Br. J. Pharmac. (1972), **46**, 602–618.

The hyperglycaemic effect of morphine

W. FELDBERG AND S. V. SHALIGRAM*

National Institute for Medical Research, Mill Hill, London NW7 1AA

Summary

1. In the unanaesthetized cat, an injection of 0·75 mg of morphine into a lateral cerebral ventricle produced strong hyperglycaemia; on intravenous injection, 10 to 30 times larger doses were required. Other effects produced with both injections were shivering, pupillary dilatation, opening of the eyes, miaowing, periods of excitation, and analgesia. Between the periods of excitation the cat did not react to objects moving in front of its eyes and it had a vacant stare.

2. Noradrenaline, adrenaline, and 5-hydroxytryptamine (5-HT) injected intraventricularly (250 μg, twice) depressed the hyperglycaemia due to intraventricular morphine, and noradrenaline also depressed the hyperglycaemia due to intravenous morphine. Adrenaline produced the strongest and 5-HT the weakest depression. 5-HT did not depress the other effects of morphine, but the catecholamines depressed most of them; only analgesia and the vacant stare appeared to be unaffected.

3. Reserpine injected intraventricularly (0·5 mg, twice) greatly accentuated the hyperglycaemia as well as the other effects produced by intraventricular morphine, but pupillary dilatation and opening of the eyes no longer occurred; the protrusion of the nictitating membranes produced by the reserpine persisted.

4. Pentobarbitone sodium injected intraperitoneally in an anaesthetizing dose practically abolished the morphine hyperglycaemia, but injected intraventricularly in a dose of a few milligrammes, it had a two fold effect: depression followed by enhancement of the morphine hyperglycaemia. The enhancement may be due to sensitization of the effect of the adrenaline released by morphine, since adrenaline hyperglycaemia was enhanced as well.

5. Morphine did not seem to act on structures in the walls of either the lateral or third ventricle when producing its hyperglycaemic effect on intraventricular injection. The action may therefore be on more caudally situated parts of the neuro-axis, on the central grey, on structures in the floor of the fourth ventricle or of the lateral recesses, or even on structures near the ventral surface of the brain stem.

Introduction

The hyperglycaemic effect of morphine has been known for many years. Morphine injected subcutaneously was found by Araki, in 1891, to produce glycosuria in unanaesthetized rabbits and dogs, and by Ross, in 1918, to produce hyper-

* Commonwealth Medical Fellow.

glycaemia in unanaesthetized dogs. Since then, the hyperglycaemic effect has been amply confirmed in experiments on unanaesthetized cats and dogs. It has been firmly established that the effect is brought about mainly by release of adrenaline from the suprarenals, and that a sympathetic discharge, apart from that to the suprarenals, contributes only to a small degree to the hyperglycaemia (Stewart & Rogoff, 1922; Bodo, Cotui & Bengalia, 1937, 1968; Vasalle, 1961). Bodo & Brooks (1937) attributed the sympathetic discharge to an action of morphine on supraspinal centres. More recently, Borison, Fishburn, Bhide & McCarthy (1962) showed that, injected into the cerebral ventricles of unanaesthetized cats, morphine produced hyperglycaemia in doses 25 to 50 times smaller than those causing hyperglycaemia on intravenous injection. From the degree of hyperglycaemia produced when the morphine was injected in different volumes into the lateral ventricle, they concluded that the morphine acts on structures in the ventricular wall that lie 'in the vicinity of the intraventricular foramen of Monro'.

Central sites of action have been proposed also for the hyperglycaemias obtained with other drugs. Barbitone and leptazol were found to produce hyperglycaemia when injected into the cerebral ventricles of unanaesthetized rabbits, but the hyperglycaemia no longer occurred after bilateral splanchnicotomy (Hasselblatt & Sproull, 1961). Sodium salicylate caused hyperglycaemia in anaesthetized cats on injection into the cerebral ventricles, or into the vertebral artery in doses which were ineffective when given intravenously, and the hyperglycaemia produced by intraventricular injection was abolished by bilateral adrenalectomy (Gaitondé, Joglekar & Shaligram, 1967).

The present paper deals with the hyperglycaemia in response to morphine injected into the cerebral ventricles of unanaesthetized cats and with the effects produced on this hyperglycaemia by noradrenaline, adrenaline, 5-HT, reserpine and pentobarbitone sodium similarly injected. An attempt was also made to localize the site where morphine acts when producing its hyperglycaemic effect.

Methods

The experiments were performed on cats of either sex, weighing between 2·4 and 4·2 kg. For injections into the cerebral ventricles, a Collison cannula was implanted aseptically into the cerebral ventricles under pentobarbitone sodium (36 mg/kg i.p.) anaesthesia. In most experiments the cannula was implanted into the left lateral ventricle near the foramen of Monro, and injections through this cannula are referred to in this paper as intraventricular injections. In a few cats the cannula was implanted into the anterior or inferior horn of the left lateral ventricle, or into the third ventricle, rostral or caudal to the massa intermedia.

The Collison cannula used was modified by Mr. A. R. J. Collins and is illustrated diagrammatically in Fig. 1. It has a hollow stainless steel shaft (22 gauge) which continues through its head to the upper end. The space between the shaft and wall of the cannula is filled with araldite. For insertion into the third ventricle, the upper end of the shaft is strengthened by a stainless steel tube (19 gauge) which is slipped over the upper end and cemented to it before it is cemented into the head of the cannula; the tube protrudes 4 mm below the head. This strengthening of the shaft prevented sideways bending of the cannula which would otherwise have been brought about by the dura surrounding the superior sagittal vein, and by the falx cerebri, because during the insertion in the mid-line, these two

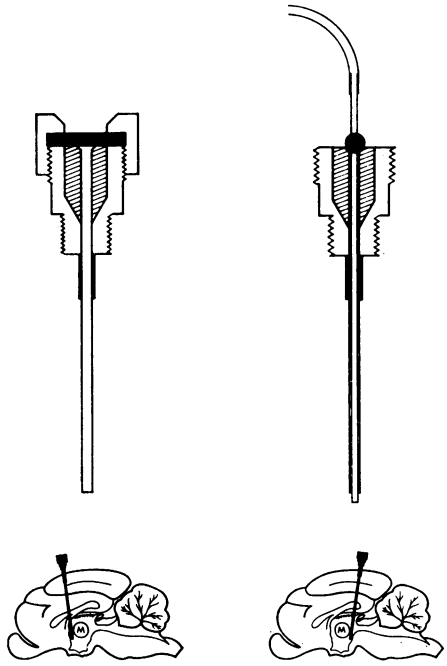

FIG. 1. Diagram of modified Collison cannula with strengthening of the upper end of the shaft. On the left, cannula with cap screwed on, on the right cannula with cap removed and inner tube inserted ready for injections. The bottom diagrams of the midsagittal sections of the cat's brain indicate position of cannula when inserted rostral or caudal to massa intermedia (M).

structures were pushed slightly to one side, so that when the cannula was in place, they exerted a sideways pressure on it. The length of the shaft varied. It was 12 mm for insertion into the lateral ventricle near the foramen of Monro, 20 mm for insertion into the anterior horn, and 22 mm for all other insertions. The cannula used for insertion into the inferior horn had a side opening near its end, but all other cannulae were open-ended, and all had a stilette, which passed through the entire length of the shaft and was removed before the injections.

The cannulae were inserted vertically. For cannulating the lateral ventricle near the foramen of Monro the point of insertion was 3 mm lateral to the midline, and 7 mm posterior to the coronal suture. This cannula was screwed into the skull through a burr hole, whereas all the other cannulae were inserted through an opening of about 8×10 mm made in the skull with a drill and nibbling forceps. For cannulating the anterior horn, the point of insertion was 1·5 mm lateral to the midline, and 2 mm posterior to the coronal suture, and the cannula was lowered 16 mm below the dura. For cannulating the inferior horn the corresponding measurements were 13·5 mm lateral to the midline, 5 mm anterior to the interaural zero plane of Snider & Niemer (1961), and 20 mm below the dura. For cannulating the third ventricle, the cannula was inserted in the midline either at a point 12·5 mm anterior to the interaural plane, if insertion was rostral, or 4·5 mm if it was caudal to the massa intermedia. The cannula was lowered 19 mm below the dura for the rostral, and 20 mm for the caudal insertion. All cannulae were fixed to the skull by acrylic cement and, except for the cannula

screwed into the skull for insertion into the lateral ventricle near the foramen, further fixation was obtained by enveloping with acrylic cement two small stainless steel screws screwed into the skull, one on each side of the hole.

The volume of fluid in which the drugs were injected was 0·15 ml for the injections into the lateral ventricle near the foramen, 0·05 ml for those into the anterior or inferior horn and 0·02 ml for those into the third ventricle, rostral or caudal to the massa.

In order to assess the spread of morphine in the ventricular cavities following its injection into the cerebral ventricles, a 0·2% bromophenol blue solution was injected through the cannula in the same volume as had been used for the morphine injection, the cat was killed 20 min later under pentobarbitone sodium anaesthesia, the head perfused with 30% formaline saline solution, the brain removed and the staining of the ventricular walls observed with the naked eye.

In a few experiments, morphine was applied in a volume of 4 μl by microinfusion through a Collison cannula into the region of the anterior or posterior hypothalamus. The method has been described in detail (Feldberg & Saxena, 1971). As the microinfusion pump used delivered 1 μl/min, the time of infusion was 4 minutes.

Collection of blood samples

For this purpose, as well as for intravenous injections, a nylon catheter (Portex) was inserted aseptically either about 8 cm deep into the right jugular vein so that its opening was lying about 0·5 cm above the clavicle, or about 20 cm deep into the right femoral vein so that its opening was lying in the inferior vena cava below the entrance of the hepatic veins. The free end of the catheter with the attached rubber cap was taken over to the back of the neck or to the outer side of the thigh and held in position with adhesive tape. The patency of the catheter was maintained by flushing it with an injection of 1 ml of 0·9% NaCl solution containing 50 u heparin through the rubber cap immediately after insertion, and again every second day. The catheter was inserted either at the same time as the implantation of the Collison cannula was carried out, or 2 to 3 days later, and then again under pentobarbitone sodium anaesthesia.

The actual experiment was carried out no sooner than six days after the implantation of the Collison cannula, the cat being kept without food, but not without water, for the last 24 hours. Between five and seven samples of 1 ml of blood were collected in each experiment and, in order to prevent the samples from containing blood that had been stagnating in the catheter, 0·75 ml blood was withdrawn before each sample and discarded. Further, to keep the catheter patent, it was flushed after collection of each sample with 0·75 ml of the heparin-saline solution. If the cat was used for several experiments, an interval of at least seven days was allowed between each experiment.

Blood glucose estimation

The blood glucose was determined by the glucose oxydase method as modified by Werner, Rey & Wielinger (1970), with the 'Testpack' supplied by Boehringer (Mannheim). Each 1 ml sample of blood withdrawn was at once placed into a test tube which contained 50 u heparin in 0·01 ml; 0·2 ml of the heparinized

blood was then transferred into 2 ml of 0·33 N perchloric acid and kept at room temperature until centrifugation. All samples were centrifuged together. Afterwards, 0·1 ml of the supernatant was added to 5 ml of the glucose oxydase colour reagent, and 35 min later the colour intensity of the mixture was read in an EEL colorimeter and compared with that of the standard glucose solution. The reading obtained with 0·1 ml of the standard solution added to 5 ml of the glucose oxydase colour reagent corresponded to 100 mg glucose/100 ml.

Drugs used

Morphine sulphate, (−)-adrenaline and (−)-noradrenaline acid tartrate, 5-hydroxytryptamine creatinine sulphate (5-HT), reserpine phosphate, pentobarbitone sodium. All values in the text refer to the salts. The drugs were dissolved in artificial cerebrospinal fluid (c.s.f.) except reserpine, which was dissolved in distilled water. Further, morphine, when injected in a volume of 0·2 ml was dissolved in 0·9% NaCl solution and when given by microinfusion in a volume of 4 μl, it was dissolved in distilled water. All drugs were freshly dissolved before the injection.

Results

Effects of morphine

The finding of Borison *et al.* (1962) was confirmed that morphine injected intraventricularly produces hyperglycaemia in unanaesthetized cats in doses that are ineffective on intravenous injection. Table 1 illustrates the hyperglycaemic response to 0·75 mg of morphine injected intraventricularly in nine unanaesthetized cats. In all of them blood glucose had risen in the 30 min sample after the injection had reached its maximum, mean values of 197 and 202 mg/100 ml, in the 60 and 120 min samples were still elevated in the 240 min sample. In one cat (not shown in Table 1) in which 0·24 mg of morphine was injected intraventricularly, the blood glucose rose from 64 to 84, 92 and 100 mg/100 ml at 30, 60 and 120 min, and was still 98 mg/100 ml 240 min after the injection. An intraventricular injection of 0·15 ml of artificial c.s.f. had no effect on blood glucose (Fig. 2, cat 9).

TABLE 1. *Blood glucose in unanaesthetized cats after intraventricular injection of morphine*

Cat No.	Weight (kg)	Min after morphine injected: 0	30	60	120	240
		Blood glucose (mg/100 ml)				
1	2·4	80	185	244	280	120
2	2·6	75	202	215	205	107
3	2·75	82	135	152	167	157
4	2·8	75	142	170	140	105
5	2·8	90	212	250	270	210
6	3·4	59	135	145	135	72
7	3·6	72	133	140	157	81
8	3·7	90	198	225	243	238
9	3·9	60	112	200	225	205
Mean		76	159	197	202	144

At zero time, 0·75 mg of morphine in 0·15 ml of artificial c.s.f. was injected into the left lateral ventricle.

To obtain with intravenously administered morphine a hyperglycaemic effect of the same order as that obtained with 0·75 mg injected intraventricularly, 10 to 30 times greater doses were required. Table 2 shows the results obtained in 3 cats weighing between 2·75 and 4·2 kg when 5 mg/kg morphine, i.e. between 14 and 21

TABLE 2. *Blood glucose in unanaesthetized cats after intravenous injection of morphine*

Cat No.	Weight (kg)	Min after morphine injection: 0	30	60	120	180	240
		Blood glucose (mg/100 ml)					
10	2·75	75	190	234	246		192
11	2·75	94	212	265	256	230	170*
12	4·2	67	207	247	255	225	175
Mean		74	203	250	252	228	179

At zero time, 5 mg/kg of morphine was injected intravenously. * Sample taken at 210 instead of at 240 min.

mg, was injected intravenously. The mean maximal hyperglycaemia was greater than in the experiments with intraventricular injection (Table 1), but in individual experiments the maximal rise after intraventricular injection was as great or even greater than that found after intravenous injection. In one cat weighing 2·5 kg, the effect of an intravenous injection of 3 mg/kg, i.e. of 7·5 mg, was examined. Blood glucose rose from 67 to 117 and 148 mg/100 ml in the samples collected 30 and 60 min after injection and then fell to 125 and 97 mg/100 ml in the 120 and 180 min samples. This hyperglycaemia was of the same order as that produced in some of the experiments of Table 1, in which 0·75 mg was injected intraventricularly.

Most of the acute behavioural effects of morphine described by Borison *et al.* (1962) were the same after intraventricular or intravenous injection. One effect produced only by intraventricular injection was vomiting; it occurred once or twice within 3 min of the injections. Other effects which occurred within the first 10 min were shivering, midriasis, opening of the palpebral fissures, vocalization, excitation and analgesia. In a few cats salivation occurred, and in one cat there was panting which began 15 min after an intraventricular injection and persisted for 7 minutes.

Shivering began sometimes within 1 min after the injection and continued for at least 1 hour. It was weak after the intravenous but vigorous and widespread after the intraventricular injections. It was not described by Borison *et al.* (1962) but has been observed in anaesthetized cats following intraventricular injections of morphine (Banerjee, Feldberg & Lotti, 1968).

Midriasis and opening of the palpebral fissure were present for hours after the injection.

Vocalization consisted of miaowing which was more pronounced and longer-lasting after intraventricular than after intravenous injection. After intraventricular injection, it began within 5 min ; at first it was not continuous, but the intervals between the miaowing periods decreased during the following 30 min and finally miaowing became continuous. It also became progressively louder and more frequent and, at the height of the effect, miaowing occurred with each expiration, i.e. nearly 40/min. One hour after the injection, miaowing was still nearly continuous, but the frequency was only about 20/minute. From then onwards, periods of

miaowing occurred at lengthening intervals and became shorter, whilst the miaowing itself became weaker and less frequent.

Excitation, too, was more pronounced after intraventricular than after intravenous injection. It followed a definite pattern. The cat, sitting on its hindquarters, suddenly moved its head abruptly from side to side and, scarcely unflexing its hindquarters, placed its forepaws first to one, then to the other side. After some minutes, such a sideways movement led to circling. At first, the cat circled once, and then sat down again ; later it circled several times without stopping. The hindquarters were partly extended but later they became fully extended during the circling, which went on with increasing speed, whilst the periods of circling became longer and more frequent. A characteristic sequence frequently seen was that, after a few circling movements, the cat would sit down abruptly and cease all movements, only to resume its compulsive circling after a second or two. Between periods of circling, the cat nearly always resumed its original posture, sitting on its hindquarters, with its pupils maximally dilated and its eyes wide open. It stared vacantly and did not react to objects moving in front of its eyes. Yet it resisted handling, without trying to bite or attack, by clinging tightly with its fully extended claws to the wire mesh of the side of the cage. Muscle tone was greatly increased. Before the circling movements became fully developed, the cat often vigorously scratched the floor or jumped against the roof or side of the cage. When jumping against the side of the cage, it clung to it and sometimes remained immobile in a nearly erect position for several minutes, as if it were cataleptic. However, when putting the cat into an abnormal position, for instance, by placing it in a nearly erect position with its forepaws over the rungs of an inverted stool, it resisted and did not maintain this posture.

Analgesia lasted for at least 4 hours. During this period the cat did not react to pinching of the skin or pressing of the paws.

Effects of noradrenaline

The intraventricular injections of noradrenaline had a pronounced depressant effect on the hyperglycaemia produced by morphine. Noradrenaline was injected in a dose of 250 μg, 15 min before and again 30 min after the morphine, and its effect on the morphine hyperglycaemia differed somewhat according to whether the morphine was injected intraventricularly or intravenously. The hyperglycaemia was greatly delayed without necessarily being much attenuated when the morphine was injected intraventricularly ; it was mainly attenuated when the morphine was injected intravenously. This difference is illustrated in Figure 2.

The upper records show the effect of noradrenaline on the hyperglycaemia produced by intraventricular morphine on 2 cats (Nos. 9 and 12). The noradrenaline delayed the onset of the hyperglycaemia for about 2 hours. The blood glucose had scarcely risen 1 h after the morphine injection ; after 2 h it was only 113 mg/100 ml in cat No. 9, and 99 mg/100 ml in cat No. 12. With morphine alone, the blood glucose was already elevated after 30 min, after 1 h the values were 200 mg/100 ml in cat No. 9, and 175 mg/100 ml in cat No. 12, and after 2 h they were 225 mg/100 ml in cat No. 9 and 270 mg/100 ml in cat No. 12. The high value in cat No. 12 was probably due to the intraventricular injection of pentobarbitone sodium given 1 h after the morphine since such an injection enhances morphine hyper-

glycaemia (see below). Three hours after a morphine injection alone i.e. without noradrenaline, blood glucose began to fall, whereas when both noradrenaline and morphine were given, the main hyperglycaemic response developed only at this time. It did not seem possible to delay its development further by additional noradrenaline. This is illustrated by the result obtained in cat No. 12 in which a third dose of 250 μg noradrenaline was injected 120 min after the morphine.

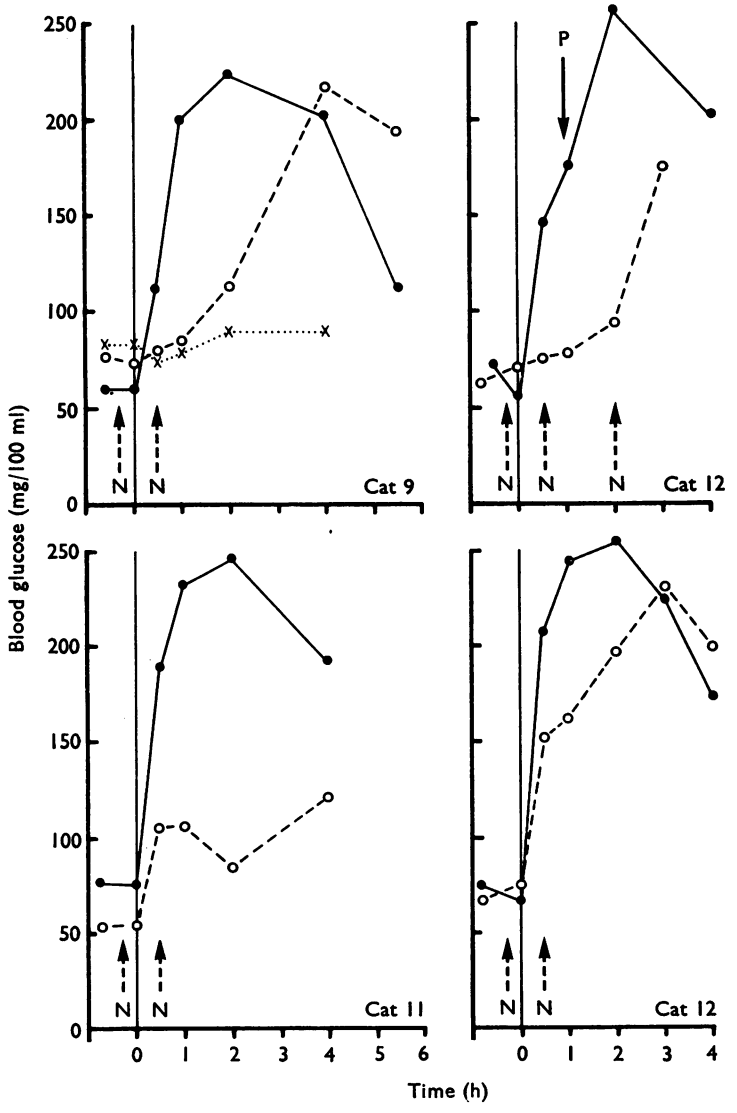

FIG. 2. Blood glucose (mg/100 ml) in three unanaesthetized cats (Nos. 9, 11 and 12, of Tables 1 and 2). Each continuous line indicates an experiment with morphine alone, each interrupted line an experiment with morphine and noradrenaline, and the dotted line an experiment with artificial c.s.f. The vertical lines at zero time indicate, for the upper records, intraventricular injection of 0·75 mg morphine, or in cat No. 9, of 0·15 ml artificial c.s.f., and for the lower records, intravenous injections of 5 mg/kg morphine. The arrows marked N indicate intraventricular injections of 250 μg noradrenaline and refer to the interrupted curves. The arrow marked P indicates an intraventricular injection of 4 mg pentobarbitone sodium and refers to the continuous line.

The lower records show the effect of noradrenaline on the hyperglycaemia produced by intravenous morphine on two cats (Nos. 11 and 12). Noradrenaline did not delay the onset of hyperglycaemia but the rise in blood glucose which developed during the first 30 min was greatly reduced. It remained reduced throughout the whole 4 h observation in cat No. 11, and during the first 2 h in cat No. 12; afterwards the hyperglycaemia in this cat was about the same as with morphine alone.

Other effects produced by morphine were also delayed, attenuated or even abolished by the intraventricular injections of noradrenaline. Noradrenaline was particularly effective when morphine was injected intraventricularly. Shivering did not occur, or occurred much later and was not vigorous. Pupillary dilatation developed more gradually. Miaowing was abolished. Excitation was greatly reduced. Periods of circling either did not occur or did so only after about 1 h; they were not as frequent as after morphine alone, nor were the movements as abrupt and rapid. On the other hand, analgesia was not abolished and most of the time the cats sat on their hindquarters and showed the typical vacant stare of morphine-treated cats.

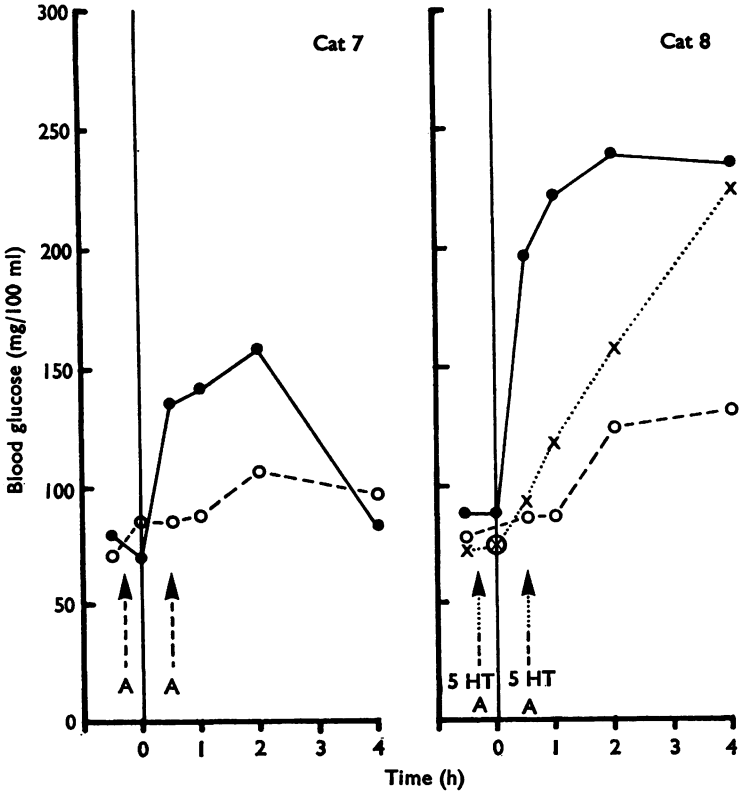

FIG. 3. Blood glucose (mg/100 ml) in two unanaesthetized cats (Nos. 7 and 8 of Table 1). Each continuous line gives an experiment with morphine alone, each interrupted line an experiment with morphine and adrenaline, and the dotted line an experiment with morphine and 5-hydroxytryptamine (5-HT). The vertical lines at zero time indicate intraventricular injections of 0·75 mg morphine. The arrows indicate intraventricular injections of 250 μg of either adrenaline (A) or 5-HT.

Effects of adrenaline

As first shown by Hasselblatt & Sproull (1961), adrenaline does not produce its hyperglycaemic effect when injected into the cerebral ventricles of cats. This finding was confirmed, and it was further shown that adrenaline depressed morphine hyperglycaemia. This is illustrated in Fig. 3 for the hyperglycaemia produced by intraventricular morphine in 2 cats (No. 7 and 8). Adrenaline was injected intraventricularly in a dose of 250 μg, once 15 min before and again 30 min after the morphine. From a comparison with the results obtained in cats No. 9 and 12 of Fig. 2, it is evident that adrenaline causes a greater depression of the hyperglycaemia than noradrenaline. The depressant effect of adrenaline was also stronger with regard to shivering, pupillary dilatation, miaowing and excitation produced by intraventricular morphine. Analgesia and the vacant stare of the morphine-treated cat, on the other hand, appeared to be unaffected.

Effects of 5-hydroxytryptamine (5-HT)

A depressant effect on the hyperglycaemia produced by morphine was also obtained with 5-HT injected intraventricularly but the effect was weaker than that of the catecholamines as shown for 5-HT and adrenaline on cat No. 8 in Fig. 3.

None of the other effects produced by morphine were depressed by 5-HT. Shivering was in fact accentuated and lasted longer. In cat No. 8, tachypnoea was produced by morphine plus 5-HT but not by morphine alone. Since 5-HT itself produces shivering and tachypnoea on intraventricular injection the effects of 5-HT may have summed with those of morphine.

Effects of reserpine

Table 3 shows the results obtained on 3 cats after pretreatment with intraventricular reserpine phosphate. In all three cats, reserpine pretreatment alone caused a mild hyperglycaemia and the subsequent morphine hyperglycaemia was greatly accentuated. Reserpine pretreatment also accentuated the shivering, miaowing and excitation produced by the intraventricular injection of morphine, and there were definite signs of catalepsy. Placed in a nearly erect position with their forepaws over the rungs of an inverted stool, the cats maintained this abnormal posture for minutes. On the other hand, the pupils did not dilate, the palpebral fissures did not open, and the protrusion of the nictitating membranes produced by reserpine persisted after the morphine injection.

TABLE 3. *Blood glucose in unanaesthetized cats pretreated with reserpine after an intraventricular injection of morphine*

Cat No.	Min after morphine injection: 0	30	60	120	240
	Blood glucose (mg/100 ml)				
1	125 (80)	256 (185)			
6	124 (59)	190 (135)	254 (145)	239 (135)	135 (72)
9	105 (60)	275 (112)	295 (200)	275 (225)	230 (205)

The cats were pretreated with 2 intraventricular injections of 0·5 mg of reserpine, one given 18 h and the other 4 h before the injection, at zero time, of 0·75 mg of morphine into the left lateral ventricle. The figures in parentheses refer to the blood glucose values given in Table 1; they were obtained in the same cats prior to reserpine pretreatment.

Effects of pentobarbitone sodium

Intraperitoneal injections. The finding of Borison *et al.* (1962) that the hyperglycaemia produced by an intraventricular injection of morphine is suppressed in pentobarbitone sodium anaesthesia, was confirmed. Even when anaesthesia was wearing off, a few hours after an intraperitoneal injection of pentobarbitone sodium (36 mg/kg) the hyperglycaemic response to an intraventricular injection of 0·75 mg

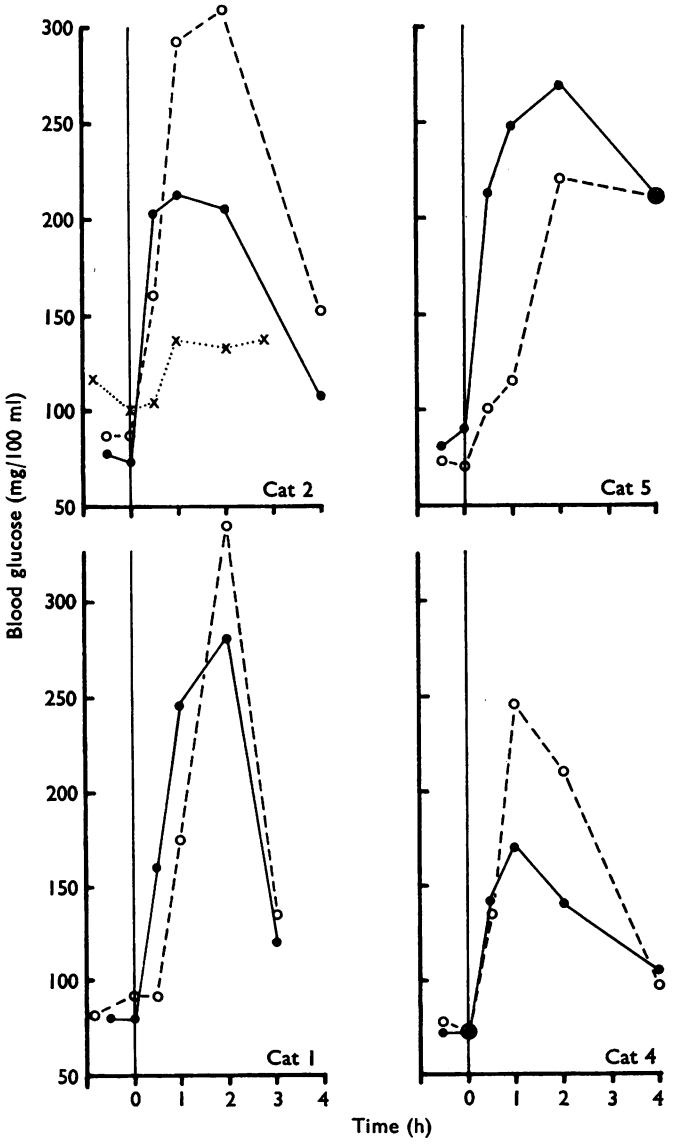

FIG. 4. Blood glucose (mg/100 ml) in four unanaesthetized cats (Nos. 2, 5, 1 and 4 of Table 1). Each continuous line indicates an experiment with morphine alone and each interrupted line an experiment with morphine and intraventricular injection of pentobarbitone sodium. The vertical lines at zero time indicate intraventricular injections of 0·75 mg morphine. The pentobarbitone sodium was injected either in a dose of 4·5 mg intraventricularly 5 min before (cats No. 2, 5 and 1) or 30 min after (cat No. 4) the morphine, or intraperitoneally in an anaesthetizing dose of 36 mg/kg, 2·5 h before the morphine (cat No. 2, dotted line).

of morphine was still very weak. This is illustrated for cat No. 2 in Fig. 4 by the dotted line. The morphine was injected 2·5 h after the onset of anaesthesia.

Intraventricular injections. In the unanaesthetized cat, an intraventricular injection of 4·5 mg of pentobarbitone sodium had either no effect on blood glucose or produced a rise not greater than 25 mg/100 ml blood. Such an injection, however, had a two-fold effect on the hyperglycaemia produced by an intraventricular injection of morphine. With the pentobarbitone sodium injected a few minutes before the morphine, the hyperglycaemia was delayed for 30 or 60 min, or reduced during this time, but afterwards the hyperglycaemia was usually enhanced. In some experiments depression, in others enhancement predominated. With the pentobarbitone sodium injected 30 min after the morphine, enhancement was the main or the sole effect. Typical results for 4 cats are illustrated in Figure 4.

In three cats, the pentobarbitone sodium was injected 5 min before the morphine. In cat No. 2, the hyperglycaemia was slightly attenuated during the first 30 min and then greatly enhanced. In cat No. 5, there was great attenuation without subsequent enhancement, and in cat No. 1, the hyperglycaemic response was delayed by 30 min, reduced during the following 30 min, but enhanced afterwards. The enhancement which occurred when the pentobarbitone sodium was injected 30 min after the morphine is shown by cat No. 4. In cat No. 12 of Fig. 2 the injection of pentobarbitone sodium was given 1 h after the morphine and was probably responsible for the steep rise in blood glucose which occurred during the following hours.

The intraventricular injection of pentobarbitone sodium did not prevent any of the other effects produced by morphine though their onset was delayed by 5 to 15 min when the pentobarbitone sodium was given 5 min before the morphine.

As the morphine hyperglycaemia is due in the main to a release of adrenaline from the suprarenal medulla, its enhancement by intraventricular pentobarbitone sodium could result from sensitization of an adrenaline effect. In that case, intraventricular pentobarbitone sodium should enhance an adrenaline hyperglycaemia as well. The results given in Table 4 show that pentobarbitone sodium appears to have this effect when injected intraventricularly but not, or at most to a small degree, when injected intravenously.

Preliminary experiments concerning the site of action of morphine

Although the injections were made into the lateral ventricle near the foramen of Monro, it was unavoidable that some of the injected morphine (0·75 mg in 0·15 ml)

TABLE 4. *Blood glucose in unanaesthetized cats after an intravenous injection of adrenaline*

Cat No.	Min after 1st injection of adrenaline: 0	15	60 (Blood glucose mg/100 ml)	Injection of pentobarbitone sodium	Min after 2nd injection of adrenaline: 0	15	60 (Blood glucose mg/100 ml)
5	76	125	85	None	74	125	85
5	76	125	95	Intravenously	80	146	110
9	63	148	111	Intravenously	91	155	97
9	72	135	117	Intraventricularly	80	180	157
12	64	118	92	Intraventricularly	81	175	148

At zero time, 40 μg/kg of adrenaline were injected intravenously. The 2nd adrenaline injection was given 2–2·5 h after the 1st; pentobarbitone sodium (4·5 mg) was injected 5 min before the 2nd injection of adrenaline. There was an interval of 12 and 8 days respectively, between the two experiments in cats 5 and 9.

did not enter the third ventricle, but remained in the lateral ventricle and spread within its anterior and inferior horn. This became evident by the staining of their walls when bromophenol blue (0·15 ml of a 0·2% solution) was injected instead. However, the hyperglycaemia did not result from an action of morphine on structures in the walls of the inferior or anterior horn because when injections were made in such a way that only a little of the injected morphine entered the third ventricle and the main part remained in the lateral ventricle, no hyperglycaemia occurred. This was the case when the dose of 0·75 mg was injected away from the foramen of Monro, i.e. into the inferior or anterior horn, and in a volume of only 50 μl. With 50 μl of a 0·2% solution of bromophenol blue injected in this way, the dye was found to be distributed mainly in the inferior and anterior horn; their walls became deeply stained, whereas there was only faint staining in the walls of the third ventricle.

From the effects obtained when 0·75 mg of morphine was injected in an even smaller volume (20 μl) into the third ventricle either rostral or caudal to the massa intermedia, it became evident that the hyperglycaemia did not result from penetrating that region of the ventricular wall which lies rostral to the massa. As shown by the two experiments of Fig. 5, no hyperglycaemia ensued when the injection was made rostral to the massa (cat A), but a strong hyperglycaemia occurred when the injection was made caudal to it (cat B); the blood glucose then rose from 62 to nearly 200 mg/100 ml within 15 min and reached 300 mg/100 ml in 1 hour. The diagrams show the staining produced in these cats by bromophenol blue (20 μl of

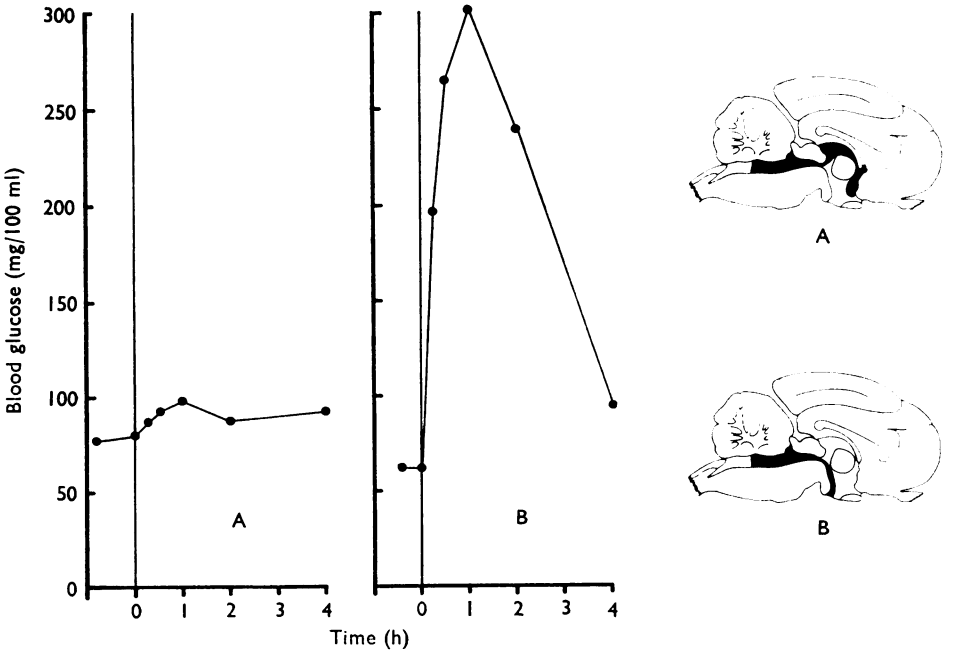

FIG. 5. Blood glucose (mg/100 ml) in two unanaesthetized cats (A and B). The vertical lines at zero time indicate intraventricular injections of 0·75 mg of morphine in a volume of 20 μl, injected through cannulae implanted into the third ventricle either rostral (A) or caudal (B) to the massa intermedia as illustrated in Fig. 1. The diagrams are midsagittal sections of the brains of the cats and indicate in black, the staining after similar injections of 20 μl of a 0·2% bromophenol blue solution through the cannulae.

a 0·2% solution) similarly injected. The injection rostral to the massa had produced deep staining of the region of the walls of the third ventricle rostral and dorsal to the massa, including the region around the foramen of Monro. Some dye had entered the left lateral ventricle and stained the walls of its anterior horn (not shown in the diagram). The aqueduct and floor of the fourth ventricle up to the lateral recesses were stained though not as deeply as the rostral and dorsal region of the third ventricle. However, there was no staining of the walls of the third ventricle caudal to the massa. In contrast, this region became deeply stained when the injection was made caudal to the massa, whereas the regions rostral and dorsal to the massa remained unstained. The aqueduct and the floor of the fourth ventricle up to the lateral recesses were also well stained, but whether the injection was made rostral or caudal to the massa intermedia, the dye was injected in such a small amount that it did not enter the subarachnoid space through the lateral recesses in a concentration sufficient to stain the ventral surface of the brain stem.

From the results shown in Fig. 5 it is evident that the morphine, when producing its hyperglycaemic effect, did not act on structures in the walls of the third ventricle rostral to the massa. This means it does not act on the anterior hypothalamus. An action on this region of the brain was also excluded by the finding that microinjections of morphine (160 μg in 4 μl) into this region of the brain did not produce hyperglycaemia. On the other hand, the strong hyperglycaemia produced with morphine injected caudal to the massa intermedia suggested an action on the posterior hypothalamus. So far only one experiment has been done with a microinjection of morphine (100 μg in 4 μl) correctly placed in this region ; a very weak hyperglycaemia ensued.

Discussion

The finding of Borison *et al.* (1962) has been confirmed, namely that morphine injected into the cerebral ventricles of unanaesthetized cats produces hyperglycaemia in doses which are much smaller than those required with intravenous injection, and that the hyperglycaemic response to intraventricular morphine is not obtained in pentobarbitone sodium anaesthesia. It was further found that the hyperglycaemic response was depressed by intraventricular injections of 5-HT, noradrenaline and adrenaline, the three monoamines which are considered to be transmitter substances of monoaminergic nerve fibres ending in the hypothalamus. 5-HT produced the weakest and adrenaline the strongest depression, although adrenaline has a strong hyperglycaemic effect when injected intravenously and, when injected into the cerebral ventricles by itself, has no effect on blood glucose, at least not in cats (Hasselblatt & Sproull, 1961).

Adrenaline and noradrenaline are known to cause anaesthesia or a sleep-like condition when injected into the cerebral ventricles ; this soporific effect is also obtained, though to a lesser extent, with 5-HT applied by the intraventricular route (Feldberg & Sherwood, 1954). If it should turn out that inhibition of morphine hyperglycaemia is a common property of hypnotics and anaesthetics then the depressant effect of the three monoamines on this hyperglycaemia would be fully explained by this property.

The same considerations apply to the other effects of morphine that were found to be inhibited by adrenaline and noradrenaline, shivering, miaowing and excita-

tion—as these effects are inhibited by anaesthetics as well. When injected into the cerebral ventricles of cats, the catecholamines inhibit not only morphine shivering, but also the shivering which occurs in a number of other conditions. This inhibitory effect on shivering is part of the mechanism by which the catecholamines exert a physiological effect on temperature regulation in cats. In this connexion it is interesting to note that an increased release by anaesthetics of catecholamines in the hypothalamus has been suggested as the cause of the hyperthermia in anaesthesia. However, the problem is more complicated in that 5-HT does not inhibit the other effects of morphine. Shivering is in fact accentuated, probably because 5-HT itself initiates shivering when injected intraventricularly into cats.

One effect of morphine which was not inhibited by intraventricular injection of any of the three monoamines was analgesia. If assessed quantitatively, it would possibly have been found to have become enhanced by the catecholamines, because they themselves have analgesic properties when injected intraventricularly.

In the past, the possibility has been considered that the temperature effects of intraventricular injections of morphine, which differ in different species, are mediated by release of the monoamines in the hypothalamus (Banerjee *et al.*, 1968 ; Banerjee, Burks, Feldberg & Goodrich, 1968) since morphine reduces the catecholamine content of the hypothalamus (Vogt, 1954 ; Holzbauer & Vogt, 1956) and in cats appears to reduce the diencephalic 5-HT as well (Turker & Akcasu, 1962). The finding that after intraventricular injections of reserpine, the morphine effects, including the hyperglycaemia, not only persisted but became enhanced, shows that they are unlikely to be mediated by the monoamines since the reserpine injections practically deplete the hypothalamus of its catecholamines and reduce its 5-HT content (Cooper, Cranston & Honour, 1967 ; Sharman & Vogt, 1969). The enhancement would rather suggest that depletion of the catecholamines by pre-treatment with reserpine had removed an inhibitory effect which the noradrenaline, through its release from adrenergic fibres ending on the hypothalamus, would otherwise have exerted on the morphine hyperglycaemia.

Although the site where morphine acts when producing its hyperglycaemic effect was not ascertained, certain sites could be excluded. Structures in the walls of the anterior or inferior horn could be excluded, because the hyperglycaemia did not occur when morphine was injected in a small volume into either horn. On the other hand, hyperglycaemia occurred under conditions in which the morphine did not enter the lateral ventricles. This happened when morphine was injected in a small volume into that part of the third ventricle which lies caudal to the massa intermedia. An action on structures in the vicinity of the foramen of Monro as assumed by Borison *et al.* (1962) could also be excluded because hyperglycaemia did not occur when the morphine was injected, again in a small volume, into that part of the third ventricle which lies rostral to the massa. The result of this experiment also excluded an action on the anterior hypothalamus ; such an action was further excluded by the finding that microinjections into this part of the brain had no hyperglycaemic effect. On the other hand, the strong hyperglycaemia which occurred when the injection of morphine, again in a small volume, was made into that part of the third ventricle which lies caudal to the massa, seemed to point to an action on the posterior hypothalamus. However, in the one experiment in which morphine was applied to this region by microinjection, only a very weak hyperglycaemic effect occurred. The possibility has therefore to be considered that mor-

phine does not act on structures in the walls of the third ventricle when producing its hyperglycaemic effect, but on more caudally situated structures of the neuroaxis. The action may be on the periaqueductal gray or on structures in the floor of the fourth ventricle, where, according to Herz, Albus, Metys, Schubert & Teschemacher (1970), morphine acts when producing its antinociceptive response (inhibition of the licking reaction elicited by electrical stimulation of the tooth pulp) on intraventricular injection into rabbits. Or the morphine may have to pass into the subarachnoid space and then acts on structures near the ventral surface of the brain stem. The finding of Borison *et al.* (1962) that a dose of morphine, which on intraventricular injection produced strong hyperglycaemia did not raise blood sugar when injected into the cisterna magna, is no evidence against this conclusion. Cats do not have a foramen Magendie at the caudal end of the fourth ventricle. Therefore substances injected into the cerebral ventricles pass into the subarachnoid space through the foramina of Luschka and reach the ventral surface of the brain stem more readily than on injection into the cisterna. Consequently, substances that act on structures near the ventral surface of the brain stem should be more effective on intraventricular than on intracisternal injection. This has recently been shown for the vasodepressor effect of pentobarbitone sodium (Feldberg & Guertzenstein, 1972) and the same situation may apply for the hyperglycaemic action of morphine.

REFERENCES

ARAKI, T. (1891). Über die Bildung von Milchsäure und Glucose im Organismus bei Sauerstoffmangel. Über die Wirkung von Morphium, Amylnitrit, Cocain. *Hoppe Seyler's Z. physiol. Chem.*, **15**, 546–561.

BANERJEE, U., BURKS, T. F., FELDBERG, W. & GOODRICH, C. A. (1968). Temperature effects and catalepsy produced by morphine injected into the cerebral ventricles of rabbits. *Br. J. Pharmac. Chemother.*, **33**, 544–551.

BANERJEE, U., FELDBERG, W. & LOTTI, V. J. (1968). Effect of body temperature of morphine and ergotamine injected into the cerebral ventricles of cats. *Br. J. Pharmac. Chemother.*, **32**, 523–538.

BODO, R. C. & BROOKS, CH.McC. (1937). The effects of morphine on blood sugar and reflex activity in the chronic spinal cat. *J. Pharmac. exp. Therap.*, **61**, 82–88.

BODO, R. C., COTUI, F. W. & BENGALIA, A. E. (1937). Studies on the mechanism of morphine hyperglycaemia. The role of the adrenal glands. *J. Pharmac. exp. Ther.*, **61**, 48–57.

BODO, R. C., COTUI, F. W. & BENGALIA, A. E. (1938). Studies on the mechanism of morphine hyperglycaemia. The role of the sympathetic nervous system with special reference to the sympathetic supply to the liver. *J. Pharmac. exp. Ther.*, **62**, 88–105.

BORISON, H. L., FISHBURN, B. R., BHIDE, N. K. & MCCARTHY, L. E. (1962). Morphine-induced hyperglycaemia in the cat. *J. Pharmac. exp. Ther.*, **138**, 229–235.

COOPER, K. E., CRANSTON, W. I. & HONOUR, H. J. (1967). Observations on the site and mode o action of pyrogens in the rabbit brain. *J. Physiol., Lond.*, **191**, 325–337.

FELDBERG, W. & GUERTZENSTEIN, P. G. (1972). A vasodepressor effect of pentobarbitone sodium. *J. Physiol., Lond.*, **224**, 83–103.

FELDBERG, W. & SAXENA, P. N. (1971). Fever produced by prostaglandin E_1. *J. Physiol., Lond.*, **217**, 547–556.

FELDBERG, W. & SHERWOOD, S. L. (1954). Injections of drugs into the lateral ventricle of the cat. *J. Physiol., Lond.*, **123**, 148–167.

GAITONDÉ, B. B., JOGLEKAR, S. N. & SHALIGRAM, S. V. (1967). The hyperglycaemic action of sodium salicylate. *Br. J. Pharmac. Chemother.*, **30**, 554–560.

HASSELBLATT, A. & SPROULL, D. H. (1961). Hyperglycaemia induced by drugs. *J. Physiol., Lond.*, **157**, 124–136.

HERZ, A., ALBUS, K., METYS, J., SCHUBERT, P. & TESCHEMACHER, H. (1970). On the central sites for the antinociceptive action of morphine and fentanyl. *Neuropharmacol.*, **9**, 539–551.

HOLZBAUER, M. & VOGT, M. (1956). Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.*, **1**, 8–11.

ROSS, E. L. (1918). Blood dextrose as affected by morphine and morphine with ether anaesthesia *J. biol. Chem.*, **34**, 335–342.

SHARMAN, D. F. & VOGT, M. (1969). Cited by Goodrich, C. A. (1969). *Br. J. Pharmac.*, **37**, 87–93.

SNIDER, R. S. & NIEMER, W. T. (1961). *A Stereotaxic Atlas of the Cat Brain*. Chicago: The University of Chicago Press.

STEWART, G. N. & ROGOFF, M. (1922). Morphine hyperglycaemia and the adrenals. *Am. J. Physiol.*, **62**, 93–112.

TURKER, K. & AKCASU, A. (1962). Effect of morphine on 5-HT of cat's brain. *New Istanb. Contr. clin. Sci.*, **5**, 89–97.

VASALLE, M. (1961). Role of catecholamine release in morphine hyperglycaemia. *Am. J. Physiol.*, **200**, 530–534.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol., Lond.*, **123**, 451–481.

WERNER, W., REY, H-G. & WIELINGER, H. (1970). Properties of a new chromogen for the determination of glucose in blood according to the GOD/POD (glucose–oxidase–peroxidase) method. *Z. analyt. Chem.*, **252**, 224–228.

(*Received February* 15, 1972)