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MORPHINE HYPERGLYCAEMIA

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SUMMARY

1. To find the site where morphine acts when producing hyperglycaemia on injection into the cerebral ventricles in unanaesthetized cats, morphine sulphate was infused or injected through an implanted Collison cannula into different parts of the liquor space in an amount of 0·75 mg except on microinfusion into the posterior hypothalamus, when the amounts were 80 or 160 μg. The glucose was determined in blood samples collected from the inferior vena cava.

2. Microinfusions of morphine into the posterior hypothalamus did not produce hyperglycaemia.

3. Infusion of morphine into the liquor space at the entrance of the aqueduct or of the fourth ventricle produced hyperglycaemia. Any structures in the walls of the third ventricle as well as the peri-aqueductal grey are thus excluded as the site of action.

4. Infusion of morphine into the subarachnoid space just above the corpora quadrigemina or below the ventral surface of the brain stem produced hyperglycaemia. With these routes the morphine does not enter any part of the ventricular cavities and the action would appear to be on structures at the ventral surface of the brain stem.

5. Injection of morphine into the cisterna magna produces hyperglycaemia when the doses are larger than those already effective on injection into the cerebral ventricles. This also suggests an action on structures at the ventral surface of the brain stem, as this surface is reached more readily from the ventricles than from the cisterna.

6. It is concluded that on injection into the cerebral ventricles, the morphine has to pass into the subarachnoid space, through the foramina of Luschka, in order to produce hyperglycaemia. It then reaches the ventral surface of the brain stem and probably acts there on structures in the upper part of the medulla oblongata.

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7. Infusion of morphine into the corpora quadrigemina near the caudal end of the superior corpora can produce profound hypoglycaemia.

8. Anaesthesia depresses the morphine hyperglycaemia, but when the dose injected into the cerebral ventricles is increased four times or more, hyperglycaemia is also produced in pentobarbitone sodium anaesthesia.

INTRODUCTION

Morphine injected into the cerebral ventricles of unanaesthetized cats produces hyperglycaemia which is antagonized by nalorphine similarly applied (Borison, Fishburn, Bhide & McCarthy, 1962). The site where morphine acts when producing hyperglycaemia is not known. Borison *et al.* concluded that it acts on structures in the ventricular wall that 'lie in the vicinity of the intraventricular foramen of Monro'. But Feldberg & Shaligram (1972) found that when morphine was injected in a small volume into the third ventricle, either close to the intraventricular foramen, i.e. rostral to the massa intermedia, or away from the foramen, i.e. caudal to the massa, hyperglycaemia occurred only on injection caudal to the massa. They pointed out that in this experiment the morphine may have passed in effective concentration into the aqueduct, or into the fourth ventricle, or even through the foramina of Luschka into the subarachnoid space. So the hyperglycaemia produced may have been due to an action on the peri-aqueductal grey or to an action on structures in the floor of the fourth ventricle, or on superficial structures at the ventral surface of the brain stem. In the present experiments, these possibilities have been examined by making infusions or injections of morphine into different parts of the liquor space.

Doses of morphine which produce hyperglycaemia when injected into the cerebral ventricles of unanaesthetized cats are known to be ineffective in anaesthetized cats. In the present experiments it was shown that by increasing the dose, a hyperglycaemic response is also obtained under anaesthesia.

Most of the results obtained have been communicated at a Meeting of the Physiological Society (Feldberg & Gupta, 1972).

METHODS

Cats of either sex weighing between 3 and 4·1 kg were used. For introducing morphine into different parts of the liquor space, a Collison cannula, as modified by Mr A. R. J. Collins, and described previously (Feldberg & Shaligram, 1972) was implanted under pentobarbitone sodium (36 mg/kg I.P.) anaesthesia in an aseptic operation. The cat, lying on its belly, had its head fixed to the ear bars and mouth-piece of a stereotaxic instrument. With the exception of cannulation of the lateral

ventricle, the Collison cannula to be implanted was held by a micromanipulator at the correct angle so that it could be moved millimetre by millimetre.

For implantation of the cannula into the lateral ventricle, the method described by Feldberg & Shaligram (1972) and for implantation into the posterior hypothalamus, that described by Feldberg & Saxena (1971), was used.

For implantation of the cannula, so that its tip lay either at the entrance of the aqueduct or at the entrance of the fourth ventricle, or above the corpora quadrigemina, or in its substance, the Collison cannula had a shaft of 20–22 mm and was inserted in the mid line.

For implantation at the entrance of the aqueduct (see diagram *A* of Fig. 1), the cannula was inserted vertically at a point 5 mm anterior to the interaural line. Before insertion, the rubber cap of the cannula was pierced by a needle attached to a syringe filled with saline solution. The cannula was first lowered to nearly the correct depth which was 19–19·5 mm below the dura. For the last part it was slowly lowered whilst the syringe was kept in a vertical position. When the tip of the cannula entered the aqueduct, the saline solution flowed freely from the syringe into the cannula.

For implantation of the cannula at the entrance of the fourth ventricle (see diagram *B* of Fig. 1) the cannula was inserted 6–6·5 mm anterior to the interaural line at an angle of 20° directed posteriorly until the tip hit the tentorium. The cannula was then moved with the micromanipulator 1–2 mm anteriorly so that its tip could pass in front of the tentorium. The cannula was then further lowered. Again the rubber cap was pierced by a needle attached to a syringe filled with saline solution so that the flow of saline solution from the syringe could be observed when the tip of the cannula reached the entrance of the fourth ventricle. This happened when the cannula had been inserted between 19 and 21·5 mm beyond the dura.

For implantation of the cannula above or into the substance of the corpora quadrigemina (see diagrams of Fig. 2) the procedure was the same as for implantation at the entrance of the fourth ventricle, but the cannula was inserted only between 15 and 18·5 mm beyond the dura.

For implantation of the cannula at the ventral surface of the brain stem (see diagrams of Fig. 3) a Collison cannula with a shaft 39 to 40 mm long was inserted in the mid line at an angle of 28° directed posteriorly. It was inserted about 7 mm anterior to the interaural line so as to pass just in front of the tentorium and lowered until it touched the basal plate of the skull. This occurred after 31–33 mm insertion beyond the dura. The cannula was then raised 1 mm. In all other details the implantation was similar to that at the entrance of the fourth ventricle.

For injections into the cisterna magna, a Collison cannula was fixed to the back of the skull as described by Feldberg, Gupta, Milton & Wendlandt (1973).

When not otherwise stated, the actual experiment of injecting of infusing morphine through the implanted cannulae was made without anaesthesia 7 days after the operation when the animals had fully recovered. The cat was kept without food, but not without water, for the last 24 hr.

The morphine used was the sulphate and all values in the text refer to the salt. The morphine was dissolved in a 0·9% NaCl solution. With the exception of the experiments in which the morphine was introduced into the cisterna magna, which was done by simple injection, the morphine was introduced into the liquor space by microinfusion. The rate of infusion varied according to the volume of fluid introduced. It was 1 μl./min when the volume was 4 μl. and 10 μl./min when it was 20 or 40 μl.

The method for applying morphine bilaterally in anaesthetized cats by means of Perspex rings to the ventral surface of the brain stem at a region just caudal to the trapezoid bodies and lateral to the pyramids, was the same as described previously

(Feldberg & Guertzenstein, 1972; Guertzenstein, 1973). The volume of morphine solution placed inside each ring was about 20 μl. and the correct placement of the rings was later ascertained by placing 0·2% bromophenol blue into the rings and washing out the dye after a few minutes and removing the rings.

In order to assess the spread of the morphine solution in the different experiments a 0·2% bromophenol blue solution was injected or infused through the implanted cannulae in the same volume as the morphine solution used. The cat was killed 20 min later under pentobarbitone sodium anaesthesia, the head perfused with 10% formalin saline solution, the brain removed and the staining of the ventricular walls and of the outer surface of the brain and the brain stem observed with the naked eye. In the experiments with microinfusion of morphine into the posterior hypothalamus, the same procedures were adopted, and the correct placement of the cannula was ascertained by making frozen sections of the formalin perfused brain.

The collection of venous blood from the vena cava for blood glucose estimation and the estimation by the glucose oxidase method were in all details the same as described by Feldberg & Shaligram (1972).

TABLE 1. Blood glucose levels expressed as percentage of normal in unanaesthetized cats following an injection into the posterior hypothalamus of 80 μg morphine or 0·9% NaCl solution in a volume of 4 μl

	Injections of morphine							Injection of 0·9% NaCl
Min after injection	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6*	Mean	Expt. 7
15–20	94	85	—	—	85	120	96	112
30	106	91	104	75	94	116	97	112
60	106	109	123	99	117	124	113	115
120	118	133	139	108	97	116	119	133
180	118	130	162	—	154	139	141	151
220–240	114	—	153	139	154	147	142	—

The injection cannula was implanted 1·5 mm lateral to mid line, 9 mm above interaural plane and either 7 mm (Expts. 1–3) or 8 mm (Expts. 4–7) anterior to the interaural line.

* Injection of 160 μg morphine.

RESULTS

Experiments in unanaesthetized cats

Infusion into the posterior hypothalamus

Feldberg & Shaligram (1972) obtained no hyperglycaemia on infusion of morphine into the anterior hypothalamus. In one experiment they infused morphine also into the posterior hypothalamus and again no hyperglycaemia occurred. The result of this single experiment has been confirmed in the present experiments.

Following the infusion of 80 or 160 μg morphine into the region of the posterior hypothalamus there was a gradual rise in blood glucose, usually preceded during the first 15–30 min by a small fall. However, a control

infusion of 0·9 % NaCl solution into the posterior hypothalamus produced a gradual rise as well, although not preceded by a fall. As shown in Table 1, the mean gradual rise obtained in six experiments with morphine was of the same order as that obtained in Expt. no. 7, with saline solution.

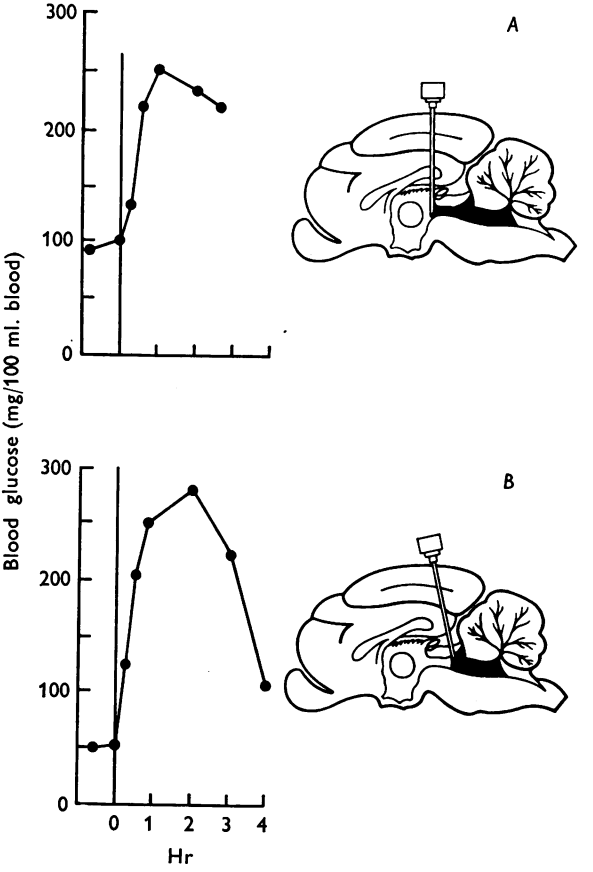

Fig. 1. Effect on blood glucose in two unanaesthetized cats of 0·75 mg morphine injected at 0 time through a permanently implanted cannula, the tip of which lay at the entrance of the aqueduct in cat *A*, and at the entrance of the fourth ventricle in cat *B*. The diagrams give the position of the cannulae and the black areas indicate the staining when bromophenol blue was injected instead of morphine (for details see text).

Infusion or injections into different parts of the liquor space

Figs. 1 and 2 show hyperglycaemic responses to 0·75 mg morphine infused into different parts of the liquor space, and the accompanying diagrams the positions of the Collison cannulae through which the infusions were made. The black and shaded areas indicate the spread of the

injected morphine. They show the regions which became stained when, at the end of the experiment, a solution of bromophenol blue was infused through these cannulae in the same volume as was used for the morphine infusion.

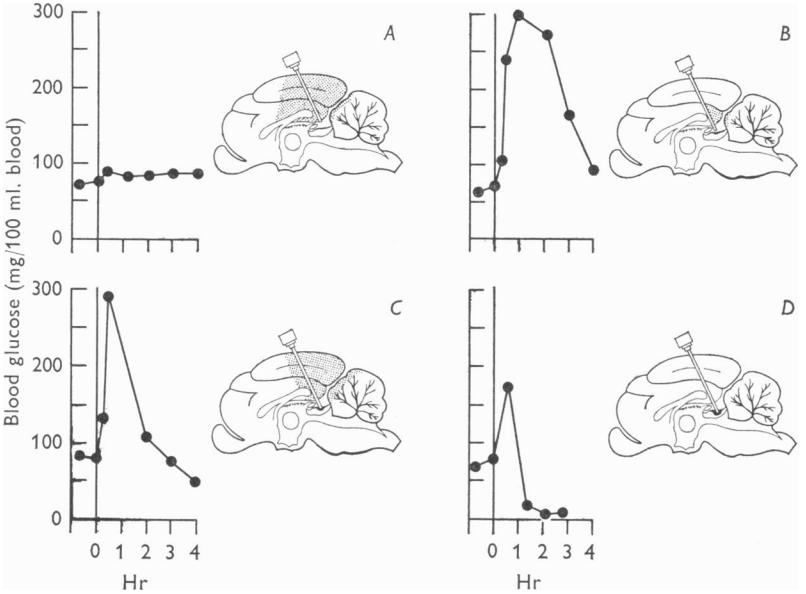

Fig. 2. Effect on blood glucose in four unanaesthetized cats of 0·75 mg morphine injected at 0 time through a permanently implanted cannula, the tip of which lay above or within the substance of the corpora quadrigemina as indicated in the diagrams. The dotted and black areas indicate the staining when bromophenol blue was injected instead of morphine (for details see text).

Aqueduct. In the Expt. *A* of Fig. 1, the tip of the Collison cannula lay just beyond the third ventricle at the entrance of the aqueduct. The morphine infused through this cannula in a volume of 20 μl. produced pronounced hyperglycaemia although it did not enter the third ventricle, the walls of which remained unstained when 20 μl. of a solution of bromophenol blue was infused through this cannula. Thus the hyperglycaemia did not result from an action on structures in the walls of the third ventricle. On the other hand, there was deep staining of the aqueduct and of the floor of the fourth ventricle, but only up to the middle of it. Here the staining ended abruptly as the dye passed through the foramina at the lateral recesses into the subarachnoid space where it produced staining of the vessels at the ventral surface of the brain stem. This proved that the dye had reached the ventral surface though in a concentration not sufficient

to stain the brain tissue itself. This occurs only with somewhat higher concentrations.

Fourth ventricle. In the Expt. *B* of Fig. 1, the tip of the Collison cannula lay beyond the aqueduct at the entrance of the fourth ventricle. The morphine infused through this cannula in a volume of 40 μl. produced strong hyperglycaemia during the following 3 hr although the morphine did not enter the aqueduct which remained unstained when 40 μl. of a solution of bromophenol blue was infused through the cannula. This finding excludes not only the third ventricle, but also the peri-aqueductal gray as the site of the hyperglycaemic action of morphine. There was again deep staining of the floor of the fourth ventricle up to the middle of it. At the ventral surface of the brain stem not only the vessels but also the brain surface itself was stained. In another, similar, experiment the hyperglycaemia lasted longer; blood glucose was still 240 mg/100 ml. 4 hr after the injection.

Above and into the corpora quadrigemina. The exact position of the tip of the cannula was critical. The result depended on whether the tip lay some distance away from, or very close to the surface of the corpora, or whether it had penetrated the corpora. This is shown by the experiments of Fig. 2. In these, the morphine as well as the bromophenol blue was infused in a volume of 40 μl.

When the tip of the cannula lay 2–3 mm above the corpora, as in the Expt. *A*, the morphine infusion had no effect on the blood glucose level. From the staining produced when bromophenol blue was infused instead, it was evident that the dye had not entered the ventricular system. It had mainly spread along the medial surface of the cerebral cortices and along the anterior surface of the cerebellum. There was some staining of the corpora but no staining of the ventral surface of the brain stem. This finding shows that an effect of morphine on the cerebral and cerebellar surface had no effect on the blood glucose level.

When the tip of the cannula lay just above the corpora, as in the Expt. *B*, the morphine infusion produced strong hyperglycaemia. From the staining produced by the bromophenol blue it was again evident that the dye had not entered the ventricular system. There was staining on the medial surface of the cerebral cortices and on the anterior surface of the cerebellum, but not as extensive and widespread as in Expt. *A*. But the corpora were deeply stained and the dye had passed along the side of the brain stem to its ventral surface which was stained. This finding shows that the morphine does not need to enter any part of the ventricular system in order to produce hyperglycaemia.

When the tip of the cannula had penetrated the tissue of the corpora quadrigemina, the infusion of morphine resulted in hyperglycaemia

followed by hypoglycaemia. The extent of the hypoglycaemia appeared to depend on the depth of penetration. This is illustrated by the Expts. *C* and *D* of Fig. 2.

In the Expt. *C*, the tip had just penetrated the caudal end of the superior quadrigemina in the mid line. After the morphine infusion the blood glucose rose from 74 to 284 mg/100 ml. within half an hour; but this high level was not maintained; 2 hr after the injection, it had fallen to nearly pre-infusion level, and after another 2 hr to below this level, to 43 mg/100 ml. After the bromophenol blue infusion, no dye was found in the ventricular system, but the surface of the corpora and the tissue around the tip of the cannula were deeply stained. The dye had spread along the medial surface of the cerebral cortices, along the anterior surface of the cerebellum and on to the ventral surface of the brain stem. In the Expt. *D*, the tip of the cannula had penetrated the corpora at about the same point as in the Expt. *C*, but the penetration was deeper. The morphine infusion produced a rise of the blood glucose level from 78 to 172 mg/100 ml. during the first half hour followed by a fall to 13 mg/100 ml. in the sample collected 80 min after the infusion. In the sample collected about 2 hr after the infusion, the glucose level fell to less than 5 mg/100 ml. and in the next sample, collected after another hour, the level was 8 mg/100 ml. Immediately after collection of this sample an I.P. injection of pentobarbitone sodium was given to anaesthetize the cat. The injection, however, resulted in death, and as the bromophenol blue infusion was made after death, the exact spread of the dye as it would have occurred during life could not be ascertained. The surface of the corpora and the tissue around the tip of the cannula were found to be deeply stained and there was no dye in the ventricles.

Ventral surface of the brain stem. The hyperglycaemic responses shown in Fig. 3 were obtained in two experiments in which the Collison cannulae were passed in the mid line through the whole substance of the brain stem so that their tips pierced the pia mater on the ventral surface. The hyperglycaemic responses produced by the morphine infused through these cannulae were not as pronounced as in the Expts. *B*, *C* and *D* of Fig. 2, or as in those of Fig. 1. This may be due to the fact that the morphine had apparently spread only, or mainly, along one side of the ventral surface of the brain stem because, in both experiments, the main staining after injection of bromophenol blue was on the left side in a region which extended laterally 5–6 mm from the mid line and caudally 7–8 mm from the trapezoid body. In the Expt. *A*, there was some staining along the lower part of the cannula tract, but nowhere in the ventricular system. In the Expt. *B*, there was some staining along the whole cannula track through the brain stem, in the rostral part of the floor of the fourth

ventricle, particularly around the entry of the cannula, and very faint staining in the distal part of the aqueduct, but none in the third ventricle.

The implantation of the cannula itself seemed to produce hyperglycaemia which varied in degree and duration. The result shown for cat *A* in Fig. 3, was obtained 5 days after implantation of the cannula. On the day following implantation the blood glucose level was 224 and 238 mg/100 ml. in two samples of blood collected at an interval of one hour, and did not rise

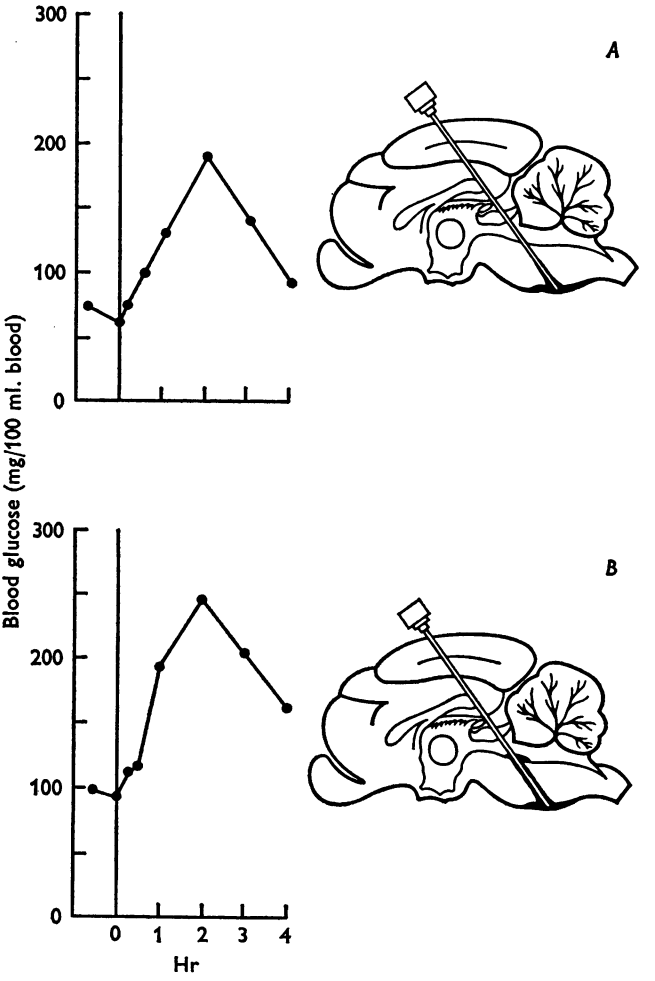

Fig. 3. Effect on blood glucose in two unanaesthetized cats of 0·75 mg morphine injected at 0 time through a permanently implanted cannula, the tip of which lay at the ventral surface of the medulla oblongata as indicated in the diagrams. The black areas indicate the staining when bromophenol blue was injected instead of morphine (for details see text).

further after an infusion of 0·75 mg morphine through the implanted cannula. The next day, the glucose blood level was down to 107 mg/100 ml. To exclude the possibility that the hyperglycaemic response shown in *A* of Fig. 3 was due to the mechanical effect of the infusion itself a control infusion of 40 μl. 0·9 % NaCl solution was given a day later (i.e. 6 days after the implantation). It produced no increase of glucose in blood samples collected at 30–60 min intervals during the following 4 hr. The result shown for cat *B* in Fig. 3 was obtained the day after implantation of the cannula. So if the implantation had produced hyperglycaemia it did not last for more than 24 hr, as in cat *A*. In a third cat in which a cannula was implanted in a similar way as in the experiments of Fig. 3, one sample of blood was taken a few minutes after the end of the operation, a second 3 hr later, when the cat was coming out of anaesthesia, and a third after another 15 hr. The glucose level in these three samples was 76, 152 and 64 mg/100 ml. So in this cat the implantation had produced a hyperglycaemia which was not present immediately after the implantation whilst the cat was deeply anaesthetized, and which had disappeared within 15 hr.

TABLE 2. Blood glucose in unanaesthetized cats before and after injection of morphine into the cisterna magna

	Min before and after morphine injection							
Morphine injected (mg)	−90 to −45	0	15	30	60	120	180	240
	Blood glucose (mg/100 ml.)							
1·5	59	54	107	161	148	183	161	105
2	57	61	180	139	115	148	183	191
2	71	74	81	152	146	136	140	
2	93	100	202	221	205	239	200	
Mean	70	72	143	168	154	177	171	148
Mean from Feldberg & Shaligram (1972)		(76)		(159)	(197)	(202)		(144)

The 1·5 mg were injected in 0·3, the 2 mg in 0·4 ml. artificial c.s.f.

Cisterna magna. Borison *et al.* (1962) found that a dose of morphine, which on intraventricular injection produced strong hyperglycaemia, did not raise blood sugar when injected into the cisterna magna. However, somewhat larger doses of morphine than those effective on intraventricular injection, were found to produce hyperglycaemia. As shown in Table 2, intracisternal injections of 1·5 and 2 mg morphine produced hyperglycaemias of the same order as previously obtained by Feldberg &

Shaligram (1972) with intraventricular injections of 0·75 mg. The figures in brackets at the bottom of the Table are taken from their paper and give the mean blood glucose values in nine experiments in which this dose of morphine had been injected intraventricularly. In none of the four experiments of Table 2, however, did the blood glucose rise as much as in the two experiments of Fig. 1, or in the Expts. *B* and *C* of Fig. 2 in which 0·75 mg morphine was infused either into the aqueduct, or into the fourth ventricle, or into the subarachnoid space above the corpora quadrigemina.

Behavioural and other effects

Previously it was shown that an intraventricular injection of morphine produced in addition to hyperglycaemia, shivering, vomiting, mydriasis, opening of the eyes, miaowing, excitation and analgesia (Feldberg & Shaligram, 1972). With the infusion or injections of morphine into the other parts of the liquor spaces some of these effects were also observed, others were absent and new effects not seen with intraventricular injections were obtained.

The infusion of morphine into the aqueduct or into the fourth ventricle, as in the experiments of Fig. 2, produced vomiting, mydriasis, miaowing and analgesia. The infusion into the aqueduct produced shivering as well. Since only minute amounts of morphine have to be present in the third ventricle to produce shivering, it is most likely that a small fraction of the infused morphine had escaped into the third ventricle, but that this did not happen on infusion into the fourth ventricle when no shivering was observed. Shivering was also absent with infusion of morphine into the other parts of the liquor space.

After an infusion of morphine 2–3 mm above the corpora quadrigemina, as in the Expt. *A* of Fig. 2, which did not produce hyperglycaemia, there occurred only mydriasis and opening of the eyes. On the other hand, the infusion of morphine just above the corpora, as in the Expt. *B* of Fig. 2, produced mydriasis, salivation, restlessness, circling movements and short-lasting convulsions. On infusions of morphine into the substance of the corpora, as in the Expts. *C* and *D* of Fig. 2, convulsions did not occur, but there was maximal mydriasis and the cats became hyperexcitable. From time to time, there were very rapid circling movements or a sudden charging ahead, which could be initiated by a clap of the hands. At a later stage, during the period of hypoglycaemia, the cats were lying on their side with rapid respiration or panting and with rigidity of their leg muscles.

An infusion of morphine into the subarachnoid space below the ventral surface of the brain stem as in the Expts. of Fig. 3, produced mydriasis and circling movements. In Expt. *B*, but not in Expt. *A*, there was panting as well, and some indication of analgesia.

After an intracisternal injection of 1·5–2 mg morphine there was mydriasis, salivation, sometimes vomiting and panting, never miaowing, but always bouts of vigorous scratching movements. When they subsided 30–45 min after the injection, there was analgesia lasting for several hours.

Experiments under pentobarbitone sodium anaesthesia

An intraperitoneal injection of anaesthetic doses of pentobarbitone sodium (33–36 mg/kg) had no definite effect on blood glucose apart from a gradual late rise which occurred a few hours after the injection when the cats had not yet come out of the anaesthesia. This late gradual rise is shown in the first three experiments of Table 3.

TABLE 3. Blood glucose in cats anaesthetized with pentobarbitone sodium before and after injection of morphine into the lateral ventricle

	Min before and after morphine injection								
Morphine injected (mg)	−120 to −80	−60 to −30	0	15	30	60	120	180	240
	Blood glucose (mg/100 ml.)								
0*	50	70	84	—	—	102	113	160	177
0	65	89	70	—	—	83	96	130	148
0	77	68	73	—	—	86	105	125	141
2	63	63	63	63	63	59	74	107	—
3	93	115	90	91	82	77	94	227	—
3	73	82	78	69	196	207	276	240	—
4	90	98	90	75	143	275	325	325	320
4	—	158	126	132	147	336	379	321	179

The first samples were taken a few minutes after the I.P. injection of pentobarbitone sodium when full anaesthesia was obtained. At zero time morphine injected in 0·15–0·2 ml. artificial c.s.f.

* In this experiment the head was fixed to the ear bars of a stereotaxic instrument.

Previously it was found (Feldberg & Shaligram, 1972) that an intraventricular injection of 0·75 mg of morphine which has a strong hyperglycaemic effect in the non-anaesthetized cat did not raise blood sugar when injected during pentobarbitone sodium anaesthesia. It was now found that even 2 mg were ineffective. However, with 3 and 4 mg hyperglycaemia was obtained; 3 mg appeared to be near the threshold dose because in one of the two experiments with 3 mg the hyperglycaemia occurred only 3 hr after the morphine injection (Expt. 5), whereas in the other it developed within 1 hr, as it did in the two experiments in which 4 mg was injected.

One of the first effects of these injections of morphine into the lateral

ventricle was vigorous shivering which occurred within a minute or two; later on, 15–30 min after the injection, there were typical scratching movements, the pupils dilated and there were sometimes periods of loud miaowing.

When morphine was applied bilaterally by means of Perspex rings to the ventral surface of the rostral part of the medulla oblongata in a concentration of 50 or 100 mg/ml., blood glucose rose to between 200 and 350 mg/ml. in ten out of thirteen experiments, but a rise of nearly the same order was obtained in six out of ten experiments without applying morphine, by exposing or cutting the dura, and particularly by placing the Perspex rings on to the ventral surface of the brain stem. It is therefore by no means certain that the rise when it occurred on morphine application was due to its action or even partly due to its action, and not in the main the result of the surgical procedures.

DISCUSSION

The results of the present experiments show that the hyperglycaemia produced by injection of morphine into the cerebral ventricles of unanaesthetized cats, is not due to an action on structures in the walls of any part of the ventricular cavities, but that the morphine has to enter the subarachnoid space through the foramina of Luschka in order to produce its hyperglycaemic effect. The results further point to superficial structures at the ventral surface of the brain stem as the site of action. The fact that Borison *et al.* (1962) did not obtain a hyperglycaemic response to morphine on its injection into the cisterna magna is not valid evidence against this conclusion. These authors only showed that a dose which produced hyperglycaemia on intraventricular injection was ineffective when injected intracisternally. The ventral surface of the brain stem, however, is reached more readily by the intraventricular than by the intracisternal route (Feldberg & Guertzenstein, 1972), so to become effective intracisternally morphine must be given in a larger dose. As shown in the present experiments it was necessary only to double the dose to reveal its hyperglycaemic effect on intracisternal injection; this dose is still far below the threshold which produces hyperglycaemia on intravenous injection (Feldberg & Shaligram, 1972).

Direct evidence for an action on structures near the ventral surface was obtained in those experiments in which the infusion of morphine was made through a cannula implanted in such a way that its tip was at the ventral surface of the brain stem. The hyperglycaemia produced in these experiments was not as pronounced as that obtained with infusion into the lateral ventricle, aqueduct or fourth ventricle. But this may well be due to

the fact that with the infusion aimed to reach the ventral surface, the morphine had spread to one side of the brain stem only, as was suggested by the staining obtained when, instead of morphine, bromophenol blue was infused through the cannula. If this interpretation is correct, then the site, though not necessarily the synapses on which the morphine acts when producing its hyperglycaemic effect, may be the same region at the rostral part of the medulla oblongata which contains various chemosensitive zones from where cardiovascular and respiratory effects are obtained with topical application of a number of drugs (for references see Feldberg & Guertzenstein, 1972, and Guertzenstein, 1973). The hyperglycaemia produced by morphine is mainly brought about by release of adrenaline through a sympathetic discharge to the suprarenal medulla (for references see Feldberg & Shaligram, 1972). The changes in arterial blood pressure produced by topical application of drugs to the chemosensitive zones at the rostral end of the medulla oblongata are also mediated via the sympathetic. Thus the results obtained with morphine can be viewed in the same context, since they, too, emphasize the role of central synapses or relay stations in the sympathetic pathway which are situated close to the ventral surface of the brain stem and readily reached from the liquor space.

There is, however, another possibility which cannot yet be excluded. The region where morphine acts may be situated more rostrally, and with the placement of the cannula on to the ventral surface of the brain stem, the infused morphine may have spread to this region in a weak concentration, which would explain the attenuation of the hyperglycaemic response. This possibility has to be considered because of the results obtained by Sproull (1963) when he investigated the hyperglycaemia produced by adrenaline injected into the cerebral ventricles or into the cisterna magna of cats. He, too, found that to produce hyperglycaemia on intraventricular injection the adrenaline had to pass through the foramina of Luschka into the subarachnoid space, and that both on intraventricular and intracisternal injection, the adrenaline had to pass to the ventral surface of the brain stem. But the effective site was not at the upper part of the medulla oblongata but rostral to the pons at the interpeduncular fossa. However, since the hyperglycaemia produced by injection of adrenaline into the liquor space is only partly a central, and mainly a peripheral effect which occurs after its absorption into the blood stream, and the absorption occurs readily from this region, it is doubtful whether his results can be used in deciding about the central site of action of morphine when producing hyperglycaemia.

To be certain about the exact site where morphine acts, experiments would have to be done by applying it topically to different regions of the

ventral surface of the brain stem. Recently a technique for such topical application was developed by using Perspex rings (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973). But this technique can only be used in anaesthesia, which depresses the morphine hyperglycaemia. The finding that by increasing the dose of morphine injected into the cerebral ventricles it was possible to obtain a hyperglycaemic response also in pentobarbitone sodium anaesthesia, raised the hope that the technique could be used for the topical application of morphine. However, the surgical procedures involved in this technique so often produced hyperglycaemia that the positive results obtained on topical application of morphine were inconclusive. It has to be seen whether, by changing the anaesthetic and/or the surgical procedures, it will be possible to use this technique also for studying the hyperglycaemic action of morphine and to obtain unequivocal results.

The first evidence that the central nervous system can influence the glucose level of the blood goes back to Claude Bernard's famous piqûre. The exact site which had to be injured or excited by the piqûre has never been established. The site may be the same as that where morphine acts when producing hyperglycaemia, and with the morphine experiments we may have imitated the piqûre by a chemical lesion. The analogy can be taken a step further. The implantation of the cannula with its passage through the brain stem to reach the ventral surface of the rostral part of the medulla oblongata produced by itself a hyperglycaemia which outlasted anaesthesia and in one cat persisted for at least 24 hr. Unwittingly we may have reproduced with the implantation of the cannula, Claude Bernard's original piqûre.

In the course of the experiments aimed at infusing morphine into the subarachnoid space above the corpora quadrigemina, the tip of the cannula had penetrated in some experiments into the corpora, and the morphine infused into the cannula would therefore be able to act in high concentration on its structures. The result was hyperglycaemia followed by hypoglycaemia. The hyperglycaemia is readily explained by escape of morphine to the ventral surface of the brain stem, but the subsequent hypoglycaemia must have resulted from an action on sites within the corpora, either by the morphine or by the salt solution injected. If the effect is produced by the morphine it may be the first instance of hypoglycaemia obtained by the action of a drug on a localized region of the brain. The mechanism of this hypoglycaemic action has not been analysed.

Infusion of morphine into different regions of the ventricular cavities or subarachnoid space may also provide a suitable method for localizing other effects produced by morphine when acting from the liquor space. No detailed analysis was made in the present experiments of the different

effects observed. But as far as the results go, they show that shivering and miaowing results from an action on structures reached from the third ventricle, whereas mydriasis, salivation, restlessness and scratching movements result from an action on structures reached from the subarachnoid space. The structures on which morphine and other drugs act when eliciting scratching movements is known to be on the dorsal surface of the upper cervical cord (for references see Feldberg, 1963).

Finally, our results support the view previously expressed that the analgesia obtained on injection into the cerebral ventricles is not due to an action on structures reached from the third ventricle. Whether its action is on the periaqueductal grey or on structures in the floor of the fourth ventricle as suggested by Herz, Albus, Metys, Schubert & Teschemacher (1970) or on structures reached from the subarachnoid space as suggested by the long lasting analgesia which followed the bouts of scratching movements on intracisternal injection, or whether several sites are involved, requires re-examination with quantitative methods for measuring analgesia.

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