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Topography of the Respiratory and Circulatory Responses to Acetylcholine and Nicotine on the Ventral Surface of the Medulla oblongata*

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Abstract

1. Acetylcholine and nicotine were superfused on the ventral medullary surface between the ponto-medullary border and C_1 in anaesthetized cats in order to determine the topical distribution of their actions on respiration and circulation.

2. Acetylcholine (10^{-4} g · ml^{-1} = $5.5 \cdot 10^{-4}$ mMol · ml^{-1}) produced an increase in respiration and a lowering of blood pressure. The magnitude and the time course of the responses varied according to the points of superfusion on the surface.

3. Nicotine (10^{-4} g · ml^{-1} = $6.2 \cdot 10^{-4}$ mMol · ml^{-1}) elicited hyperventilation and more often an increase in arterial pressure on unilateral superfusion of the surface. In some cases, however, a drop in blood pressure was also observed.

4. The responsive regions of the surface on which nicotine acted and elicited hyperventilation, bear a close resemblance to the regions responsive to acetylcholine.

5. The topographical distribution of the respiratory effects elicited by the above-mentioned drugs were similar to the distribution of the responses to changes in pH on the ventral medullary surface or to electrical stimulation.

6. Procaine ($2 \cdot 10^{-2}$ g · ml^{-1} = $7.3 \cdot 10^{-2}$ mMol · ml^{-1}) applied bilaterally in the intermediate zone (S) caused profound inhibition of respiration and of arterial pressure. Procaine at this concentration also inhibited respiratory hyperventilation caused by nicotine (10^{-4} g · ml^{-1} = $6.2 \cdot 10^{-4}$ mMol · ml^{-1}) applied to the caudal and rostral areas.

Key words: Medulla oblongata – Respiration – Circulation – Acetylcholine – Nicotine.

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Introduction

The role of hydrogen ion and other cations in the extracellular fluid (ECF) in changing cardiovascular and respiratory patterns has been studied by several authors (Loeschke and Koepchen, 1958; Schläfke et al., 1970; Berndt et al., 1972; Borison et al., 1972). For reviews readers are referred to Leusen (1972) and Loeschke (1974).

As to the site of action of hydrogen ions in the ECF, superficial areas on the ventral surface of the medulla oblongata have been proposed (Mitchell et al., 1963; Loeschcke et al., 1970; Feldberg and Guertzenstein, 1976). Application of acid buffers on the ventral surface of the medulla causes an increase in ventilation without an effect on arterial pressure (Loeschcke et al., 1958). Also Loeschcke et al. (1970) described two areas on the ventral surface of the medulla oblongata, the electrical stimulation of which gave rise to increased ventilation and may be identical with the chemosensitive structure. Electrical stimulation also raises the arterial pressure.

Since potentiating effects of acid on the response of several cholinergic systems to extrinsic acetylcholine has been shown by Brassfield and Gesell (1942) in the heart of the turtle, the submaxillary gland of the dog and the gut of the rabbit, acid base alterations may well modify activities of cholinergic neurones in the CNS. The presence of acetylcholine within synaptic nerve endings in the CNS has been shown for example by Ryall (1963). Widely scattered neurones reacting to acetylcholine and physostigmine in the medulla oblongata were also described by Salmoiraghi and Steiner (1963). These neurones were inhibited by hexamethonium or by Dihydro-β-erythroidine but not by atropine. Gesell and Hansen (1942) observed gross changes of ventilation after injection of acetylcholine into the cerebral arteries.

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However, little information is available concerning the central action of cholinergic drugs. Intracerebroventricular injection of cholinomimetic drugs has been shown to evoke excitatory cardiovascular responses in dogs (Sinha et al., 1967; Lang and Rush, 1973) and variable effects – excitatory, inhibitory and biphasic – in cats (Armitage and Hall, 1967; Feldberg and Guertzenstein, 1967). The same applies to respiration. The methodical differences have led to interpretational difficulties regarding the role of central cholinoceptors in cardiovascular and respiratory regulation. Observation of increased ventilation by Mitchell et al. (1963) who first applied acetylcholine on the rostral chemosensitive area, may suggest a possible role of cholinergic transmission in the surface layer of the ventral medulla oblongata. Guertzenstein (1973) observed a fall of arterial pressure after application of carbachol to the ventral medullary surface.

The following study was carried out to determine the respiratory and cardiovascular effects of superfusion of cholinergic drugs on the ventral surface of the medulla oblongata and to find out their site of action. The experimental results may yield evidence as to whether or not cholinergic substances have the same locus of action as pH changes in CSF and if they would contribute to the regulation of respiration and circulation.

Method

Anaesthesia and Surgery. Cats of either sex weighing 1.9 – 4.5 kg (av. 2.4 kg) were lightly anaesthetized with chloralose-urethane ($0.04\,g \cdot kg^{-1}$ α-glucochoralose, $0.2\,g \cdot kg^{-1}$ urethane) following induction with a mixture of N_2O (80 %) and O_2 (20 %). This anaesthesia lasted for hours and only in experiments of long duration supplementation by an additional dose was necessary. This anaesthesia is light with well maintained cardiovascular reflexes, relatively high arterial pressure and moderately diminished sensitivity of respiration to CO_2.

The trachea was cannulated. The rostral part of the trachea and of the oesophagus were then reflected rostrally. Afterwards the condylus os occipitalis, the pars basilaris of the os occipitale and the ventral portion of the atlas with the processus odontoideus of the epistropheus were removed. The dura was opened along the midline and reflected laterally by sutures.

The body temperature of the cat was kept constant throughout the experiment at 37 ± 0.5° C by an automatic thermocontroller. The animal was placed in supine position, with the head fixed in a Horseley-Clarke stereotaxic apparatus The animal inhaled pure oxygen throughout the experiment. This was considered to be favorable to the experiment because the peripheral chemoreceptors may be expected to discharge at a low rate which is almost independent of variations of p_{O_2}.

Plan of the Experiment. The chemode system used to superfuse mock CSF as well as test solutions on the medulla oblongata has been described by Schläfke et al. (1970). All solutions were maintained at a constant temperature of 37° C and equilibrated with 5 % CO_2 in air. The pH of the solutions was kept in between 7.30 – 7.34. The perfusion of control and test solutions flowed at a rate of $1\,ml \cdot min^{-1}$ and the counter perfusion with mock CSF at a rate of $10\,ml \cdot min^{-1}$. The inner tubing of the probe had a diameter of 1.4 mm. Since the perfusion fluid outside of this ring was diluted tenfold the area superfused with the test solutions was approximately $1.5\,mm^2$. The superfusions in all cases were applied in the rostro-caudal direction of the ventral surface in steps of 2 mm in a distance of 3 mm lateral to the midline of the medulla. This avoids superfusion of the basilar artery. The branches of this artery may in some superfusions have been included in the superfused area. Never any additional effect of this on respiration or circulation has been observed. The level of the middle of the hypoglossal root was taken as a reference.

In another series of experiments, a ring-method was used. This method is a slightly modified form of the method used by Feldberg and Guertzenstein (1976). In our experiments plexiglas rings with an inner diameter of 2 mm were placed bilaterally on the areas under investigation. Vaseline was applied to the opening of the ring before it was put on the medulla and liquid agar-gel was poured around the periphery of the rings in order to secure their positions throughout the experiment. Constant volumes (10 µl) of drug solutions were applied inside each of the rings and the drugs were washed out by mock cerebrospinal fluid after the test-period without injuring or disturbing the medullary surface.

Measurements. Respiratory tidal volume and frequency were recorded by a low-inertia Krogh spirometer with an inductive transducer. Expiratory P_{CO_2} was recorded with an infrared analyser. Heart frequency was continuously recorded by using a frequency meter (Luttmann and Mückenhoff, 1974) and femoral arterial blood pressure was monitored via a cathether in the femoral artery with a strain gauge.

Materials. Acetylcholine was used as chloride, Nicotine as the base, Procaine as hydrochloride and Physostigmine as the base.

Experimental Protocol. In the control period mock CSF was superfused. The drug solutions were only superfused when the cat exhibited a steady-state of breathing and arterial pressure in the control phase. The superfusion of the drug solution lasted 60 s. Cardiovascular and respiratory parameters were evaluated before and continuously for the first 2 min after the beginning of the superfusion. The respiratory and cardiovascular effects were described by the ventilation – and the arterial pressure ratios respectively. This is the ratio of the test value over the control value.

Statistical Methods. The mean ($\bar{x}$) and standard error of the mean (s_m) of arterial pressure, ventilation and rise or fall in these variables were determined at each time interval of 10 s and the significance of difference investigated by the use of Fisher's *t*-test (Pätau, 1943; Fisher, 1963) and Students distribution.

Results

Acetylcholine. Following the acetylcholine chloride superfusion ($10^{-4}\,g \cdot ml^{-1} = 6.2 \cdot 10^{-4}\,mMol \cdot ml^{-1}$), the cats increased their ventilation. The cardiovascular effects consisted of an increase of the heart rate and a fall in arterial blood pressure.

In the most caudal part (−4 up to −6 mm) the changes in respiration and arterial pressure after the superfusion of acetylcholine were negligible. Rostral to this zone, 9 among 14 cats show a transient inhibition of respiration in the first 10 s followed by an increased ventilation maintained over up to 80 – 100 s. In the other 5 cats the response started with hyperventilation.

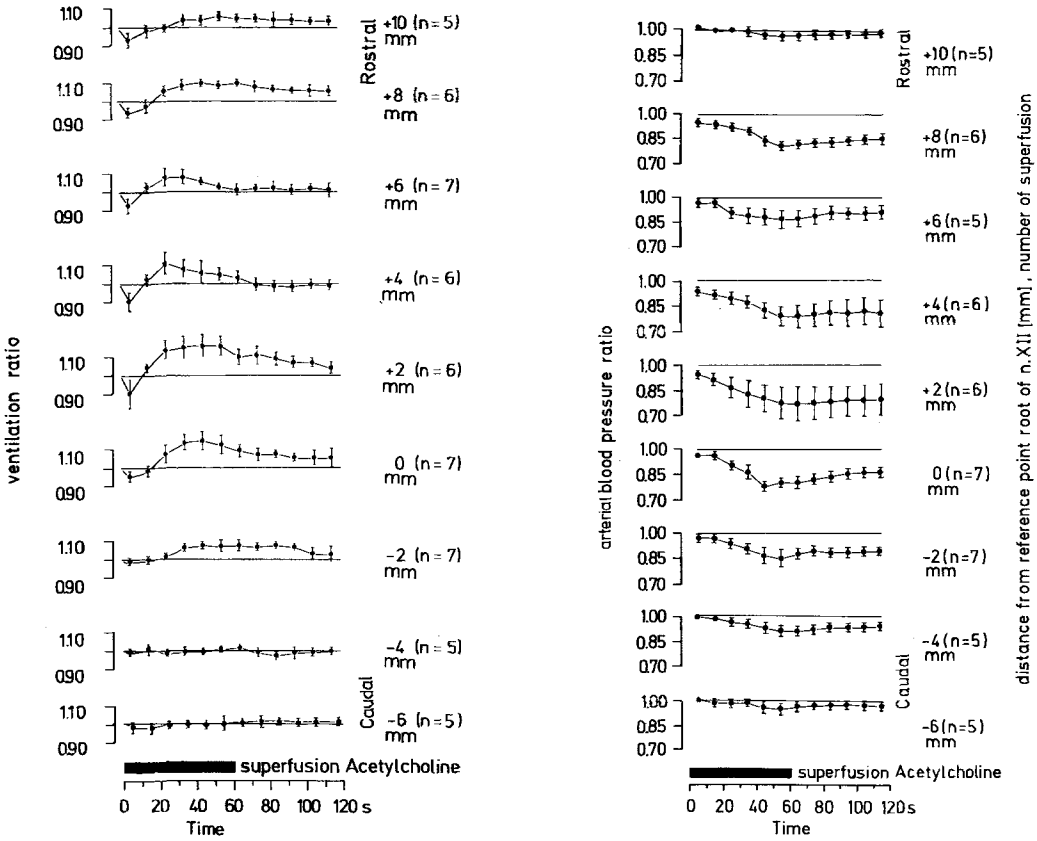

Fig. 1. Response of ventilation ratio (left) and the arterial blood pressure ratio (right) to local superfusion of acetylcholine chloride (10^{-4} g · ml^{-1} = 5.5 · 10^{-4} mMol · ml^{-1}) on the ventral surface of the medulla oblongata in distances of 2 mm in rostro-caudal direction (upwards down) relative to the reference point (0 mm, middle of hypoglossal root) as indicated on the right side. All superfusions were exerted unilaterally at 3 mm lateral distance from the mid-line. Mean values of 54 superfusion tests in 14 cats for ventilation and of 52 tests in 14 cats for arterial pressure are shown. The vertical bars show the standard error of the mean

This was observed in a zone extending from 2 mm caudal of the reference point (middle of hypoglossal root) to the foramen caecum. Figure 1 shows the magnitude of the ventilatory response to superfusion of acetylcholine in different parts of the ventral surface.

The respiratory response persisted over the period of superfusion (60 s) and reached a maximum in between 40 – 50 s and then subsided gradually to return to control in about 120 s. The ventilatory amplitude in the region of −2 and 0 was highly significant ($P = 0.004$ and 0.0015 respectively). In the intermediate zone, the increase in ventilation caused by acetylcholine was not statistically significant. Whereas in the region of +6 and +8 the magnitude of the ventilatory response again reached statistically significant values ($P = 0.0015$ and 0.0018 respectively). The regional distribution of the ventilatory response to acetylcholine and the distribution of the probability of the zero hypothesis are shown in Fig. 2. The absolute and relative data for the change in ventilation are compiled in Table 1.

In contrast to respiration, the cardiovascular effect of localised superfusion of acetylcholine was monophasic – it caused a decrease in arterial pressure (Fig. 1). The drop in blood pressure caused by acetylcholine persisted longer than the change in respiration. The region in which a maximal drop in blood pressure was observed, was at the rostral end of the hypoglossal root (+2 mm). In the rostral part of the medulla, the reduction in arterial pressure was of lower magnitude. The regional sensitivity of the medullary surface to acetylcholine to elicit blood pressure responses is presented in Table 1. The time-course relationship of the blood pressure response is shown in Fig. 1 and the relative magnitude of the arterial pressure response and also the p-value for the change in mean arterial pressure on local superfusion of acetylcholine is represented graphically in Fig. 2.

Nicotine. In a concentration of 10^{-4} g · ml^{-1} = 6.2 · 10^{-4} mMol · ml^{-1} nicotine increased ventilation (Fig. 2) with an accompanying increase in arterial

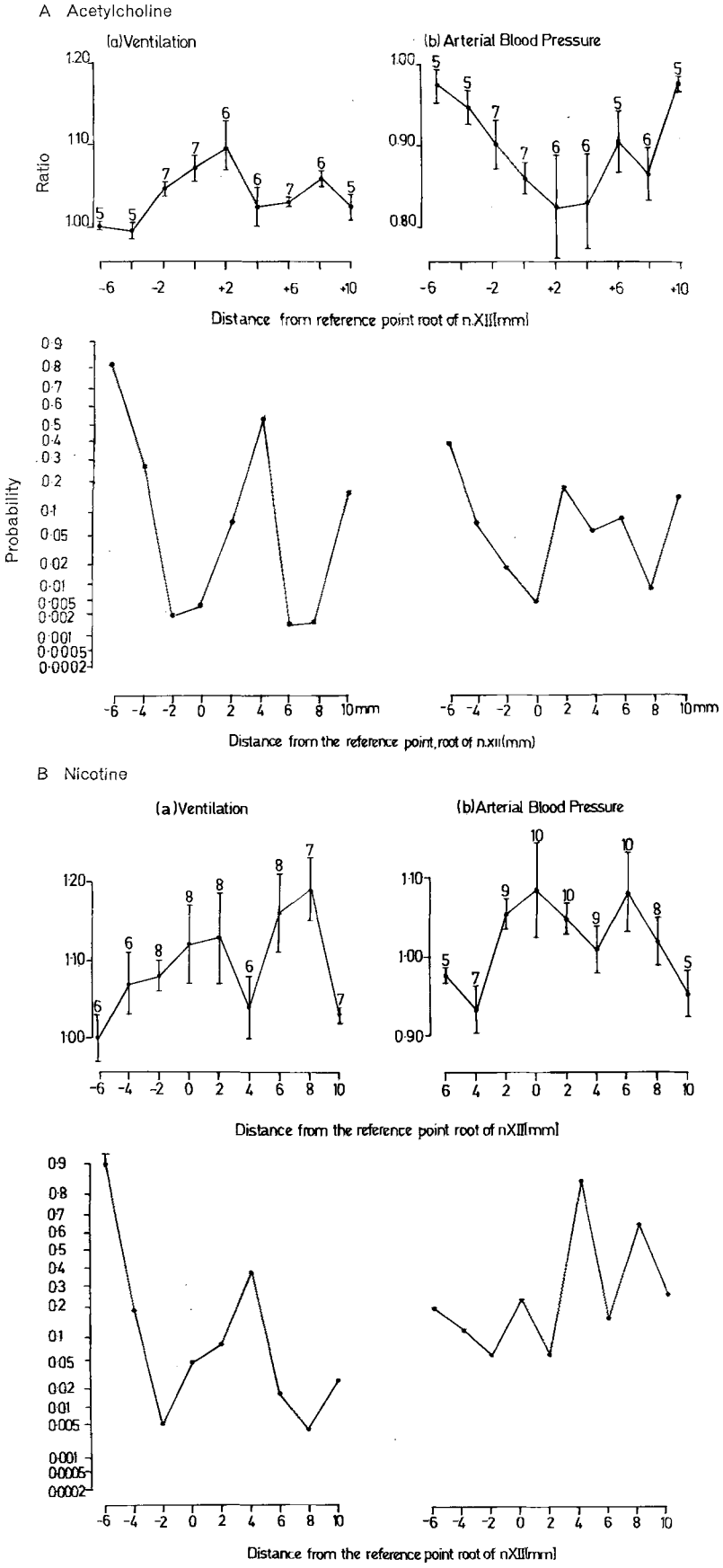

Fig. 2
A Topographical distribution of the ventilation ratio (**a**) and mean arterial pressure ratio (**b**) in superfusion of acetylcholine chloride (10^{-4} g · ml^{-1} = 5.5 · 10^{-4} mMol · ml^{-1}) at different localisations from 10 mm rostral up to 6 mm caudal of the reference point (middle of the hypoglossal root) and topographical distribution of the probabilities of the Null-hypothesis. All points were at 3 mm distance lateral to the midline.
B The same in superfusion of nicotine (10^{-4} g · ml^{-1} = 6.2 · 10^{-4} mMol · ml^{-1}). The data show the mean value and the standard error of the mean. The number of the tests are written to the points. Results out of 14 cats are given

Table 1. Mean-values of ventilation and of the mean arterial pressure, absolute and relative to the control-value in different chemode-positions (3 mm lateral of the midline) caused by local superfusion of acetylcholine chloride (10^{-4} g · ml^{-1} = 6.2 · 10^{-4} mMol · ml^{-1}). Reference point (0 = middle of hypoglossal root). Results out of 54 superfusion tests in 14 animals. The data show the mean-value over 120 s after the beginning of the superfusion with acetylcholine. Duration of superfusion – 60 s

	Caudal		CHEMODE-POSITION [mm from the reference point]														Rostral	
	−6		−4		−2		0		+2		+4		+6		+8		+10	
	c	s	c	s	c	s	c	s	c	s	c	s	c	s	c	s	c	s
Ventilation absolute [ml.min^{-1}]	547	548	608	606	597	624	675	724	672	737	593	609	502	518	550	584	505	516
Relative ±s_m	1·00	1·00 ±0	1·00	0·99 ±0·04	1·00	1·04 ±0·01	1·00	1·07 ±0·02	1·00	1·09 ±0·04	1·00	1·02 ±0·04	1·00	1·03 ±0	1·00	1·06 ±0·01	1·00	1·02 ±0·01
n	5	5	5	5	7	7	7	7	6	6	6	6	7	7	6	6	5	5

	Caudal		CHEMODE-POSITION [mm from the reference point]														Rostral	
	−6		−4		−2		0		+2		+4		+6		+8		+10	
	c	s	c	s	c	s	c	s	c	s	c	s	c	s	c	s	c	s
Mean arterial Pressure absolute [KPa]	21·12	20·6	18·62	17·65	18·42	16·62	21·00	18·08	19·00	15·69	22·0	18·50	18·24	16·41	21·94	16·58	19·84	19·34
Relative ±s_m	1·00	0·98 ±0·02	1·00	0·95 ±0·02	1·00	0·90 ±0·03	1·00	0·86 ±0·03	1·00	0·83 ±0·09	1·00	0·84 ±0·06	1·00	0·90 ±0·04	1·00	0·86 ±0·03	1·00	0·98 ±0·01
n	5	5	5	5	7	7	7	7	6	6	6	6	5	5	6	6	5	5

Table 2. Left: Ventilation ratio and right: Mean arterial pressure ratio obtained in different chemode-position on the surface of the medulla oblongata on application of nicotine (10^{-4} g · ml^{-1} = 6.2 · 10^{-4} mMol · ml^{-1}). Mean-values obtained over 120 s are given. Duration of superfusion – 60 s. Results from 8 (left) and 9 (right) cats

Ventilation ratio

CHEMODE-POSITION [mm from the reference point]								
Caudal								Rostral
−6	−4	−2	0	+2	+4	+6	+8	+10
1·08	1·00	1·12	1·40	1·13	1·02	1·47	1·13	1·00
1·00	1·01	1·15	1·20	1·15	1·01	1·02	1·28	1·00
1·01	1·10	1·07	1·04	1·00	1·13	1·02	1·14	1·04
0·91	1·19	1·10	0·99	1·11	0·92	1·15	1·16	1·10
1·00	1·05	0·91	1·23	1·57	1·03	1·27	1·26	1·00
1·02	1·00	1·15	1·04	0·97	1·14	1·04	1·20	1·06
		1·10	1·06	1·07		1·12	1·14	0·98
		1·05	1·03	1·07		1·19		

Mean arterial pressure ratio

CHEMODE-POSITION [mm from the reference point]								
Caudal								Rostral
−6	−4	−2	0	+2	+4	+6	+8	+10
1·00	1·06	1·11	1·06	1·11	1·04	1·06	1·15	1·00
1·00	1·00	1·05	1·10	1·00	1·00	1·06	1·01	1·00
1·00	1·00	1·00	1·43	1·05	1·09	1·04	1·11	1·03
0·92	0·83	1·07	1·45	1·13	1·00	1·50	1·00	0·84
0·96	0·95	1·13	1·09	1·06	1·00	1·00	1·02	0·88
	0·79	1·15	1·00	1·00	1·00	1·13	1·12	
	0·90	1·04	1·02	1·14	1·00	1·00	0·88	
		1·00	0·92	1·00	1·16	1·12	0·85	
		0·93	0·79	0·96	0·77	1·00		

pressure, but not invariably so. Sometime, a small reduction in arterial pressure was observed. The increase in ventilation caused by nicotine has a very short latency and the ventilatory peak was reached in between 20 – 50 s after the beginning of the superfusion. The respiratory response usually had two components, i.e., the increase in the amplitude of the inspiration and in many cases, a deepening of the expiration. There was a highly significant difference between the ventilatory amplitude in the control period and after the superfusion of nicotine in the caudal part of the medulla (0 +2 mm) and at +8 mm rostral of the hypoglossal root. The individual ventilation ratios are shown in Table 2. The averages and the regional p-values have been plotted in Fig. 2b. They are highly significant in the rostral and caudal areas of the investigated region. In the average the arterial pressure increased (Fig. 2b). The p-values for the increase of arterial pressure almost touch the line of significance. That there is no clear significance is due to the fact that in a minority of experiments arterial pressure dropped. All the effects, however, are reproducible in the single animal.

Procaine. In 8 cats, the effects of procaine hydrochloride applied bilaterally in the intermediate zones were investigated with the ring method. Though in 5 among 8 cats a drop of arterial pressure was observed the effect of procaine in this location (2 · 10^{-2} g · ml^{-1} = 7.3 · 10^{-2} mMol · ml^{-1}) on the arterial pressure was not significant in these experiments. There was, however, a significant decrease in ventilation.

In a further series of experiments, the effect of nicotine on ventilation in the presence of procaine solution in the intermediate zones has been investigated. Under the influence of procaine solution (2 · 10^{-2} g · ml^{-1} = 7.3 · 10^{-2} mMol · ml^{-1}) on the intermediate area the magnitude of the ventilatory response to local application of nicotine (10^{-4} g · ml^{-1} = 6.2 · 10^{-4} mMol · ml^{-1}) to the rostral or the caudal area was inhibited to a considerable degree. The inhibition of the ventilatory response to nicotine on the rostral and the caudal areas is presented in Fig. 3.

Discussion

In order to localize the effects on respiration or circulation of physiological or pharmacological stimuli applied to the ventral surface of the medulla oblongata three different methods have been applied. Mitchell et al. (1963a, b) used pledgets of filter paper soaked with the solutions under investigation which they applied under the guidance of the eye. Among the substances investigated was acetylcholine. The rostral area of chemosensitivity was described as the area from which responses of the ventilation to buffers of varying pH were observed. This method has the advantage to be

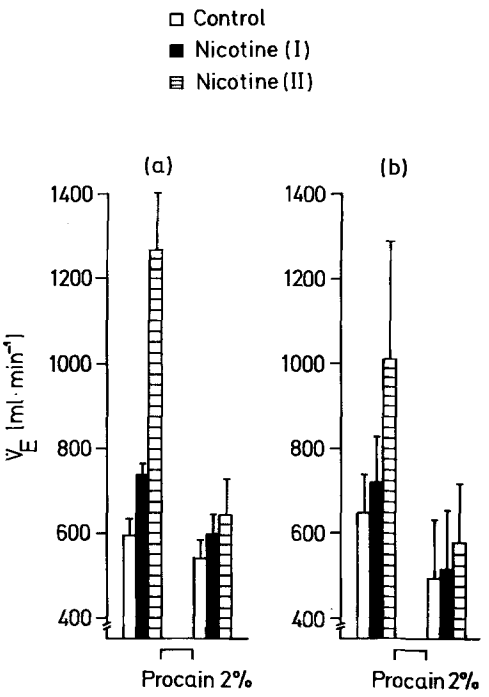

Fig. 3. Comparison of the ventilatory response to nicotine (10^{-4} g · ml^{-1} = 6.2 · 10^{-4} mMol/l^{-1}) applied to rostral area (M) and caudal area (L) bilaterally, before and after application of procaine (2 · 10^{-2} g · ml^{-1}) to the intermediate zones (S). Left (a): Area M. Right (b): Area L. Values shown are the mean values ± standard error of the mean. Before application of nicotine. Mean of first 15 breaths (nicotine I). Mean of 16th to 30th breaths (nicotine II). Duration of nicotine application – 60 s. Each column represents the mean of four experiments in four animals

simple. The localization, however, could not be very precise because it must be assumed that the substances will spread on the surface by diffusion. Schläfke et al. (1970) therefore, developed their method of counter-superfusion where the fluid investigated was brought to the surface in a continuous flow through a tube with a small diameter directed to the medullary surface perpendicularly in such a way that the opening approached the surface closely i.e. to a fraction of a mm. There was a wider tube around this inner tube through which a rinsing fluid (mock CSF) was superfused with a flow tenfold in comparison to the inner tube flow. The fluid from the inner tube was diluted and washed away by this means. The area exposed to the undiluted fluid, therefore, is approximately equal to the cross sectional area of the inner tube. In this investigation the diameter was 1.4 mm. The spatial resolution of this method was estimated to be better than 2 mm. It turned out in the experiments that marked differences in response were obtained if the "chemode" was applied in steps of two millimeters so confirming the estimation. Since in the medullary tissue the fluid would spread sideways while diffusing into the depth a smaller chemode probably would not have improved the spatial resolution and probably the limit of any topographical method from the surface is reached by this method. A shortcoming is that because of the small area superfused by the inner tube the responses may be smaller than with the following method.

Guertzenstein (1973) put perspex rings on the surface. These rings were then filled with the fluid to be investigated. The ring method was also adapted in this study for some investigations. The diameter of the rings (2 mm) was larger than that of the superfusion probe and also it turned out to be difficult to keep the rings tightly attached to the surface. It must be taken into account that some loss of fluid may take place spreading on the medullary surface. In this study the ring on it's side of attachment was greased with vaseline and to keep it in place and to avoid spreading in most cases it was surrounded with agar gel. As it seems this method for localization purposes is of limited value. It, however, may give higher responses than the superfusion probe and for this reason it may be preferable for pharmacological investigations.

It seemed to us that for purposes of localization the counter-superfusion method should be preferred and preference should be given to results obtained with this method.

The main result of this investigation is that the distribution of the actions of acetylcholine and nicotine on respiration is almost identical with the distribution on the medullary surface of the action of variations of pH (Schläfke et al., 1970). This suggests that these drugs act on the chemosensitive structures. The distribution also coincides with the distribution of ventilatory responses to electrical stimulation (Loeschcke et al., 1970). The concept emerges that in the caudal and rostral areas there are structures projecting to the respiratory centers which respond to electrical stimulation, to an acid shift of pH, and to cholinomimetic drugs by driving the respiratory centers. That these cells are cholinoceptive may mean that in their normal function they are driven by cholinergic synapses. It may be mentioned that parallel observations on the cellular level in surviving tissue slices of this region have been described by Fukuda and Loeschcke (1979) and also that it was shown in a second paper (Dev and Loeschcke, 1979) that chemosensitivity (response to inhaled CO_2) could be strongly diminished by local application of atropine to the chemosensitive areas. This directly demonstrates the involvement of a cholinergic mechanisms in the functional process of central chemosensitivity. Furthermore Schläfke and See (1978a) demonstrated the increase of discharge of cells in the superficial layer of the medulla reacting to pH changes if acetylcholine was applied to the surface.

The interpretation of the effects on circulation is more difficult. Clearly acetylcholine in the dose applied

(10^{-4} g · ml^{-1} = 5.5 · 10^{-4} mMol · ml^{-1}) causes a drop of arterial pressure and this is in agreement with the result of Feldberg's group (Guertzenstein, 1973; Feldberg and Guertzenstein, 1976; Feldberg, 1976) who investigated the action on arterial pressure of cholinomimetic drugs applied to the same region. In our study the distribution of the effect over the medullary surface is similar to that of the action on ventilation. Also the nicotine effect seems to be similarly distributed. Nicotine, however, in the majority of the tests raised the arterial pressure which means that it acted antagonistically to acetylcholine. The effects were of strongly varying amplitude and they did not reach the level of statistical significance. This is why this observation will not be further discussed here. However, a similar antagonism is between acetylcholine and electrical stimulation. Loeschcke et al. (1970) and Trouth et al. (1973) regularly observed increase of arterial pressure during local electrical stimulation of the caudal and rostral areas. Schläfke and See (1978a, b) underneath the intermediate area picked up potentials from units which did not react to pH changes and therefore did not belong to the respiratory chemosensitive system. These same neurons increased their discharge when a hypotensive drug was applied and decreased it in response to a hypertensive drug. The authors, therefore, believed that these neurons belonged to the cardiovascular control system. They clearly responded to locally applied acetylcholine with an increase of impulse frequency. Furthermore Bousquet et al. (1975) observed that the hypotensive action of clonidine given intravenously disappeared after coagulation of the intermediate area. These observations together suggest that acetylcholine drives a type of neurons which inhibit the vasomotor tone. This may either be an action on central medullary vasomotor drive or it could also be an action on descending neurons projecting to the sympathetic chain (Amendt et al., 1978). Also the descending pathway mediating the "defense reaction" (Hilton, 1975) which seems to have a relay in the same ventral location of the medulla oblongata may be involved. Why electrical stimulation has the opposite effect remains unclear, possibly another neuron inhibiting the neuron reacting to acetylcholine is stimulated or there are two independent systems one facilitating and the other inhibiting the vasomotor tone centrally or peripherally.

It was already discussed by Loeschcke and Koepchen (1958) that the neurons projecting to the respiratory system were not identical with those projecting to the vasomotor system. First of all a pH change on the medulla acts on the ventilation alone not on arterial pressure. An other argument was that some drugs acted synergistic (e.g. veratridine) and others antagonistic (e.g. cyanide) on ventilation and circulation. Acetylcholine belongs to the second group and nicotine (where it raises arterial pressure) to the first one.

Furthermore Loeschcke and Koepchen (1958) observed different time courses of the effects of procaine on ventilation and on arterial pressure indicating that procaine applied to the surface reaches the elements acting on ventilation first and those acting on vasomotor tone later. In the experiments of this paper the baroreceptor nerves were intact allowing feedback regulation while in the experiments of Loeschcke and Koepchen (1958) carotid sinus nerves and vagi were cut.

Feldberg and Guertzenstein (1976), Feldberg (1976) investigated the cardiovascular effects of carbachol, physostigmine and nicotine in cats. Carbachol acts similar to acetylcholine with the main difference that it is less hydrolysed by the esterases. The carbachol effect may be compared with the acetylcholine effect in this paper. The authors described two areas A and B on the ventral surface of the medulla. It is difficult to relate Feldbergs areas A and B to the rostral and caudal chemosensory areas described by Loeschcke et al. (1970). This is because in the drawing of Feldberg and Guertzenstein the distances from the foramen coecum (or the rostral end of the trapezoid body to the root of the hypoglossal nerve is 12 mm. In our experiments this distance in the average was 10 mm. From the drawing it may, however, be suspected that area A is approximately the same as the area intermediate between the rostral and the caudal chemosensitive areas and area B corresponds with the caudal area. In the experiments of Feldberg and coworkers from both areas a drop of arterial pressure was elicited by carbachol and this corresponds with the effects of acetylcholine described in this paper. Nicotine was found to act on area B only also causing a drop of arterial pressure. This is at variance with our results. In our experiments nicotine given in a relatively small dose raised the arterial pressure. As will be shown in a following paper (Dev and Loeschcke, 1979) this is a matter of dose since with higher concentrations also a drop of arterial pressure was observed insofar confirming Feldberg (1976). However, nicotine acted as well on the rostral chemosensitive area in small dosis increasing and in high dosis decreasing the arterial pressure. Furthermore Feldberg and Guertzenstein (1976) did not see effects on ventilation as we did. This is possibly a matter of anaesthesia because the central chemosensitivity may be easily abolished by anaesthetics and in order to obtain the respiratory effects it is necessary to keep the anaesthesia light.

Another topographical problem was approached by the experiment in which procaine was applied to the intermediate and nicotine to either the caudal or to the

rostral area. Procaine on this location blocked the nicotine effects on ventilation elicited from either chemosensitive area almost completely demonstrating that the signals originating from the rostral and caudal chemosensitive areas have to pass the intermediate area at least in their majority. This is a confirmation of earlier observations (Schläfke et al., 1969; Loeschcke, 1973) where the effect of electrical stimulation of the rostral or caudal areas was blocked by procaine on the intermediate area or focal cooling, a result which led to the convergence hypothesis stated above. The direct effect of procaine to the intermediate area was in the same direction (diminishing ventilation) as in the experiments of Schläfke et al. (1969) though less marked and similar to the observation of Cozine and Ngai (1967). This difference is partly due to the experimental condition inasmuch as in Schläfke et al.'s experiments the carotid sinus nerves and the vagi were severed while in the experiments of this study they were intact.

It may finally be tried to reconcile the observations on respiratory chemosensitivity with the concept of Hilton (1975) who described a descending pathway starting in the amygdala mediating the defense reaction. Hilton recently (1978) proposed that this system is active also under resting conditions and provides drive not only to cardiovascular sympathetic neurones but also to ventilation. At least as far as ventilation is concerned it must be objected that in a midcollicularly decerebrated animal resting ventilation remains unchanged and regulation of ventilation as it is usually tested is unimpaired. Possibly to a lesser extent this objection holds also for circulation. From this basic experiment (decerebration) it must be concluded that respiration at least in the resting state is not continuously driven to any appreciable extent by the descending fibers albeit this concept in the case of an emergency appears to be quite acceptable.

The central chemosensory drive since unaffected by decerebration must be assumed to function independently of an descending impulse traffic. However it seems to be quite possible that impulses from descending pathways converge in the region of the intermediate area with chemosensory impulses, together with these in emergency situations providing a tonic drive transmitted in a common pathway to the respiratory centers.

A candidate for such a convergence is the paragigantocellular nucleus with one part situated underneath the intermediate area, where Schläfke (1978) demonstrated the existence of neurons activated by hydrogen ions and also by afferent signals from the carotid sinus nerve or from the limbs, probably muscles. Other neurons in Schläfkes experiments (1978) which were located slightly deeper and did not react to hydrogen ions but to hypertensive or hypotensive drugs may well be part of the vasocular projection of the descending defense system. Their projection on the sympathetic system has already been shown by Schläfke (1978). However, the connection of the descending pathway with these two groups of neurons remains to be clarified experimentally.

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