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Respiratory responses to chemical pulses in the cerebrospinal fluid of cats

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Summary

1. In cats anaesthetized with pentobarbitone, the fluid spaces in and around the brain stem were perfused from the third ventricle to the foramen magnum with artificial cerebrospinal fluid (c.s.f.) flowing usually at the rate of 5 ml/minute. Test solutions were substituted for the artificial c.s.f. without switching artifact for periods varying from 5 to 60 seconds. Observations were made on respiratory excursions, end-expiratory % CO_2 and arterial blood pressure.

2. Perfusion with sucrose solution equiosmolar with the c.s.f. produced no respiratory or cardiovascular response. Replacement of sodium with potassium (60 to 133 mM) resulted in a prompt but mild respiratory stimulation and a delayed fall in blood pressure associated with a slowing of the heart beat. Replacement of sodium with magnesium (40 to 131 mM) resulted in a late prolonged apneustic depression of breathing and in an early but slight reduction in blood pressure.

3. Procaine (1 to 50 mg/ml) elicited a respiratory response similar to that of excess magnesium; however, an initial rise in blood pressure to as high as 200 mmHg was evoked with procaine. Nicotine (0·05 to 0·5 mg/ml) produced an immediate brief bradypnea followed by a vigorous and slowly reversing hyperpnea accompanied most often by a fall in blood pressure. Tachyphylaxis was observed in the response to nicotine. Noradrenaline (0·001 and 0·1 mg/ml) did not produce any effect, and it did not alter the responses elicited by procaine and nicotine given by perfusion either simultaneous with or subsequent to the noradrenaline. Acetylcholine (0·5 mg/ml) produced weak transient respiratory stimulation and a small fluctuation in blood pressure which disappeared in repeated tests. Methacholine (1 mg/ml) caused a brief hyperpnea and a fall in blood pressure both of which were abolished after atropine (0·2 mg) was injected into the third ventricle. Pilocarpine (10 mg/ml) elicited no change in respiration or blood pressure. Respiratory and cardiovascular effects produced by strychnine (1 mg/ml) were attributable non-specifically to convulsive movements of the animal. Ethamivan (1 mg/ml) produced a single deep breath and a slowly reversing rise in blood pressure. Cyanide (0·5 mg/ml) barely stimulated the respiration but it produced a long lasting rise in blood pressure. Ethyl alcohol (0·1 ml/ml) elicited brisk though brief respiratory stimulation and a short lasting fall in blood pressure.

4. It was shown that the effects of procaine and nicotine were not qualitatively altered when the perfusion effluent was collected through a ventral craniotomy instead of the cisterna magna.

5. It is concluded that the brain surfaces are insensitive to the substances tested and that the observed effects resulted from movement of the agents into the brain parenchyma.

Introduction

The present work was undertaken to determine the effects of rapid alteration in the chemical environment of the brainstem by perfusing the cerebrospinal fluid (c.s.f.) space surrounding it with fluid of different composition at a rate of a few millilitres per minute. The experiments were carried out on anaesthetized cats. In previous studies of this kind, the rate of perfusion was of a much lower order, approximately 0·1 ml/minute. For a c.s.f. space of about 0·5 ml this meant that 5 min would be required for simple volume replacement and a considerably longer time for a complete turnover in chemical content because of the slow mixing of perfusate with the relatively large pool of the fluid into which it is delivered. With a flow rate of a few millilitres per minute, the times required for volume replacement and complete turnover in chemical content are much reduced, consequently peak concentration in the perfused spaces is attained more rapidly, and this makes for a better characterization of centrally induced responses. This method of rapid perfusion and altering the ionic composition of the perfusing fluid or adding drugs to it was used to examine the postulated respiratory chemosensitivity of the brainstem surface (Loeschcke & Koepchen, 1958a, b; Winterstein, 1961; Mitchell, Loeschcke, Massion & Severinghaus, 1963). In addition, the arterial blood pressure was recorded to find out whether the rapid changes produced in this way in the composition of the fluid surrounding the brainstem would result also in cardiovascular responses.

Methods

Experiments were performed on 14 cats anaesthetized with pentobarbitone sodium (40 mg/kg) injected intraperitoneally. The trachea was cannulated low in the neck, a catheter was placed in a femoral vein for systemic administration of drugs, and a catheter was placed in a femoral artery for recording the blood pressure. Respiratory excursions were transmitted to an inflated infant blood pressure bag held against the thorax and abdomen of the cat by means of a body sleeve made from an elastic stocking. Blood pressure and respiration were recorded through appropriate strain-gauge transducers connected to a Brush rectilinear polygraph. End-expiratory intratracheal concentration of CO_2 was recorded simultaneously through a Beckman infra-red analyzer. Body temperature was measured with a rectal thermometer and maintained at approximately 38° C with a heating pad.

In all experiments a cannula was placed stereotaxically in the third ventricle of the brain as described by McCarthy & Borison (1966). In twelve of the experiments, ventriculo-cisternal perfusion was carried out as shown in Figure 1. Artificial c.s.f. and the test drug solution were pumped at near equal flows through separate ports in the temperature regulated valve manifold. Switching between solutions was accomplished within a fraction of a second. A side port was available for direct injection into or sampling from the ventricular input stream. Deadspace from the valve switching point to the end of the ventricular cannula

was not more than 0·15 ml. The highest flow delivered was 16 ml/min, but perfusion was kept routinely, and if not otherwise stated, at 5 ml/min to minimize the risk of pressure build-up in the system. It was found that with the conventional method of making cisternal taps by needle puncture the point of greatest resistance to perfusion flow was at the cisternal exit. This bottleneck was relieved by the surgical procedure of exposing the foramen magnum, removing the atlanto-occipital membrane, and securing a large-bore flanged plastic cannula against the bony orifice with sutures placed in the overlying muscles (see Fig. 1). Two cats were turned to the supine position after installation of their ventricular cannulae. The oesophagus and trachea were then divided and reflected, the ventral aspect of the cranium was freed of overlying muscle and the basi-occipital bone was trephined between the tympanic bullae. Finally, the dura was incised to expose the medulla and to permit escape of the c.s.f. which was continuously removed by suction during perfusion.

Test solutions were prepared by dissolving drugs in artificial c.s.f. Concentrations refer to weight of the salt (when given) per unit volume of solution. The drugs used were acetylcholine iodide (Nutritional Biochemicals), ethamivan (U.S. Vitamin Corporation), methacholine chloride (Merck), nicotine hydrochloride (K & K Chemicals), noradrenaline bitartrate (Levarterenol, Winthrop), pentylenetetrazol (Knoll), pilocarpine nitrate (Nutritional Biochemicals), procaine hydrochloride (Merck), strychnine sulphate (Merck). Concentration of ethyl alcohol (U.S.P.) is expressed on a volume/volume basis. The composition of artificial c.s.f. was in millimoles (mM): NaCl, 130; $NaHCO_3$, 20; KCl, 3·5; $CaCl_2 \cdot 2H_2O$, 1·3; $MgCl_2 \cdot 6H_2O$, 1·14; NaH_2PO_4, 0·51; urea, 2·17; glucose, 3·4. In those experiments in which potassium or magnesium concentration was elevated, osmolality of the perfusate was maintained with an equivalent reduction in sodium concentration.

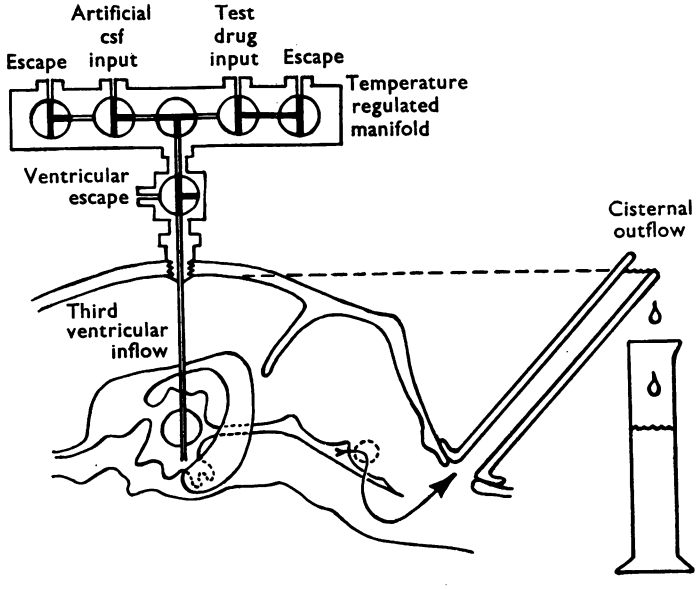

FIG. 1. Diagrammatic representation of the ventriculocisternal perfusion. Ventricular and cranial configurations obtained radiographically. The arrow passing through the foramen of Luschka follows the path of egress around the ventral surface of the lateral lobe of the cerebellum to the foramen magnum. Switching between artificial c.s.f. and test solution was performed by rotating the two input valves simultaneously.

Results

Ionic composition. Replacement of all ions with sucrose (300 mM) in the perfused artificial c.s.f. was done initially to examine the immediate importance for control of the respiration of the salt content of the fluid surrounding the brainstem. It is evident from Fig. 2 that elimination of the normal ionic medium from the brainstem surfaces did not produce any respiratory or cardiovascular changes in the short run of 60 s, even though such an interval represents a critical period of control in blood gas regulation. This experiment also demonstrates that the switching manoeuvres between perfusion with artificial c.s.f. and the interjected test solution did not produce functional artifacts.

Effects of excess potassium in the c.s.f. were not evident until its concentration was raised to 60 mM when mild stimulation of respiration and a slight fall in blood pressure resulted after approximately 15 s of perfusion. This is shown in the upper panels of Fig. 3 whereas the lower panels are from an experiment in which the concentration of potassium was increased so as to replace sodium entirely in the artificial c.s.f., that is to a total of 133 mM. Perfusion with such a solution produced after a latency of only a few seconds, a stronger, though still mild stimulation of the respiration and a large fall in arterial blood pressure associated with pronounced slowing of the heart. The figure illustrates also that a 5 s perfusion with such a solution was sufficient to produce these effects, and that the main recovery on subsequent perfusion with normal artificial c.s.f. required 1 to 2 min depending on the duration of perfusion with the potassium-enriched solution.

An increase in the concentration of magnesium in the artificial c.s.f., with a corresponding decrease in sodium ions, caused a depression of respiration and a fall in blood pressure, but not until the concentration was increased to between 40 and 80 mM. Even when all of the sodium ions in normal artificial c.s.f. were replaced with magnesium (total of 131 mM), the respiratory effect of the modified c.s.f. solution began after a latency of 15 to 30 s and was characterized by slowing of breathing and holding of respiration in inspiration. The fall in blood pressure

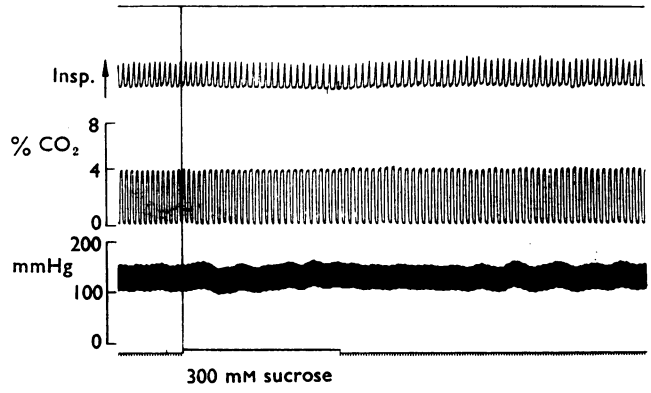

FIG. 2. Respiratory excursions (top record), intra-tracheal CO_2 concentration (middle record) and arterial blood pressure (bottom record) of a cat anaesthetized with pentobarbitone sodium during perfusion from third ventricle to cisterna magna with artificial c.s.f. at the rate of 5 ml/minute. The peak values of CO_2 correspond to alveolar CO_2. Time marker at the bottom in 1 s signals. During the one minute in which the time signals were omitted starting at the vertical line, perfusion was with 300 mM sucrose instead of with artificial c.s.f.

by as much as 50 mmHg, appeared earlier than the respiratory effect, but both effects lasted for up to 10 min after return to perfusion with normal artificial c.s.f. The respiratory effect produced by excess magnesium was similar to that obtained with procaine.

Procaine. Ventriculo-cisternal perfusion with procaine in concentrations ranging between 0·2 and 0·5 mg/ml was examined. Although, at the lowest concentration, procaine produced in one of two experiments a late and slight reduction in amplitude of breathing, 1 mg/ml was required to produce regularly a detectable slowing of the respiration, and 10 mg/ml resulted in apneustic breathing after a minute of perfusion as illustrated in the top panel of Figure 4. The respiratory effect was preceded by the onset of a continuing large rise in blood pressure. The maximal effect on respiration and blood pressure was reached after the return to perfusion with normal artificial c.s.f. The respiratory effect persisted for approximately 2 min and the blood pressure subsided slowly to the resting level within the next 3 to 5 minutes. The middle and bottom panels of Fig. 4 show that perfusion with 50 mg/ml for 15 s and for only 5 s produced a more intense and longer lasting apneustic response. The main effect on blood pressure was no longer a monophasic rise, but a delayed long lasting fall which with the perfusion lasting 15 s was preceded by a small rise. The rate of restoration to the control levels of these effects resulting from brief perfusion with 50 mg/ml procaine could be modified by altering the subsequent perfusion flow of normal artificial c.s.f. Thus, depressed respiration was re-instituted simply by slowing or stopping the post-drug

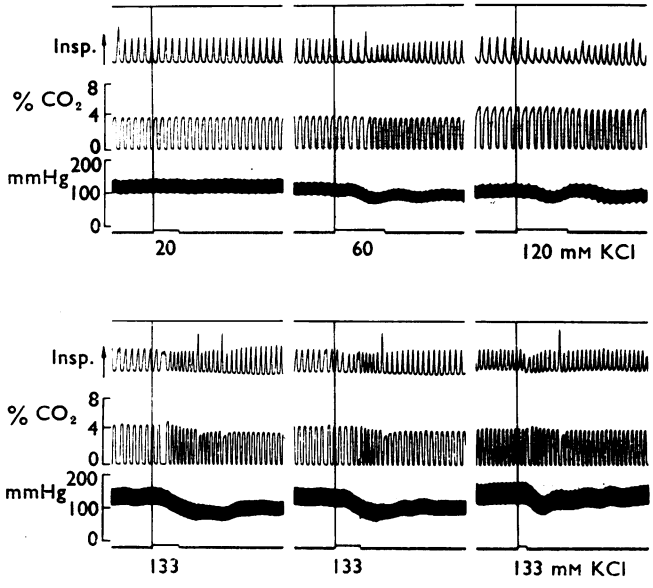

FIG. 3. The records in the upper and lower panels were obtained from different cats; those in the lower panels were from the same cat as in Fig. 2. Respiratory excursions (top records) intra-tracheal CO_2 concentration (middle records) and arterial blood pressure (bottom records) of cats anaesthetized with pentobarbitone sodium during perfusion from third ventricle to cisterna magna with artificial c.s.f. at the rate of 5 ml/minute. Time marker at the bottom in 1 s signals. During the periods of 5 to 30 s in which the time signals were omitted starting at the vertical lines, sodium in the artificial c.s.f. was replaced by various concentrations of KCl as indicated in mM KCl.

artificial c.s.f. perfusion, and the rate of recovery of respiration and blood pressure was restored on returning the perfusion flow to its former rate.

In two cats in which the perfusion path was changed so that the outflow was on the ventral side of the cranium, perfusion with procaine at 50 mg/ml for periods of 5 and 15 s produced the same responses as obtained by use of the cisternal outflow. In one of these experiments, an infusion of 50 mg/ml procaine solution was delivered for 30 s through a fine soft vinyl catheter inserted through the ventral craniotomy into the left medullo-cerebellar recess and it produced prompt but slight stimulation of the respiration followed by a rise in blood pressure, both of which persisted for 2 to 3 minutes.

These results confirm the apneustic pattern of breathing obtained by Rosenstein, McCarthy & Borison (1968) with ventricular and bilateral hindbrain subarachnoid injections of procaine in anaesthetized cats, and are in agreement with the pattern of slower and deeper breathing associated with a rise in blood pressure reported by

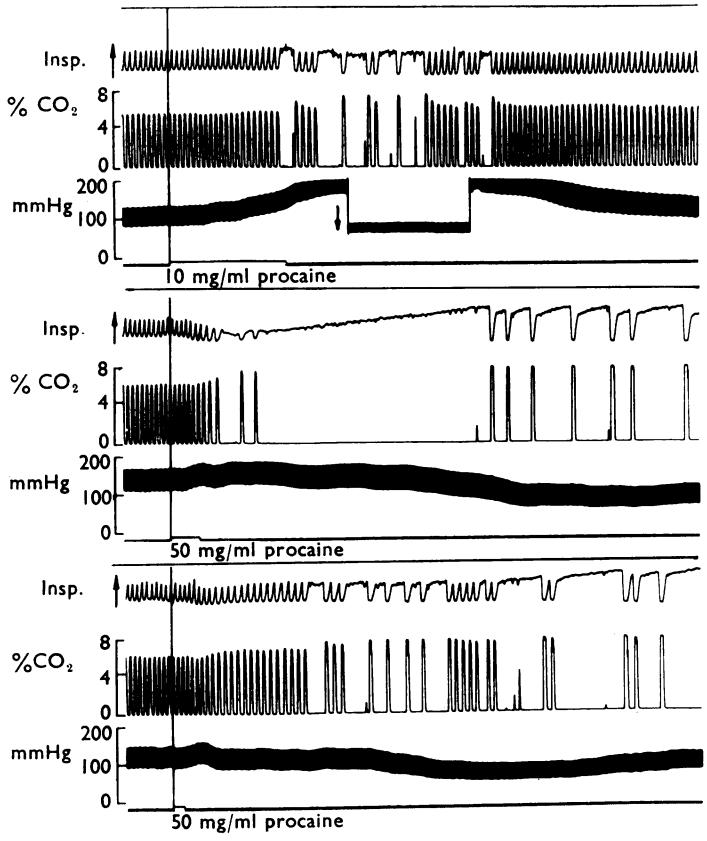

FIG. 4. Respiratory excursions (top records), intra-tracheal CO_2 concentration (middle records) and arterial blood pressure (bottom records) of a cat anaesthetized with pentobarbitone sodium during perfusion from third ventricle to cisterna magna with artificial c.s.f. at the rate of 3·8 ml/minute. The interval between top and middle panel 35 min; between middle and bottom panel 30 minutes. Time marker at the bottom of each panel in 1 s signals. During the periods in which the time signals were omitted starting at the vertical lines, perfusion was with 10 mg/ml procaine for 1 min (top panel), with 50 mg/ml for 15 s and 5 s (middle and bottom panels). At the arrow, the sensitivity of the amplifier for recording blood pressure was reduced to 0·4 of the calibrated scale for over 2 minutes.

Haranath & Venkatakrishna-Bhatt (1968) on ventriculo-cisternal perfusion with procaine in anaesthetized dogs.

Nicotine. No change in breathing or blood pressure was observed when nicotine was administered by ventriculo-cisternal perfusion in the concentration of 0·01 mg/ml for a period of 60 seconds. With 0·1 mg/ml perfused for 30 s there was a definite slowing followed by a weak stimulation of the respiration and an accompanying slight fall in the blood pressure. The results obtained with 0·5 mg/ml nicotine perfused for only 5 s in repeated tests are shown in Figure 5. There was prompt strong slowing followed by quickening of the respiration with a consequent fall in end-expiratory % CO_2 which lasted for 1 to 2 minutes. The blood pressure fell by as much as 50 mmHg, and remained low for the duration of the respiratory effect. Some variability of the blood pressure response was evident as a result of tachyphylaxis that developed when the tests were repeated at short intervals. The respiratory response to nicotine was also diminished by tachyphylaxis but it appeared to be more resistant than the vasodepressor effect. Recovery of full sensitivity to nicotine was obtained by allowing an interval of at least 15 min between tests. Twitching of the ears, as described by Armitage, Milton & Morrison (1966) and by Hall & Reit (1966), was regularly observed with all concentrations of nicotine effective for eliciting respiratory responses. Nicotine, 0·5 mg/ml, produced the same effects when perfused for 5 s through the ventral escape route as through the cisternal path. However, a pledget soaked in 0·5 mg/ml nicotine solution topically applied for 5 s to the exposed ventral surface of the brainstem, elicited no response.

Noradrenaline. Ventriculo-cisternal perfusion of noradrenaline in concentrations ranging between 0·001 and 0·1 mg/ml for periods up to 60 s did not affect respiration or blood pressure, and did not alter the responses elicited by procaine or nicotine given by perfusion either simultaneous with or subsequent to the noradrenaline.

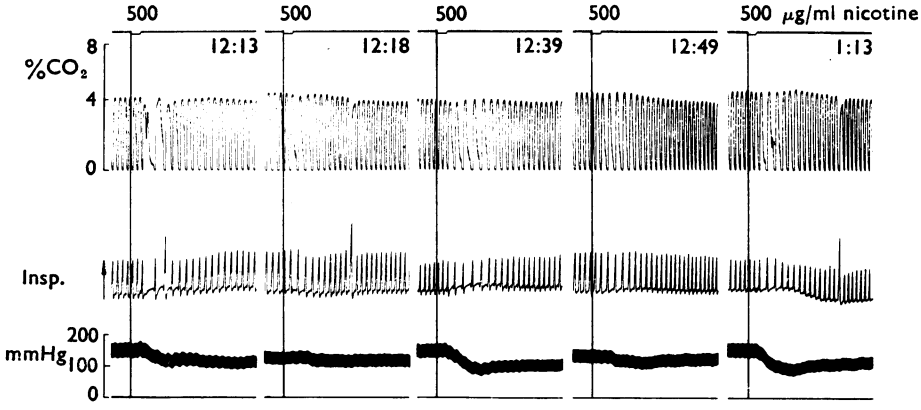

FIG. 5. Intra-tracheal CO_2 concentration (top records), respiratory excursion (middle records) and arterial blood pressure (bottom records) of a cat anaesthetized with pentobarbitone sodium during perfusion from third ventricle to cisterna magna with artificial c.s.f. at the rate of 5 ml/minute. Each panel shows the effects of a separate test in a repeated sequence with nicotine, 500 μg/ml, perfused for 5 s starting at the vertical line. Time marker at the top of the panels in 1 sec signals. Clock time for each test is given in the upper right hand corner of the panel.

Acetylcholine was remarkably ineffective by ventriculo-cisternal perfusion. Figure 6 shows the transient stimulating effect on respiration and the small fall in blood pressure produced on perfusion with 0·5 mg/ml which was about the minimal effective concentration. The figure also illustrates rapid development of acute tolerance since the effect of a second perfusion of acetylcholine 8 min later was hardly detectable.

Methacholine, when perfused in concentrations of 0·1 or 1·0 mg/ml for 15 or 30 s, caused a brief stimulation of respiration and a fall in blood pressure. These effects were eliminated after 0·2 mg atropine in 0·1 ml of solution was injected into the third ventricle.

Pilocarpine, the third cholinomimetic drug examined, did not produce any effect on respiration or blood pressure when perfused in concentrations up to 10 mg/ml.

Pentylenetetrazol, strychnine and ethamivan. Of these three excitants examined by ventriculo-cisternal perfusion, pentylenetetrazol was found to have no effect on respiration and blood pressure when perfused in a concentration of 1 mg/ml for 60 seconds. Strychnine, 1 mg/ml, produced convulsions with consequent disturbances in the mechanics of breathing, but apparently produced no definable respiratory effect *per se* ; the blood pressure changes which occurred were apparently also non-specific. Ethamivan, when perfused for 30 to 60 s at 1·0 and 5·0 mg/ml produced a moderate slowly reversing rise in blood pressure and, early in the perfusion, a single deep breath which did not significantly affect the end-expiratory CO_2 level.

Cyanide. Ventriculo-cisternal perfusion of sodium cyanide in concentrations of 0·1 and 0·5 mg/ml for 60 and 30 s, respectively, produced towards the end of the perfusion period a barely perceptible stimulation of the respiration and a coincident rise in blood pressure of approximately 40 mmHg which lasted for 2 to 3 minutes.

Ethyl alcohol. When perfused in a concentration of 0·1 ml/ml for 5 or 15 s duration from third ventricle to cisterna, the main respiratory effect of ethyl alcohol

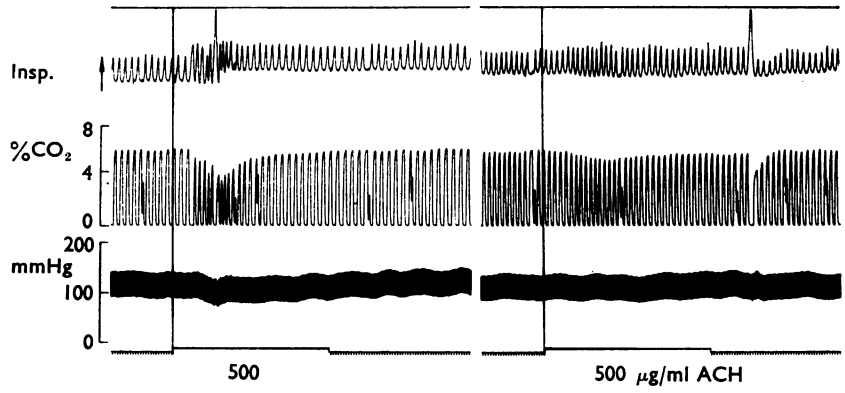

FIG. 6. Respiratory excursions (top record), intra-tracheal CO_2 concentration (middle record) and arterial blood pressure (bottom record) of a cat anaesthetized with pentobarbitone sodium during perfusion from third ventricle to cisterna magna with artificial c.s.f. at the rate of 3·8 ml/minute. Time marker at the bottom in 1 s signals ; during the periods in which the time signals were omitted starting at the vertical lines perfusion was with 500 μg/ml acetylcholine for 60 s in both instances. An interval of 8 min elapsed between the first and second perfusion.

was a short lasting brisk stimulation immediately following an initial momentary slowing of the respiration. This response is illustrated in Fig. 7 which also shows that the alcohol perfusion resulted in a short lasting fall in blood pressure. In the same animal, the response to repeated perfusions with alcohol was consistent, but the response varied from animal to animal in that the respiratory and cardiovascular effects did not on occasion last as long as the period of perfusion or even did not occur.

Discussion

The purpose of the present study was to characterize the central effects of a variety of substances placed transitorily in the fluid medium surrounding the lower brainstem. This was achieved by perfusing the substances at a rapid rate (usually 5 ml/min), from the cannulated third ventricle to cisterna magna. Since the dead space of the perfusion system was 0·15 ml and the c.s.f. space from the ventricular cannula to cisterna, according to McCarthy & Borison (1966), was between 0·25 and 0·5 ml, the turnover time with complete volume displacement would be between 3 and 6 seconds. At the perfusion rate of 5 ml/min, a 5 s perfusion period would thus allow a complete exchange of test solution for artificial c.s.f. and, conversely, a complete washout of the test solution upon switching back to artificial c.s.f.

Three mechanisms that could account singly or in combination for the central effects of substances introduced into the CSF are as follows: (1) direct contact

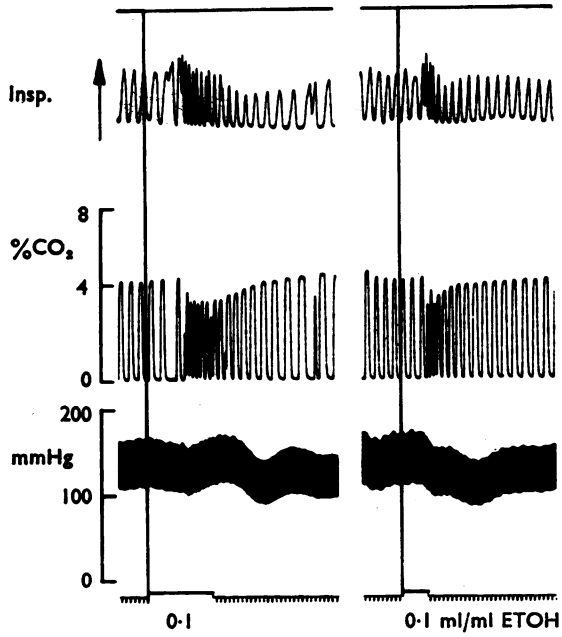

FIG. 7. Respiratory excursions (top record), intra-tracheal CO_2 concentration (middle record) and arterial blood pressure (bottom record) of a cat anaesthetized with pentobarbitone sodium during perfusion from third ventricle to cisterna magna with artificial c.s.f. at the rate of 5 ml/minute. Time marker at the bottom in 1 s signals. During the periods in which the time signals were omitted starting at the vertical lines perfusion was with 0·1 ml/ml ethyl alcohol for 15 and 5 seconds. Same cat as in Fig. 2.

with receptors that may lie on the brain surface or are located in the meninges and exposed blood vessels; (2) access through diffusion to neural structures situated immediately below the brain surface; (3) access through transport, whether by fluid movement in extracellular channels or by absorption into the local blood supply, to neural structures located within the brain parenchyma.

Rapid onset of effect indicates either that the perfusate brings the test substance into direct contact with its responding element or that the substance is promptly transported to the site of action. Diffusion to a subsurface receptor site could not by itself be effective because of the long time required (Pollay & Kaplan, 1970). Rapid termination of response means rapid elimination of the agent which further implies minimal tissue storage; it does not, however, obviate the role of an intermediate transport step. Ethyl alcohol was the only substance tested which showed both a rapid onset and a rapid termination of response.

Substances whose actions were rapid in onset but terminated slowly should presumably arrive quickly at their sites of action. On the other hand, slow decline of response indicates that deposition of the agents must have taken place in the test interval. Since with very short perfusion periods, insufficient time is available for surface penetration by diffusion, persistence of the response during washout is best explained by parenchymal storage associated with a fast transport process. The respiratory stimulant agents that produced effects with rapid onset and slow decline were nicotine (the most striking) and potassium in high concentrations. Acetylcholine acted promptly but its effect was short-lasting probably owing to inactivation by brain tissue acetylcholinesterase.

The actions of substances like procaine, and magnesium in high concentrations, were both slow to start and slow to terminate. This combination of characteristics could be explained by a mechanism requiring limited diffusion through the surface of the brain. On the other hand, a rapid transport to a deep repository but with slower penetration to the final receptive target would provide a more satisfactory explanation. It is interesting that the only substances (of those tested) whose effects developed slowly and terminated slowly were the depressant agents, which indicates that they act at different sites from the stimulatory agents. Nevertheless, the character of the depressant responses as well as the dependence of the decline in response upon rate of washout suggest that deep sites and tissue storage are involved in the actions, thereby favouring the participation of a transport process.

The mechanisms by which substances are normally eliminated from the c.s.f. are still only poorly understood (Davson, 1967). The striking vascularity of the pial surface and the known absorption of adrenaline (Feldberg, 1963) and procaine (Cohen, 1968) from the subarachnoid space support the contention that the blood supply to the brainstem plays an important part in the transport of substances from c.s.f. into the brain parenchyma for production of physiological responses. Indeed, it is becoming increasingly evident that the brain surface vasculature performs an exchange function which may well participate in the regulation of c.s.f. composition (Rall, 1968; Van Harreveld & Ahmed, 1968; Pollay & Kaplan, 1970). The crucial hypothetical question concerning the present experiments is how far into the brain parenchyma the capillary circulation can carry substances that are picked up from the c.s.f. before these pass into the venous outflow. This would necessarily be decided for different sites by the number of interconnexions between capillary plexuses in the various parts of the central nervous system (Crosby,

Humphrey & Lauer, 1962). The area postrema and its homologous vascular ependymal proliferations constitute a special system with a unique relationship to the blood-brain barrier (Davson, 1967). While the area postrema is known to perform a chemoceptive role in vomiting (Borison, 1959), it has not yet been established how emetic substances penetrate into this structure nor has its discrete chemoreceptor element been identified morphologically.

There are two important findings reported by Katzman, Graziani & Ginsburg (1968) that support the interpretation given to the present work. First, the clearance of potassium from the subarachnoid space occurs more rapidly than from the ventricular space and at a rate several times that of c.s.f. formation. Second, the flux of potassium between c.s.f. and brain is considerably larger than that of magnesium. In addition, the distribution of radioactive potassium which they determined within the medulla following ventriculo-cisternal perfusion favours deeper penetration from the subarachnoid surface than from the ventricular surface.

In conclusion, it is evident that no single mode of action, as on a universal pial receptive site for example, can account for the various effects produced by the substances tested. The results of the present experiments indicate that the regular ependymal and pial surfaces of the brainstem are insensitive to the given changes in c.s.f. composition and that the observed effects on respiration and blood pressure resulted from movement of the test substances into the brain parenchyma. It is suggested that a transport mechanism, possibly effected through the blood supply, participated in the delivery of most if not all of the agents to their respective sites of action in the brain.

Dr. Haranath was a Visiting Scientist of the World Health Organization at Dartmouth Medical School in the Fall and Winter of 1968 to 1969. His present address is the Department of Pharmacology, Kurnool Medical College, Kurnool, A.P. India. This investigation was supported by U.S. Public Health Service Grant NS 04456. A preliminary report was presented to the American Physiological Society at Davis, California in August, 1969.

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