Br. J. Pharmac. (1968), **34**, 408–416.

Procaine perfused into cerebral ventricles and subarachnoid space in conscious and anaesthetized dogs

P. S. R. K. HARANATH AND H. VENKATAKRISHNA-BHATT

Department of Pharmacology, Kurnool Medical College, Kurnool, A.P. India

1. Perfusion of 1% procaine into the cerebral ventricles of conscious dogs produced mild paresis, defaecation, vomiting, jerky movements of eyelids, brisk nystagmus, increase in amplitude of respiration and sometimes loss of consciousness. Procaine 2% produced paralysis, loss of consciousness and sometimes respiratory depression.

2. Procaine 2% perfused into the cerebral ventricles of dogs under chloralose anaesthesia produced an initial increase in amplitude of respiration, which preceded its final depression, which is due primarily to procaine and only partly to a change in pH.

3. The site of action for the initial increase in amplitude of respiration was in the fourth ventricle, for it did not occur on perfusion of procaine into the cranial subarachnoid space.

4. Perfusion of spinal subarachnoid space with procaine is enough to cause respiratory failure even when the procaine does not reach the medulla.

Haranath, Naseem Ayesha Begum & Sitaramayya (1965) described the effects of procaine injected into the cerebral ventricles of conscious dogs and its effects on perfusion into the cerebral ventricles of dogs under chloralose anaesthesia. They observed an initial increase in the amplitude of respiration and a rise of blood pressure, both of which were later depressed. In the present experiments the effect of procaine perfused into the cerebral ventricles of conscious dogs was studied and an attempt was made to determine its site of action.

It is known that perfusion of the fourth ventricle with solutions of acidic pH causes an increase in respiratory volume (Loeschcke, Koepchen & Gertz, 1958). The hydrochloride of procaine used in the present experiments lowers the pH of the artificial cerebrospinal fluid in which it is dissolved. To what extent the change in pH is responsible for the increase in amplitude of respiration was also investigated.

Methods

Dogs weighing 5.5–17 kg were used. Bitches were employed for experiments in the conscious state because they are docile and can be trained to be quiet.

Conscious dogs

A Collison cannula was implanted into the lateral ventricle of bitches under intravenous pentobarbitone anaesthesia (30 mg/kg), under aseptic conditions according to the methods described by Feldberg & Sherwood (1953). At the same time a cannula was placed into the upper cervical subarachnoid space after removing the laminae of the two lower cervical or upper lumbar vertebrae according to the method described by Haranath (unpublished). A tracheotomy was performed at the same time and a Fuller's tracheal cannula with a winged outer tube and an inner tube was placed therein. At the time of recording respiration the inner tube was connected to a tambour.

During the week after recovery the cerebral ventricles were perfused with sterile artificial cerebrospinal fluid or procaine solutions at 0.1 ml./min from the cannula in the lateral ventricles. The outflow was from the cervical subarachnoid cannula. The artificial cerebrospinal fluid had the following composition (g/l.): NaCl 8.1, KCl 0.25, $CaCl_2$ 0.14, $MgCl_2$ 0.11, $NaHCO_3$ 1.76, NaH_2PO_4 0.07, urea 0.13 and glucose 0.61 (Merlis, 1940).

Anaesthetized dogs

Dogs were anaesthetized with 1% chloralose in saline (110 mg/kg) given intravenously. Blood pressure was recorded from the femoral artery with a mercury manometer and respiration was recorded with a tambour connected to the cannulated trachea.

Perfusion of cerebral ventricles. The cerebral ventricles were perfused with artificial cerebrospinal fluid or procaine solution from lateral ventricles to cisterna with a continuous slow injector according to the method described by Bhattacharya & Feldberg (1958) for cats. The usual speed of infusion was 0.1 ml./min.

Perfusion of cranial subarachnoid space. After implanting a cannula into the lateral ventricle, the cisterna magna was exposed. The margin of the occipital bone was chipped off and the cistern was laid open. A fine polythene tube PE 10 was introduced in the recess between cerebellum and the medulla and was pushed as far as possible along the side of the medulla. It was held in position with a clamp. Procaine solution and methylene blue 1% (at the end of the experiment) were injected through this cannula at 0.1 ml./min and the fluid escaped out of the open cisterna. To prevent this fluid from entering the fourth ventricle a counter perfusion with artificial cerebrospinal fluid was made at double the speed from the lateral ventricle.

Perfusion of spinal subarachnoid space. The laminae of the upper two lumbar vertebrae were removed and the spinal cord with its meningeal coverings was exposed. A small hole was made into the spinal subarachnoid space and a polythene tube with a tapered end was pushed into this opening so that it served both as an inlet for the perfusion fluid and also fitted the hole in the meninges tightly to prevent leakage of fluid. The cisterna magna was exposed and the first cervical vertebral arch was removed. The cistern and the subarachnoid space now exposed were laid open. Procaine alone or with 0.5% methylene blue was perfused into the lumbar subarachnoid space at 0.1 ml./min and was allowed to flow out through the cisterna magna. To prevent this fluid from reaching the cranial subarachnoid space, the head was fixed at a higher level in a head holder and artificial cerebro-

spinal fluid was perfused at double the speed from the lateral ventricle. Post-mortem examination confirmed that the dye given with procaine did not reach the cranial subarachnoid space.

Perfusion of cervical subarachnoid space. With the animal in a prone position, a lumbar subarachnoid cannula was introduced as described earlier, and a Collison cannula was placed in the lateral ventricle. The subarachnoid space under the first and second cervical vertebrae, exposed by removing the vertebral arches, and the cisterna magna were laid open. Along the side of the exposed spinal cord, a polythene tube was pushed down the subarachnoid space till its tip was at the sixth or seventh cervical vertebra. The animal was then turned on its back and its legs and head were raised high so as to leave the cervical subarachnoid space at the lowest level. Procaine with dye was perfused through the cannula already placed in the cervical subarachnoid space. It entered at the lower cervical region and escaped from the opening in subarachnoid space at the second cervical vertebra. To prevent this fluid from reaching the thoracic region a counter perfusion of artificial cerebrospinal fluid at double the speed was set up through the lumbar subarachnoid cannula. In the same way, to prevent the procaine from reaching the cranial subarachnoid space, another counter perfusion with artificial cerebrospinal fluid was made from a cannula in the lateral ventricle. Post-mortem examination revealed that the staining did not extend either into the cranial or thoracic regions.

Solutions. Procaine (Hoechst, India) was used as its hydrochloride and the doses and concentrations refer to its salt.

Solutions of procaine were made in artificial cerebrospinal fluid. The pH of the artificial cerebrospinal fluid was usually 8.0, but procaine when dissolved to make a 2% solution lowers the pH of artificial cerebrospinal fluid to about 7.0. In the experiments in which the influence of pH on the respiration was studied, artificial cerebrospinal fluid was acidified with hydrochloric acid to the same pH as 2% procaine solution, for control observations. When it was attempted to raise the pH of procaine solutions to that of artificial cerebrospinal fluid either large amounts of sodium bicarbonate were necessary or precipitation of procaine base resulted on complete alkalinization with sodium hydroxide. Hence in some experiments the pH of procaine solutions was partly raised to about pH 7.4 by alkalinizing with 0.5 ml. 1N-NaOH to 100 ml. of 2% procaine solution. In such experiments the control artificial cerebrospinal fluid was acidified with hydrochloric acid only to this pH.

Results

Perfusion in conscious dogs

Procaine 1% or 2% in artificial cerebrospinal fluid was perfused for an hour at 0.1 ml./min into the lateral ventricle in conscious dogs and the effluent emerged from the cervical subarachnoid cannula.

Perfusion with 1% procaine produced the following effects. Jerky movements of the eyelids and brisk nystagmus appeared within 3–5 min and were present throughout except when the animals became unconscious. For about 10–15 min the animal was restless and it defaecated, retched and vomited. Then it became quiet, but the gait was paretic. Later it lay down on its side and closed its eyes. In two out of six experiments the animals became unconscious after about half an hour of per-

fusion. The respiratory movements were regular, slow and deep except during defaecation and vomiting, when they became irregular and rapid. Figure 1 shows a record from one such experiment. The animal was unconscious after half an hour and respiration became slow and its amplitude increased, first in the expiratory phase and later in the inspiratory phase.

When 2% procaine was perfused, nystagmus, defaecation and vomiting occurred much more quickly and paresis was more marked. Three out of four animals lost consciousness 15 min after perfusion was started. The animal remained unconscious until the end of perfusion and during this period the knee jerk reflex was exaggerated and eyeball movements and the corneal reflex were absent. Respiration progressively became very slow and deep, as can be seen in Fig. 2. There was no respiratory depression even after 1 hr of perfusion at 0.1 ml./min. When the speed was doubled, respiratory depression occurred in about 17 min.

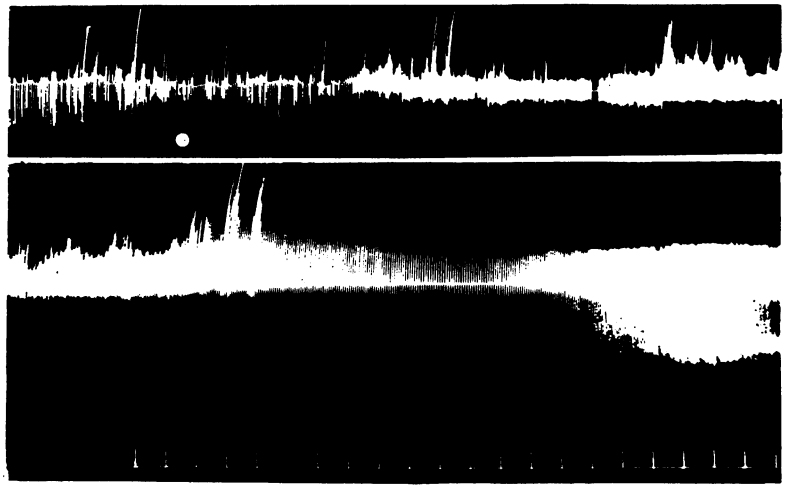

FIG. 1. Record of respiration of unanaesthetized dog. At the white dot perfusion with 1% procaine into the lateral ventricle started. Lower record continuation of top. Time marks, 1 min.

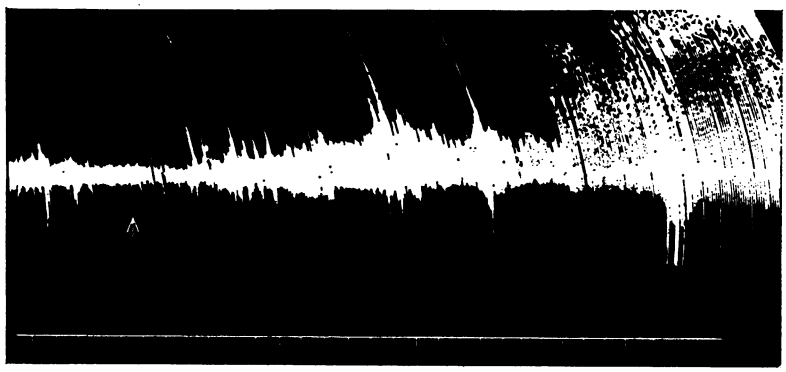

FIG. 2. Record of respiration of unanaesthetized dog. At the arrow perfusion with 2% procaine into the lateral ventricle started. Time marks, 1 min.

Perfusion in anaesthetized dogs

In animals under chloralose anaesthesia, the cerebral ventricles were perfused with 2% procaine from a lateral ventricle to the cisterna. This produced slowing and increase in amplitude of respiration followed by depression. Blood pressure rose soon after the commencement of perfusion and later fell. These effects were described earlier by Haranath *et al.* (1965). To determine the site of action for these effects on respiration and blood pressure, the cranial subarachnoid space around the brain stem, the spinal subarachnoid space and the cervical subarachnoid space were separately perfused in different experiments. The role of the acidity of procaine solutions in bringing about these changes in respiration was investigated by using control solutions of similar pH. The results in detail are as follows.

Perfusion from lateral ventricle to cisterna. Artificial cerebrospinal fluid when perfused into the cerebral ventricles produced no effect on respiration and blood pressure. In five experiments artificial cerebrospinal fluid acidified to the same pH as 2% procaine solution was perfused from lateral ventricle to cisterna. In three experiments it produced an increase in amplitude of respiratory movements which was sometimes delayed. But when procaine 2% was subsequently perfused there was initially greater increase in amplitude and respiration was finally depressed. This shows that procaine has an effect of its own in increasing the amplitude of respiration in addition to the effect of the change in pH of its solution. Acidified artificial cerebrospinal fluid had no effect on blood pressure, whereas 2% procaine generally caused a rise.

In seven other experiments, procaine solutions partly alkalinized to pH 7.4 and artificial cerebrospinal fluid acidified to the same pH were perfused. It was observed that the acidified cerebrospinal fluid solutions produced only very slight increase in the amplitude of respiration. Procaine solutions, however, caused a greater increase in the amplitude of respiration, as can be seen in Fig. 3. Respiratory depression occurred later in four of seven experiments. Blood pressure did not show the usual initial rise with perfusion of procaine in four of seven experiments.

Perfusion of cranial subarachnoid space. In eleven experiments 2% procaine was perfused into the subarachnoid space around the brain stem and its entry into the

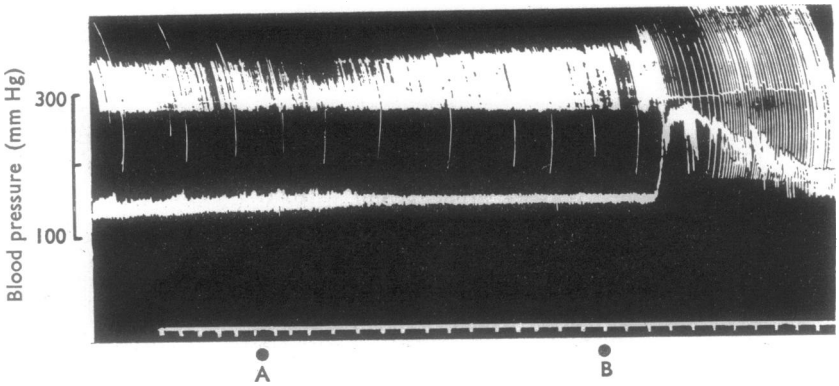

FIG. 3. Record of respiration and blood pressure of dog under chloralose anaesthesia. At A, perfusion from lateral ventricle to cisterna was changed from artificial cerebrospinal fluid (pH 7.8) to the same solution acidified to pH 7.4. This was later changed at B to 2% procaine alkalinized to pH 7.4. Time marks, 1 min.

fourth ventricle was prevented by a counter perfusion from the lateral ventricle with artificial cerebrospinal fluid. Respiration rapidly decreased in amplitude and sometimes slowed. No increase in amplitude of respiration occurred in any of the experiments. On continued perfusion respiration stopped in about 5–6 min. In some experiments perfusion with procaine was discontinued after respiration was depressed and the counter-perfusion with artificial cerebrospinal fluid was continued. Respiration returned to normal, respiratory movements regained slowly their amplitude and usually increased beyond the original level. When respiration again became normal, 2% procaine was perfused—this time from the lateral ventricle. Respiration increased in amplitude and slowed. This suggests that the site of action for the increase in amplitude of respiration is not in the subarachnoid space but in the fourth ventricle. Figure 4 represents the respiration and blood pressure changes in one such experiment on perfusion with procaine first into subarachnoid space and later into cerebral ventricles.

With subarachnoid perfusion of procaine, blood pressure did not vary much but usually fell slowly. The rise of blood pressure observed with perfusion of procaine from the lateral ventricle is therefore probably due to its action in the fourth ven-

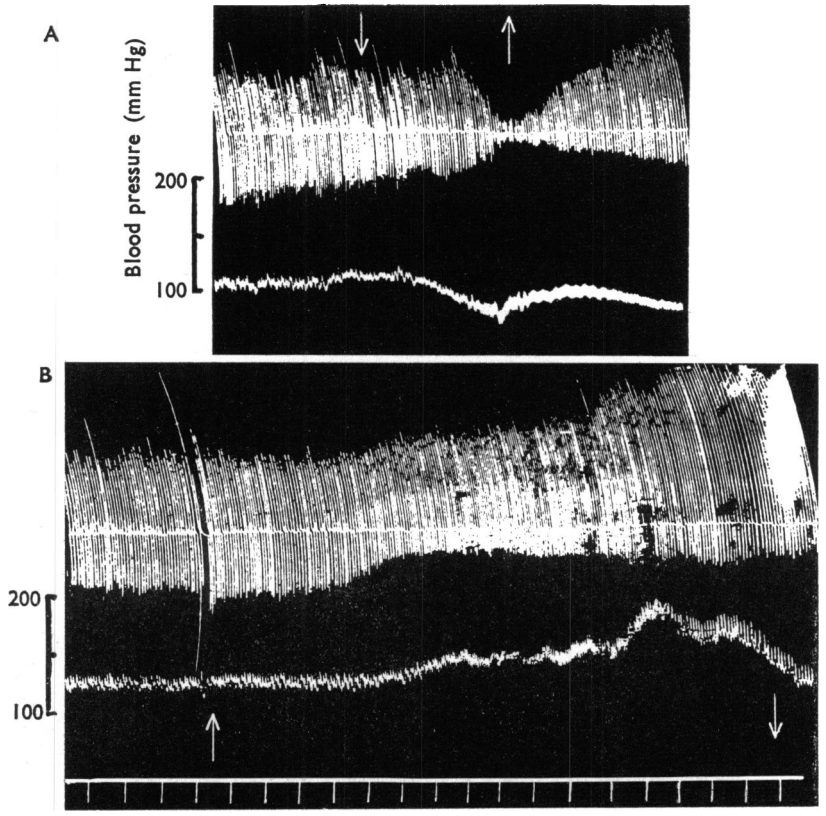

FIG. 4. Record of respiration and blood pressure of dog under chloralose anaesthesia. In A, the cranial subarachnoid space was perfused with 2% procaine. In B, perfusion was with 2% procaine from lateral ventricle to cisterna. Arrows denote the start and end of the perfusions. Time marks, 1 min.

tricle and not in the subarachnoid space. At the end of the experiment, methylene blue was perfused in the same way as procaine through the subarachnoid cannula and the counter perfusion with artificial cerebrospinal fluid from the lateral ventricle was continued. On post-mortem examination there was no staining in the fourth ventricle or at the lateral recesses. The staining was usually at the sides and under surface of the medulla and pons.

Perfusion of spinal subarachnoid space. The spinal subarachnoid space was perfused from a lumbar subarachnoid cannula in seven experiments. There was no change in respiration and blood pressure when the perfusion fluid was artificial cerebrospinal fluid with or without 0.5% methylene blue dissolved in it. When the perfusion fluid contained 2% procaine, however, the blood pressure started to fall in about 2–3 min. Respiration increased in amplitude for about 2 min, coinciding with the decline in blood pressure. Later the respiration progressively diminished in amplitude and finally stopped, as can be seen in Fig. 5. During this period intercostal respiration progressively diminished and respiration became more abdominal in nature. Towards the end, gasping respiration occurred with the muscles of the neck and jaw taking part. Procaine alone in artificial cerebrospinal fluid was perfused in two experiments and in others 0.5% methylene blue was also added. It took about 10–20 min for the procaine and the dye to reach the cisterna magna. Respiration progressively declined and finally stopped 1–2 min after procaine reached the level of first cervical vertebra. Post-mortem examination revealed that the staining of the spinal cord with the dye was limited to below the level of first cervical vertebra.

Perfusion of cervical subarachnoid space. In three experiments the cervical subarachnoid space was perfused with 2% procaine to determine whether there is any specific chemosensitive area in the cervical cord producing immediate depression of respiration. A progressive diminution of the respiration occurred and finally

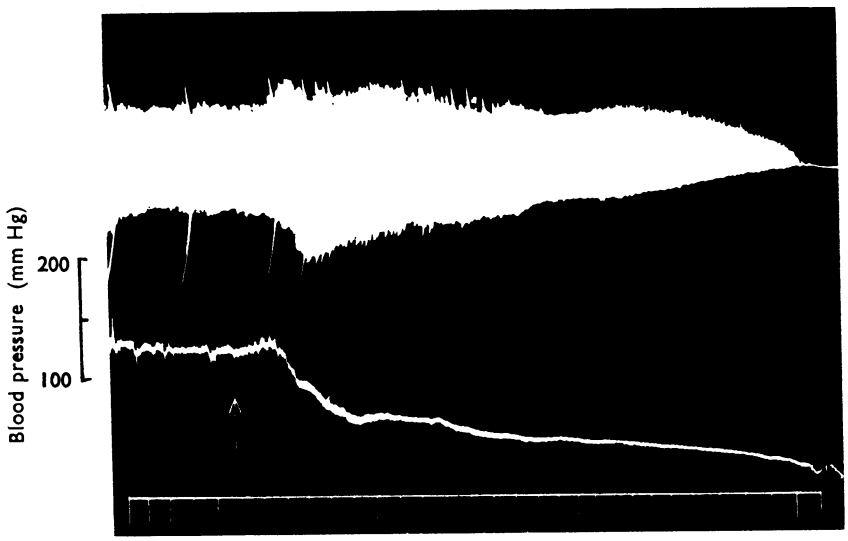

FIG. 5. Record of respiration and blood pressure of dog under chloralose anaesthesia. Perfusion of spinal subarachnoid space from lumbar cannula. At the arrow, perfusion was changed from artificial cerebrospinal fluid to 2% procaine. Time marks, 30 sec.

stopped in about 5–20 min. The effect was in no way different from that produced by perfusion of the whole spinal cord from a lumbar subarachnoid cannula.

Discussion

Loeschcke & Koepchen (1958a, b) observed depression of respiration when procaine was injected into the fourth ventricle in dogs under morphine-urethane-chloralose anaesthesia and in cats under chloralose-urethane anaesthesia with the peripheral chemoreceptors denervated. They found no change in respiration when 2% procaine, soaked into a cotton pledget, was applied at the obex of the fourth ventricle, but depression of respiration occurred when 0.01 ml. of the procaine solution was injected into the lateral recesses. The site of action for this inhibition of respiration was localized to a chemosensitive area at the ventro-lateral surface of the medulla (Mitchell, Loeschcke, Massion & Severinghaus, 1963; Severinghaus, Mitchell, Richardson & Singer, 1963). Haranath *et al.* (1965) observed an initial increase in amplitude and subsequent depression of respiration on perfusion of the cerebral ventricles with procaine from lateral ventricle to cisterna in dogs under chloralose anaesthesia. They did not observe these effects when procaine was perfused from lateral ventricle to aqueduct, so they excluded the lateral and third ventricles as possible sites of action. They suggested that the site of action for the increase in amplitude of respiration could be in the fourth ventricle. The present experiments confirm this suggestion by excluding the cranial subarachnoid space as the possible site of action.

The experiments perfusing the spinal subarachnoid space can be compared with an upward spread of spinal anaesthetic solutions when administered clinically. Death occurred in these animals on continued perfusion due to a progressive fall in blood pressure and respiratory failure. The fall in blood pressure was immediate and was due to interruption of vasomotor impulses passing via the spinal cord. The respiratory failure was due to progressive paralysis of the intercostal muscles and finally the diaphragm. Procaine need not reach the medullary centres to produce respiratory failure. When it reaches the medulla it can produce either respiratory depression due to an action on the chemosensitive areas in the subarachnoid space or an initial increase in respiratory amplitude if it reaches the fourth ventricle. There seem to be no chemosensitive areas in the cervical portion of the spinal cord.

Experiments with perfusion of cerebral ventricles in conscious dogs show that 1% procaine given at 0.1 ml./min for 1 hr did not depress the respirations but caused an increase in amplitude. Even with 2% procaine respiratory depression was observed only when the perfusion speed was doubled. The respiratory depressant effects of procaine injected into cerebral ventricles appear to be greater in anaesthetized than in conscious animals.

The initial increase in amplitude of respiration observed with procaine could be due either to the action of procaine itself, or to the acidity of its solutions, or both. The present experiments show that, although some increase in amplitude of respiration could be due to acidity of the solutions, procaine itself predominantly produces this effect. Haranath & Venkatakrishna-Bhatt (1968) observed similar effects with other local anaesthetics. They found that cinchocaine hydrochloride (1 : 200 or 1 : 1,500) does not lower the pH of the artificial cerebrospinal fluid in which it is

dissolved but produces an increase in amplitude of respiration before it is finally depressed. They have also shown that 0.5% lignocaine hydrochloride in artificial cerebrospinal fluid produces a marked increase in amplitude of respiration, when artificial cerebrospinal fluid acidified to the same pH does not have any effect on respiration.

This work was carried out with the aid of a grant from Indian Council of Medical Research, which is gratefully acknowledged.

REFERENCES

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

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

HARANATH, P. S. R. K., NASEEM AYESHA BEGUM & SITARAMAYYA, G. (1965). The effects of procaine injected into the cerebral ventricles of conscious and anaesthetized dogs. *Br. J. Pharmac. Chemother.*, **24**, 339–347.

HARANATH, P. S. R. K. & VENKATAKRISHNA-BHATT, H. (1968). Studies on cinchocaine and lidocaine administered into cerebral ventricles of conscious and anaesthetized dogs. *Indian J. med. Res.*, **56**, 217–226.

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

LOESCHCKE, H. H. & KOEPCHEN, H. P. (1958b). Versuch sur Lokalisation des Angriffsortes der Atmungs und Kreislaufwirkung von Novocain im Liquor cerebrospinalis. *Pflugers Arch. ges. Physiol.*, **266**, 628–641.

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

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

MITCHELL, R. A., LOESCHCKE, H. H., MASSION, W. H. & SEVERINGHAUS, J. W. (1963). Respiratory responses mediated through superficial chemosensitive areas on the medulla. *J. appl. Physiol.*, **18**, 523–533.

SEVERINGHAUS, J. W., MITCHELL, R. A., RICHARDSON, B. W. & SINGER, M. M. (1963). Respiratory control at high altitude suggesting active transport regulation of CSF pH. *J. appl. Physiol.*, **18**, 1155–1166.

(*Received June* 14, 1968)