrosine. On the right: histogram showing the mean effect elicited in five experiments by 25 μM-tyrosine; same arrangement as above.

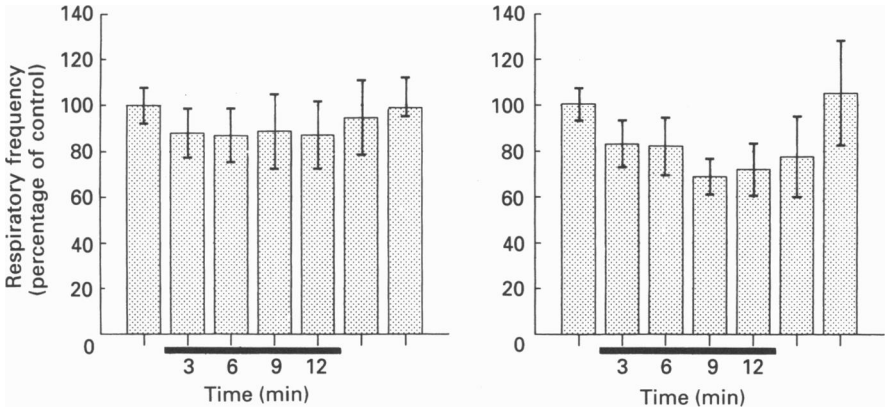

Fig. 8. Pargyline reveals the respiratory effects of tyrosine at a weak concentration. Same arrangement as before. Histograms showing the mean effect plotted on the basis of experiments performed without (on the left, $n = 7$) and with (on the right, $n = 5$) pargyline (100 μM) pre-treatment prior to exposure to 10 μM-tyrosine medium (horizontal black bar). Tyrosine medium elicited respiratory changes only after pargyline pre-treatment.

In seven experiments where a weaker concentration of tyrosine (10 μM) was used, no significant changes in respiratory frequency were observed (Fig. 8), even when the tyrosine bathing lasted for more than 12 min. After previous bathing with pargyline medium (100 μM, $n = 5$), however, a significant decrease in respiratory frequency was then elicited by tyrosine medium (10 μM) in all the preparations tested (Fig. 8). The control respiratory frequency (100%) was determined after 9 min of pargyline

bathing as previously with weak concentration of NA. Tyrosine (10 μM) then induced significant decreases in respiratory frequency which reached a maximum within 9–12 min (around 30% of the control value).

When prior to tyrosine (25 μM), a medium containing the α_2-antagonist piperoxane (100 μM, $n = 5$) was used for 9–12 min to block the α_2-receptors, the tyrosine medium no longer elicited a significant decrease in respiratory frequency.

When prior to tyrosine (25 μM), a medium containing the NA synthesis inhibitor, α-methyltyrosine, was used as bathing medium for 9 min (50 μM, $n = 5$), a subsequent 12 min application of tyrosine medium did not decrease the respiratory frequency in three of five experiments and resulted in only weak decreases in the two other experiments. The mean effect was not statistically significant.

DISCUSSION

The above results confirm previous reports on the noradrenergic modulation of the respiratory rhythm generator (Errchidi *et al.* 1988; Hilaire *et al.* 1989) and demonstrate that (i) the mechanisms involved in the biosynthesis of noradrenaline (NA) continue to function in the isolated newborn rat brain stem and (ii) the pontine structures release endogenous NA which slows down the activity of the medullary respiratory generator.

The isolated newborn rat brain stem continues to elaborate a central respiratory activity *in vitro* (Suzue, 1984; Murakoshi & Otsuka, 1985; Murakoshi *et al.* 1985; Smith & Feldman, 1987). It has been suggested previously (Errchidi *et al.* 1988; Hilaire *et al.* 1989) on the basis of section, coagulation and stimulation experiments that the medullary respiratory generator might be tonically inhibited by a structure located in the caudal ventrolateral pons. Pharmacological studies have indicated that the inhibition was noradrenergic in nature and originated from the pontine noradrenergic area A5. Previous studies on adult animals have already reported that NA has a depressant effect on respiratory frequency (Bolme & Fuxe, 1973; Bolme, Corrodi, Fuxe, Hökfelt, Lidbrink & Goldstein, 1974; Eldridge & Millhorn, 1981).

The large increase in respiratory frequency described herein after ponto-medullary transection confirms previous reports (Errchidi *et al.* 1988; Hilaire *et al.* 1989) and suggests the existence of a permanent pontine inhibitory drive impinging on the medullary respiratory rhythm generator. Adding yohimbine and piperoxane (α_2-antagonists which block the effects of any endogenous NA) to the normal medium increased the respiratory frequency of ponto-medullary preparations to the level reached after transection. On the contrary, adding desipramine and pargyline (which potentiate the effects of endogenous NA by inhibiting NA uptake and monoamine oxydase activity, respectively; Titus & Spiegel, 1962; Glowinski & Axelrod, 1965) to the normal medium decreased the respiratory frequency. Spector, Gordon, Sjöerdsma & Udenfriend (1967) have reported that pargyline increases the endogenous NA levels in the brain; concomitantly with the increase in NA levels which may have occurred when pargyline was applied to the isolated brain stem in the present experiments, the respiratory frequency decreased. Exogenous NA added to the bathing medium elicited decreases in the respiratory frequency which were blocked by α_2-antagonists and potentiated by pargyline. Pharmacological data have suggested that endogenous NA may modulate the activity of the respiratory rhythm

generator. Since yohimbine, piperoxane, desipramine and pargyline did not elicit any significant respiratory changes after elimination of the pons, the noradrenergic modulation is likely to have originated from the pons.

Fluorescence histochemical studies have shown that central monoamine neurons develop early in fetal life (Olson & Seiger, 1972) and radioenzymatic assays have confirmed that NA biosynthesis is already functioning at birth (McNamara & Lawson, 1984). The changes in respiratory frequency elicited by either NA, tyrosine or α-methyltyrosine demonstrate that NA biosynthesis continues to occur under these *in vitro* experimental conditions. Moreover, our results argue in favour of a permanent release of endogenous NA which modulates the resting respiratory frequency. Tyrosine, which is the first precursor of NA decreased the respiratory frequency when added to the bathing medium; this effect was potentiated by pargyline and suppressed by α_2-antagonists. This may mean that tyrosine was transformed into NA which in turn slowed down the respiratory frequency. It is unlikely that tyrosine acted directly *per se*, since after pre-treatment with the NA synthesis inhibitor α-methyltyrosine, tyrosine no longer significantly affected the respiratory frequency. The results obtained with α-methyltyrosine are always complex however and should be interpreted with caution. On the one hand, α-methyltyrosine blocks NA synthesis by inhibiting tyrosine hydroxylase (Euler, 1972) and concomitantly increases the respiratory frequency and suppresses the depressant effects of tyrosine. On the other hand, (i) the increases in frequency elicited by α-methyltyrosine were weaker than those evoked by ponto-medullary transection or α_2-block, (ii) tyrosine was still able to decrease the frequency after α-methyltyrosine poisoning in two of five experiments, and moreover (iii) α-methyltyrosine had differential effects, depending upon whether it was applied to the whole brain stem or the pons. The complex results obtained with α-methyltyrosine may be due to several factors. Hence, α-methyltyrosine may have only attenuated but not totally suppressed NA synthesis (Spector, Sjöerdsma & Udenfriend, 1965; McMillen & Shore, 1977). Secondly, stored NA may have been utilized instead of newly synthesized NA. Furthermore, notwithstanding its inhibitory effect, α-methyltyrosine may be to some extent itself transformed into α-methyl-NA (Euler, 1972) which is a potent α_2-noradrenergic agonist, and might therefore tend to depress the respiratory frequency. As stated by Millhorn (1986), all the available pharmacological approaches to the central respiratory mechanisms have their own limits and it is necessary with the present model to allow for the fact that the drug(s) added to the bathing medium may act on several sites. The resulting changes therefore involve complex interacting mechanisms depending on the nervous structures affected, their relative accessibility to the drug(s), their relative efficiency when activated (or inactivated), etc. The possibility cannot therefore be excluded that the respiratory changes observed with α-methyltyrosine may involve not only pontine structures, but also other medullary catecholaminergic nuclei (Hökfelt, Märtensson, Björklund, Kleinau & Goldstein, 1984). Likewise, even if the main effect of NA application on medullary preparations is a decrease in respiratory frequency, this effect may result from various actions on several sites, for example on the rather superficial medullary catecholaminergic nuclei, and on the raphe structures, as well as on the respiratory nuclei (Saether, Hilaire & Monteau, 1987; Hilaire *et al.* 1990) which are sensitive to NA (Champagnat, Denavit-Saubie, Henry & Leviel, 1979). Although NA clearly and

unquestionably has depressant effects on respiratory frequency, it is not yet possible to fully elucidate the mechanisms and sites of actions involved.

The results reported herein confirm the recently proposed hypothesis that the medullary respiratory rhythm generator is subject to pontine noradrenergic modulation (Hilaire *et al.* 1989). As the main catecholaminergic pontine nucleus is the A6 nucleus, and as A6 noradrenergic neurons send extensive axonal processes to the whole brain as well as receiving information from a large number of regions (Amaral & Sinnamon, 1977; Foote, Bloom & Aston-Jones, 1983), A6 might be suspected of being responsible for the respiratory effects reported herein. However, other evidence shows that this may be unlikely, since the dorsal part of the pons where A6 is located appears to be devoid of any respiratory function in the rat (Monteau, Errchidi, Gauther, Hilaire & Rega, 1989) and its elimination entails no changes in the respiratory frequency (Hilaire *et al.* 1989). In the present study, it is not definitively established that the pontine modulation originates from area A5, since only transection experiments were performed, but it is certainly very likely that A5 was involved. A5 neurons send axons towards the ventral reticular formation (Byrum & Guyenet, 1987) and the decrease in respiratory frequency elicited by NA may have resulted, at least partly, from the activation of NA receptors located on the target of these A5 axons. Likewise, the effects of yohimbine, piperoxane, pargyline and desipramine may be interpreted as reflecting a modulation of the efficiency of the NA released from these axons, since the effects were suppressed when the A5 axons were severed by ponto-medullary transection. Finally, the data obtained with tyrosine and α-methyltyrosine are not incompatible with the idea that NA may be synthesized by A5 neurons.

One implication of the possible modulation of the medullary rhythm generator by endogenous NA is that dysfunction of NA biosynthesis may result in respiratory changes which might be dramatic in newborn infants. Abnormal urinary catecholamine levels have been reported in pre-term infants with apnoea (Kattwinkel, Macs, Farnoff & Klaus, 1976). On the other hand, modulation of this kind might be part of a process integrating respiratory and cardiovascular regulation. The A5 nucleus has been assumed to be mainly involved in cardiovascular regulation, since the A5 neurons send axonal processes towards all the areas involved in cardiovascular regulations and receive afferent inputs from major cardiovascular centres (Byrum & Guyenet, 1987). These neurons are sensitive to α_2-noradrenergic tensive agents (Andrade & Aghajanian, 1982), and to changes in blood pressure (Guyenet, 1984), and their activation leads to hypotension (Close, Neil & Loewy, 1982). If the A5 neurons do modulate medullary respiratory activity (Hilaire *et al.* 1989), functional interactions between respiratory and cardiovascular regulations might be, at least partly, mediated by A5.

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