J. Physiol. (1972), **224**, *pp.* 83–103
With 7 text-figures
Printed in Great Britain

A VASODEPRESSOR EFFECT OF PENTOBARBITONE SODIUM

BY W. FELDBERG AND P. G. GUERTZENSTEIN*
From the National Institute for Medical Research, Mill Hill, London NW7 1AA

(*Received 22 November 1971*)

SUMMARY

1. In anaesthetized cats under artificial ventilation, a few milligrams of pentobarbitone sodium injected into the cerebral ventricles produced a pronounced fall in arterial blood pressure, which was central in origin and resulted from inhibition of vasomotor tone.

2. Pentobarbitone sodium was more effective in lowering blood pressure when injected into the cerebral ventricles than when injected into the cisterna magna, yet the pentobarbitone sodium did not act on structures in the ventricular wall, but acted on structures reached from the subarachnoid space.

3. To produce its vasodepressor effect, the pentobarbitone sodium had to pass through the foramina of Luschka into the subarachmoid space beneath the medulla oblongata and to penetrate its ventral surface in a region caudal to the trapezoid bodies and lateral to the pyramids. This was the outcome of experiments in which the pentobarbitone sodium was injected into or perfused through the cerebral ventricles with or without an outflow cannula inserted into the aqueduct or into the fourth ventricle, and of experiments in which pentobarbitone sodium solutions were applied by means of Perspex rings to this region of the exposed ventral surface of the medulla. Whereas the application of pentobarbitone sodium to this region on one side had a weak vasodepressor effect only, its application on both sides produced a pronounced fall in arterial blood pressure.

4. The region where pentobarbitone acted on topical application covers the region where nerve cells are found in the marginal glia immediately under the pia mater. The possibility is discussed that these cells are the morphological substrate on which the pentobarbitone acts, that arterial blood pressure is maintained by their activity which is suppressed by the pentobarbitone sodium.

* Supported by Fundação Amparo A Pesquisa Do Estado De Sâo Paulo.

INTRODUCTION

An injection of a few milligrams of pentobarbitone sodium into the cerebral ventricles of anaesthetized cats under artificial ventilation regularly produced a fall in arterial blood pressure. The effect was a central one, because it did not occur when the injection was made intravenously. In order to find out which part of the liquor space had to be reached by the pentobarbitone sodium to produce the effect, different methods of introducing it into the liquor space were used: it was injected into the cerebral ventricles with or without cannulating the aqueduct or the fourth ventricle, it was injected into the cisterna magna, and it was perfused from a lateral ventricle either to the opened cisterna or to the cannulated aqueduct, or to the cannulated fourth ventricle. The results obtained with these methods all pointed to the subarachnoid space beneath the medulla oblongata as being the site which had to be reached by the pentobarbitone sodium. Consequently, its action had to be on structures close to the ventral surface of the medulla oblongata. This was proved by experiments in which the pentobarbitone sodium was perfused from the pontine cisterna to the opened cisterna magna, or applied by microinfusion into the subarachnoid space beneath the medulla or into the medulla itself close to its ventral surface. Finally, the exact site was verified by topical application of pentobarbitone sodium on to the exposed ventral surface of the medulla.

METHODS

The experiments were carried out in anaesthetized cats under artificial ventilation. In most experiments, anaesthesia was with pentobarbitone sodium given by I.P. injection (30 mg/kg), supplemented whenever required later in the experiment by an I.V. injection of 12 or 18 mg pentobarbitone sodium. In a few experiments, anaesthesia was with I.V. chloralose (50–60 mg/kg) after inducing anaesthesia with ethyl chloride and ether to allow cannulation of the left femoral vein. To record arterial blood pressure, a cannula was inserted into a femoral artery and for artificial ventilation the trachea was cannulated. The head of the cat was fixed to the ear bars and mouthpiece of a Dell–Moruzzi stereotaxic instrument with the cat lying on its belly except when the pentobarbitone sodium was applied to the ventral surface of the medulla oblongata. In these experiments the cat was put on its back.

The methods used for injection into a lateral cerebral ventricle through an implanted Collison cannula, or for perfusion from the cannulated lateral ventricle to aqueduct, fourth ventricle or cisterna magna, were the same as originally described by Feldberg & Sherwood (1953) and Bhattacharya & Feldberg (1958).

For injections into the cisterna magna, the skin over the neck and the muscles covering the atlanto-occipital membrane were divided in the mid line, and a tube (21 gauge) with an indwelling stilette was inserted into the cisterna and kept firmly in position. For the injections the stilette was replaced by a tube (27 gauge) connected by polyethylene tubing to a 1 ml. syringe filled with the pentobarbitone solution. The volume of injection was 0·2–0·4 ml.

For the perfusion from the pontine cisterna to the opened cisterna magna, a tube (20 gauge) with an indwelling stilette was used. It was inserted by means of a micromanipulator from a point on the dorsal surface of the right cerebrum through the cortex and the mid-brain, in such a way that its tip rested in the pontine cisterna in the mid line, about 5 mm caudal to the interpeduncular fossa. The insertion itself produced either no change, or a slight transient rise in blood pressure. The point of insertion was 1 cm lateral from the mid line at the inter-aural zero line: the tube was inclined backwards at an angle of 12°. For the perfusion the stilette was replaced by a tube inserted through the entire length of the cannula and connected by polyethylene tubing to a 20 ml. syringe driven by a Palmer slow infusion pump at a rate of 0·1 or 0·2 ml./min.

For the microinfusions into the subarachnoid space beneath the medulla or into the medulla itself, two 21-gauge tubes served as guide tubes. They were mounted on to a holder, parallel to and 6 mm apart from each other, and then inserted together by a micromanipulator of the stereotaxic instrument through cerebellum and brain stem, so that their tips rested either in the subarachnoid space or in the ventral part of the medulla. The insertion produced a rise in blood pressure which lasted for a few minutes only. For the infusion, each of the guide tubes carried an inner tube (27 gauge). The inner tubes were filled with the solution to be injected and connected by polyethylene tubing to a 1 ml. syringe before they were inserted through the entire length of the guide tubes. The 1 ml. syringe was driven by a Palmer slow infusion pump delivering through both inner tubes a total of 2·5 or 5 μl./min.

For the topical application of the pentobarbitone sodium solution on to the ventral surface of the medulla the basal plate of the skull and the dura covering the medulla were removed and two Perspex rings, 3·0 mm in diameter and 7 mm high, fixed to a holder were then lowered on to the medulla so that their lower siliconed rims rested under light pressure on the surface of the medulla. When the rings were filled with the solutions to be tested, care was taken that no air bubbles remained between brain surface and fluid.

To find the exact sites reached by the pentobarbitone sodium solution when infused either into the subarachnoid space beneath the medulla or into the medulla itself, a 0·2% bromophenol blue solution was infused subsequently through the cannulae at the same rate as the pentobarbitone sodium solution for the same, or for a shorter time. Ten minutes later, the cat was killed, the head perfused from the aorta with formalin saline solution and the brain removed. The stained areas at the surface of the medulla or, when it was cut, inside in the plane of the needle tracts, indicated the regions previously reached by the pentobarbitone sodium. In the experiments with topical application, the Perspex rings were filled at the end of the experiment with the same volume of 0·8% bromophenol blue solution as previously used for the pentobarbitone sodium solution, the bromophenol blue solution was removed after 5–10 min and the rings were washed out several times with artificial c.s.f. Afterwards the cat was killed. The stained areas on the ventral surface of the medulla indicated the sites reached previously by the pentobarbitone sodium solution similarly applied.

The arterial blood pressure was recorded by means of a transducer connected through a Cambridge preamplifier (Type 72342) to a Smith's Servoscribe potentiometric recorder. The artificial c.s.f. used was that described by Merlis (1940). The pentobarbitone sodium used for studying its vasodepressor effect when acting from the liquor spaces was Nembutal sodium powder (Abbott Laboratories Ltd). It was freshly dissolved in artificial c.s.f. before use. Solutions containing 200, 100 or 50 mg/ml. made in this way had a pH of 9·9, 9·8 and 9·5 respectively. To test the effects of control solutions with the same alkalinity, buffer solutions were made with 0·2 N-KH_2PO_4 and 0·2 N-NaOH according to Britton & Welford (Britton, 1955).

RESULTS

Pentobarbitone sodium affected both blood pressure and respiration when acting from the liquor space. For instance, when injected into the cerebral ventricles in doses which lowered blood pressure, respiration became depressed and respiratory standstill could occur which required artificial ventilation. In all experiments therefore artificial ventilation was given from the beginning so as to be able to study the blood pressure effects without interference from the effects produced on respiration.

Intraventricular and intracisternal injections

In cats anaesthetized with intraperitoneal pentobarbitone sodium, injections into the lateral ventricle of 4–12 mg pentobarbitone sodium, in a volume of 0·2–0·6 ml., produced a fall in arterial blood pressure which amounted usually to between 50 and 80 mm Hg; sometimes it was not greater than 20 or 30 mm; in one experiment it was as great as 120 mm. In the few experiments under chloralose anaesthesia the blood pressure fell between 50 and 70 mm Hg after the intraventricular injections of pentobarbitone sodium. The depressor effect usually began within a minute of the injection and reached its maximum in a few minutes; the blood pressure then returned to its pre-injection level within half an hour. The depressor effect was not due to absorption of pentobarbitone sodium into the blood stream because the blood pressure did not fall when 4–12 mg of pentobarbitone sodium were injected I.V.

The pentobarbitone sodium injections usually produced some bradycardia, but this was not the cause of the fall which occurred when there was no slowing of the heart or when the slowing was prevented by a preceding I.V. injection of 2 mg atropine sulphate. In some experiments the fall was preceded by a short-lasting rise in pressure varying between 10 and 30 mm Hg.

The same doses of pentobarbitone sodium which lowered blood pressure on intraventricular injection either had no effect when injected into the cisterna magna, or produced a much smaller fall, or a fall preceded by a rise, or solely a rise. A pressor response was more frequently obtained on cisternal than on intraventricular injection, and occurred also in those cats in which intraventricular pentobarbitone sodium produced solely a fall in blood pressure.

Fig. 1 illustrates an experiment in which 6 mg pentobarbitone sodium injected intraventricularly had a pure depressor effect, lowering the blood pressure by about 60 mm (top record), whereas injected into the cisterna magna, 40 min later, it first produced a rise of about 20 mm followed by a fall to about 20 mm below the pre-injection level (middle record). A

renewed intraventricular injection of 6 mg, after another 30 min, again produced a fall, this time of about 70 mm Hg (bottom record).

Fig. 2 was obtained from another cat. Again the pentobarbitone sodium (4 mg) injected intracisternally scarcely affected the blood pressure (top record) and injected intraventricularly produced purely a fall (upper

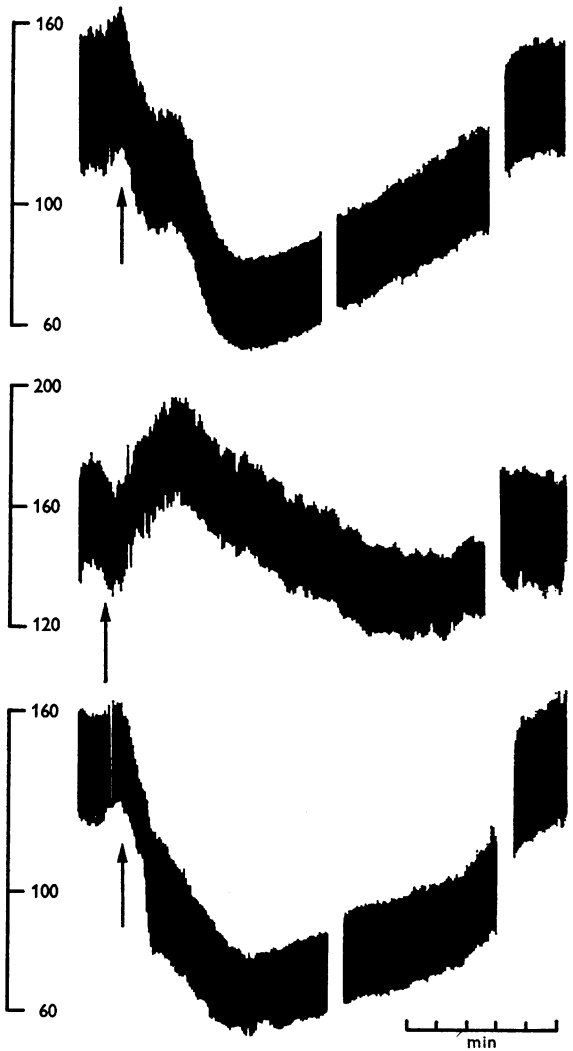

Fig. 1. Arterial blood pressure of a 3·7 kg cat anaesthetized with I.P. pentobarbitone sodium under artificial ventilation. At the arrows, injection of pentobarbitone sodium (6 mg in 0·3 ml.) into the cannulated left lateral ventricle (top and bottom records) or into the cisterna magna (middle record). Each break in the records represents an interval of 5 min. Blood pressure in mm Hg.

middle record). The depressor effect, however, no longer occurred on intraventricular injection when the cisterna magna was opened and an outflow cannula was passed along the floor of the fourth ventricle into the middle of the aqueduct (lower middle record). The site where pentobarbitone sodium acted when producing its depressor effect was therefore not reached from the lateral or third ventricle or from the rostral part of the aqueduct.

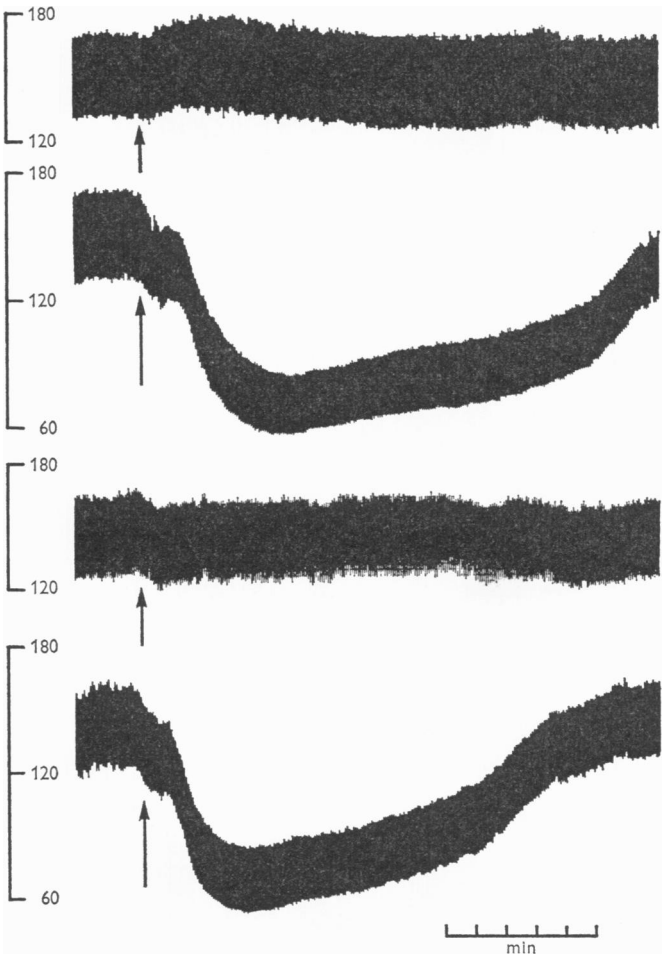

Fig. 2. Arterial blood pressure of a 3·4 kg cat anaesthetized with I.P. pentobarbitone sodium under artificial ventilation. At the arrows, injection of pentobarbitone sodium (4 mg in 0·2 ml.) into the cisterna magna (top record) or into the cannulated left lateral ventricle with cisterna magna opened (upper middle record) with the tip of an outflow cannula in the middle of the aqueduct (lower middle record), and with the tip of the outflow cannula withdrawn and lying at the caudal end of the fourth ventricle (bottom record). Blood pressure in mm Hg.

The depressor effect reappeared on intraventricular injection (bottom record) when the aqueductal cannula was withdrawn so that its tip was lying at the caudal end of the fourth ventricle. In other similar experiments it was found that once an outflow cannula had been inserted into the middle of the aqueduct and the depressor effect had disappeared, it did not return, or it became greatly attenuated when the cannula was withdrawn to the caudal end of the fourth ventricle or was removed.

When following up these observations obtained with intraventricular injections it was found (*a*) that just opening the cisterna magna did not reduce the depressor effect, (*b*) that the presence of an outflow cannula with its tip in the middle of the aqueduct regularly abolished it, but (*c*) that irregular results were obtained, even in the same cat, on repeated injections, with the tip of an outflow cannula lying in the middle or at the caudal end of the floor of the fourth ventricle. In some experiments it was sufficient to make a kind of artificial foramen of Magendie, by introducing a cannula into the fourth ventricle below the vermis and then withdrawing it after a few minutes, for the depressor effect to disappear.

These findings made it seem unlikely that the pentobarbitone exerted its depressor effect by an action on structures lining the fourth ventricle. They suggested that the pentobarbitone left the ventricular system through the lateral recesses and acted either on structures lining these recesses or, after having entered the subarachnoid space, on structures close to the lateral or ventral surface of the brain stem. An action on structures lining the lateral recesses was unlikely because injections of pentobarbitone sodium into the cisterna magna sometimes produced a depressor effect, although the pentobarbitone sodium injected in this way would not have entered the lateral recesses. An action on structures close to the dorsal surface of the brain stem was excluded by the fact that pentobarbitone sodium was less effective in lowering blood pressure on intracisternal than on intraventricular injection. This site was further excluded by the finding that after opening the cisterna magna, the depressor effect of the intraventricular injection was regularly obtained, although under this condition c.s.f. often did not reach the exposed dorsal surface of the brain stem. These conclusions are supported by the results obtained in experiments in which the pentobarbitone was perfused through different parts of the liquor space.

Perfusion of the cerebral ventricles and the subarachnoid space

A strong depressor effect was regularly obtained when solutions containing 20–50 mg/ml. pentobarbitone sodium were perfused from lateral ventricle to opened cisterna magna for a sufficiently long time, depending on the rate of perfusion. No depressor effect was obtained when the outflow

was no longer from the opened cisterna, but from the cannulated aqueduct, and again, no fall, or only a small one, occurred with the outflow from the cannulated fourth ventricle.

Fig. 3 illustrates typical effects obtained in two experiments. In both, pentobarbitone sodium was perfused in a concentration of 20 mg/ml. but the rate of perfusion was 0·1 ml./min in the one (records 1 and 2) and 0·2 ml./min in the other (records 3 and 4). On perfusion from lateral ventricle to cisterna magna the arterial blood pressure fell in both experiments but with the slower rate, the onset of the depressor effect was delayed. The latency was about 10 min when the rate was 0·1 ml./min (record 1) as compared to about 3 min when it was 0·2 ml./min (record 3). The pentobarbitone sodium perfusion no longer produced its depressor effect after cannulating the aqueduct (record 2) or the fourth ventricle (record 4). The small initial fall obtained on perfusion to the aqueduct occurred before the onset of the pentobarbitone sodium perfusion during perfusion with artificial c.s.f. It was probably due to a mechanical effect since it was associated with short stoppage of the outflow, which began again as soon as the position of the aqueductal cannula was slightly changed.

To produce a depressor effect with pentobarbitone sodium it did not have to pass through any part of the ventricular system. A depressor effect was regularly obtained when it was perfused from the pontine cisterna to the opened cisterna magna. With this method of perfusion the depressor effect was obtained with smaller amounts of pentobarbitone sodium and occurred earlier in the perfusion, than when perfusion was from the lateral ventricle to the opened cisterna. This is illustrated in Fig. 4. In this experiment a 5 min perfusion of a solution of pentobarbitone sodium (50 mg/ml.) at a rate of 0·1 ml./min had scarcely any effect when the perfusion was from the lateral ventricle (record 1) but blood pressure began to fall steeply after about 1·5 min when the perfusion was from the pontine cisterna (record 2). To obtain, in this cat, a fall in blood pressure on perfusion from the lateral ventricle, the perfusion with pentobarbitone sodium had to be continued beyond 5 min (record 3). This experiment also illustrates that absorption of pentobarbitone into the blood stream plays no part in the depressor effect since, infused at the same rate intravenously, pentobarbitone did not lower the blood pressure (record 4). The depressor effect is also not due to the strong alkalinity of the pentobarbitone sodium solution used for perfusion (pH about 9·5) since a buffer solution of the same pH did not lower the arterial blood pressure when perfused from the pontine cisterna to the opened cisterna magna.

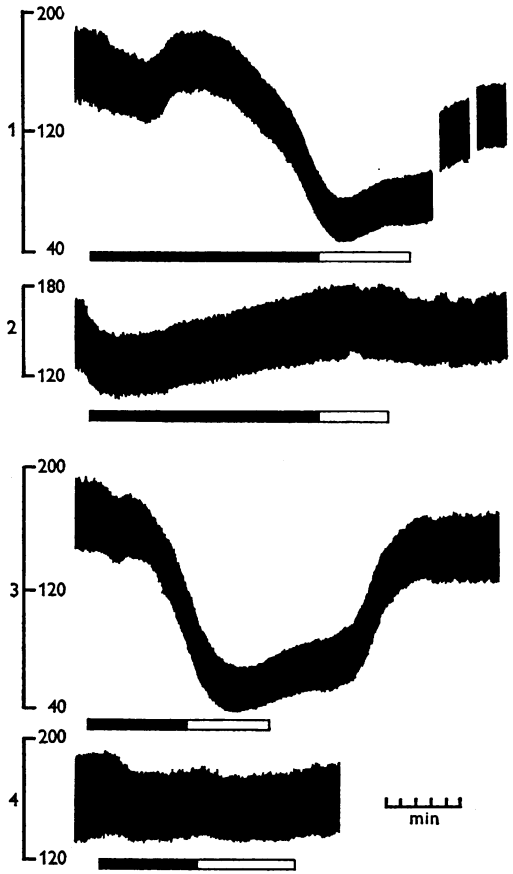

Fig. 3. Arterial blood pressure of two cats anaesthetized with I.P. pentobarbitone sodium under artificial ventilation. Records 1 and 2 from a cat weighing 3·4 kg, records 3 and 4 from a cat weighing 3·3 kg. The black bar under each record indicates the period of perfusion with artificial c.s.f. containing pentobarbitone sodium (20 mg/ml.) and the open bar the subsequent period of perfusion with artificial c.s.f. alone. Rate of perfusion was 0·1 ml./min in records 1 and 2, and 0·2 ml./min in records 3 and 4. Perfusion was from lateral ventricle to open cisterna (records 1 and 3), to cannulated aqueduct (record 2) and to cannulated fourth ventricle (record 4). When perfusion was to aqueduct or fourth ventricle, the pentobarbitone sodium perfusion was preceded (not indicated in the records) by a few minutes perfusion with artificial c.s.f. Each break in the top record represents an interval of 5 min. Blood pressure in mm Hg.

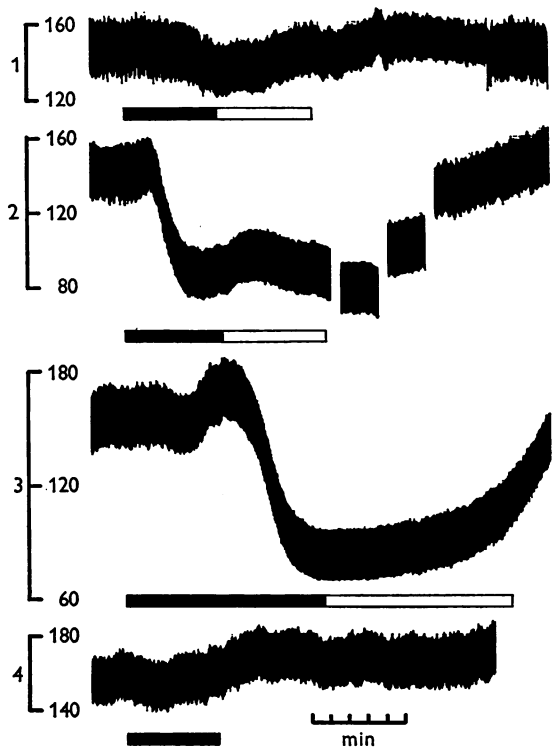

Fig. 4. Arterial blood pressure of a 2·9 kg cat anaesthetized with I.P. pentobarbitone sodium under artificial ventilation. The black bars under records 1–3 indicate periods of perfusion with artificial c.s.f. containing pentobarbitone sodium (50 mg/ml.) and the open bars subsequent periods of perfusion with artificial c.s.f. alone. Rate of perfusion 0·1 ml./min. Records 1 and 3, perfusion from lateral ventricle; record 2 from pontine cisterna to open cisterna magna. The black bar under record 4 indicates period of I.V. infusion of pentobarbitone sodium solution (50 mg/ml.) at a rate of 0·1 ml./min. The breaks in record 2 indicate intervals of 10 min. Blood pressure in mm Hg.

Topical application of pentobarbitone sodium to different regions on the ventral surface of the medulla oblongata

The site where pentobarbitone sodium acts when entering the subarachnoid space appears to be a relatively small area at the ventral surface of the medulla oblongata. This area is situated lateral to the pyramids and just caudal to the trapezoid bodies. This area is covered under the circle No. 2 of Fig. 6*A*.

The first evidence for this site of action was obtained in experiments in which pentobarbitone sodium solution (50 mg/ml.) was infused at a rate of 5 μl./min through each of two micro-injection cannulae inserted at

corresponding sites from the mid line into the subarachnoid space below the ventral surface of the medulla oblongata. The insertion of the cannulae themselves sometimes had a transient pressor effect, otherwise the blood pressure remained steady until the pentobarbitone sodium solution was infused. This produced a fall in arterial blood pressure dependent on the placement of the cannulae. A difference of 2 mm in the anterior posterior direction could change a steep, almost immediate fall, into a gradual one which did not begin until several minutes after the onset of infusion. The upper record of Fig. 5 illustrates the steep almost immediate fall when the infusions into the subarachnoid space were made lateral to the pyramids and about 1 mm caudal to the trapezoid bodies. The blood pressure fell within the first minute, before 0·5 mg pentobarbitone sodium had entered the subarachnoid space; it was still low 30 min later, and had not fully recovered after another 30 min. The long-lasting action is probably due to the fact that there was no subsequent washing out of the pentobarbitone sodium solution. The sites of infusion are shown in the diagram *A* of Fig. 5. The two black spots correspond to the regions of the ventral surface of the medulla found to be stained after 0·2 % bromophenol blue had been infused for 2 min at a rate of 5 μl./min through each of the two microcannulae.

Further evidence for the site of the depressor action was obtained in experiments in which the micro-infusion cannulae were lowered only so far that their tips remained about 1 mm above the ventral surface of the medulla oblongata. This avoided any spread of pentobarbitone sodium in subarachnoid space along the ventral surface of the medulla. The lower record of Fig. 5 was obtained from such an experiment and shows the steep and long-lasting fall produced by 1 mg pentobarbitone sodium applied by this method. The pentobarbitone sodium was infused in a concentration of 200 mg/ml. at a rate of 1·25 μl./min for 2 min through each of the two micro-infusion cannulae. In the inset *B* which is a diagram of a frontal section in the plane of the cannulae, the black areas correspond to the regions found to be stained blue, when instead of the pentobarbitone solution, 1·25 μl./min of a 0·2 % bromophenol blue solution had been infused for 2 min through each cannula. On the left side some of the dye had escaped along the needle tract. This, however, had not been the cause of the fall, because with injections made 2 mm more dorsal from the ventral surface of the medulla, the depressor effect was greatly attenuated or absent. The inset *C* is a diagram of the ventral surface of the medulla and the two circles indicate the regions where the dye was shining through. When the micro-injections were made 2 or 3 mm more rostral or more caudal they no longer lowered the blood pressure.

Finally, the site of action was ascertained by applying the pentobarbitone sodium solution on to the ventral surface of the medulla in two

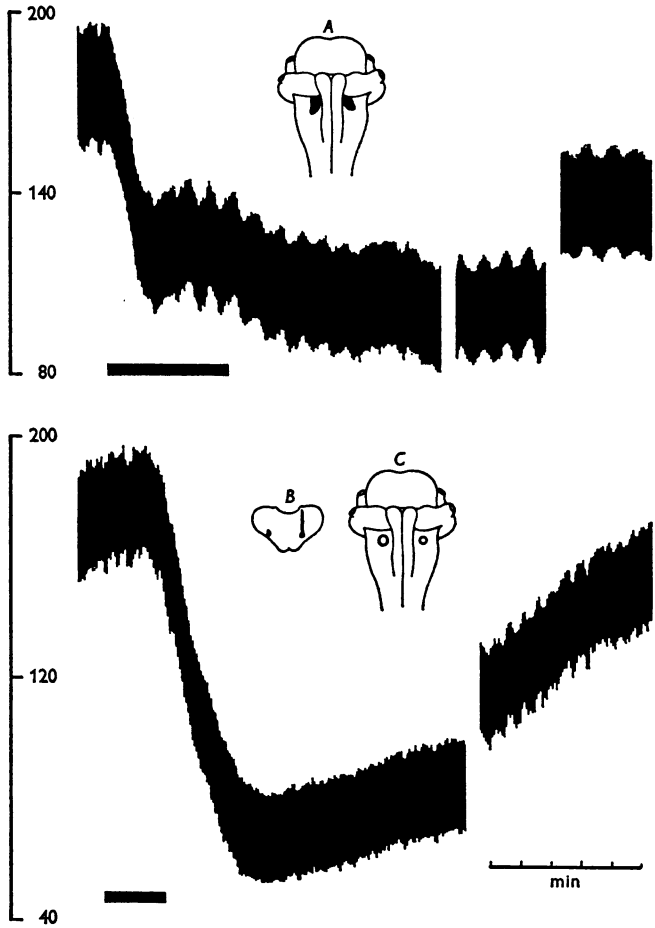

Fig. 5. Arterial blood pressure of two cats anaesthetized with I.P. pentobarbitone sodium under artificial ventilation. Upper record from a 3·2 kg cat; black horizontal bar indicates 4 min bilateral infusion at a rate of 5 μl./min on each side of a pentobarbitone sodium solution (50 mg/ml., total amount 2 mg) into the subarachnoid space beneath the medulla; the black areas in inset *A* indicate the stained regions on the ventral surface of the medulla after a similar infusion for 2 min of a 0·2% bromophenol blue solution. Lower record from a 3·4 kg cat; black horizontal bar indicates 2 min bilateral infusion at a rate of 1·25 μl./min on each side of a 200 mg/ml. pentobarbitone sodium solution (total amount 1 mg) into the central parts of the medulla; the black areas in inset *B* indicate stained regions in a frontal section in the plane of the needle tract after a similar infusion of 0·2% bromophenol blue solution through each cannula, and the circles in the inset *C*, the regions where dye was shining through on the surface. The breaks represent intervals of 23 min in the upper, and of 8 min in the lower record. Blood pressure in mm Hg.

Perspex rings placed at corresponding points on each side of the mid line. The diagram of Fig. 6*A* gives the three positions of the Perspex rings that were tested. When the rings covered the area 1 or 3, the pentobarbitone sodium solution (100 mg/ml.) did not lower the blood pressure, but when they covered the areas 2, which lie just caudal to the trapezoid bodies and lateral to the pyramids, the pentobarbitone sodium produced a steep fall in arterial blood pressure. When only one of the two Perspex rings covering the areas 2 was filled with the pentobarbitone sodium solution, the fall in blood pressure was much reduced, or even absent. Such an experiment is

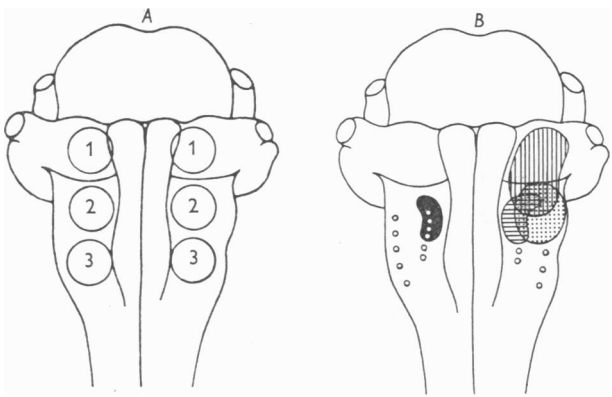

Fig. 6. Two diagrams of the ventral surface of the medulla of the cat. The circles 1, 2 and 3 in diagram *A* represent the areas covered by the Perspex rings through which the pentobarbitone sodium was applied. Diagram *B* is taken from Petrovický (1968). The black area on the left indicates the region where he found nerve cells immediately under the pia mater. The areas on the right indicate: (▤) the thermosensitive zone of Schläfke & Loeschcke (1967); (▥) the respiratory chemosensitive zone of Mitchell *et al.* (1965) and (▦) the respiratory chemosensitive zone as suggested by Petrovický from the experiments of Loeschcke & Koepchen (1958) and Loeschcke *et al.* (1958).

illustrated in Fig. 7. The effect of a 100 mg/ml. pentobarbitone sodium solution applied to the area 2 of one side is illustrated by the middle record, and to the areas 2 of both sides by the bottom record, which was obtained a few minutes later. When first one, then the other Perspex ring was filled with the pentobarbitone sodium solution, the fall in blood pressure was minimal as long as the pentobarbitone sodium acted on one area 2 only, but when it acted on both, the blood pressure fell steeply to a low level. This is shown in the upper record of Fig. 7 obtained about 2 hr earlier than the middle record. During the first 2·5 min of the 5 min period indicated by the horizontal bar below the record, one Perspex ring was filled with

the pentobarbitone solution, during the second 2·5 min both rings were filled with it.

With repeated bilateral application of pentobarbitone sodium in a concentration of 100 mg/ml. the fall often varied and decreased after the first application; this did not happen in the experiment of Fig. 7. A reduction

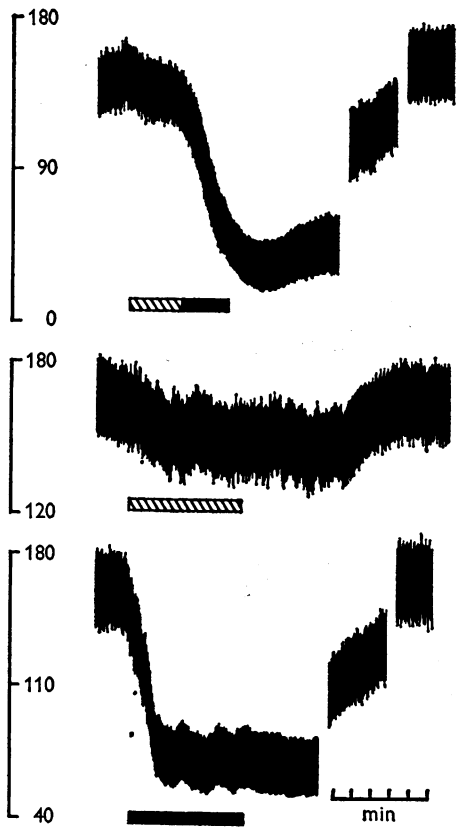

Fig. 7. Arterial blood pressure of a 3·6 kg cat anaesthetized with I.P. pentobarbitone sodium under artificial ventilation. The horizontal bars indicate times of unilateral (striped bars) or bilateral (black bars) application of pentobarbitone sodium solution (100 mg/ml.) through Perspex rings to the ventral surface of the medulla covering the areas represented in Fig. 6 by the circles No. 2. In top and bottom records the first break represents an interval of 15 and the second of 6 min. Blood pressure in mm Hg.

in the concentration of the pentobarbitone sodium to 50 mg/ml. greatly diminished the depressor effect, and a further reduction to 25 mg/ml. abolished it, except in one experiment in which this solution produced a small fall in blood pressure, but only when applied for the first time.

The steep fall produced when pentobarbitone sodium was applied to both areas 2 in a concentration of 100 mg/ml. cannot be attributed to the alkalinity of the solution, which was buffered and had about the same pH as the four times weaker solution which was ineffective.

DISCUSSION

The fall in arterial blood pressure produced when pentobarbitone sodium acts from the liquor space is due to central inhibition of vasomotor tone. It is not a peripheral effect after absorption of the pentobarbitone sodium into the bloodstream, nor does it result from activation of cholinergic vasodilator fibres. This became evident at the very beginning of the investigation when it was found that the fall was atropine resistant and that doses of pentobarbitone sodium which lowered blood pressure on injection into the cerebral ventricles did not do so when injected I.V.

The finding that pentobarbitone sodium was more potent in lowering arterial blood pressure when injected into the cerebral ventricles than when injected into the cisterna magna, but acted on structures reached from the subarachnoid space, is of general interest with regard to the problem of where a drug acts when introduced into the liquor space. It shows that it does not necessarily act on structures in the ventricular walls when it is more potent on intraventricular than on intracisternal injection. There are two reasons why this is so, and why it applies to the vasodepressor effect of pentobarbitone sodium. First, its action is on structures situated near the ventral surface of the medulla, and second, cats, like dogs and rabbits, but unlike man and other primates, lack a foramen Magendie (for references see Davson, 1956). The c.s.f. therefore leaves the ventricular cavities entirely through the foramina of Luschka at the lateral recesses of the fourth ventricle and then passes directly over the ventral surface of the medulla. This region is thus particularly favourably situated to come into contact with and to respond to substances present in the ventricular c.s.f. when this fluid passes into the subarachnoid space. Drugs injected in a small volume into the cerebral ventricles seem to reach this region in a stronger concentration than when injected into the cisterna magna. This is the reason why pentobarbitone is more potent on intraventricular than on intracisternal injection.

The search for a chemosensitive zone at the ventral surface of the medulla began in 1958 when Loeschcke, Koepchen & Gertz, followed up the observation of Leusen (1954*a*, *b*) that changes in the CO_2 tension of the c.s.f. affected respiration. After having found that the respiratory effects were brought about not by the specific stimulus of CO_2 itself, but by the changes produced in hydrogen concentration they showed that the

hydrogen ions did not act on the conventional respiratory centre in the floor of the fourth ventricle, but that they acted on some superficial zone in the region of the lateral recesses and in their neighbourhood at the base of the brain. The evidence was obtained in experiments in which solutions of different pH were either applied topically to the floor of the fourth ventricle, when they did not affect respiration, or infused into the fourth ventricle so that they passed through the lateral recesses into the subarachnoid space beneath the base of the brain, when they affected respiration; blood pressure effects were not observed in these experiments. Some drugs, however, affected arterial blood pressure as well. Veratridine increased tidal volume and produced a rise in blood pressure when it reached the chemosensitive zones on infusion into the fourth ventricle, but it was ineffective when applied topically to its floor. Opposite effects were obtained with lobelin, whereas the infusion of sodium cyanate caused respiratory arrest with a rise in blood pressure (Loeschcke & Koepchen, 1958*a*). Procaine was examined in more detail; it caused depression of respiration and a fall in arterial blood pressure, when infused into the fourth ventricle, when injected directly into the lateral recesses or when applied to the ventral surface of the medulla (Loeschcke & Koepchen, 1958*b*, *c*).

During this earlier work as well as in later experiments, emphasis was on the respiratory effects, whereas the changes in blood pressure were treated more as a side issue. The reactive areas were regarded as 'respiratory chemosensitive areas' and their role in respiratory regulation was discussed. By applying pledgets of filter paper soaked in various solutions to the ventral surface of the medulla, the respiratory chemosensitive areas were identified by Mitchell, Loeschcke, Severinghaus, Richardson & Massion (1963) as paired areas, bounded medially by the pyramidal tracts, laterally by the nerve roots of 8 to 11, rostrally by the pons, and extending caudally 5–6 mm. Applied to these areas, pledgets soaked in c.s.f. with a high p_{CO_2}, or with a high H^+, or containing nicotine or acetylcholine, produced hyperpnoea but did not affect arterial blood pressure, and pledgets soaked in c.s.f. containing procaine produced respiratory depression. On the other hand, Armitage & Hall (1967) obtained a fall in arterial blood pressure by an action of nicotine on the ventral surface of the brain stem. More recently, Schläfke & Loeschcke (1967) studied the effects on respiration and blood pressure of localized cooling of the ventral surface of the medulla with a thermode by which a circular area of 2 mm^2 could be cooled down to between 8 and 5° C within 30 sec. With this method they identified paired thermosensitive areas which were much smaller, each about 9 mm^2, than the chemosensitive areas described by Mitchell *et al.* The cooling resulted in a reduction of the tidal volume and in a fall of arterial blood pressure.

Petrovický (1968) seems to have identified the morphological substrate of the thermosensitive area because he found that this area corresponded closely to a region in which groups of nerve cells are lying immediately under the pia mater within the marginal glia, i.e. in close proximity to the liquor. The nerve cells belong to the nucleus paragiganto cellularis. In other regions of the medulla, nerve cells do not lie so close to the surface. Fig. 6*B*, which is a diagram of the ventral surface of the medulla, is from his paper. It gives on the left side, in black, the region where the nerve cells were found in the marginal glia, and on the right side, for comparison, three areas: the thermosensitive area of Schläfke & Loeschcke, the large chemosensitive area described by Mitchell *et al.* and the smaller chemosensitive area which, according to Petrovický, is the region from where Loeschcke and his co-workers obtained the respiratory effects in 1958, when their solutions passed from the lateral recesses into the subarachnoid space. Petrovický suggests that the nerve cells in the marginal glia may be the morphological substrate not only of the thermosensitive but also of the chemosensitive area, which may not be as large as described by Mitchell *et al.* because, with the method they used, exact localization was scarcely possible.

With the exception of part of the chemosensitive area described by Mitchell *et al.* the areas shown in Fig. 6*B* lie in the region which in Fig. 6*A* is covered by the circle no. 2; it is the region from where the vasodepressor effect was obtained with pentobarbitone sodium which may, therefore, act on the nerve cells which lie immediately under the pia mater and belong to the nucleus paragiganto cellularis. Pentobarbitone sodium lowered blood pressure also when applied by micro-infusion into this region about 1 mm above the ventral surface of the brain stem. Applied in this way, its action may be on nerve cells of the magno-cellular field of Berman (1968) which lie in this region. And if on topical application the pentobarbitone sodium were to penetrate deeply enough to reach these cells its action would be on these cells as well and not only on the cells belonging to the nucleus paragiganto cellularis. Further, when pentobarbitone sodium is injected into the cerebral ventricles, its action may not be confined to the chemosensitive area on the ventral surface of the medulla and perhaps to the cells of the magno-cellular field of Berman. It may have an additional action on chemosensitive areas in the lateral recesses where the morphological substrate would again be superficially situated nerve cells. Fleischhauer & Petrovický (1968) discovered such nerve cells immediately underneath the ependyma, i.e. in close proximity to the liquor, in the floor of the entire lateral recess where they form an extremely thin nucleus.

The effects produced by cooling the ventral surface of the medulla, are probably due to an action on the same superficially situated nerve cells on

which the pentobarbitone sodium acts. This would apply also to other substances which, like cooling, affect respiration and lower blood pressure when acting from the ventral surface of the medulla. It would apply to procaine, the vasodepressor action of which has been described in the early experiments of Loeschcke & Koepchen and was confirmed by Rosenstein, McCarthy & Borison (1968) and by Berndt, Berger & Trouth (1970). These authors found, in addition, that potassium applied to this region raised arterial blood pressure, although it produced the same respiratory effects as procaine.

The situation is not clear with regard to CO_2 and hydrogen ions. According to Loeschcke *et al.* (1958) and Mitchell *et al.* (1963) they do not affect arterial blood pressure when acting on the chemosensitive areas. In the present experiments alkaline solutions were found to be ineffective, too, but Trzebski, Zielinski, Lipskin & Majcherczyk (1971) obtained a rise in blood pressure when p_{CO_2} saturated artificial c.s.f. was applied to the ventrolateral surface of the medulla. Since a respiratory effect can be obtained with and without an effect on blood pressure by drugs acting on the chemosensitive areas, it would appear that the morphological substrate on which drugs act when affecting respiration and when affecting blood pressure is different, and if the superficially situated nerve cells are the morphological substrate, this would imply that different nerve cells are acted upon to produce respiratory and blood pressure effects.

However, the action of pentobarbitone sodium may not be on nerve cells, but may be on nerve fibres. This possibility has not been excluded; it could account for the vasodepressor effect of procaine and of cooling as well since both procedures block nervous conduction. But synaptic transmission is more susceptible to these procedures and also to pentobarbitone sodium (Richards, 1971). Therefore, the finding that there is a close correspondence between the thermosensitive area of Schläfke & Loeschcke (1967) and the region where Petrovický found nerve cells in the marginal glia favours the view that the action is on these cells. It would then have to be assumed that they are not excited, but depressed, by the action of pentobarbitone sodium, procaine, and cooling. Excitation of these cells, on the other hand, might account for the pressor responses produced by potassium cyanide and veratridine, or these pressor responses might result from an action on other cells inside or outside the thermosensitive area of Schläfke & Loeschcke. In this connexion it is interesting to note that these authors obtained pressor responses on localized cooling of a region rostral to this area.

If depression or inactivation of a localized group of nerve cells results in a steep, long-lasting fall in arterial blood pressure, the group of cells must play a role in maintaining vascular tone. Such a function, i.e. a continuous

sympathetic discharge to the blood vessels may therefore be exerted by the nerve cells in the marginal glia on the ventral surface of the medulla oblongata. The finding that pentobarbitone sodium had scarcely any vasodepressor action when applied to only one side of the ventral surface of the medulla could further suggest that the nerve cells of one side are sufficient to maintain this function.

It has been known for about a century that structures in the rostral portion of the medulla oblongata are responsible for maintaining arterial blood pressure, but the actual sites have not been identified. First, they were thought to be located near the dorsal surface in the floor of the fourth ventricle, then to be distributed more or less throughout the entire substance of the medulla, and now it is suggested that an influence is exerted from cells located near its ventral surface. The earlier literature on this subject has been reviewed by Alexander (1946).

The idea that the sites or 'centres' are located in the floor of the fourth ventricle is based on the finding that discrete pressor and depressor sites could be mapped out when exploring the floor with stimulating needle electrodes. However, destruction of these sites by cautery did not interfere with normal pressor and depressor functions. The idea that the sites are distributed throughout the medullary substance is based on the results obtained when the brain stem was explored from pons to decussation of the pyramids and from the dorsal to the ventral surface with fine stimulating electrodes. Large pressor and depressor fields were found nearly throughout the entire substance and extending in some parts close to the ventral surface (Wang & Ransom, 1939; Monnier, 1939; Alexander, 1946). Alexander himself, however, pointed out that 'it is impossible to determine whether the electrodes are stimulating afferent, association, or efferent elements'.

The results obtained with pentobarbitone sodium now suggest that arterial blood pressure may be influenced by the activity of cells situated close to the ventral surface of the medulla. These cells could act as relay stations in the central vasomotor pathway. Or they could be the origin of a tonic influence on the sympathetic discharge to the blood vessels. Or the dendrites of these cells which reach the medullary surface may have an additional function as chemoreceptors responding to changes in the composition of the c.s.f. and thereby influencing the activity of the cells. This would resemble the function of the chemoreceptor in the aortic and carotid bodies which respond to changes in the composition of the blood and through the activity in the aortic and carotid nerves, influence the medullary centres and thereby respiration and blood pressure.

REFERENCES

ALEXANDER, R. S. (1946). Tonic and reflex functions of medullary sympathetic cardiovascular centres. *J. Neurophysiol.* **9**, 205–217.

ARMITAGE, A. K. & HALL, G. H. (1967). Effects of nicotine in the systemic blood pressure when injected into the cerebral ventricles of cats. *J. Neuropharmac.* **6**, 143–149.

BERMAN, A. L. (1968). *The Brainstem of the Cat.* Madison: University of Wisconsin Press.

BERNDT, J., BERGER, W. & TROUTH, C. O. (1970). Respiratory and circulatory effects of 100 mg/l. potassium or 2% procaine in the cerebrospinal fluids of cats. *Pflügers Arch. ges. Physiol.* **321**, 346–363.

BHATTACHARYA, B. K. & FELDBERG, W. (1958). Perfusion of cerebral ventricles: effects of drugs on outflow from the cisterna and the aqueduct. *Br. J. Pharmac.* **13**, 156–162.

BRITTON, H. T. S. (1955). *Hydrogen Ions.* London: Chapman Hall.

DAVSON, H. (1956). *Physiology of the Ocular and Cerebrospinal Fluid.* London: J. and A. Churchill.

FELDBERG, W. & SHERWOOD, S. L. (1953). A permanent cannula for intraventricular injections in cats. *J. Physiol.* **120**, 3*P*.

FLEISCHHAUER, K. & PETROVICKÝ, P. (1968). Ueber den Bau der Wandungen des Aquaeductus cerebri und des IV. Ventrikels der Katze. *Z. Zellforsch. mikrosk. Anat.* **88**, 113–125.

LEUSEN, I. R. (1954*a*). Chemosensitivity of the respiratory center. Influence of CO_2 in the cerebral ventricles on respiration. *Am. J. Physiol.* **176**, 39–44.

LEUSEN, I. R. (1954*b*). Chemosensitivity of the respiratory center. Influence of changes on the H^+ and total buffer concentrations in the cerebral ventricles on respiration. *Am. J. Physiol.* **176**, 45–51.

LOESCHCKE, H. H. & KOEPCHEN, H. P. (1958*a*). Ueber das Verhalten der Atmung und des arteriellen Drucks bei Einbringen von Veratridin, Lobelin und Cyanid in den Liquor cerebrospinalis. *Pflügers Arch. ges. Physiol.* **266**, 586–610.

LOESCHCKE, H. H. & KOEPCHEN, H. P. (1958*b*). Beeinflussung von Atmung und Vasomotorik durch Einbringen von Novocain in die Liquorraemume. *Pflügers Arch. ges. Physiol.* **266**, 611–627.

LOESCHCKE, H. H. & KOEPCHEN, H. P. (1958*c*). Versuch zur Loakisation des Angriffsortes der Atmunds- unde Kreislaufwirkung von Novocain im Liquor cerebrospinalis. *Pflügers Arch. ges. Physiol.* **266**, 628–641.

LOESCHCKE, H. H., KOEPCHEN, H. P. & GERTZ, K. H. (1958). Ueber den Einfluss von Wasserstoffionenkonzentration und CO_2-Druck im Liquor cerebrospinalis auf die Atmung. *Pflügers Arch. ges. Physiol.* **266**, 569–585.

MERLIS, J. K. (1940). The effect of changes in the calcium content of the c.s.f. on spinal reflex activity in the dog. *Am. J. Physiol.* **131**, 67–72.

MITCHELL, R. A., LOESCHCKE, H. H., SEVERINGHAUS, J. W., RICHARDSON, B. W. & MASSION, W. H. (1963). Regions of respiratory chemosensitivity on the surface of the medulla. *Ann. N.Y. Acad. Sci.* **109**, 661–681.

MONNIER, M. (1939). Les centres végétatifs bulbaires (effets de l'excitation faradique du bulbe sur la respiration, la tension artérielle, les pouls, la vessie et la pupille chez le chat). *Archs int. Physiol.* **49**, 455–463.

PETROVICKÝ, P. (1968). Über die Glia marginalis und oberflächliche Nervenzellen im Hirnstamm der Katze. *Z. Anat. EntwGesch* **127**, 221–231.

RICHARDS, C. D. (1971). The selective depression of evoked cortical EPSPs by pentobarbitone. *J. Physiol.* **217**, 41–43*P*.

ROSENSTEIN, R., MCCARTHY, L. E. & BORISON, H. L. (1968). Respiratory effects of ethanol and procaine injected into the cerebrospinal fluid of brain stem in cats. *J. Pharmac. exp. Ther.* **162**, 174–181.

SCHLAEFKE, MARIANNE & LOESCHCKE, H. H. (1967). Localisation eines an der Regulation von Atmung und Kreislauf beteiligten Gebietes an der ventralen Oberflaeche der Medulla oblongata durch Kaelteblockade. *Pflügers Arch. ges. Physiol.* **297**, 201–220.

TRZEBSKI, A., ZIELINSKI, A., LIPSKI, J. & MAJCHERCZYK, S. (1971). Increase of the sympathetic preganglionic discharges and of the peripheral vascular resistance following stimulation by H^+ ions of the superficial chemosensitive areas on the medulla oblongata in cats. *Proc. int. Union Physiol. Sci.* **9**, 571.

WANG, S. C. & RANSOM, S. W. (1939). Autonomic responses to electrical stimulation of the lower brain stem. *J. comp. Neurol.* **71**, 437–455.