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A pharmacological study on respiratory rhythm in the isolated brainstem-spinal cord preparation of the newborn rat

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1 An *in vitro* brainstem-spinal cord preparation of the newborn rat was used to examine the effects of neurotransmitters and transmitter candidates on respiratory frequency.

2 Spontaneous periodic depolarization of the spinal ventral roots of the 4th or 5th cervical segment was observed at a frequency of 5–15 min^{-1} constantly for more than 5 h. The frequency of this depolarization was monitored as an index of the respiratory frequency.

3 An elevation of the concentration of Ca^{2+} or Mg^{2+} caused a decrease in the respiratory frequency, whereas an elevation of K^+ concentration caused an increase. The frequency was also increased by a reduction of pH. The highest frequency was observed at 27–28°C.

4 Dopamine, 5-hydroxytryptamine, histamine, acetylcholine, glutamic acid, substance P, and thyrotropin releasing hormone accelerated the respiratory frequency when applied by perfusion to the brainstem, whereas noradrenaline, γ-aminobutyric acid, glycine, and [Met^5] enkephalin and [Leu^5] enkephalin slowed the frequency. Experiments with antagonists suggested that the stimulant effect of acetylcholine on respiratory frequency was mediated mainly by muscarinic receptors and the depressant effect of noradrenaline was mediated by α-adrenoceptors.

Introduction

Respiratory rhythm is generated by the respiratory centre in the lower brainstem, and the afferent inputs from peripheral organs modulate this process (Widdicombe, 1964; Cohen, 1979). In order to investigate the mechanisms of action of drugs on the respiratory centre, drugs have usually been administered to whole animals via intravenous, intracerebroventricular, and intracisternal routes, or by direct microinjection into the brainstem (Eldridge & Millhorn, 1981; Mueller *et al.*, 1982). However, these methods have the following drawbacks: (1) it is difficult to distinguish the effects of the drugs on the respiratory centre from their effects on peripheral organs such as the aortic or carotid chemoreceptors; (2) respiratory activity is easily affected by general conditions of the animals, e.g., blood pressure, body temperature, etc., and especially the level of anaesthesia; and (3) it is also difficult to estimate the precise effective concentrations of drugs at the sites of action in the brain.

Recently a method was developed to isolate the brainstem and spinal cord from the newborn rat and maintain its physiological functions *in vitro*. In this preparation, spontaneous respiratory activity can be maintained and the periodic depolarization can be recorded from some cranial nerves and cervical ventral roots (Suzue *et al.*, 1983; Suzue, 1984). This preparation consists only of the brainstem and the cervical spinal cord separated from peripheral organs. The whole preparation is perfused by physiological solution so that it can be maintained in an anaesthetic-free environment and any drugs can be applied by perfusion in controlled concentrations. Therefore, this preparation is suitable for investigating the actions of drugs on the respiratory centre.

In this paper we describe the effects of temperature, some cations and pH and in particular, the effects of neurotransmitters and transmitter candidates on the respiratory frequency of this preparation.

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Methods

Brainstem-spinal cord preparation

The procedures for isolating the preparation are essentially the same as described in previous papers (Suzue *et al.*, 1983; Suzue, 1984). The brain and spinal cord were isolated from 0 to 4 day-old Wistar rats under ether anaesthesia. Decerebration was performed at the intercollicular level and cerebellum was also removed. As shown in Figure 1a, the preparation was placed in a 0.5–1.0 ml bath perfused with artificial cerebrospinal fluid (CSF) that was equilibrated with 95% O_2 and 5% CO_2 and maintained at 28 ± 0.5°C. The composition of the artificial CSF was as follows (mM): NaCl 129, KCl 3.35, $CaCl_2$ 1.26, $MgCl_2$ 1.15, $NaHCO_3$ 21.0, NaH_2PO_4 0.58, glucose 30.0. The glucose concentration was set at 30 mM instead of the 10 mM that was used for the spinal cord preparation (Otsuka & Yanagisawa, 1980), because a high concentration of glucose was needed to maintain viability of the preparation. The perfusion bath was made of silicone and had two compartments, one for the brainstem and the other for the spinal cord, so that the two parts could be perfused separately. The perfusion rate was kept at 6–7 ml min^{-1} in each compartment. The solution was drained by aspiration from the groove on the partition wall.

Electrical activity was recorded extracellularly from the 4th or 5th cervical ventral root and the phrenic nerve with tightly fitting suction electrodes (internal diameters of tips: 100–150 μm). The potentials were recorded through a preamplifier on an oscilloscope. Output of the preamplifier was fed to a pen-recorder via high-pass filters (time constants of the filters were 0.12 s for Figures 2 and 4, and 2.0 s for Figure 7). Drugs were dissolved in artificial CSF and applied by perfusion.

In some experiments, the brainstem-spinal cord preparation was isolated together with a dorsal part of the thorax, and one of the ribs was connected to a force-displacement transducer with a thread.

Vagotomy in whole animals

Eight rats (4 to 27 days old) were anaesthetized with ether and fixed in a supine position. Subcutaneous temperature was maintained at 32–34°C with a heat lamp. The vagal nerves were exposed bilaterally at the level of the thyroid cartilage. The respiratory movement was monitored by a force-displacement transducer connected to the skin of the lower thorax via a thread.

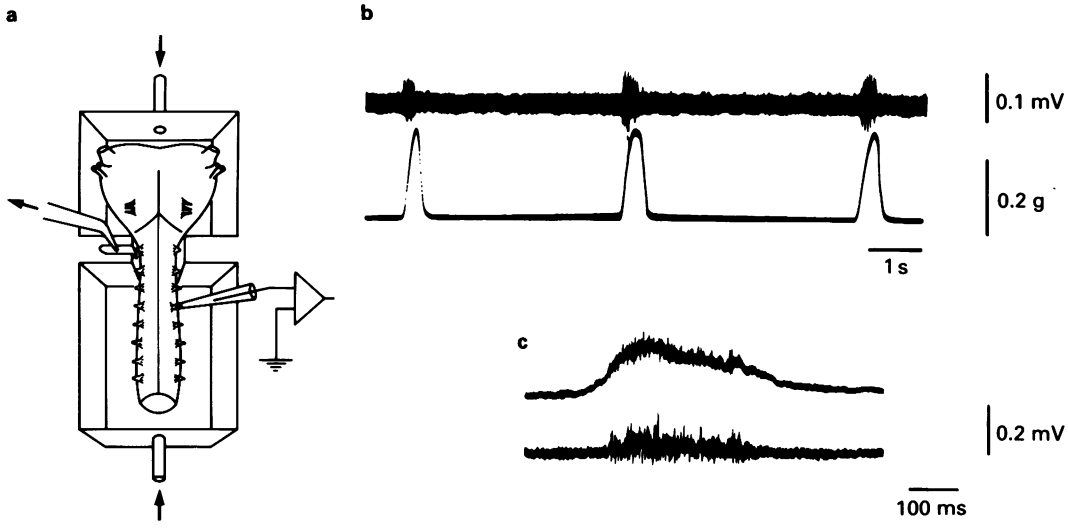

Figure 1 The experimental set-up and the spontaneous periodic activity. (a) An isolated brainstem-cervical spinal cord preparation placed in a bath with its ventral side up. One of the ventral roots (C4 or C5) was used for extracellular recording with a glass capillary suction electrode. The flow of the perfusion solution is indicated by arrows. (b) Spike activity recorded from a phrenic nerve (upper trace), and simultaneously recorded thoracic movements (lower trace). The upward deflections in the lower trace show the rostral movements of the 11th rib. Movements of the 11th rib were monitored with a force-displacement transducer connected to the rib by a thread. (c) Depolarization of the 4th cervical ventral root (upper trace) and spike activity of a phrenic nerve (lower trace). In (b) and (c), potentials were recorded extracellulary from the nerves with suction electrodes and the records were displayed on an oscilloscope.

Results

Spontaneous respiratory activity

The isolated brainstem-spinal cord preparation of the newborn rat exhibited periodic spontaneous activity at a frequency of 5–15 min^{-1} which could be recorded from some cranial nerves (IX–XII) and ventral roots (C1–Th10). In a preparation isolated with the phrenic nerve and the thorax, the spike discharges recorded from the phrenic nerve synchronized with the contraction of the inspiratory intercostal muscles (Figure 1b), and the depolarizing phase of the 4th or 5th cervical ventral root also synchronized with the phrenic nerve discharges (Figure 1c). Therefore, the frequency of this depolarization of the 4th or 5th cervical ventral root was used as an index of the respiratory frequency in the present study.

The effect of vagotomy

The respiratory frequency observed in the isolated preparation was much lower than the respiratory frequency observed in intact newborn rats under light ether anaesthesia (ca. 90–100 min^{-1}). Furthermore, the spontaneous activity of the isolated preparation consisted of a short-lasting inspiratory phase (400–500 ms) and a much longer expiratory phase (3–11 s), and it therefore resembled gasping. One possible reason for this low frequency may be an unfavourable condition of the respiratory centre in the brainstem due to lack of blood circulation. But another important cause might be the lack of sensory input from the periphery, particularly via the vagal nerves. This was supported by the following observations.

As shown in Figure 2, the effects of vagotomy on respiration were studied in intact rats of three different ages under light ether anaesthesia. Figure 2a illustrates sample records showing the effect of unilateral and bilateral vagotomy on the respiratory frequency and Figure 2b summarizes the results obtained from eight animals. The average frequency in the intact rats was $97 \pm 4\ min^{-1}$ (mean ± s.e.mean, $n = 8$). After unilateral vagotomy the frequency decreased to about half that of the control in five out of eight animals. After bilateral vagotomy the animals exhibited gasping-like respiration and the respiratory frequency was

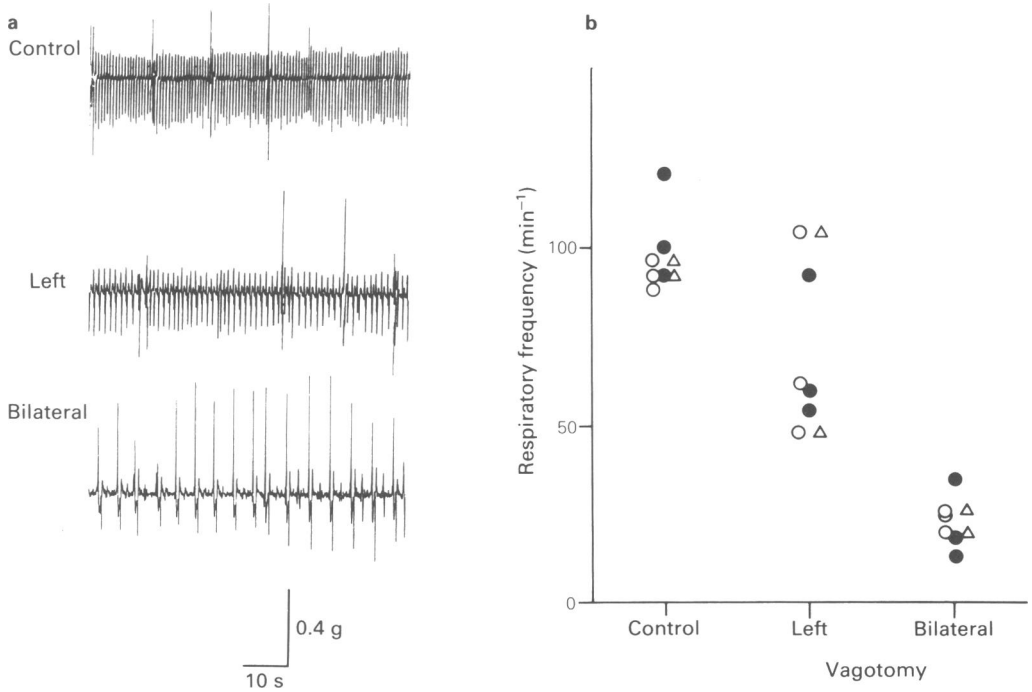

Figure 2 The effects of vagotomy on the respiratory frequency observed in whole animals. (a) Thoracic movements of a 9 day-old rat. Both right and left vagal nerves were intact in the upper trace; the middle trace shows a record after left unilateral vagotomy; and the lower trace after bilateral vagotomy. Movements of lower ribs were recorded with a transducer connected to the skin of the thorax by a thread. Upward deflections indicate elevation of the ribs. (b) Effects of unilateral and bilateral vagotomy on the respiratory frequency in 4 day-old (○), 9 day-old (●), and 27 day-old (△) rats. Each point represents the value obtained from one animal.

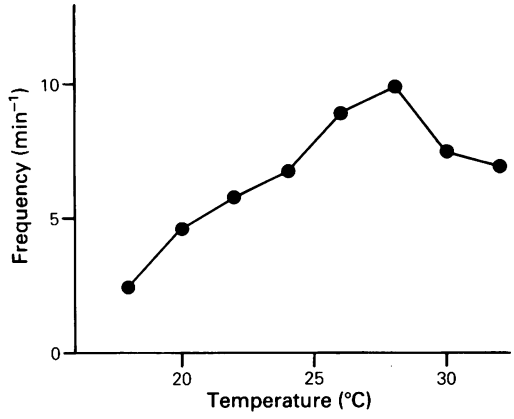

Figure 3 The relationship between the respiratory frequency and the temperature of the perfusing solution. The temperature was measured with a thermister probe located near the preparation in the flow of the solution.

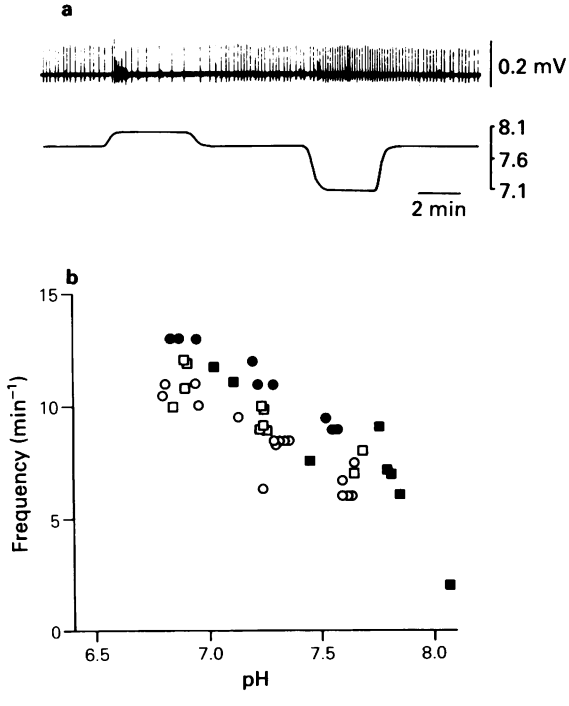

Figure 4 The effects of pH on the respiratory frequency. The pH of the medium perfusing the brainstem was varied by changing HCO_3^- concentration while P_{CO_2} was kept constant at 33 mmHg. In (a), the upper trace shows a recording of the spontaneous periodic depolarization of the 4th cervical ventral root, and the lower trace shows the simultaneously monitored pH of the perfusion medium. (b) The relationship between the respiratory frequency and pH of the medium. Four preparations from 2 day-old rats were used and the data from each preparation is represented by one type of symbol.

$23 \pm 2\,min^{-1}$. This frequency is similar to that of spontaneous rhythmic activity observed in the isolated brainstem-spinal cord preparation.

The effects of the temperature

We usually kept the preparation at 28 ± 0.5°C because this temperature was found to be optimal for the maintenance of electrical activity of the preparation. Since the low temperature might be another reason for the relatively slow respiratory rhythm, we studied the effects of the temperature. The temperature of the perfusion fluid was changed to various levels. As shown in Figure 3, the respiratory frequency was the highest at 27–28°C, and both raising and lowering the temperature resulted in a decrease in frequency. This was consistently observed in all of 3 preparations examined.

The effects of Ca^{2+}, Mg^{2+} and K^+

Elevation of the concentration of Ca^{2+} from 1.26 mM to 3.0 mM in the solution perfusing the brainstem resulted in a decrease in the respiratory frequency from 5.5 to $2.0\,min^{-1}$. Raising the concentration of Mg^{2+} from 1.15 to 2.7 mM also caused a decrease in the frequency from 9.0 to $3.0\,min^{-1}$. Lowering the Ca^{2+} concentration from 1.26 mM to 0.5 mM increased the respiratory frequency from 5.5 to $11\,min^{-1}$. The effects of Ca^{2+} and Mg^{2+} were concentration-dependent within the range 0.5 to 3.0 mM for Ca^{2+}, and 1.15 to 2.7 mM for Mg^{2+}. The elevation of K^+ concentration from 3.35 mM to 8.4 mM resulted in a slight increase in the frequency from 5.0 to $7.5\,min^{-1}$. When the concentrations of these ions in the solution perfusing the spinal cord were varied, the respiratory frequency was not altered.

The effect of pH

As observed by Suzue (1984), the respiratory frequency was affected by the pH of the perfusing fluid. Figure 4a shows the record from the 4th cervical ventral root when the pH was varied by changing the bicarbonate ion concentration in the medium while keeping the CO_2 tension constant (33 mmHg at 28°C). At the control pH level of 7.85 the respiratory frequency was $7\,min^{-1}$. When the pH of the solution perfusing the brainstem was lowered to 7.12, the frequency was increased to $11\,min^{-1}$, and when the pH was elevated to 8.07 the frequency was decreased to $2\,min^{-1}$. The relationship between the respiratory frequency and pH observed in 4 preparations is shown in Figure 4b. A similar relationship between the respiratory frequency and the pH was observed when the pH was varied by changing P_{CO_2} while keeping the bicarbonate concentration constant (21.0 mM).

Alteration of pH of the medium perfusing the spinal cord did not affect the respiratory frequency.

Effects of neurotransmitters and related drugs

(1) *Catecholamines and other amines* Noradrenaline (NA; 10–30 μM) caused a decrease in the respiratory frequency when applied to the brainstem (Figure 5a). After the removal of NA a transient rebound acceleration of the respiratory rhythm followed. Figure 5d illustrates the relation between the concentration of NA and its effect on the respiratory frequency. When applied to the spinal cord, NA exerted a depolarizing action on motoneurones that was observed as a slow positive shift of the ventral root potential (Figure 5b, cf. Evans & Watkins, 1978). Application of NA to the spinal cord, however, did not affect the frequency of the spontaneous respiratory activity (Figure 5b). The depressant effect of NA on respiratory frequency was abolished by the α-adrenoceptor antagonist, phentolamine, at a relatively high concentration (60 μM, Figure 5c). After pretreatment of the preparation with phentolamine, NA occasionally (in 5 out of 12 preparations) accelerated the respiratory frequency. In the remaining 7 preparations, phentolamine simply abolished the effect of NA. The effect of adrenaline (10μM) on the respiratory frequency was similar to that of NA (number of preparations; $n = 5$). When applied to the brainstem, a β-adrenoceptor agonist, isoprenaline (16 μM), did not affect the respiratory frequency in 4 preparations and decreased the frequency in one preparation. A β-adrenoceptor antagonist, propranolol (20 μM), did not block the action of NA or adrenaline applied to the brainstem ($n = 5$). These findings suggest that the depressant effect of NA on respiratory frequency is mediated by α-adrenoceptors. Dopamine, 10 μM, increased the respiratory frequency (Figure 6a) and this effect disappeared rapidly after the removal of dopamine. At a higher concentration (30 μM) dopamine exerted a biphasic effect, first increasing the frequency transiently and then reducing it below the control level during the continued presence of dopamine. The stimulant effect of dopamine was antagonized by pretreatment with haloperidol (10 μM) and the depressant effect was antagonized by phentolamine (60 μM, $n = 4$).

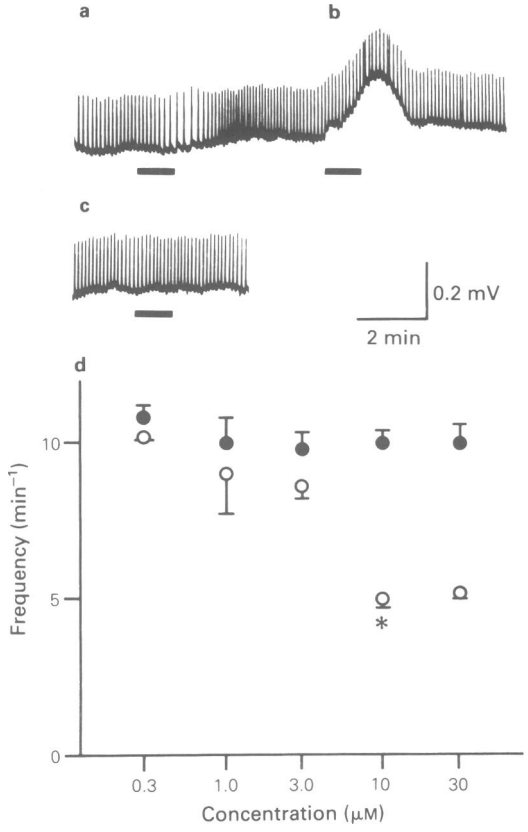

Figure 5 The effect of noradrenaline (NA) on the respiratory frequency. In (a) and (c), NA (10 μM) was applied to the brainstem, and in (b) to the spinal cord, during the periods indicated by black bars under the records. In (c) phentolamine (60 μM) was added to the perfusion medium for 1 min before the application of NA and also during the period of application of NA. (d) Relation between the concentration of NA and its effect on the respiratory frequency. For each concentration, the control respiratory frequency (●) was measured first, and then NA at the concentration given on the horizontal axis was added and the respiratory frequency (○) was monitored. Each point represents the mean (vertical lines show s.e.mean) obtained from 3–10 preparations. The asterisk indicates a significant difference from the control value ($P < 0.05$, paired t test).

5-Hydroxytryptamine (5-HT; 30 μM) exerted a biphasic effect, i.e., the drug induced an initial high-frequency phase followed by a low-frequency phase during the continued presence of 5-HT ($n = 4$). Histamine (10–30 μM) also increased the respiratory frequency (in 3 out of 4 preparations). The effects of 5-HT and histamine were much less marked than that of dopamine.

(2) *Acetylcholine* Acetylcholine (ACh; 3–30 μM) applied to the brainstem increased the respiratory frequency (Figure 6b). This effect of ACh was markedly diminished but not completely abolished by atropine (10 μM, in 5 out of 8 preparations). A nicotinic antagonist, dihydro-β-erythroidine (5 μM), did not significantly reduce the effect of ACh ($n = 4$), but when dihydro-β-erythroidine was applied together with atropine (10 μM), the stimulant effect of ACh was completely abolished in 6 out of 7 preparations. We therefore assume that the stimulant effect of ACh is mediated mainly by the muscarinic receptors, while nicotinic receptors contribute a minor part.

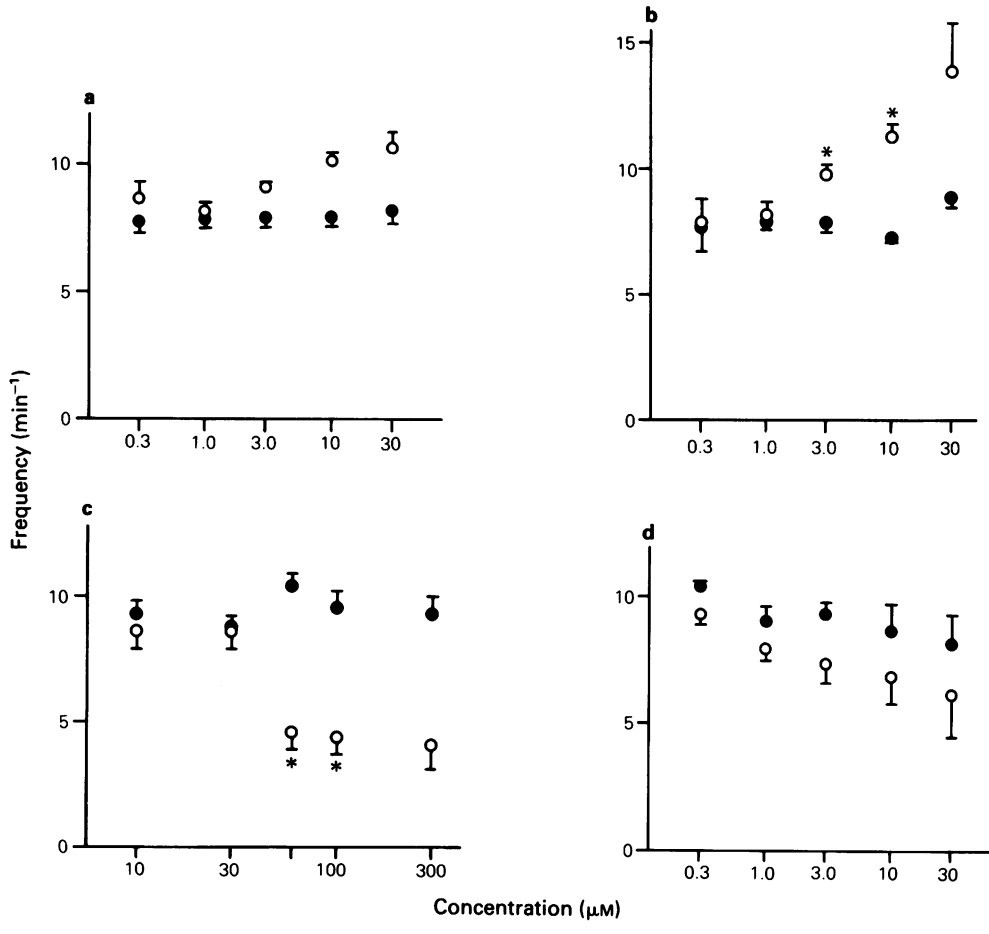

Figure 6 The effects of dopamine (a), acetylcholine (b), GABA (c), and [Met^5] enkephalin (d) on the respiratory frequency. The drugs were applied to the brainstem by perfusion. For each concentration, the control respiratory frequency (●) was measured first, and then the drug at the concentration given on the horizontal axis was added and the respiratory frequency (○) was monitored. Each point represents the mean (vertical lines show s.e.mean) obtained from 3–15 preparations. Asterisks indicate significant difference from the control values ($P<0.05$, paired t test).

Pretreatment with edrophonium (5 μM) potentiated the acceleratory effect of ACh ($n = 3$). Edrophonium (5 μM) alone also increased the respiratory frequency in 2 out of 4 preparations, suggesting that an endogenous cholinergic mechanism is functioning in the brainstem to increase the respiratory frequency.

(3) *γ-Aminobutyric acid, glycine, and glutamic acid* Application of γ-aminobutyric acid (GABA; 60–300 μM, Figure 6c) and glycine (10–300 μM, $n = 8$) to the brainstem decreased the respiratory frequency. The spontaneous periodic activity was sometimes totally abolished by these inhibitory amino acids at a relatively high concentration (300 μM) and usually a transient rebound acceleration of the respiratory rhythm was observed after the removal of the amino acids. Glutamic acid (30–100 μM, $n = 7$) induced an increase in the respiratory frequency when applied to the brainstem (e.g., from 4.8 to 7.3 min^{-1} at 100 μM).

(4) *Enkephalins and morphine* [Met^5]enkephalin (3–30 μM, Figure 6d) and [Leu^5]enkephalin (3–30 μM) decreased the respiratory frequency when applied to the brainstem (in 4 out of 5 preparations). A stable enkephalin analogue, [D-Ala^2, Met^5] enkephalinamide at relatively low concentrations (0.2–3 μM, $n = 8$) also decreased the respiratory frequency. The effects were reversed immediately after the removal of the peptides. When these peptides were applied to the spinal cord the respiratory rhythm was not altered. The effect of morphine in relatively high concentrations (30–100 μM, $n = 6$) on the respiratory frequency was

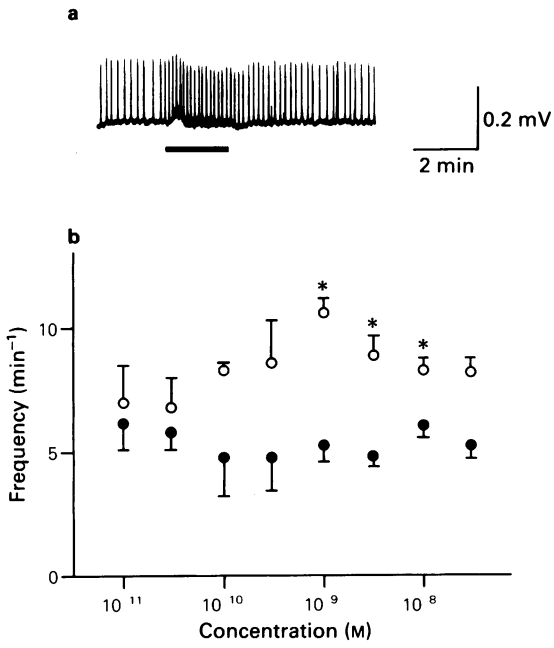

Figure 7 The effect of substance P (SP) applied to the brainstem on the respiratory frequency. (a) SP (10 nM) was applied during the period indicated by the black horizontal bar. (b) Relation between the concentration of SP and its effect. For each concentration, the control respiratory frequency (●) was measured first, and then SP at the concentration given on the horizontal axis was added and the respiratory frequency (○) was monitored. Each point represents the mean (vertical lines show s.e.mean) obtained from 3–13 preparations. Asterisks indicate significant difference from the control values ($P<0.05$, paired t test).

similar to that of enkephalins except that the effect lasted more than one hour after the removal of the drug. The effects of these opioid peptides and morphine were completely abolished by a specific opiate receptor antagonist, naloxone (1–2 μM, $n = 7$).

(5) *Substance P and thyrotropin releasing hormone* Substance P (SP; 0.1–30 nM, Figure 7) and thyrotropin releasing hormone (TRH; 3–100 nM, $n = 5$) applied to the brainstem increased the respiratory frequency. SP was the most potent of all the drugs so far studied on a molar basis. We occasionally observed in some preparations slow and irregular spontaneous rhythm, which was probably due to deterioration. In such preparations, application of SP restored the regular and faster rhythmic activity. When SP was applied to the spinal cord, a depolarizing response was recorded from the ventral root (Konishi & Otsuka, 1974), whereas the respiratory frequency was unaffected. Capsaicin is known to induce a release of SP from primary afferent neurones and its effect on the respiratory activity was examined. When applied to the brainstem, capsaicin (0.6 μM) produced a stimulant effect on the respiratory frequency and this accelerated rhythm lasted more than ten minutes after the removal of the drug ($n=3$).

Discussion

In the present study, a systematic screening of pharmacological effects of neurotransmitters and transmitter candidates on respiratory rhythm was performed using the *in vitro* brainstem-spinal cord preparation of the newborn rat. In this preparation, the spontaneous and periodic depolarization of the cervical ventral roots (C4–C5) occurred synchronously with the discharges of the phrenic nerve and the contraction of the inspiratory intercostal muscles. We therefore assumed that the frequency of this rhythmic depolarization represents the respiratory frequency.

There are two major differences between the results obtained from the present *in vitro* preparation and those from the *in vivo* experiments. First, the frequency of the respiratory rhythm in the *in vitro* preparation was much lower than that of intact rats. Second, although the tidal volume is another important factor in respiratory activity and is altered by various agents in intact animals, the amplitude of depolarization of the cervical ventral roots in this *in vitro* preparation which may correspond to the tidal volume, was almost constant when the frequency was altered by drugs or other factors. In a few experiments, we measured the area delineated by the depolarizing potential curve recorded from the cervical ventral root and the baseline. This area, which probably parallels the integrated phrenic nerve activity and therefore the tidal volume (Eldridge, 1971, see Figure 1c), was not altered by changes in pH of the perfusion fluid whereas the respiratory frequency was markedly altered. This is in contrast to the findings that in intact animals the tidal volume rather than the respiratory frequency is altered by pH changes of the brain extracellular fluid (Loeschcke, 1974; Schlaefke, 1981). These discrepancies between the *in vitro* preparation and intact animals were discussed and some explanations were given in the previous paper such as differences in experimental conditions and animals (Suzue, 1984). The slow and deep respiration observed in the present preparation may correspond to gasping, i.e., respiration in the pathological state, and might have been caused by hypoxia of the CNS. However, from the present results on the effect of vagotomy in intact animals (Figure 2), the main cause of the slow and deep respiration appears to be deafferentation, rather than unfavourable conditions of the CNS. Further-

more, the effects of changes in the pH or cation concentrations of the perfusion fluid in this *in vitro* preparation were in the same direction as changes in the respiratory minute volume seen in intact animals (Leusen, 1972; Loeschcke, 1974; Schlaefke, 1981). These results suggest that the basic properties of the respiratory rhythm generation are probably the same between the present preparation and intact animals.

Extensive studies have been made on the effects of drugs on respiration using whole animals (for reviews, see Eldridge & Millhorn, 1981; Mueller *et al.*, 1982). These studies consistently showed that GABA (Hedner *et al.*, 1981a; Yamada *et al.*, 1982), glycine (Sgaragli & Pavan, 1972; Wessberg *et al.*, 1983), and opioid peptides (Pazos & Flórez 1983; Flórez & Mediavilla, 1977) exert an inhibitory effect on the respiratory activity, whereas SP (von Euler & Pernow, 1956; Yamamoto *et al.*, 1981), and TRH (Hedner *et al.*, 1981b; Yamamoto *et al.*, 1981) exert a facilitatory effect. The results of the present *in vitro* experiments agree with these previous results. On the other hand, there seems to be no general agreement on the effects of NA (Bolme & Fuxe, 1973; Mediavilla *et al.*, 1979), dopamine (Bolme *et al.*, 1977; Hedner *et al.*, 1982) and 5-HT (Feldberg & Sherwood, 1954; Lundberg *et al.*, 1980) in whole animal experiments. For instance, Bolme & Fuxe (1973) described an inhibitory effect of adrenoceptor agonists on the respiratory rhythm, but Mediavilla *et al.* (1979) observed a stimulant effect. Such disagreement can be ascribed to variable anaesthetic conditions, to species differences among experimental animals, or to possible effects of the drugs on the peripheral organs. In our isolated CNS preparation, the indirect action of drugs on respiration through peripheral organs was completely excluded, enabling us to study the direct effect of drugs on the respiratory centre in the brainstem in an anaesthetic-free environment. It was also possible to observe the effects of the drugs under completely controlled experimental conditions such as temperature, pH, O_2 tension, the concentrations of various ions, and other humoral factors. Therefore, the inconsistency of results due to variations in experimental conditions can be minimized. Furthermore, by the use of antagonists it was possible to reveal that the stimulant effect of acetylcholine on the respiratory frequency is mediated mainly by muscarinic receptors, and that the inhibitory action of noradrenaline is mediated by α-adrenoceptors. The inhibitory action of dopamine was presumably mediated by α-adrenoceptors since it was blocked by phentolamine. In this respect, McDonald & Goldberg (1963) described an α-adrenoceptor-mediated effect of dopamine on the artery.

The distribution of neurotransmitters and transmitter candidates in the brainstem has been extensively studied using histochemical methods and quantitative measurements. The nucleus tractus solitarii, nucleus retroambigualis, and nucleus parabrachialis medialis are considered to be the key structures for the generation of respiratory rhythm (Cohen, 1979). And in nucleus tractus solitarii and nucleus parabrachialis medialis, NA (Fuxe, 1965; Palkovits & Jacobowitz, 1974), dopamine (Fuxe, 1965), 5-HT (Fuxe, 1965; Maley & Elde, 1982), [Met^5] enkephalin (Williams & Dockray, 1983), SP (Ljungdahl *et al.*, 1978; Cuello & Kanazawa, 1978; Maley & Elde, 1982), and TRH (Hökfelt *et al.*, 1975) were demonstrated to be within nerve terminals. The existence of NA, ACh and GABA was also indicated by the presence of their synthesizing enzymes, dopamine-β-hydroxylase for NA (Swanson & Hartman, 1975), choline acetyltransferase for ACh (Kimura *et al.*, 1981), and glutamic acid decarboxylase for GABA (Pérez de la Mora *et al.*, 1981). These results suggest that these substances function as neurotransmitters in the central control of respiration in the brainstem. This suggestion based on histochemical studies was further supported by the present study from a functional point of view. In particular, the fact that the anticholinesterase, edrophonium, alone exerted an acceleratory effect on the respiratory rhythm similar to that of exogenously applied ACh, suggests the involvement of an endogenous cholinergic mechanism in the control of the respiratory rhythm. As for the control of respiration by endogenous opioid peptides, naloxone has been found to activate ventilation in normal animals that had not received opioid compounds (Lawson *et al.*, 1979). However, in the present study we could not observe any conspicuous effects of naloxone on the respiratory frequency in fresh preparations. On the other hand, capsaicin noticeably augmented the respiratory frequency. Gamse *et al.* (1979) and Theriault *et al.* (1979) showed that capsaicin causes a release of substance P from primary afferent nerve terminals. Therefore, the stimulant effect of capsaicin on the respiratory rhythm might be due to the action of endogenous substance P released from the central terminals of sensory fibres of cranial nerves such as the glossopharyngeal and vagal nerves.

The present study demonstrated that the brainstem-spinal cord preparation of the newborn rat is suitable for studying the mechanisms of action of drugs on the respiratory centre. When combined with more refined techniques such as single cell recording with a microelectrode and microinjection of drugs into more restricted areas of the brainstem, this preparation may provide an excellent opportunity for analysing the neural control of respiratory activity and the mechanism of action of a drug.

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