Brit. J. Pharmacol. (1965), **24**, 339–347.

THE EFFECTS OF PROCAINE INJECTED INTO THE CEREBRAL VENTRICLES OF CONSCIOUS AND ANAESTHETIZED DOGS

BY

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(*Received March* 5, 1964)

The present experiments examined the effects of procaine injected into the cerebral ventricles of conscious dogs or perfused through the cerebral ventricles of anaesthetized dogs.

The effects of procaine introduced into the cerebrospinal fluid spaces have not been uniform. According to Koster & Kasman (1929) 2.5% procaine applied to the medulla and the upper cervical cord of anaesthetized guinea-pigs and cats had no great effect on respiration. On the other hand, Hill & MacDonald (1935) obtained respiratory depression with injections of procaine (10 mg; 0.1 ml. of 10%) into the cisterna or into the fourth ventricle (5 mg; 0.1 ml. of 5%) of cats anaesthetized with chloralose with morphine as premedication. In dogs and cats anaesthetized with chloralose and urethane with the cerebellum sucked away, Loeschcke & Koepchen (1958) found no change in respiration when applying 2% procaine, soaked into a cotton pledget, at the obex of the fourth ventricle, but depression of respiration occurred when injecting 0.01 ml. of the procaine into the lateral recesses. The site of action has recently been located (Mitchell, Loeschcke, Massion & Severinghaus, 1963; Severinghaus, Mitchell, Richardson & Singer, 1963). Topical application of procaine containing pledgets revealed on each side of the ventrolateral surface of the medulla a well-defined region lying between the eighth and eleventh cranial nerve from which apnoea was produced by the procaine.

METHODS

Dogs weighing between 3.25 and 10.8 kg were used.

Procedures in conscious dogs

A Collison cannula was implanted into the lateral ventricle during pentobarbitone sodium anaesthesia and aseptic conditions according to the method described for cats by Feldberg & Sherwood (1953). Injections were made in a volume of less than 1 ml. during the week after recovery. The fluid used for dissolving procaine was artificial cerebrospinal fluid, the one introduced by Merlis (1940) and later used by Leusen (1949). Its composition is as follows (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.

To record respiration, tracheotomy was performed a day or two earlier using pentobarbitone sodium anaesthesia and aseptic conditions, and a Fuller's tracheal cannula consisting of a winged outer tube and an inner tube was inserted and kept in position. At the time of recording the inner tube was connected to a tambour. In addition, the chest movements of respiration were recorded with an improvised stethograph by placing on the dog a device shown in Fig. 1. Two plywood planks 42 cm long and 8 cm wide were

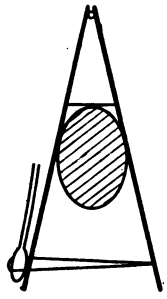

Fig. 1. Diagrammatic representation of the arrangement for recording chest movements in conscious dogs in standing position. The obliquely hatched oval represents the cross section of the thorax of the dog. For further description, see text.

hinged at the top and a rubber band fixed to the planks at a distance of about 16 cm from the hinge. This band rested on the back of the animal with the planks lying astride. To the outer surface of one of the planks a balloon was fixed and connected to a tambour. A tape fixed to the end of the opposite plank was passed over the balloon so that with each inspiratory expansion of the chest the planks drew apart and the balloon was compressed against the plank by the tape and a record obtained on a slowly moving smoked drum.

For recording the blood pressure in conscious animals the carotid artery was brought into a skin loop in an aseptic operation during pentobarbitone sodium anaesthesia. In some experiments the artery was cut between ligatures high in the neck, and the cardiac segment was drawn into a 3-mm-wide polyethylene tube closed at the end which was left projecting outside the wound. At the time of recording the artery was punctured with a No. 20 needle through the skin or the polyethylene tube and was attached to a mercury manometer. In the experiments where the artery was kept in the polyethylene tube the blood pressure was recorded on the day after the operation as delay resulted in clot formation in the blind artery. The dog was raised from the ground by slings passed under all the four limbs and was kept on a restraint stand when recording the blood pressure and respiration.

Procedures in anaesthetized dogs

The dogs were anaesthetized with intravenous 1% chloralose (110 mg/kg). The arterial blood pressure was recorded from a femoral artery and respiration was recorded by means of a tambour connected to the cannulated trachea.

Perfusion of cerebral ventricles. Cerebral ventricles were perfused with artificial cerebrospinal fluid or with procaine solution at a rate of 0.1 ml./min with a continuous slow infusion pump. Perfusion was from lateral ventricle to cisterna or to aqueduct. The method was essentially similar to that described by Bhattacharya & Feldberg (1958) for cats. The aqueductal cannula had to be introduced under direct vision after lifting the cerebellum with a curved spatula, since in dogs the aqueductal opening is at a higher level than the floor of the fourth ventricle and the axis of the aqueduct is at an angle with the floor of the fourth ventricle.

For perfusion of the fourth ventricle, the cerebellum was exposed by removing the atlanto-occipital membrane as well as the part of the occipital bone which forms the upper margin of the foramen magnum. By gently lifting the vermis, a soft polyethylene cannula was then pushed into the middle of the fourth ventricle. In order to prevent the procaine (introduced at a rate of 0.075 ml./min through this cannula) from reaching the aqueduct and third ventricle, a counter-perfusion of artificial cerebrospinal fluid was made through a cannula in the lateral ventricle at a faster rate of 0.1 ml./min. The perfusion fluid escaped either from the sides of the cannula or through the foramena of Luschka. When at the end of the experiment methylene blue was introduced in the same way as procaine, *post mortem* examination confirmed that the dye had not reached the aqueduct.

Phrenicotomy. In experiments where phrenicotomy was done, the roots of the phrenic nerves arising from the 5th and the phrenic-root-bearing 6th and 7th cervical nerve trunks were exposed and cut.

Solutions. Isotonic solutions of procaine (Hoechst, India), 0.5, 1.0 and 2%, were made by first dissolving the procaine in 8 ml. of distilled water for each 0.3 g and later making up the volume with artificial cerebrospinal fluid to the desired strength—a procedure recommended by Sprowls (1949) for preparation of isotonic solutions with 0.9% saline. For comparison the procaine was diluted in some experiments with artificial cerebrospinal fluid without reference to isotonicity.

RESULTS

Injections of procaine into the lateral cerebral ventricles of unanaesthetized dogs

Control injections of 1 ml. or less of either 0.9% saline or artificial cerebrospinal fluid or 1.8% saline (isotonic with 10% procaine) had no apparent effect. The effects of increasing doses of procaine injected intraventricularly are summarized in Table 1. A dose of 40 mg

TABLE 1

EFFECTS OBSERVED IN CONSCIOUS DOGS AFTER INJECTION OF PROCAINE INTO THE LATERAL CEREBRAL VENTRICLES

Dose (mg)	Motor system	Eye	Respiration	Vomiting	Defaecation and urination
0.2–1	—	—	—	—	Urination
5	Mild paresis	Pupils dilated	—	+	Urination
10–20	Mild paresis	Pupils dilated; nystagmus	Irregular; deep	+	Defaecation and urination
40	Paresis; crawling or cycling movements	Pupils dilated; nystagmus; twitching of lids	Deep	++	Defaecation and urination
100	Paralysis	Pupils widely dilated; nystagmus; twitching of lids	Failure		Defaecation and urination

(0.4 ml. of 10%) resulted within 1 min in retching, twitching of lids, brisk nystagmus and dilatation of the pupils. The animals became unable to hold up their heads or to stand, staggered to the ground and remained in this condition without moving for 15 to 25 min. The corneal reflex was absent but the conjunctival reflex was present. The withdrawal response was obtained on deep pressure over the limbs. Respiration gradually increased in depth, sometimes preceded by slight diminution of amplitude. Movements occurred within 10 to 25 min, consisting sometimes of pedalling; recovery occurred gradually within 15 to 45 min. Generalized convulsions, however, were not observed. Haley (1956) injected procaine amide (5.1 to 7.1 mg/kg) into the third ventricle in unanaesthetized dogs and observed defaecation, slight twitching of the facial musculature and stage III plane III

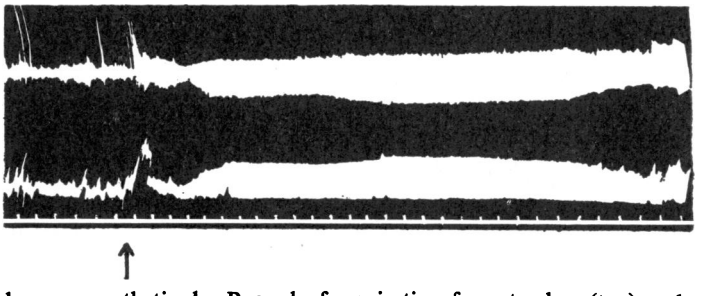

Fig. 2. Dog, 5 kg, unanaesthetized. Record of respiration from trachea (top) and chest movements (bottom). Down strokes in the top and upstrokes in the bottom traces denote inspiration. At the arrow 40 mg (0.4 ml. of 10%) of procaine was injected into the lateral cerebral ventricle. Time marks, 1 min.

anaesthesia. These effects were also observed with the procaine injections, but were of short duration.

Fig. 2 shows the effects on respiration and chest movements obtained with 40 mg procaine given into the lateral ventricle. Before procaine was injected, the record showed occasional movements of the animal superimposed on the respiratory records. Immediately after injection of procaine, the chest movement record showed abrupt changes due to retching. The respiratory amplitude was at first slightly diminished, but later gradually increased. At the end of 25 min (towards the end of the record) voluntary movements once more appeared. But the increase in depth of respiration persisted for nearly 1 hr after the animal had recovered from the motor effects. With large doses of procaine (100 mg) the respiratory amplitude and rate showed an immediate diminution followed by arrest of respiration. If artificial respiration was applied the animal regained natural breathing in 5 to 10 min.

In four experiments in which the blood pressure was recorded from the carotid artery in the conscious animal, a pronounced rise of blood pressure followed the intraventricular injection of 40 mg procaine. Such an experiment is illustrated in Fig. 3.

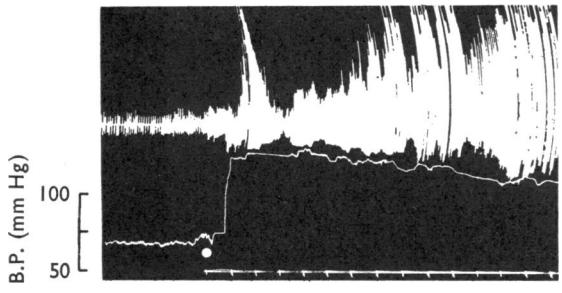

Fig. 3. Dog, 4.75 kg, unanaesthetized. Record of respiration from trachea and carotid blood pressure (B.P.). At the white dot, 40 mg (0.4 ml. of 10%) of procaine was injected into the lateral cerebral ventricle. Time marks, 1 min.

Perfusion of cerebral ventricles with procaine in anaesthetized dogs

Perfusion from lateral ventricle to cisterna. Perfusion with 0.5% procaine had no effect on blood pressure and caused only a slight increase in depth of respiration without any change of rate after about 1 hr of perfusion. Perfusion with 1% procaine produced some slowing of respiration and an increase in depth after about 30 min of perfusion. In addition, the arterial blood pressure rose. On perfusion with 2% procaine, effects on respiration and blood pressure began within 2 to 10 min. Respiration slowed to a different degree in different animals, the amplitude of respiration increased nearly twofold and the arterial blood pressure rose. On continued perfusion with this concentration of procaine respiration later became depressed and finally ceased.

Fig. 4 illustrates the changes in respiration and arterial blood pressure produced on perfusion with 2% procaine. In this experiment the increase in depth of respiration lasted for 16 min, then respiration became depressed and finally ceased (not included in the record). In some experiments in which the onset of the respiratory changes was delayed, the increase in amplitude of respiration continued for 1 to 1.5 hr before depression set in. Usually the blood pressure began to rise before the changes in respiration set in. The

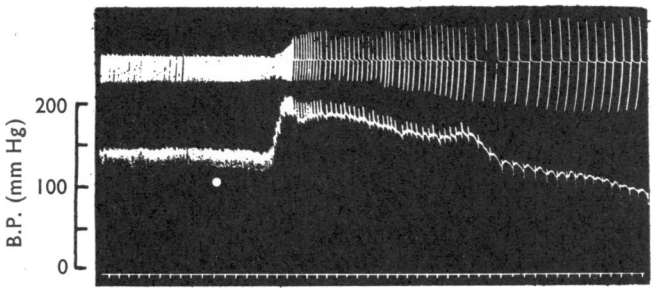

Fig. 4. Effects of perfusion of procaine from lateral ventricle to cisterna on respiration and blood pressure (B.P.) in a dog during chloralose anaesthesia. At the white dot the perfusion was changed from artificial cerebrospinal fluid to 2% procaine solution. Time marks, 30 sec.

rise in blood pressure lasted for about 10 min and then fell below the original level. In some experiments no rise in blood pressure occurred and there was only a progressive fall. The effects on respiration and blood pressure were reversible. When the perfusion with 2% procaine was stopped and replaced by perfusion with artificial cerebrospinal fluid respiration and blood pressure returned to normal within 30 min.

In three dogs which were anaesthetized with intravenous pentobarbitone sodium (30 mg/kg) instead of chloralose, perfusion with 2% procaine produced no rise in arterial blood pressure but a gradual fall. Respiration slowed in all three experiments; in two, depth of respiration first increased slightly and then decreased, in the third there was a progressive decrease in depth of respiration from the beginning.

Perfusion with 4% procaine produced slowing of respiration associated during the first 5 min with a decrease then with an increase in depth of respiration. The blood pressure rose immediately but later fell. Perfusion with 8% procaine caused pronounced slowing and deepening of respiration and respiratory arrest within 6 min. The blood pressure showed an immediate rise followed by a steep fall.

In none of these perfusion experiments did procaine produce general convulsions.

Perfusion from lateral ventricle to aqueduct. Perfusion with 2% procaine caused no slowing or deepening of respiration. The only effect was a slight, very gradual, diminution in amplitude of respiration.

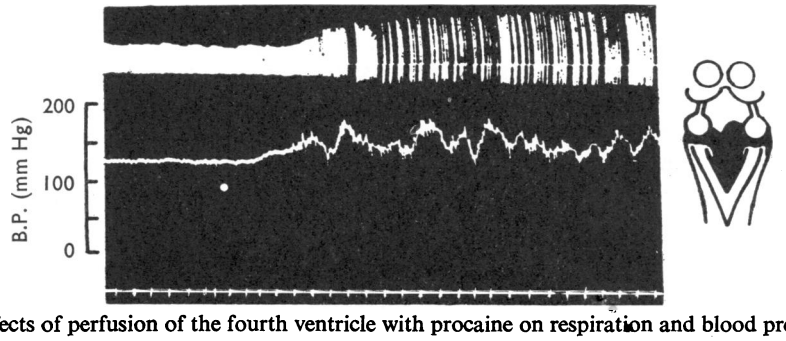

Fig. 5. Effects of perfusion of the fourth ventricle with procaine on respiration and blood pressure (B.P.). At the white dot perfusion was changed from artificial cerebrospinal fluid to 2% procaine solution. On the right is indicated the area perfused with procaine as shown by staining with methylene blue (instead of procaine) at the end of the experiment. Time marks, 1 min.

Perfusion of fourth ventricle. Perfusion with 2% procaine caused slowing and deepening of respiration; the effect began within 2 min and lasted for about 30 min. In addition, there was in several but not in all experiments a gradual rise in arterial blood pressure with large fluctuations. Such an experiment is illustrated in Fig. 5. The diagram on the right shows the area of the floor of the fourth ventricle which was stained with methylene blue which at the end of the experiment was perfused instead of the procaine. The dye, and thus the procaine, had not entered the aqueduct.

Bilateral vagotomy. Cutting the vagi is immediately followed by pronounced slowing and deepening of respiration. The effect partly subsides within the first hour. Fig. 6,*a* shows the changes in respiration immediately and 1.5 hr after bilateral vagotomy. In five experiments perfusion of 2% procaine from lateral ventricle to cisterna was begun 1 to 3 hr after vagotomy. The effect on respiration varied. In two experiments, one of which is illustrated in Fig. 6,*b*, the first effect was a large increase in amplitude of respiration. Then

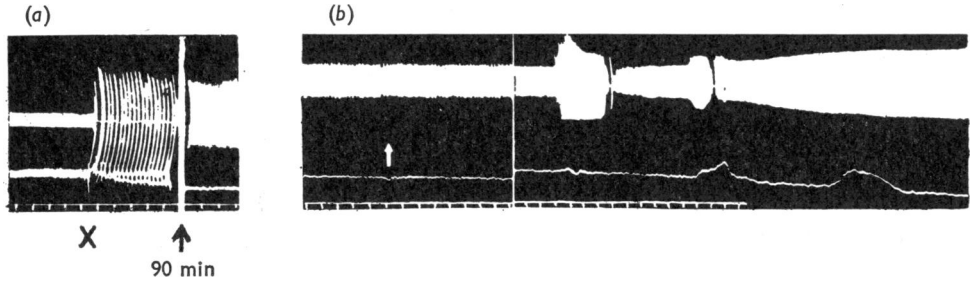

Fig. 6 (*a*). Effects of bilateral vagotomy on respiration and blood pressure in a dog during chloralose anaesthesia. At X the vagi were cut, 90-min interval at the arrow. (*b*) Effects of perfusion of procaine from lateral ventricle to cisterna on respiration and blood pressure in a dog during chloralose anaesthesia, with both vagi cut 3 hr before. At the arrow perfusion was changed from artificial cerebrospinal fluid to 2% procaine. (*a*) and (*b*) are from two different animals. Time marks, 1 min.

the amplitude returned to normal to be followed by a second gradual increase. In two experiments the first effect was a diminution in amplitude with slowing of respiration, but after about 10 min the amplitude increased and became greater than before the previous perfusion; in one experiment there was only a diminution in amplitude of respiration.

In two experiments in which the fourth ventricle was perfused with 2% procaine after bilateral vagotomy, the slowing of respiration was more pronounced than in the experiments with vagi intact. In addition, the procaine perfusion caused an increase in depth of respiration for the first 30 min, then the amplitude of respiration decreased more and more until respiration finally stopped. In both experiments the arterial blood pressure first rose and then fell.

Phrenicotomy. Hill & MacDonald (1935) observed an increase in amplitude of thoracic movements with injections of procaine 10 mg (0.1 ml. of 10% solution) into the upper cervical subarachnoid space and this was explained as being compensatory to phrenic nerve paralysis. In two experiments, in which the phrenic roots were cut in the neck on both sides, we found that perfusion of 2% procaine from lateral ventricle to aqueduct still caused an increase in amplitude of thoracic movements recorded with a stethograph

as well as of the respirations recorded through the trachea. Thus the cause of the increase in amplitude of respiration is not compensatory to phrenic nerve paralysis.

Infusion of procaine into the carotid artery. To assess the effect of procaine reaching the brain through the circulation in comparison with effects obtained on intraventricular administration, 2% procaine was infused at a rate of 0.1 ml./min into the carotid artery of a dog during chloralose anaesthesia, by means of a fine polyethylene tube inserted through the thyroid branch. The blood pressure did not show any definite changes; the record of the respiratory movements became irregular due to the convulsions which started in about 30 min to 1 hr after the commencement of the infusion. The convulsions were initially limited to the muscles of the neck, lower jaw, eyeballs and facial muscles, but later extended to the whole body.

DISCUSSION

Our experiments show that procaine injected into the cerebral ventricles or perfused from a lateral ventricle to the cisterna magna produces pronounced changes in respiration. These changes do not result from an action of procaine on structures lining either the lateral or third ventricle because they do not occur when only these parts of the ventricular system are perfused with procaine. They do, however, occur on perfusion of the fourth ventricle. As the procaine perfused through this ventricle also passes through the openings in its lateral recesses to the subarachnoid spaces, the structures on which the procaine may act could be reached from the fourth ventricle or from the subarachnoid space or from both. The vital respiratory centre is situated deep in the floor of the fourth ventricle. The procaine may act on this part of the brain since substances perfused through the cerebral ventricles pass the ependyma and penetrate the brain tissue (Draskoci, Feldberg, Fleischhauer & Haranath, 1960; Feldberg & Fleischhauer, 1960). But the procaine may also reach the chemosensitive superficial structures at the ventrolateral surface of the medulla from where profound changes in respiration can be produced. The changes in respiration produced by intraventricular procaine appear to result from an action at both sites. Applied to the chemosensitive area at the ventrolateral surface of the medulla, procaine produced only respiratory depression, but no deepening of respiration. It would therefore appear that the depression of respiration produced by intraventricular procaine also results from an action on these extracranial chemosensitive structures. For the deepening of respiration one would have to assume another site of action which may be the centre in the floor of the fourth ventricle. The effect could not, however, be the result of blockade by procaine of vagal afferent impulses to this centre because vagotomy did not abolish the effect.

Another site of action must be postulated for the short-lasting loss of consciousness produced by intraventricular injections of procaine into unanaesthetized dogs, an effect which had previously been observed by Haley (1956) on injection of procainamide into the third ventricle of dogs. During the condition of loss of consciousness the dogs did not respond to painful stimuli, but they were not paralysed because deep pressure over the limbs evoked a withdrawal response. In man, too, procaine may produce loss of consciousness. Koster & Wolf (1930) performed mastoid surgery during spinal anaesthesia. Amongst their twenty-seven patients there were fourteen children under the age of eight who did not receive any premedication. These children fell asleep on the operation table. Many substances, and not only anaesthetics, for instance adrenaline, noradrenaline and

calcium chloride, produce sleep or an anaesthesia-like condition when injected intraventricularly. Feldberg (1959), in discussing the clinical and experimental evidence on the problem of general anaesthesia and loss of consciousness, suggested that these substances produce their effect by an action on the diencephalon and in particular on the reticular activating system. Procaine probably also acts on these parts of the brain reached from the third ventricle and aqueduct when producing loss of consciousness on intraventricular injection.

Finally, there is a striking difference in the symptomatology of procaine when it reaches the brain through the blood stream or when applied by the intraventricular route. It is well known that when procaine is absorbed and passes into the blood stream it produces general convulsions. In the present experiments they occurred when the procaine was infused into the carotid artery but not when the procaine was applied by the intraventricular route. Therefore, structures lying in the ventricular walls or below the surface of the brain stem are not the site where procaine acts when producing this effect; the site may be the cerebral cortex.

SUMMARY

1. Procaine (40 mg) injected into the lateral cerebral ventricles of conscious dogs produced loss of consciousness, muscle paresis, nystagmus, retching, vomiting, urination and defaecation, a pronounced increase in depth of respiration and a rise in arterial blood pressure, but no general convulsions. Doses below 5 mg were ineffective, and 100-mg doses quickly depressed respiration and were fatal.

2. Perfusion of 2% procaine from lateral ventricle to cisterna in dogs during chloralose anaesthesia produced a slowing of respiration and an increase in amplitude later followed by depression. Perfusion with higher concentrations of procaine (4 to 8%) rapidly depressed respiration.

3. The changes in respiration did not occur when the procaine was prevented from entering the fourth ventricle and subarachnoid space, that is when perfusion was from a lateral ventricle to the aqueduct. The changes occurred, however, on perfusion of the fourth ventricle when the procaine could also pass into the subarachnoid space.

4. The increase in depth of respiration may result from an action of procaine on the respiratory centre situated in the floor of the fourth ventricle, and the depression of respiration from an action on the extracranial chemosensitive superficial structures on the ventrolateral surface of the medulla reached from the subarachnoid space.

5. The loss of consciousness may result from an action on diencephalic and mesencephalic structures reached from the third ventricle and aqueduct.

6. Whereas no general convulsions occurred when procaine was applied by the intraventricular route, they occurred when the procaine was infused into a carotid artery. The site of action is probably the cerebral cortex.

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