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METHODS FOR PERFUSING DIFFERENT PARTS OF THE CAT'S CEREBRAL VENTRICLES WITH DRUGS

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To determine where a drug acts when passing through the cerebral ventricles and how the responses resulting from its penetration into structures surrounding one part of the ventricular system may affect those from another, methods are required whereby a drug perfusing the ventricles may be either excluded from, or limited to, any given part. The present paper gives a description of such methods.

The first approach to this kind of localization was the exclusion of the subarachnoid space and fourth ventricle from the perfusion. This was achieved by collecting the effluent from a cannula passed through the opened cisterna magna and along the floor of the fourth ventricle into the middle of the aqueduct, instead of from a cannula in the cisterna (Bhattacharya & Feldberg, 1958).

The next modification was the exclusion of the perfused drugs from one lateral ventricle. For this purpose both lateral ventricles were cannulated and then perfused through the two cannulae with separate injectors, one containing the drug and the other artificial c.s.f. The outflow was from the aqueduct. By this arrangement drugs are prevented from entering the lateral ventricle perfused with c.s.f. as the fluid perfused through one lateral ventricle does not enter the other, but passes straight through the third ventricle to the aqueduct (Feldberg & Fleischhauer, 1962). This procedure still leaves large areas of grey matter exposed to the perfused drugs. As indicated in Fig. 1, in the lining of the anterior horn of the lateral ventricle are situated the olfactory grey matter, septum and caudate nucleus, and in the lining of the inferior horn, the hippocampus and amygdala. From the third ventricle perfused drugs may penetrate into nuclei of the hypothalamus and of the thalamus, particularly the massa intermedia and the habenula. From the aqueduct they may enter the central grey matter.

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One further procedure has been described for restricting the perfused area, i.e. the exclusion of the posterior half of the lateral ventricle by compression of its narrow lumen behind the foramen of Monro as well as the implanted cannula, thereby preventing drugs from reaching the hippocampus and amygdala (Carmichael, Feldberg & Fleischhauer, 1963).

Other restrictions have been encountered fortuitously. At the end of each experiment the dye bromophenol blue was substituted for the drugs in order to stain those parts of the ventricular system which had been reached by the drugs; in this way it was found that on occasion drugs had had access to only the third ventricle, or parts of it (Carmichael, Feldberg & Fleischhauer, 1962).

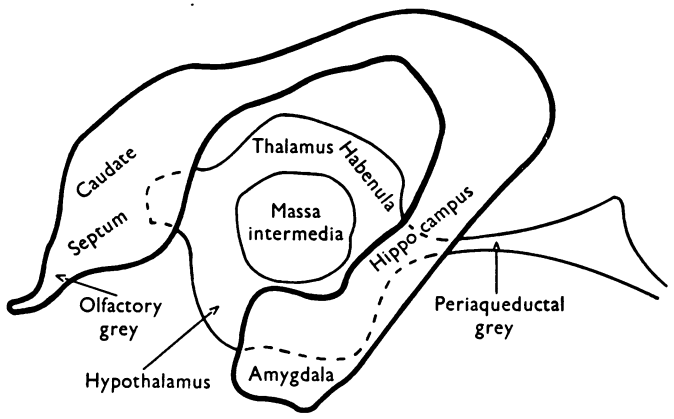

Fig. 1. Diagram of the ventricular system of the cat's brain to illustrate the regions bordering the lateral and third ventricles and aqueduct. Only one (the left) lateral ventricle is shown.

In the present experiments a method of multiple cannulation of the ventricular system has been adopted to exclude drugs from, or limit their perfusion to, a given part. Four or five cannulae are used, each inserted into a different part. One cannula acts as an outflow, the others are attached to separate injectors to serve as inflow cannulae; usually only one delivers the drug, the others, artificial c.s.f. As drugs are denied access to any part cannulated and perfused with artificial c.s.f., variation in choice of cannulae for infusing either drugs or artificial c.s.f., as well as for collecting the effluent, makes it possible to perfuse with drugs separately, or in combination, the anterior horn, the inferior horn and the third ventricle. The perfused drugs may also be limited to the part of the third ventricle lying ventral or dorsal to the massa intermedia.

METHODS

The experiments were carried out on 2·3–3 kg cats anaesthetized with ethyl chloride and ether followed by chloralose (60–65 mg/kg) injected into the cannulated left femoral vein or, in a few experiments, with intraperitoneal pentobarbitone sodium (36 mg/kg). After cannulation of the trachea, with the cat lying on its belly, the head was fixed to the ear bars and the mouthpiece of a Horsley–Clark stereotaxic instrument. The surface of the skull was widely exposed and the muscles covering the atlanto-occipital membrane were dissected away.

Four types of cannulae have been used. (1) *A fine polythene tube*, 2 mm *o.d.*, for cannulation of the aqueduct as described by Bhattacharya & Feldberg (1958). Previously this cannula has been used as the outflow, but in some of the present experiments, when attached to an injector delivering either artificial c.s.f. or drug, it served as an inflow. (2) *Collison cannulae* as described by Feldberg & Sherwood (1953) and modified so that there was only one side hole about 1 mm from the closed tip (Bhawe, 1958), were screwed into the skull for cannulation of the body of each lateral ventricle. To test if the opening of the cannula is in the ventricle, a fine hypodermic needle attached to a tuberculin syringe filled with artificial c.s.f. is inserted through the rubber cap. If the opening is correctly placed, and the syringe is held perpendicular, the artificial c.s.f. in it flows readily by gravity into the ventricle. The cannula on the right serves as an inflow for artificial c.s.f. to exclude the drug from this ventricle, and that on the left either as an inflow or as an outflow. To act as an outflow, the cap with the rubber diaphragm is replaced by one through the central opening of which a tube of 18 gauge has been soldered, projecting 1 cm outside and 3 mm inside the cap. A leak-proof joint with the cannula is ensured by a rubber washer through which the inside tube protrudes. The outside tube is connected to some 10 cm of polythene tubing, the opening of which is so arranged as to be below the level of the aqueduct. (3) *Stainless-steel cannulae* for introduction into the third ventricle and the left anterior and inferior horns. By means of micromanipulators these cannulae are inserted vertically and held in place throughout the experiment. The cannula for the insertion into the third ventricle is 6 cm long, made of hypodermic stainless steel tubing, gauge 22 (0·7 mm *o.d.*, 0·5 mm bore) with either a side opening at its closed tip or an open end. Those for introduction into the anterior and inferior horns of the lateral ventricle always have the side opening. For the inferior horn tubing of 18 gauge (1·2 mm *o.d.*, 0·7 mm bore) is used, and for the anterior horn either 18 or 22 gauge. (4) *Double bore stainless-steel cannula* for insertion into the third ventricle to allow separate perfusion of the parts lying dorsal or ventral to the massa intermedia. This cannula is also inserted vertically by means of a micromanipulator which holds it in place during the experiment. It is shown diagrammatically in Fig. 5. The inner tube is of 22 gauge, projects beyond the outer tube and has a side opening at its closed end. The outer tube of 18 gauge has a side arm at its upper end for attachment to an injector, and at its lower end there is an opening 7·5 mm above that of the inner tube and pointing in the same direction. The cannula has been designed and constructed by Mr A. R. J. Collins.

The holes in the skull for the Collison and the stainless-steel cannulae are drilled at sites plotted with reference to the mid line and to either the coronal suture or a zero line drawn across the exposed skull in the frontal plane above the centres of the ear bars. This line is a projection onto the skull of the inter-aural line of Jasper & Ajmone-Marsan (1954) which connects the centre of each external auditory meatus.

The fluid used for perfusion and for dilution of drugs is the artificial c.s.f. described by Merlis (1940). Its composition is (g/l.): NaCl 8·1; KCl 0·25; $CaCl_2$ 0·14; $MgCl_2$ 0·11; $NaHCO_3$ 1·76; Na_2HPO_4 0·07; $CO(NH_2)_2$ 0·13; and glucose 0·61. The fluid is delivered by each injector at a rate of either 0·05 or 0·1 ml./min.

At the end of every experiment the areas which have been exposed to the drugs are stained with bromophenol blue. For this purpose a 0·2 % solution of this dye, prepared as described

by Feldberg & Fleischhauer (1960), is substituted for the drug and perfusion is continued at the same rate for a further 15 min, which is sufficient for the dye to stain the brain tissue. The solution of dye in the cavities is subsequently washed out by substituting artificial c.s.f. for the bromophenol blue and continuing perfusion until, within a few minutes, the outflow becomes colourless. To fix the brain the head is perfused first with 0·9% NaCl solution, then with 10% formalin through the cannulated aorta after clamping off the heart and cutting the jugular veins. Following exposure of the brain and dissection of the ventricular cavities, the areas stained by the dye and the sites of the opening of the different cannulae within the ventricles are confirmed by inspection.

RESULTS

Figures 2–5 illustrate the methods used. The drug is always prevented from entering the right lateral ventricle by its perfusion with artificial c.s.f. delivered through an implanted Collison cannula. The cannula in this ventricle is not shown in the diagrams, which give the outline of the left lateral ventricle, third ventricle and aqueduct. The arrangements of the other cannulae and the directions of flow used in order to restrict the drugs to a given part of these cavities are shown in the diagrams. Outflow and inflow cannulae are marked by correspondingly directed arrows. The cannula or cannulae delivering drugs are shown in solid black and the parts of the ventricular system exposed to drugs by the shaded areas.

Perfusion with drugs of the whole left lateral ventricle (Fig. 2a)

A Collison cannula, measuring 9–10 mm from the opening in the polythene tubing to the base of the threaded portion, is implanted in each lateral ventricle through the skull at a point 7–9 mm anterior to the zero line and 3–5 mm lateral to the mid line. The openings of the cannulae are directed medially and lie posterior to the foramina of Monro, where the fimbriae become the fornices. Next the aqueduct is cannulated and the flow of artificial c.s.f. is established from both lateral ventricles. After perfusing in this direction for a few minutes a retrograde flow through the third ventricle is initiated by adapting the left ventricular cannula to act as the outflow and by connecting the aqueductal cannula to an injector delivering artificial c.s.f. In order to prevent movements of the aqueductal cannula which may lead to its displacement, it is connected to the polythene tubing attached to the injector by a 2 cm length of metal tubing firmly fixed to a stand. Finally, one cannula is inserted into the anterior, and another into the inferior horn of the left ventricle, and each is attached to an injector delivering artificial c.s.f. To reach the anterior horn the cannula with its opening directed anteriorly is introduced vertically through the skull at a point 1·5–2 mm lateral to the mid line, and level with, or up to 2 mm posterior to, the coronal suture. It is lowered to a depth of 14–16 mm from the dura, where its opening is deep in the anterior horn. The inferior horn is reached by inserting the cannula with its opening directed posteriorly

through the skull at a point 12·5–13 mm lateral to the mid line and 3·5–4·5 mm anterior to the zero line. When lowered vertically to a depth of 16–17 mm below the dura, the opening of the cannula is sited in the extreme end of the inferior horn.

The inflow of artificial c.s.f. is arranged so that 0·1 ml./min is delivered through both the anterior and inferior horn cannulae, and 0·05 ml./min through the cannula in the right lateral ventricle and aqueduct.

After perfusion for some minutes, the artificial c.s.f. delivered through the anterior and inferior horn cannulae is replaced by the solution containing the drugs and perfusion is continued at the same rate.

At the end of the experiments, after perfusing bromophenol blue instead of the drug through the cannulae in both horns, no dye was found to have entered the third or the right lateral ventricle. The absence of staining in these parts was in striking contrast to the deep staining of the surfaces of the left lateral ventricle.

A modification of this method is as follows: instead of perfusing artificial c.s.f. in a retrograde direction through the third ventricle, the aqueductal cannula is closed after a through flow to the lateral ventricles has been established; it is then kept closed throughout the experiment. The rate of artificial c.s.f. delivered through the right lateral ventricle is 0·1 ml./min instead of 0·05 ml./min.

Staining with bromophenol blue reveals that with this method, too, no dye reached the third or the right lateral ventricle. It is essential, however, that during the perfusion the aqueductal cannula remains in its proper position for if there are slight movements of the cannula, or if it does not fit the aqueduct snugly, some of the drug delivered into the left lateral will enter the third ventricle.

Perfusion with drugs of either the anterior or posterior part of the left lateral ventricle (Fig. 2b and c)

The arrangement of cannulae is the same as for perfusion of the whole left lateral ventricle, but if the drugs are to be restricted to the posterior part they are delivered only through the inferior horn cannula at 0·05 or 0·1 ml./min, whereas artificial c.s.f. at 0·1 ml./min is perfused through the anterior horn. For perfusion of drugs through the anterior part alone, this procedure is reversed. The modification of closing the aqueductal cannula as used for perfusion of the drug through the whole lateral ventricle is also applicable to perfusion of its anterior or posterior parts.

Staining by bromophenol blue at the end of these experiments showed that the dye had not entered the third ventricle or the opposite right lateral ventricle. When the dye was perfused through the inferior horn there was no staining anterior to the outflow cannula, but the hippocampus and

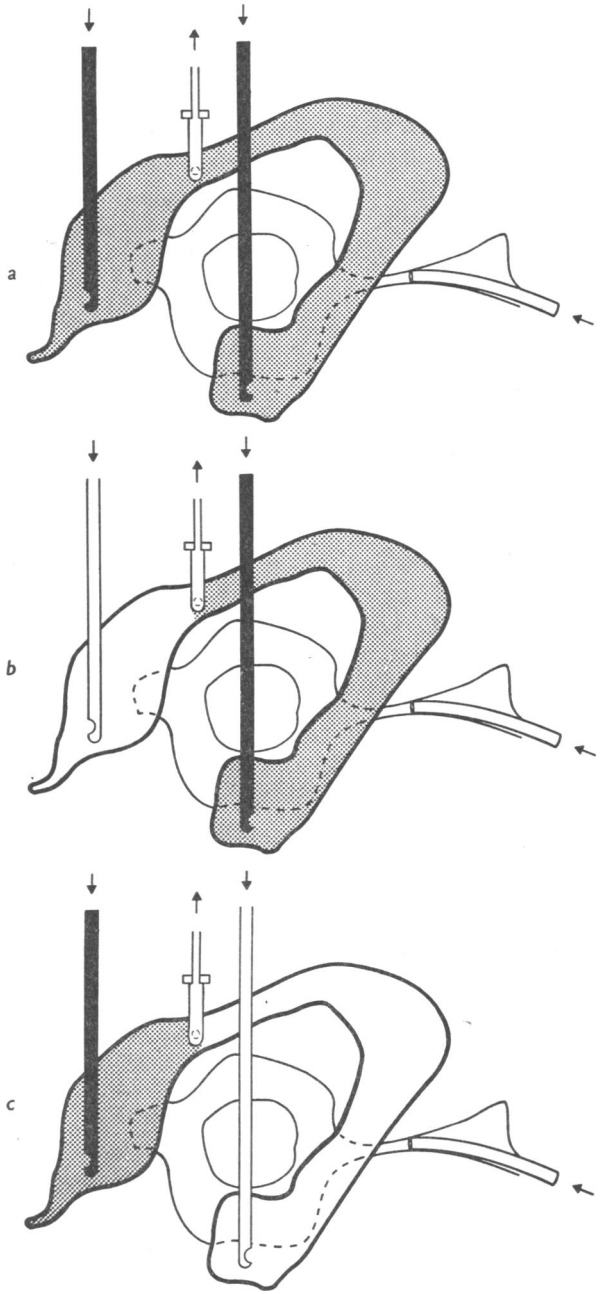

Fig. 2. Diagrams to illustrate perfusion with drugs of the whole (*a*), the anterior (*b*), and posterior part (*c*), of the left lateral ventricle. Outflow and inflow cannulae marked by arrows correspondingly directed. Cannulae delivering drugs in solid black and parts perfused with drugs shaded.

amygdala were stained deeply. With the perfusion of dye through the anterior horn, there was no staining in the left ventricle, posterior to the outflow cannula, but the walls anterior to it, including the olfactory recess,

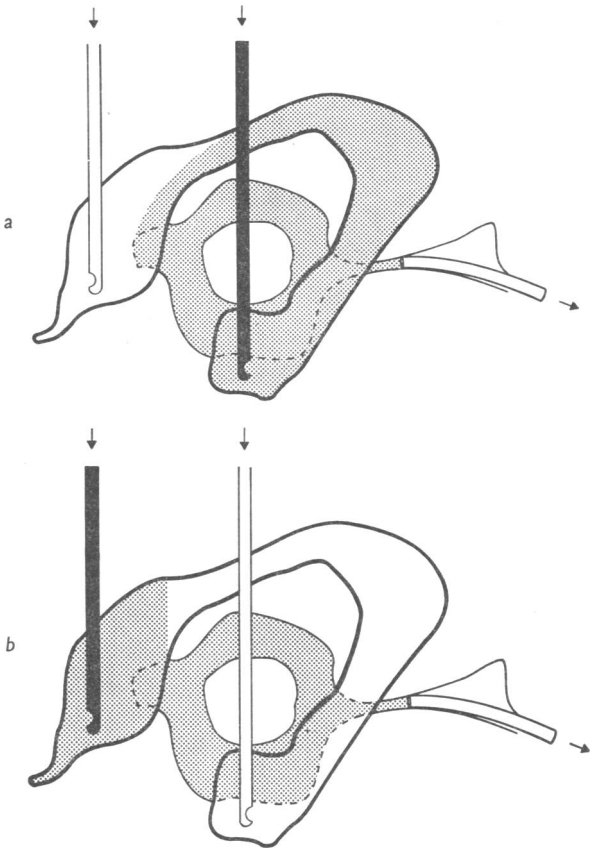

Fig. 3. Diagrams to illustrate perfusion with drugs of either the anterior (*a*) or posterior (*b*) part of the left lateral ventricle together with the third ventricle. Outflow and inflow cannulae marked by arrows correspondingly directed. Cannulae delivering drugs in solid black and parts perfused with drugs shaded.

were deeply stained. However, immediately anterior to the foramen of Monro a small area in the opposing surfaces of the septum and of the caudate nucleus was unstained or only lightly stained.

Perfusion with drugs of either the anterior or posterior part of the left lateral ventricle and the third ventricle (Fig. 3a and b)

The purpose of these experiments is to exclude the drug from either the anterior or the inferior horn of the left lateral ventricle. Two cannulae are

inserted into this ventricle, one into each horn, by the technique described in the previous section. A Collison cannula in the right lateral ventricle serves for perfusion with artificial c.s.f. at 0·05 ml./min and a cannula in the aqueduct as outflow.

In order to perfuse drugs through the posterior part of the left lateral ventricle and through the third ventricle an injector delivering the solution containing the drug at 0·05 or 0·1 ml./min is connected to the inferior horn cannula, and another delivering artificial c.s.f. at 0·1 ml./min to the anterior horn cannula. In order to perfuse drugs through the anterior part of the left lateral ventricle and through the third ventricle the procedure is reversed. In some of these experiments the cannula in the inferior horn was replaced by a Collison cannula inserted in the body of the left ventricle.

Final perfusion of bromophenol blue through the inferior horn cannula stained the hippocampus and amygdala; the walls of the anterior horn were unstained with the exception of a small area of the caudate nucleus and septum near the foramen of Monro. Dye perfused through the anterior horn deeply stained the olfactory recess, the whole of the septum and the caudate nucleus, but no structure in the inferior horn was stained. In both instances the rostral end of the aqueduct and the third ventricle were stained but in the third ventricle the staining was uneven. When the dye was perfused through the anterior horn cannula, that part of the third ventricle ventral to the massa intermedia was deeply stained, while that dorsal to it was lightly stained, often not at all. The distribution of staining was reversed when the dye entered from the cannula in the inferior horn or from that in the body of the left ventricle. Then that part dorsal to the massa was deeply stained, while that ventral slightly or not at all. This uneven staining of the third ventricle indicated that fluid entering it from a point anterior to the foramen of Monro tends to flow on its way to the aqueduct ventral to the massa intermedia, while fluid entering from a point posterior to the foramen flows mainly dorsal to the massa.

Perfusion with drugs of the third ventricle (Fig. 4a and b)

Two procedures have been developed. In the first (diagram *a*) the drugs are perfused in a retrograde direction from the aqueduct to an outflow cannula in the body of the left lateral ventricle. The arrangement of the cannulae is essentially the same as described for drug perfusion through a whole lateral ventricle (p. 357) but instead of perfusing the drug through the anterior and inferior horn it is delivered through the aqueductal cannula. Further, the Collison cannula in the left lateral ventricle for collecting the outflow is inserted more anteriorly at a point 9–12 mm anterior to the zero line and 5 mm lateral to the mid line. In addition, instead of cannulating the inferior horn, it is sufficient to perfuse the posterior part

of the left lateral ventricle from a Collison cannula inserted at a point 3 mm anterior to the zero line and 7 mm lateral to the mid line.

Before drug perfusion through the aqueductal cannula, artificial c.s.f. is delivered through it and the other inflow cannulae. Inflows are set at 0·1 ml./min into the aqueduct and into the anterior horn, and at

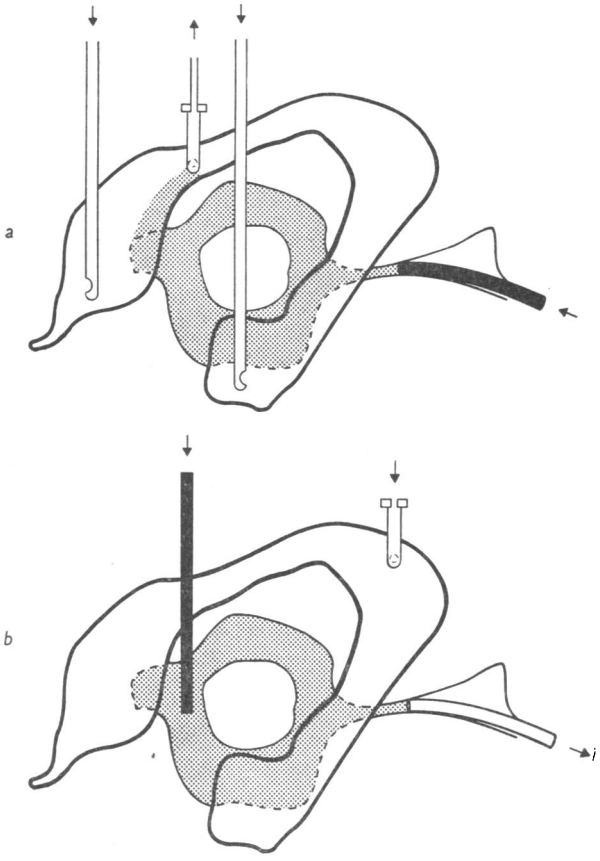

Fig. 4. Diagrams to illustrate two methods for perfusing the entire third ventricle with drugs. Outflow and inflow cannulae marked by arrows correspondingly directed. Cannulae delivering drugs in solid black and parts perfused with drugs shaded.

0·05 ml./min into the inferior horn and the right ventricle. After a few minutes of perfusion the injector delivering artificial c.s.f. to the aqueduct is changed for one delivering the drug solution at the same or half the rate. Drugs will then pass undiluted through the rostral part of the aqueduct and the third ventricle except for the small region between the foramina of Monro where the artificial c.s.f. passing from the right to the left ventricle causes some dilution.

When dye was substituted at the end of the experiment, the rostral part of the aqueduct and the walls of the third ventricle were deeply stained. In addition, a small region of white matter between the left foramen of Monro and the outflow cannula was lightly stained, including parts of the fornix, of the stratum subcallosum and of the stria terminalis. With the exception of this small area, the walls of the lateral ventricles remained unstained, indicating that the perfused drugs would not have reached the nuclear masses lining the lateral ventricles.

In the second method (diagram *b*) the cannula delivering the drug is inserted into the third ventricle, the outflow is through the aqueduct while the entrance of the drug to both lateral ventricles is prevented by their perfusion with artificial c.s.f. A Collison cannula is implanted in each lateral ventricle through the skull at a point 3 mm anterior to the zero line and 7 mm lateral to the mid line. Artificial c.s.f. (0·05 ml./min) is perfused through each cannula. The outflow is through the aqueductal cannula. Once perfusion has been established the third ventricle is cannulated. The point of insertion of the cannula is in the mid line 11–12 mm in front of the zero line. A rectangular opening, 6×8 mm with its longer axis parallel to the mid line, is made in the skull mainly to the left side but extending slightly to the right across the sagittal suture so that the superior sagittal sinus becomes visible. The dura is opened over the left hemisphere and reflected to expose the cleft between the falx and the medial surface of the left hemisphere. After gentle retraction of the sagittal sinus to the right, the cannula filled with artificial c.s.f. is lowered with the micromanipulator either 15–17 mm or 20–22 mm from the dura. The opening of the cannula should now lie in the third ventricle anterior to, and either above or below the massa intermedia. In diagram *b* an intermediate position is shown. An injector delivering artificial c.s.f. at 0·1 ml./min is then attached to this cannula. Should the outflow not increase immediately, the cannula is raised or lowered 1–2 mm as its opening may have impinged on the anterior edge of the massa intermedia. After perfusion with artificial c.s.f. for some minutes the drug solution is perfused at the same rate.

Bromophenol blue perfused at the end of the experiments stained the third ventricle and the rostral part of the aqueduct, but did not enter the lateral ventricles. The regions stained in the third ventricle depended to a certain extent on the position of the opening of the cannula. With the opening above the massa intermedia there was intense staining of the thalamus, the habenula, and particularly the upper rim of the massa, but the staining of the hypothalamus varied and was sometimes absent. With the opening below the massa the hypothalamus and the lower rim of the massa were intensely stained but the staining of the parts above the massa varied and was sometimes absent.

If the drug was perfused through the cannula in the third ventricle without simultaneous irrigation of the lateral ventricles with artificial c.s.f., some of the drug reached these ventricles as revealed by subsequent perfusion with bromophenol blue.

Perfusion with drugs of the dorsal and ventral halves of the third ventricle

Two methods have been used. One is based on the finding that fluid entering the third from a lateral ventricle tends to pass dorsal or ventral to the massa intermedia on its way to the aqueduct according to whether the inflow cannula is situated anterior or posterior to the foramen of Monro. This method, based on directional flow, has not given consistent results. The other method makes use of the double bore cannula which, on insertion into the third ventricle, allows two streams of fluid to perfuse separately the regions dorsal and ventral to the massa intermedia.

Perfusion by directional flow. To perfuse drugs through the dorsal half of the third ventricle the drug solution is delivered, at 0·1 ml./min, from a Collison cannula lying in the body of the left ventricle posterior to the foramen of Monro. This cannula is implanted through the skull at a point 7·5–8 mm in front of the zero line and 5 mm lateral to the mid line. Artificial c.s.f. is perfused at the same rate from a cannula in the anterior horn and at half the rate from both a cannula in the inferior horn and a Collison cannula in the right lateral ventricle; the outflow is from the aqueductal cannula. Perfused bromophenol blue was taken up mainly by the walls of the third ventricle dorsal to the massa intermedia. Frequently, however, the staining involved the full circumference of the massa which on a mid-sagittal section was outlined by a blue ring. On the other hand, the walls ventral to the massa were usually lightly or not stained. The only other parts stained were the rostral end of the aqueduct and a small region of white matter in the left lateral ventricle situated between the cannula delivering the dye and the foramen of Monro.

To perfuse drugs through the ventral half of the third ventricle the arrangement of cannulae is the same except that the inferior horn is not cannulated, and the drugs are delivered through the cannula in the anterior horn, which is, however, inserted through the skull at a point 2–3 mm posterior to the coronal suture so as to bring its opening (directed posteriorly) close to the foramen of Monro. On perfusion of bromophenol blue the walls of the third ventricle lying ventral to the massa intermedia were deeply stained; dorsal to the massa the staining varied from none at all to deep. Even though the cannula in the anterior horn has its opening close to the foramen of Monro, some staining of parts of the left caudate nucleus and septum is unavoidable.

Perfusion with the double bore cannula (*Fig. 5*). The procedure for inserting

the cannulae is the same as described for perfusion of the third ventricle through a single bore cannula (see p. 360). First, both lateral ventricles are cannulated with Collison cannulae, and flow of artificial c.s.f. at 0·05 ml./min is established from each cannula to the aqueduct. Then the double bore cannula filled with artificial c.s.f. is lowered with the micromanipulator 22–23 mm from the dura. The opening of the inner tube should now lie in the third ventricle ventral, and that of the outer tube dorsal, to the massa intermedia (Fig. 5).

If, on starting perfusion with artificial c.s.f. at 0·1 ml./min through the inner tube the outflow from the aqueduct does not increase immediately,

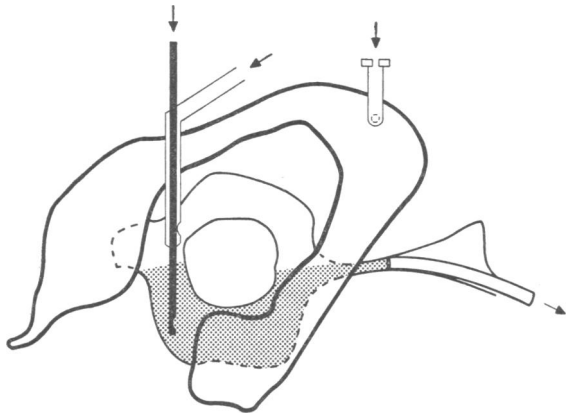

Fig. 5. Diagram to illustrate perfusion with drugs of the part of the third ventricle lying ventral to the massa intermedia. Outflow and inflow cannulae marked by arrows correspondingly directed. The inner tube cannula delivering drugs in solid black and the part perfused with the drug shaded.

raising or lowering the cannula 1–2 mm establishes the correct increase in outflow. Next, artificial c.s.f. at 0·05 ml./min is delivered to the outer tube. Usually this is quickly followed by the appropriate increase in outflow from the aqueduct. Should this increase not occur small readjustments in the depth of the cannula of < 1 mm are sufficient to bring this about without impeding the flow from the inner tube.

For perfusion of drugs through the ventral half of the third ventricle the artificial c.s.f. perfused through the inner tube at 0·1 ml./min is replaced by the drug solution at the same rate. On the other hand, to perfuse drugs through the dorsal half the artificial c.s.f. delivered to the outer tube at 0·05 ml./min is replaced by the drug solution and perfusion is continued at the same rate.

Drugs perfused through either the inner or the outer tube do not enter the lateral ventricles as seen from the absence of their staining with dye at

the end of these experiments. Dye perfused through the inner tube stained the walls of the third ventricle lying ventral to the massa intermedia, but the parts lying dorsal to it remained unstained. The staining also included the rim of the ventral part of the massa intermedia and the rostral end of the aqueduct (Fig. 5). Dye perfused through the outer tube stained those parts which had remained unstained on perfusing the dye through the inner tube. In some of these experiments the whole circumference of the massa was stained and stood out as a blue ring when the brain was cut sagittally in the mid line. The staining of the parts dorsal to the massa varied; the regions containing the habenulae were stained deeply, lightly, or not at all. The rostral part of the aqueduct was stained, though unevenly, often the dorsal surface was more deeply stained than the ventral, but occasionally vice versa.

There are certain pitfalls associated with the insertion of the double bore cannula into the third ventricle. Owing to the variations in size and shape of the massa intermedia the cannula may be found at post-mortem to have pierced the massa, or injured its anterior edge. When this had occurred, it was found that the dye from the outer tube had penetrated the grey matter of the massa from the site of injury. In other experiments the cannula was found at post-mortem to have injured the septum and dye from the outer tube had penetrated this structure from the injured site. In such experiments the perfused drugs must have reached the stained areas as well.

DISCUSSION

The principle used in the present experiments to limit perfusion of drugs through a given part of the ventricular system is based on the finding that drugs are prevented from entering the other parts by their perfusion with artificial c.s.f. The technique consists of multiple cannulation of the ventricular system, the cannulae being inserted into different parts and attached to separate injectors, but only one or two delivering the drug, the others artificial c.s.f. With this technique it is possible to limit the action of drugs to structures lining one or the other part of the ventricular system. By allowing the drug to perfuse through more than one part it is further possible to find out how the effects produced by its action on one part are influenced by its action on the other part. On the other hand, the method does not differentiate between the various structures which lie in the walls of the same part of the ventricular system, any of which may be the site of action. However, from knowledge available of the function of these structures it may be possible to decide which one must have been affected by the drug; such precise localization may further be achieved by combining the method with electrical recording from the structures in contact with the perfusing drug.

To determine the sites of drug action in the brain is a cardinal problem of the pharmacology of the central nervous system. With the method of multiple cannulation the site of action of a drug producing a particular effect following its injection into the cerebral ventricles can be readily determined. Even though precise localization is not attainable, the number of likely structures on which the drug may be acting is greatly reduced. Finally, the deductions made about the sites of drug action on perfusion of the ventricles are valid in the unanaesthetized cat when, on intraventricular injection, the signs are similar.

SUMMARY

1. Methods are described by which, in the anaesthetized cat, parts of the cerebral ventricles may be perfused with a drug.

2. For this purpose several cannulae are inserted, each into a different part of the ventricular system.

3. All cannulae, except one acting as an outflow, are attached to separate injectors and serve as inflows. One, at most two, deliver the drug, the others artificial c.s.f.

4. As the drug is denied access to any part cannulated and perfused with artificial c.s.f., variations in the choice of cannulae for perfusing either the drug or artificial c.s.f., as well as for collecting the outflow, make it possible to perfuse separately or in combination the anterior part, the posterior part of the lateral ventricle, and the third ventricle, or its ventral or dorsal half.

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