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A Cholinergic Mechanism Involved in the Respiratory Chemosensitivity of the Medulla oblongata in the Cat*

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Abstract. 1. Cholinomimetic and adrenomimetic substances were tested on the chemosensitive zones of the ventral surface of the medulla oblongata using a plexiglas ring method. Tidal volume and respiratory frequency, arterial pressure and heart frequency were observed.

2. The increase of ventilation and the depression of arterial blood pressure by locally applied acetylcholine could be blocked by previous local application of atropine. It is therefore assumed that the acetylcholine receptors have muscarinic properties.

3. Nicotine in a small dose raises arterial pressure and with higher doses a drop is observed. The responses of respiration and of arterial pressure to nicotine were blocked by previous intravenous administration of hexamethonium.

4. Local application af atropine in the caudal (L) and rostral (M) chemosensitive zones reduced resting ventilation and the slope of the ventilatory response to CO_2-inhalation. Physostigmine in these areas enhanced resting ventilation leaving unchanged the slope of the ventilatory response to CO_2-inhalation.

5. With high concentrations of (L)-noradrenaline and (L)-adrenaline a slight increase of arterial pressure was seen while serotonin caused a drop.

6. These results together with those of Fukuda and Loeschcke (1978) suggest that a cholinergic transmission in the surface layer of the ventral medulla is a component in the respiratory and circulatory control systems.

Key words: Medulla oblongata – Cholinomimetic drugs – Respiration – Circulation – Central chemosensitivity.

Introduction

In the previous paper it was reported that there are 2 zones on the ventral surface of the medulla oblongata, particularly sensitive to acetylcholine and nicotine, giving rise to hyperventilation. These zones are similar or identical with the zones where application of acidic buffers caused hyperventilation (Schläfke et al., 1970). Several of the very early accounts as well as the recent ones report on the effects of cholinergic substances affecting respiration (Diskhit, 1934; Phillipot, 1937; Gesell and Hansen, 1945; Schweitzer and Wright, 1937; Mitchell et al., 1963; Metz, 1966; Dev and Loeschcke, 1976; Trzebski, 1977). Apart from producing respiratory change, these substances also affect circulation (Borison et al., 1972; Dev and Loeschcke, 1976; Feldberg and Guertzenstein, 1976; Schläfke et al., 1977). The same chemosensitive zones are highly sensitive to pH changes in the extracellular fluid of the brain (ECF_B) (Loeschcke et al., 1958; Berndt et al., 1972; Fukuda and Loeschcke, 1977; Trzebski, 1977) and also by electrical stimulation of these areas similar responses may be obtained (Loeschcke et al., 1970; Trouth et al., 1973; Davies and Loeschcke, 1977). The mechanism responsible for the changed activity in respiration, however, has remained obscure.

It has been shown by Fukuda and Loeschcke, 1977, that an alteration of Ca^{2+} and Mg^{2+} content of the bathing medium could alter the response of the neuronal firing to H^+ in an in vitro preparation. This suggests a possible involvement of synaptic transmission. For example such an effect has been described on the cholinergic synapses of the superior cervical ganglion (Hutter and Kostial, 1954) and at the neuromuscular junction (del Castillo and Engbaek, 1954).

In this paper it was planned on the ventral surface of the medulla to test the effects on respiration and circulation of substances inhibiting cholinergic mechanisms like atropine and hexamethonium and also to inhibit the activity of acetylcholinesterase. Furthermore the effect of sympathomimetics was to be studied. The experiments were conducted parallel to experiments of Fukuda and Loeschcke (1979) who investigated the action of the same substances on an in vitro preparation of the ventral superficial layer of the

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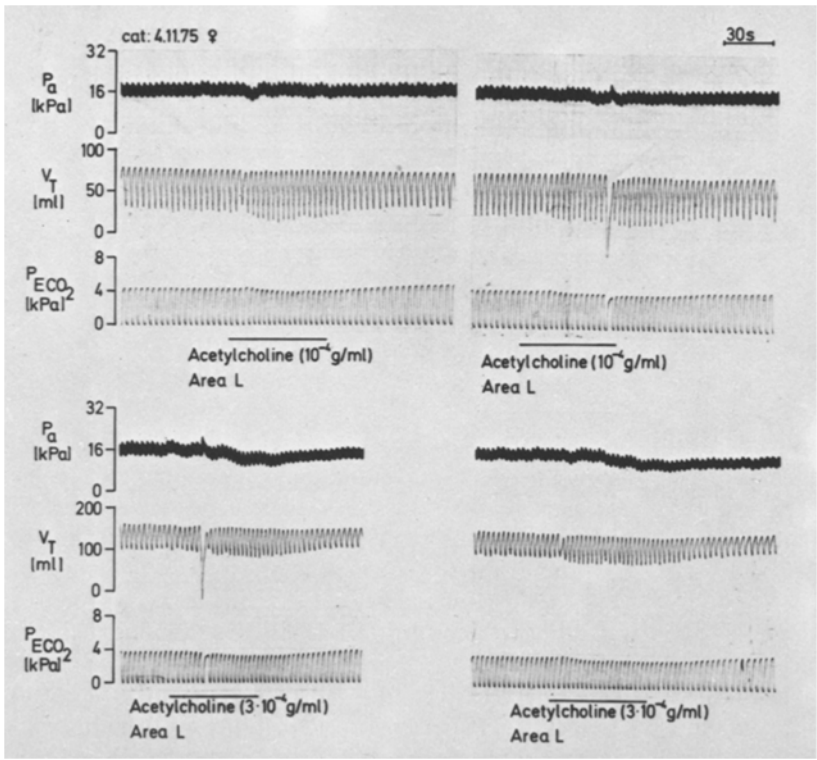

Fig. 1
Effects of two different concentrations of acetylcholine applied locally onto the caudal area bilaterally. Applications of acetylcholine (10^{-4} g · ml^{-1} = 5.5 · 10^{-4} mMol · ml^{-1}) produced only a respiratory augmentation (inspiration downward) without modifying arterial blood pressure (top records), whereas, applications of acetylcholine ($3 \cdot 10^{-4}$ g · ml^{-1} = 16.5 · 10^{-4} mMol · ml^{-1}) elicited an increase in ventilation as well as a drop in arterial blood pressure (lower records). P_a = arterial pressure, V_T = tidal volume, P_{ECO_2} = expiratory P_{CO_2}

medulla oblongata with neurophysiological techniques.

Methods

For anaesthesia, surgery, experimental set up and recording, see the previous article.

Drug Application. The method applied is a slightly modified form of the method used by Guertzenstein (1973). Plexiglas rings with an inner diameter of 2 mm were placed bilaterally on the 4 chemosensitive zones on the ventral surface of the medulla oblongata. Vaseline and liquid agar-gel was put around the periphery of the rings in order to secure their position throughout the experiment. Constant volumes (10 µl) of drug solutions were applied inside the rings and the drugs were washed out by mock cerebrospinal fluid after the test period without injuring or disturbing the medullary surface.

CO_2-Inhalation. Alveolar P_{CO_2} was raised by inhalation of different concentrations of CO_2 in the inspired air, prepared by injecting CO_2 into the inspiratory side of the circulating system. In these experiments P_{CO_2} was in between 2.7 and 9.3 kPa (20–70 Torr). After 7 min of CO_2-inhalation at a particular P_{CO_2}, the breathing was considered to be in the steady state. The end-expiratory CO_2 tension was controlled manually by fine adjustment of the CO_2-flow rate. Throughout the experiment oxygen was inhaled.

Data Analysis. Average tidal volume, frequency and ventilation in the pre-stimulus or 'control period' were computed. The average values of a response period were calculated strictly for the time between the beginning and the end of application of the drug even if there were latencies of the reaction. Responses were expressed as the ratio of test response/control response. By this procedure the control response becomes always 1. In order to demonstrate the time courses the parameters were averaged over much shorter periods. Ratios obtained from different experiments were pooled and data presented as the mean ratio ± standard error of the mean. In the type of anaesthesia used the parameters of ventilation and circulation in periods up to an hour were fairly constant. On the long run i.e. in several hours there was always a tendency of an increase of the respiratory frequency and a decrease of the tidal volume and also of a slow drop of the arterial pressure. Data were obtained from several animals in order to provide an estimate of the population response. For the linear range of the CO_2-response curve, a regression line was fitted by the method of least squares and the regression equation $Y = m X + c$ was used to define the response.

Results

Responses of Ventilation and Arterial Pressure to Application of Acetylcholine and Nicotine to the Caudal Chemosensitive Zone

Acetylcholine and nicotine were effective stimulants of the chemosensitive zone, except when anaesthesia was very deep (P_{ACO_2} = 6 kPa). Responses elicited by acetylcholine had a longer latency to onset and lasted shorter than comparable responses to nicotine.

The experiment of Fig. 1 shows such an effect of acetylcholine. A small dose of acetylcholine (10^{-4} g · ml^{-1} = 5.5 · 10^{-4} mMol · ml^{-1}) on the caudal area bilaterally hardly produced any change of blood pressure but a respiratory excitation occurred. An in-

creased dose ($3 \cdot 10^{-4}$ g · ml^{-1} = $16.5 \cdot 10^{-4}$ mMol · ml^{-1}), however, not only triggered an increase of the respiratory activity but also a drop in the systemic arterial pressure. The effects are well reproducible.

In 5 experiments, in 5 different cats, acetylcholine ($3 \cdot 10^{-4}$ g · ml^{-1} = $16.5 \cdot 10^{-4}$ mMol · ml^{-1}), was locally applied in the caudal area bilaterally and as expected a fall in arterial pressure and an increase in respiration was observed. The increase in respiration was mainly mediated by an increase in the tidal volume. With the drop in arterial pressure, also a tachycardia was observed, the duration of which was as long as the reduction in arterial pressure persisted. This tachycardia is probably mediated by a baroreceptor feed back. The hypotensive action of acetylcholine lasted over 5 min and the arterial pressure afterwards tended back to normal. The drop in blood pressure was maximum at about 30 s after application and was highly significant (P = 0.0002). The increase in ventilation caused by acetylcholine was significant at the level of P = 0.0027.

Interaction of Atropine with Acetylcholine

Local application of atropine (10^{-3} g · ml^{-1} = $3.5 \cdot 10^{-3}$ mMol · ml^{-1}) on the caudal area bilaterally for 10 min before acetylcholine application, counteracted or abolished acetylcholine induced effects on ventilation and on arterial pressure as shown in Fig. 2. However, such a local application of atropine did not interfere with the response to i.v. administered acetylcholine. The duration of the atropine effect was relatively long and on average persisted for more than 50 min. A quantitative analysis shows that the inhibition of acetylcholine induced effects by atropine is significant at the level of P = 0.0002 for n = 5. The acetylcholine effect returned completely after about 65 min of washout of the area with artificial cerebrospinal fluid. The respiratory response recovered earlier than the arterial pressure response.

Interaction of Nicotine with Hexamethonium

An intensive change in breathing and arterial blood pressure was observed on application of nicotine ($5 \cdot 10^{-5}$ g · ml^{-1} = $3.1 \cdot 10^{-4}$ mMol · ml^{-1} to 10^{-3} g · ml^{-1} = $6.2 \cdot 10^{-3}$ mMol · ml^{-1}) on the caudal area bilaterally. A small dosage of nicotine in this area invariably produced a respiratory excitation with or without an increase in arterial pressure. However, a large dose always caused a reduction in the arterial pressure with associated bradycardia and an increase in ventilation. Figure 3 presents the various effects on arterial blood pressure and respiration caused by nicotine in different concentrations in the caudal area.

Injection of hexamethonium ($2 \cdot 10^{-3}$ g · ml^{-1} = $9.9 \cdot 10^{-3}$ mMol · ml^{-1}) i.v. significantly diminished the hyperventilation elicited by tests with a small dose of nicotine and also the increase in arterial blood pressure triggered from the caudal area. Hexamethonium itself caused hypotension and an initial increase in tidal volume which, however, returned to the control level about 10 min after injection. Quantitative data from 5 experiments in 5 cats are presented in Table 1 and a typical example is shown in Fig. 4. The effect of hexamethonium on the response to nicotine as far as arterial pressure is concerned may also be the consequence of a blockade of the peripheral ganglion. This criticism, of course, cannot be applied to the effects on respiration.

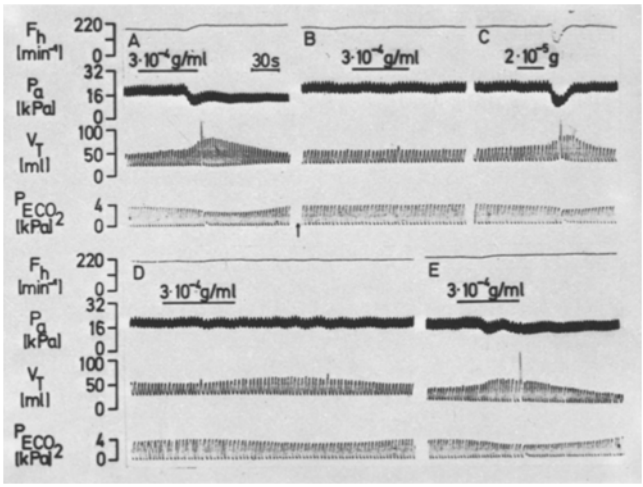

Fig. 2. Interaction of acetylcholine and atropine. Heart frequency, arterial blood pressure, tidal volume and end-expiratory CO_2-partial pressure in a cat during the application of acetylcholine ($3 \cdot 10^{-4}$ g · ml^{-1} = $16.5 \cdot 10^{-4}$ mMol · ml^{-1}) onto the caudal area bilaterally (record A). Local application of atropine (10^{-3} g · ml^{-1} = $3.5 \cdot 10^{-3}$ mMol · ml^{-1}) in the same position blocked hypotensive reaction and respiratory augmentation caused by acetylcholine (record B). Atropine was applied in between A and B for a period of 10 min. In contrast, the effect of i.v. injected acetylcholine was not visibly modified in the presence of atropine on the surface (record C). Recording 25 (D) and 35 min (E) respectively after wash out of atropine from the surface shows that the acetylcholine effects on ventilation and arterial pressure reappear. F_h = heart frequency, P_a = arterial pressure, V_T = tidal volume, P_{ECO_2} = expiratory P_{CO_2}

Interaction Between Physostigmine and Inhaled CO_2

Physostigmine (10^{-4} g · ml^{-1} = $3.6 \cdot 10^{-4}$ mMol · ml^{-1}) locally applied onto the caudal or the rostral area bilaterally produced an increase in ventilation with or without a concomitant change in the systemic arterial blood pressure. However, increasing the dose up to $2 \cdot 10^{-2}$ g · ml^{-1} = $7.2 \cdot 10^{-2}$ mMol · ml^{-1} invariably produced a fall in arterial pressure accompanied by an increase in ventilation. The fall in arterial pressure and

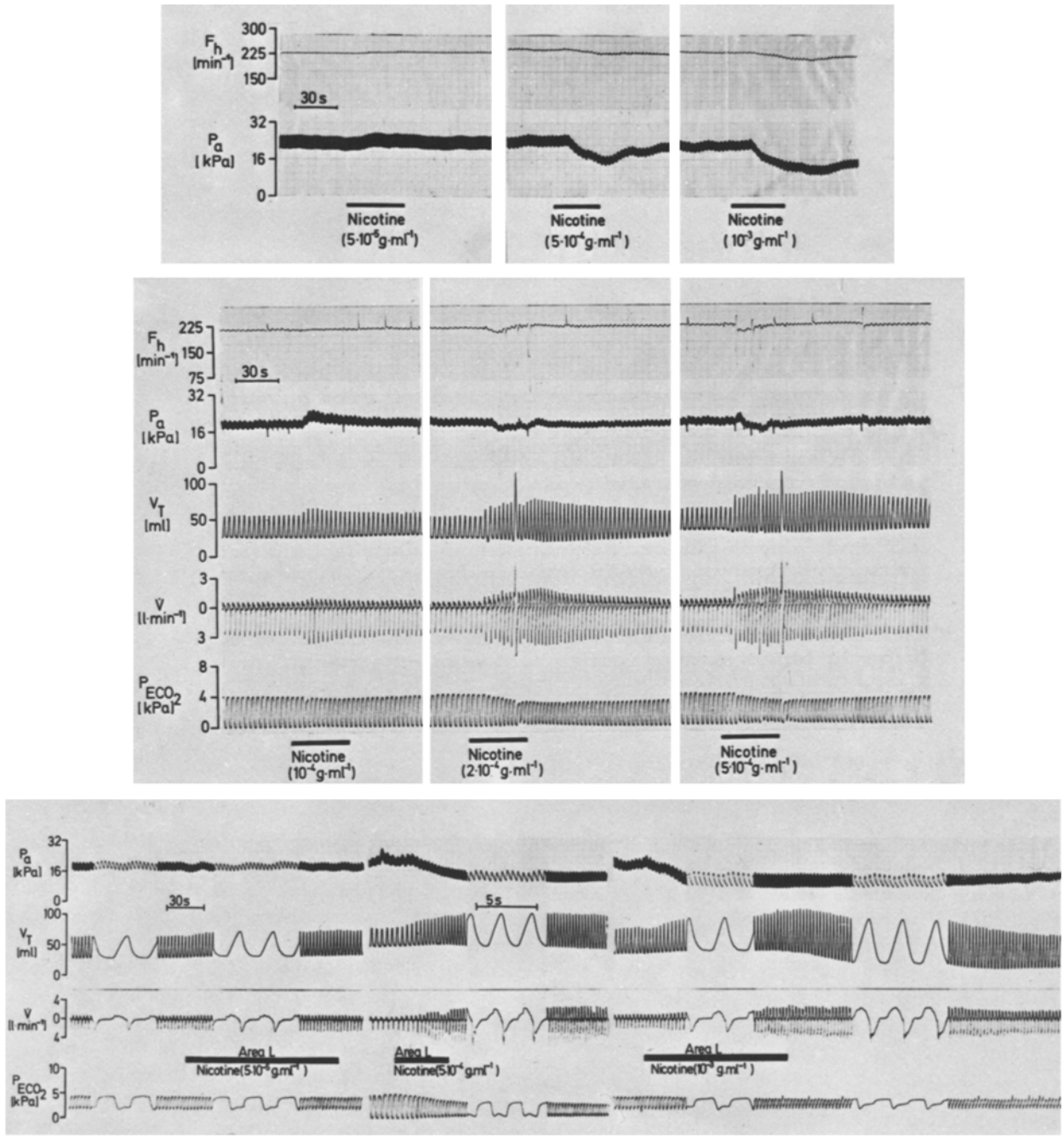

Fig. 3. Effects of nicotine on circulation and respiration, applied onto the caudal area bilaterally at different concentrations. Recordings were obtained from 3 cats. Upper panel: cat 16.3.78, 2.62 kg; Middle panel: cat 21.3.78, 2.72 kg; Lower panel: cat 30.3.78, 2.99 kg. Note the small dosage of nicotine elicits hypertension. Large dosage invariably produces hypotension with bradycardia. In contrast to different cardiovascular responses elicited by nicotine in different dosage, it always shows a marked respiratory excitation in the dose-regime investigated. Inspiration upwards. F_h = heart frequency, P_a = arterial pressure, V_T = tidal volume, $\dot{V}$ = tidal flow, P_{ECO_2} = expiratory P_{CO_2}

increase in ventilation differed somewhat from that observed after acetylcholine in so far as the drop in arterial pressure and increase of ventilation occurred after a longer latency after physostigmine application and the duration of the response to physostigmine was longer. The effect of physostigmine in inducing respiratory modification is shown in Fig. 5. Since in these experiments, the tidal volume was clearly increased in the presence of physostigmine, the response to inhaled CO_2 was studied in the presence of physostigmine. After the application of physostigmine to the chemosensitive zones, the respiratory tidal volume in all

Table 1. Effect of $2 \cdot 10^{-3}$ g · kg^{-1} = $9.9 \cdot 10^{-3}$ mMol · ml^{-1} hexamethonium on nicotine ($2 \cdot 10^{-4}$ g · ml^{-1} = $1.2 \cdot 10^{-3}$ mol · ml^{-1}) induced change in tidal volume and arterial pressure on application at the caudal area bilaterally

		Control	Nicotine	Hexamethonium	Hexamethonium and nicotine
Tidal volume	Mean ± s (ml)	36.0 ± 6.0	58.3 ± 12	37.0 ± 3.2	39.0 ± 5.4
	Ratio ± s_m		1.57 ± 0.15		1.05 ± 0.04
	P		0.02 for $n = 5$		0.34 for $n = 5$
Arterial blood pressure	Mean ± s (kPa)	18.0 ± 3.5	19.7 ± 1.9	15.7 ± 3.6	16.3 ± 3.46
	Ratio ± s_m		1.10 ± 0.04		1.03 ± 0.06
	P		0.05 for $n = 5$		0.64 for $n = 5$

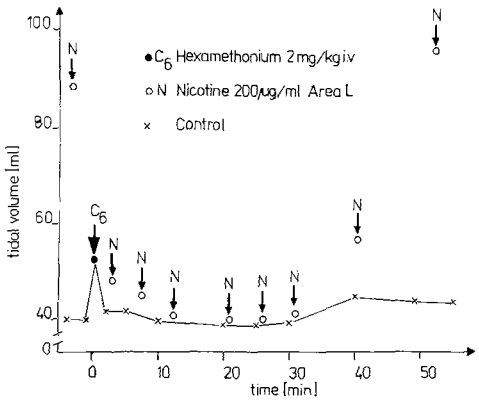

Fig. 4. Influence of i.v. injected hexamethonium (C_6, $2 \cdot 10^{-3}$ g · kg^{-1} = $9.9 \cdot 10^{-3}$ mMol · ml^{-1}) on respiratory response elicited by nicotine (N, $2 \cdot 10^{-4}$ g · ml^{-1} = $1.2 \cdot 10^{-3}$ mMol · ml^{-1}) applied onto the caudal area bilaterally. Data presented show the average tidal volume response over a 60 s period on the ordinate. C_6 was injected at 0 min and followed by a transient hyperventilation. Before C_6 nicotine on the caudal area increased tidal volume, subsequently to i.v. C_6 injection the nicotine effect was inhibited and returned after 30 min to be fully reversible after 60 min

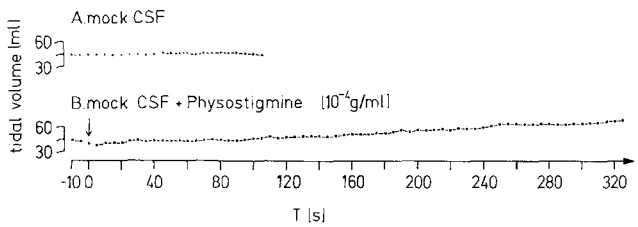

Fig. 5. Effect of physostigmine on tidal volume. In A, ventral surface of medulla oblongata superfused with mock CSF at the caudal and rostral areas bilaterally (control). In B, ventral surface superfused with mock CSF containing physostigmine (10^{-4} g · ml^{-1} = $3.6 \cdot 10^{-4}$ mMol · ml^{-1}) at the same location. Physostigmine in the course of 5 min raises the tidal volume markedly

experiments was higher at a given P_{ACO_2} level, causing a change in the intercept of the CO_2-response curve but not of the slope. This is shown in 4 cats, Fig. 6. The respiratory frequency was unchanged or diminished. The diminution could in no case balance the increase of tidal volume.

Response to Inhaled CO_2 Before and After Atropine

In 6 cats CO_2-response curves were obtained before and after application of atropine at the caudal and rostral areas bilaterally. All preparations reacted to an increase of P_{ACO_2} by an augmentation of the tidal volume and a slight increase in respiratory frequency. Application of atropine at various concentrations (10^{-2} g · ml^{-1} = $3.5 \cdot 10^{-2}$ mMol · ml^{-1} up to $3 \cdot 10^{-2}$ g · ml^{-1} = $10.5 \cdot 10^{-2}$ mMol · ml^{-1}) depressed the resting value of respiration. The increase of ventilation elicited by CO_2 was also diminished in the presence of atropine (Fig. 7). Atropine decreased the intercept and depressed the slope of the CO_2-response curve. Quantitative data are presented in Table 2. The relative slope of the CO_2-response curve in the presence of atropine in the average was reduced by 48%, significant at the level of $P = 0.0002$ for $n = 6$. The decrease in the intercept value of the CO_2-response curve was 37%, significant at $P = 0.0015$ for $n = 6$.

Respiratory and Cardiovascular Responses to Catecholamines

Application of noradrenaline, adrenaline and serotonin in concentrations ranging between $10^{-4} - 5 \cdot 10^{-3}$ g · ml^{-1} for a period up to 60 s in the caudal area (L) hardly affected respiration or arterial pressure in five experiments. In another three experiments both (L)-noradrenaline and (*l*)-adrenaline in concentrations of $5 \cdot 10^{-3}$ g · ml^{-1} = $3.0 \cdot 10^{-2}$ mMol · ml^{-1}, $2.7 \cdot 10^{-2}$ mMol · ml^{-1}, respectively) produced a slight increase in arterial pressure and no significant change either in the tidal volume or in the respiratory frequency. With serotonin at the same concentration ($5 \cdot 10^{-3}$ g · ml^{-1} = $3.2 \cdot 10^{-2}$ mMol · ml^{-1}) a fall in arterial pressure was invariably the case. The onset latency for the arterial pressure effect was variable between 40 – 80 s.

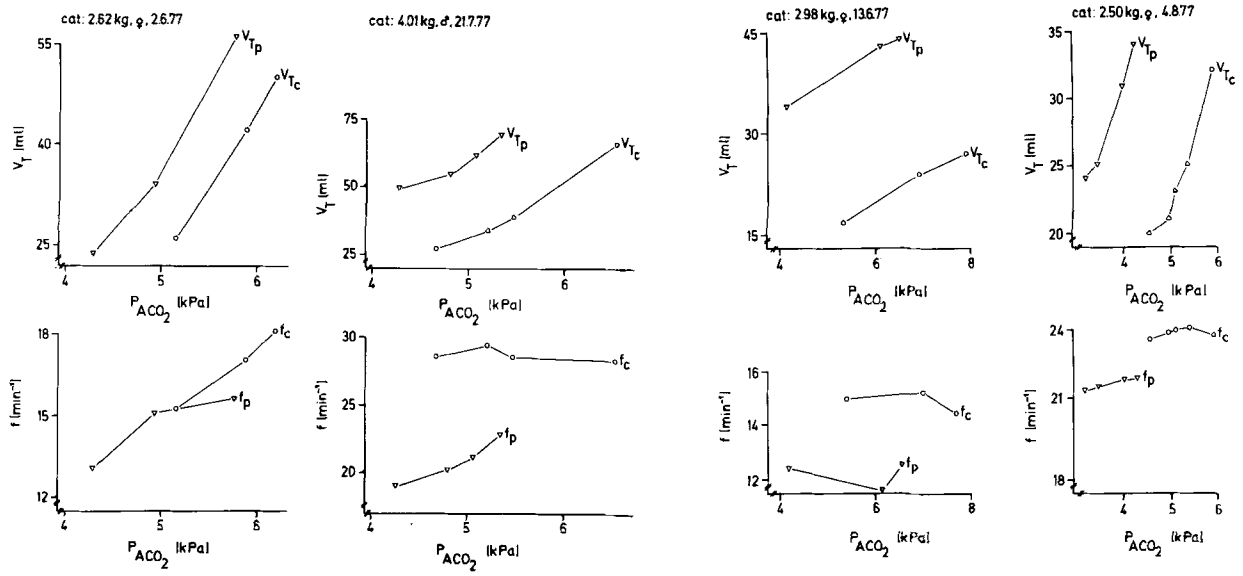

Fig. 6. CO_2 responses curves showing the effect of different P_{ACO_2} on tidal volume (upper panel) and respiratory frequency (lower panel) before (open circle) and after application of physostigmine ($2 \cdot 10^{-2}$ g · ml^{-1} = $7.2 \cdot 10^{-2}$ mMol · ml^{-1}) at the caudal and rostral area bilaterally (triangle). Results from 4 cats. Parallel shift of CO_2-response curves. Decrease of respiratory frequency in the average. V_T = tidal volume, P_{aCO_2} = endtidal pCO_2, f = respiratory frequency

Table 2. Effects on the CO_2-response curves of different concentrations of atropine applied onto the caudal and rostral areas bilaterally

Cat experiment number	Experimental conditions	Dosage (g · ml^{-1})	Control		Atropine	
			Slope (s) l · min^{-1} · kPa^{-1}	Intercept (B) kPa at $\dot{V}_E = 0$	Slope (s) l · min^{-1} · kPa^{-1}	Intercept (B) kPa at $\dot{V}_E = 0$
1	Sinus nerves and vagi denervated	$3 \cdot 10^{-2}$	0.93	2.77	0.33	1.88
2	,,	$3 \cdot 10^{-2}$	0.60	1.74	0.28	0.75
3	,,	$3 \cdot 10^{-2}$	1.02	2.36	0.62	1.74
4	,,	$1.5 \cdot 10^{-2}$	0.58	3.12	0.24	2.17
5	Sinus and vagi intact	$2 \cdot 10^{-2}$	0.63	2.36	0.40	2.98
6	,,	10^{-2}	0.73	3.21	0.31	1.83

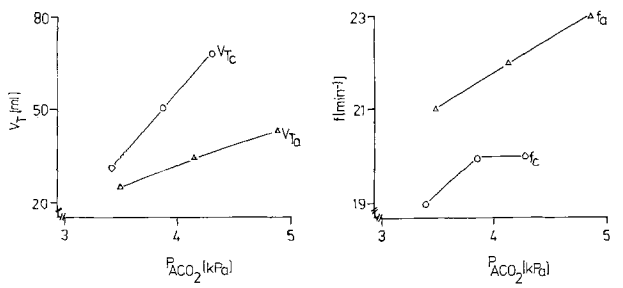

Fig. 7. CO_2-response curves showing effect of different P_{ACO_2} on ventilatory response before (open circle) and after application of atropine at the caudal and rostral areas bilaterally (triangle). Left: Tidal volume response. Right: Respiratory frequency response. Cat 29.10.76, 3.08 kg. Decrease of tidal volume slope, increase of frequency. Compare also Table 2. V_T = tidal volume, P_{aCO_2} = endtidal pCO_2, f = respiratory frequency. See also Table 2

Discussion

The concentrations of the drugs used in these experiments have been relatively high which by diffusion might have influenced activity throughout the neuronal networks of the ventral part of the medulla oblongata. It is, however, difficult to estimate the drug concentration at the receptor site, but presumably it is much lower than on the surface because these substances have to pass the pia membrane and some tissue layer containing glial cells and neurones before they could reach the chemosensitive substrate and along this way the capillary uptake process has to be considered. Destruction by enzymatic inactivation would also play a role. It is known that the brain contains specific and unspecific cholinesterase (Koelle, 1954). The fact, how-

ever, that, as was described in a preceding paper, the drug action could be referred to well defined spots on the surface which in addition were identical with the location of chemosensitivity to hydrogen ions, does indicate that the effects described cannot be considered as diffuse actions on an undefined matrix of brain tissue.

The blockade of the response of ventilation to locally applied acetylcholine and reduction of the CO_2-sensitivity by atropine suggests that muscarinic receptor sites are involved in the respiratory drive from the chemosensitive zones. Muscarinic receptor sites on Renshaw cells of the cats spinal cord have been reported by Curtis and Ryall (1966). Muscarinic inhibition or excitation of brainstem neurones have also been shown by Bradley et al. (1966).

In the present experiments the resting respiration was enhanced by the local application of physostigmine at the caudal and rostral area. This suggests that cells in these regions are synaptically driven by cholinergic fibres and the enhancement of the resting respiration is due to the inhibition of the hydrolysis of the transmitter. The presence of cholinesterase and cholineacetylase and the roles of acetylcholine in some central synaptic processes have been demonstrated by Feldberg (1950) and Krnjevic and Phyllis (1963). Increase of $[H^+]$-ion concentration in the extracellular fluid of the brain as during CO_2-inhalation (Loeschcke and Sugioka, 1969; Ahmad et al., 1977) may contribute to inactivation of cholinesterase present in the surface layer. Such inactivation of cholinesterase by increased $[H^+]$-ion in the glomus cell of the carotid body has been reported by Augustinsson (1948) and by MacIntosh and Perry (1950) and it has been demonstrated in vitro by Lüllmann and Peters (1967).

Since both physostigmine and atropine produced a change in the unstimulated resting ventilation, this suggests that the respiratory drive at rest is continuously under the tonic influence of a cholinergic input. The decrease in the slope of the CO_2-response curve under local atropine indicates that also under the condition of CO_2-inhalation the drive of ventilation can be inhibited. However, the theoretical apnea point after the application of atropine moved towards the left, suggesting that atropine in hypocapnic conditions could stimulate ventilation. Physostigmine shifted the CO_2-response curve to higher tidal volumes. This shift was approximately parallel. Fukuda and Loeschcke (1979) could show that increased spike discharge under acidic pH could be inhibited by atropine and hexamethonium in tissue slices of the medulla oblongata in rats.

The blockade by hexamethonium of the hyperventilation and of the increase in blood pressure caused by nicotine suggests that also nicotine sensitive synapses exist at the caudal area (L).

The question whether or not the described respiratory and circulatory effects are mediated by the same structure as for example a single cell population has been discussed in the preceding paper (Dev and Loeschcke, 1979). It seems that all the results cannot be explained by a single population of cells projecting on the respiratory as well as on the circulatory system. It seems also that a pressor and a depressor substrate must be assumed.

Studies using models of experimental neurogenic hypertension have shown that increased arterial pressure is associated with increased turnover of norepinephrine in bulbospinal catecholaminergic nerves (Chalmers, 1975). However, other workers have concluded that excitation of central α-adrenoreceptors enhances inhibitory activity and lowers arterial pressure (van Zwieten, 1973; de Jong, 1976). The slight increase in arterial pressure, seen after the application of higher dosage of noradrenaline and adrenaline casts doubt on the hypothesis that the hypotension induced by clonidine from the ventral surface of the medulla oblongata would be mediated through the stimulation of central α-adrenoreceptors. Cholinergic or serotoninergic involvements in the action of clonidine applied to the ventral surface of the medulla oblongata, therefore, are possible alternatives.

The finding that it is possible to influence respiration and circulation by cholinergic substances on the ventral surface of the medulla oblongata of anaesthetized cats forms the basis of a new concept according to which respiration and circulation may be regulated by the release and destruction of such substances. They may continuously be released under the influence of some so far unidentified neural input and the normal respiration and vasomotor tone may be the outcome of a balance between the release and destruction. In the respiratory system the hydrogen ion concentration may either act on the release, the destruction or the action on the postsynaptic membrane of the transmitter, acetylcholine.

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