The effects on respiration in the cat of the sudden excitation of cerebral vascular nociceptors by carbon dioxide

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Summary

1. Injection of CO₂-saturated saline in a distal direction into either a vertebral artery or an internal maxillary artery in pentobarbitone-anaesthetized cats produced abrupt changes in respiration. Vertebral-artery injections produced a transient inhibition of respiration, followed by a stimulation of it. Internal-maxillary-artery injections produced only the inhibition.

2. Injections during inspiration usually shortened that inspiration, reduced its volume and prolonged the following expiration. In the first 30% of an expiration they prolonged that expiration, but given in the next 50% they shortened it. In the last 20% of expiration internal-maxillary-artery injections again slightly prolonged the expiration.

3. Phenyl diguanide injected into either a vertebral or an internal maxillary artery also produced abrupt effects on respiration.

4. The effects of CO₂-saturated saline were abolished by intravenous acetazolamide, suggesting that nociceptors may be affected by a change in local pH.

5. The effects may arise from the excitation of vascular nociceptors, and our observations may suggest a way of studying in animals the receptors responsible for headache.

Key words: respiratory timing, vascular nociceptors, cerebral arteries, headache, carbon dioxide, phenyl diguanide, meningeal blood vessels.

Introduction

In a previous study [1] we gave injections of high Pco₂ saline into a vertebral artery in anaesthetized cats, intending to produce a sudden increase in the excitation of the medullary chemoreceptors, as had earlier been done with the peripheral chemoreceptors [2]. The first reflex effects of the injection were produced on the phase of respiration in which the injection was given. The reflex effect was an inhibition of respiration, followed by a stimulation of it. In this paper we examine the question of whether this initial inhibition is due to the early intense excitation of medullary chemoreceptors, or to the excitation of some other receptors by the high Pco₂ or low pH of the injections. Such receptors might be nociceptors in the walls of blood vessels, especially those of the dura, which are said to be pain-sensitive in man [3]. In the cat the dura of the posterior fossa is supplied by the vertebral arteries. The dura rostral to this is supplied by the middle meningeal arteries, which leave the internal maxillary arteries before the carotid plexus, through which blood passes to supply the rostral part of the brain, the internal carotid arteries being insignificant in the cat. Injections of CO₂-saturated saline into an internal maxillary artery in a distal (i.e. rostral) direction may thus reach nociceptors in the walls of the vessels which it supplies, but should not reach the area of the central chemoreceptors in the medulla. The aim of the present study was therefore to see whether injections of CO₂-saturated saline into an internal maxillary artery would elicit the same short-term reflex effects on respiration as would such injections into a vertebral artery.
Methods

Preparation of animals

We used 18 cats of either sex weighing 2-4 kg anaesthetized with pentobarbitone (Sagatal; May and Baker Ltd), 40 mg/kg intraperitoneally with further intravenous doses as required. A femoral vein was cannulated for injections. The trachea was cannulated in the neck and the animals were allowed to breathe air spontaneously. In four experiments in which the vagi were to be cut later, loops of suture were placed around them in the neck. In 14 cats an internal maxillary artery was cannulated and the cannula pushed distally (i.e. rostrally) as far as possible. Heparin was given intravenously (2500 units in 0.5 ml; Weddell Pharmaceuticals Ltd). In nine experiments, blood flow through the internal maxillary artery was preserved by using a loop, the proximal end of which was pushed about 1 cm retrogradely into the external carotid artery, and which was left open except during injections. The total volume of the loop was 0.15 ml, and a T-piece in it permitted injections and recording of blood pressure. In the other five experiments the internal maxillary artery was tied proximal to the point of cannulation, but a pulsatile pressure could still be recorded from the distal end of the artery, suggesting good anastomotic connection. Injections of 0.5-1.0 ml were given into the distal portion of the internal maxillary artery, or into the arterial loop with the proximal side of the loop clamped. The injections were given over 0.5 s and would travel as a bolus through the distribution of the artery. This has been confirmed for the meningeal branches by direct observation after craniotomy in other experiments.

In three cats with a loop placed in the left internal maxillary artery, the right vertebral artery was cannulated between the first and second cervical vertebrae with a cannula pointing distally. In a further four cats only a vertebral artery was cannulated. Body temperature was monitored with a rectal thermometer and maintained at 37 ± 0.5°C with a table heater.

Recording methods

Pressure was measured from the injection cannula (Consolidated Electronics Ltd or Watco Services Ltd) and recorded on one channel of a chart recorder (Devices Ltd or George Washington Ltd). This record gave the timing of injections. Airflow at the trachea was measured with a differential transducer (Ether Ltd or Furness Controls Ltd) as the pressure difference across a simple capillary resistance pneumotachometer head and was recorded on a second channel of the chart recorder. Tidal volume was derived from the pneumotachometer signal by integration (circuit of Deno et al. [4]) and was also recorded on the chart in some experiments. Gas was sampled from a needle inserted into the tracheal cannula and analysed for CO₂ and O₂ (Beckman LB2 and OM11 analysers or VG Micromass model 201 mass spectrometer). In most experiments tracheal fractional CO₂ concentration was also recorded on the chart.

Injections

The majority of injections were of 0.5 ml of modified Ringer solution of composition NaCl 154 mmol/l, KCl 5.6 mmol/l, CaCl₂ 2.2 mmol/l, NaHCO₃ 1.2 mmol/l. This was bubbled with 100% CO₂ and is referred to as 'CO₂ saline'.

In some tests phenyl diguanide (obtained from Dr D. Trenchard; available from Pfaltz and Bauer Inc., Flushing, NY, U.S.A.), 2 μg/ml-1 mg/ml in Ringer, was injected, and in some HCl (1-10 mmol/l) in Ringer (pH 2-3) was injected.

Acetazolamide (Sigma) was given as a single intravenous dose of 50-100 mg/kg body weight, dissolved in a few drops of NaOH (100 mmol/l) and diluted to 10 ml with Ringer solution.

Results

Effects of internal-maxillary-artery injections of CO₂ saline

Fourteen cats were studied. The reflex effects on respiration of CO₂ saline injections were qualitatively similar in all nine cats when an arterial loop was used and in the five cats in which the internal maxillary artery was ligated proximal to the point of cannulation. In the latter group the size of the reflex effects appeared to decrease with time, and the most stable preparations were those in which internal maxillary artery flow was preserved between injections. When this was done, up to 120 injections were made without apparent deterioration of the preparation. During these trials end-tidal CO₂ remained between 4.5 and 6.3% and mean arterial blood pressure remained above 90 mmHg, except in one experiment in which mean arterial pressure at the beginning was 75 mmHg; it was still 75 mmHg several hours later after 75 injections had been made. Some CO₂ saline injections into the internal maxillary artery produced a transient fall in mean arterial blood pressure of 5-15 mmHg which lasted for up to 20 s.
The effects on respiration described below were similar whether or not this fall occurred, and a similar fall was produced by injection of saline alone, which produced only slight effects on respiration.

Fig. 1 illustrates the pattern of reflex effects observed in all experiments. The effects on respiration were pronounced, but depended on the point during the respiratory cycle at which the injection was given. An injection given late in an

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**Fig. 1.** Effects of excitation of cerebral vascular nociceptors on respiration in a pentobarbitone anaesthetized cat. Pneumotachograph traces, expiratory flow upwards. Traces 1–7 show the effects on respiratory timing of injections of 1 ml of 100%-CO₂-bubbled saline given in a distal direction into an internal maxillary artery at the times shown by the arrows. Traces 8 and 9 show effects of injections of 1 ml of saline (S) alone, for comparison.
inspiration or very early in an expiration delayed the onset of the subsequent inspiration (traces 1 and 7), but injections given later in an expiration brought in inspiration early (traces 2 and 3). It was possible to establish a point in early or mid expiration at which this ‘switch’ in effect occurred and to predict whether an injection would lengthen or shorten expiration.

An injection early in an inspiration abruptly reduced airflow for about 500 ms and then airflow was resumed (Fig. 1, traces 4 and 5), but after a later injection (Fig. 1, trace 6) airflow was not resumed following the reduction and expiration started early, often being precipitated quite sharply. The volume of inspiration ($V_I$) was always reduced. Injections of saline alone, given as forcibly as possible, produced only small effects on respiration (Fig. 1, traces 8 and 9).

The shortest latency of an effect of a CO$_2$ saline injection was seen when the effect was a reduction in inspiratory airflow: thus in Fig. 1, trace 5, the latency is 200–250 ms. This latency depended on the volume of the injection given and, in experiments using an internal-maxillary-artery loop, it was increased by leaving the proximal side of the loop open during the injection.

Figs. 2(a) and 2(b) show the averaged data from an experiment where 100 tests were performed. The effects on inspiratory duration ($T_I$) or expiratory duration ($T_E$) are shown as fractions of the mean durations for the three breaths preceding each test. Despite variations in $T_E$ and $T_I$ over the several hours of such an experiment, presumably owing to changes in the depth of anaesthesia, the patterns seen for single tests in Fig. 1 can also be seen to hold for the averaged data of Fig. 2.

In four cats the effects of CO$_2$ saline injections into the internal maxillary artery were compared before and after bilateral vagotomy. No qualitative difference was found either in the effects of an injection made during expiration on $T_E$ or of the effects of an injection made during inspiration on $T_I$ despite increases in total respiratory cycle time, $T_{tot}$, of about 70% after vagotomy. Similar observations were made after the administration of supplementary doses of pentobarbitone, which also increased $T_{tot}$.

**Comparison with vertebral-artery injections**

In three cats a comparison was made of the effects of injections of CO$_2$ saline into an internal maxillary artery with those of injections of CO$_2$ saline into a vertebral artery. Some results from one experiment are shown in Fig. 3. Qualitatively the immediate effects of an injection given in expiration on $T_E$ (Fig. 3a) or the effects of an injection given during inspiration on $T_I$ (Fig. 3b) were similar for the two types of test. The increases or decreases in $T_E$ were greater for the internal-maxillary-artery injections than for the vertebral-artery injections, but we do not know that the intensities of the stimuli were similar.

![Fig. 2. Pooled data from 100 internal-maxillary-artery injections of CO$_2$ saline, showing the effects on the phase of respiration in which the injection was given and on the subsequent phase. (a) Effect of the timing of an injection given during inspiration or during the previous expiration on the duration of that test inspiration, $T_{I_{test}}$, as a fraction of the average control inspiratory duration, $T_{I_{control}}$. (b) Effect of the timing of an injection given during expiration or during the previous inspiration on the duration of that test expiration, $T_{E_{test}}$, as a fraction of the average control expiratory duration, $T_{E_{control}}$. The control durations of inspiration and expiration have been normalized for all tests and the relative durations of the phases are indicated on the abscissa. The timing of an injection in a phase was calculated as a fraction of the average duration of the phase for the three breaths which immediately preceded the test. Normalized expiratory duration is divided into 10 bins, normalized inspiratory duration into 5 bins. Each point is the mean ± SEM for all tests falling into that bin. Broken lines represent the fraction of control inspiration or expiration remaining, and thus the maximal possible shortening at any point if the phase were to be terminated immediately after injection was given.](image-url)
injection was no larger than the hyperpnoea produced in the steady state by adding 3% CO$_2$ to the air inspired by the cat. When 0.5 ml of a saline bubbled with various fractions of CO$_2$ was injected into the vertebral artery in early inspiration, it was found that only saline equilibrated with more than 15% CO$_2$ produced a transient inhibition of inspiratory flow followed by a hyperpnoea.

The latency of the responses to internal-maxillary-artery and to vertebral-artery injections differed in two of the three cats in which these latencies were compared. As noted above, a major determinant of the latency of the response to an internal-maxillary-artery injection was injection volume, and so in one of the two cats this volume was altered so that the latencies of the responses to the two types of test were similar. When the responses were then compared the transition point in expiration, i.e. the time for starting an injection at which an injection changed its effect from increasing to decreasing $T_E$, was 0.2 of control $T_E$ for the two types of test.

Effects of acetazolamide

In a previous study [5] it was reported that intravenous administration of acetazolamide greatly reduced the respiratory responses to vertebral-artery injections of CO$_2$ saline. Both the initial inhibition and the later stimulation of breathing were affected. In the present study, we found that in three cats acetazolamide (50–100 mg/kg body weight, intravenously) also abolished the responses to internal-maxillary-artery injections of CO$_2$ saline within 120 min. These responses were of course simply inhibitory.

**Effects of injecting phenyl diguanide or acidified saline**

In three cats injections of 1 ml of saline containing 10–100 µg of phenyl diguanide were made into the distal side of the internal maxillary artery loop. The drug produced immediate effects on respiration which were qualitatively similar to those of CO$_2$ saline, although smaller in size. They were followed by secondary effects, usually tachypnoea. The vagi were intact in these experiments. Tachyphylaxis prevented sufficient repetition of the tests for a thorough study to be made. Inhibitory effects on respiration were also produced by injecting 1 ml of saline acidified with HCl to pH 2, but saline acidified only to pH 4, approximately the pH of the CO$_2$ saline, had the same negligible effect as saline of pH 7.4.

In four cats injections of 0.5 ml of saline containing 1–500 µg of phenyl diguanide were made distally into a vertebral artery. Again effects on the respiratory phase could be shown,
especially a transient inhibition of inspiratory flow from an injection given early in inspiration, but often a short apnoea ensued. Tachyphylaxis to doses as high even as 500 μg occurred. Effects on respiration from vertebral-artery injections of phenyl diguanide have also been reported for the rabbit [6].

Discussion

Receptors involved in the responses

In this study we have been able to produce similar initial effects on respiration by injecting CO₂ saline in a distal direction into either an internal maxillary artery or a vertebral artery. This suggests that the reflex effects from vertebral-artery injections may not be due only to excitation of medullary chemoreceptors. The response to these vertebral-artery injections is composed of an initial inhibition of respiration, followed by a stimulation to produce a transient hyperpnoea [1]. Since an increase in the Pco₂ of the blood perfusing the medulla produces an increase in ventilation [7, 8, 9], it seems reasonable to attribute the secondary hyperpnoea to stimulation of the medullary chemoreceptors. The Pco₂ at these receptors must rise to much less than the Pco₂ of the CO₂ saline, as the hyperpnoea produced was no larger than that produced in the steady state by adding 3% CO₂ to the inspired air. Stimuli would not be expected to reach the chemoreceptors rapidly from the internal maxillary arteries, which supply mainly the cerebral hemispheres, and indeed CO₂ saline injected into an internal maxillary artery did not produce a hyperpnoea. It did, however, produce a transient inhibition of respiration. The initial inhibitory effect on respiration from either an internal-maxillary-artery or a vertebral-artery injection of CO₂ saline cannot therefore be attributed to excitation of medullary chemoreceptors. Since for vertebral-artery injections the inhibition precedes a hyperpnoea and is short-lived, it may arise from some receptors situated nearer than the chemoreceptors to the CO₂ saline in the blood vessels, and which are thus exposed for a short time to a very high Pco₂. The initial inhibition was only produced by saline equilibrated with more than 15% CO₂. Such a high Pco₂ is likely to be a nociceptive stimulus. Since the surface of the brain is said to be insensitive and the only sensation elicitable from the meninges in man is pain [3, 10], it is likely that the high Pco₂ of our injections did in fact stimulate nociceptors. These nociceptors may be in the walls of meningeal blood vessels and they may be the nerve endings of unmyelinated afferents, since phenyl diguanide is thought to stimulate unmyelinated afferents specifically [11, 12] and it produced similar immediate effects on respiration to those of CO₂ saline.

Our observation that acetazolamide abolished the response to CO₂ saline injected into either an internal maxillary artery or a vertebral artery suggests that the receptors involved possess carbonic anhydrase and that in our experiments their response to CO₂ depended on the rapid hydration of CO₂ by the enzyme, as for the medullary chemoreceptors [5].

Carbon dioxide must diffuse rapidly from the injection in the capillaries to the receptors, be hydrated and cause a decrease in local pH which affected the receptors. This explanation is supported by the observation that saline of a lower Pco₂, but acidified to about the pH of 100%-CO₂ saline, was ineffective in producing the initial inhibition. This suggests that the receptors lie beyond a barrier which CO₂ can cross rapidly but H⁺ ions cannot. Pain is said to arise from dural blood vessels [3, 10], so the barrier may be in the walls of these vessels, but at present we cannot be more precise.

If putative vascular nociceptors as well as medullary chemoreceptors may be stimulated by vertebral-artery injections of CO₂ saline this technique cannot easily be used to study the effects of rapid increases in medullary chemoreceptor activity, since initially these effects are masked by a transient inhibition of respiration from excitation of the nociceptors. One is forced instead to consider the effects on respiratory timing of sudden reductions in Pco₂ at the chemoreceptors (P. C. G. Nye, M. A. Hanson and R. W. Torrance, unpublished work).

The important finding which emerges from this paper is that it gives an easy method of repeatedly exciting the receptors which probably cause a headache and a simple method of recording a reflex response to them. Pain is said to arise from the dura rather than from the brain itself. The vertebral artery is pain-sensitive in man [3, 10] and also supplies the dura of the posterior fossa. The supratentorial dura in the cat is, however, supplied by the middle meningeal artery, and this leaves the internal maxillary artery before the carotid plexus. Pial and cerebral arteries are said not to be pain-sensitive in man [3, 10], and if this is true in the cat it suggests that part of our internal-maxillary-artery injections passed into the middle meningeal artery, presumably because the cannula was not small or flexible enough for it to impact beyond the origin of the artery. In any case there must have been good anastomotic
connection, because arterial pressure could always be recorded from the distal end of the internal maxillary artery. This method for producing a brief excitation of nociceptors might prove useful in the study of the pathogenesis of headache, and it is primarily for this reason that we report it.

The internal-maxillary-artery injections did nevertheless alter the pattern of breathing. When given early in inspiration they changed $T_i$ little, although they did reduce early inspiratory flow. This would be achieved simply by inhibition of transmission from the respiratory rhythm generator to inspiratory motor neurones. But injections given later in inspiration shortened $T_i$ and also the next $T_e$ was altered by all injections in inspiration. This shows that the afferent discharge produced must have affected the respiratory rhythm generator itself.

Finally, Mikulski & Trzebski [13] have recently reported that in cats superfusion of the medulla with acidic artificial cerebrospinal fluid produces abrupt termination of phrenic activity when the stimulus is applied during inspiration and a prolongation of $T_e$ when the stimulus is applied during expiration. These results are similar to those produced by our vertebral-artery injections of $CO_2$ saline. They have not, however, considered the possible role of nociceptors in the medullary meningeal vessels in their responses.

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