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ANALGESIA PRODUCED BY MORPHINE WHEN ACTING FROM THE LIQUOR SPACE

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1 In cats analgesia was produced by morphine sulphate introduced into different parts of the liquor space in doses too small to be effective on intravenous injection. Analgesia was measured with the tail pinch method of Russell & Tate (1975).

2 On infusion into the fourth ventricle or into the subarachnoid space beneath the ventral surface of the brain stem caudal to the pons, doses of 100 to 200 μg of morphine sulphate were sufficient to produce strong long-lasting analgesia. On injection into the cisterna magna somewhat larger doses (400 to 800 μg) were required.

3 It is concluded that the site where morphine acts when producing analgesia in all three circumstances is at the ventral surface of the brain stem.

4 The possibility is discussed that the structures acted upon are tryptaminergic nerve fibres. They arise from the raphe nuclei, belong to a descending inhibitory pathway, and on their way to the spinal cord, reach the ventral surface of the brain stem lateral to each pyramid, where they could be reached and acted upon by the morphine. This theory postulates a morphine sensitivity of tryptaminergic nerve fibres.

Introduction

There are probably several sites at which morphine can act when producing analgesia, but one site appears to be the ventral surface of the brain stem. This conclusion is the outcome of the present experiments on cats in which the analgesic effect of morphine was examined on its introduction into different parts of the liquor space.

Recently, the hyperglycaemia produced by morphine injected into the cerebral ventricles of cats was found to result from an action on structures at the ventral surface of the brain stem, and the possibility was discussed that the same site, though not necessarily the same structures may be responsible for the analgesia that morphine produces on intraventricular injection (Feldberg & Gupta, 1974; Dey & Feldberg, 1975a; Dey, Feldberg & Wendlandt, 1975). There are in fact two observations on the analgesic action itself which could be readily explained in this way, those of Herz, Albus, Metys, Schubert & Teschemacher (1970) and those of Satoh & Takagi (1971). When viewed in conjunction, these two observations suggest the ventral surface of the brain stem as a site of the analgesic action, and perhaps not only when the morphine is injected into the cerebral ventricles, but also when injected intravenously.

Herz *et al.* (1970) showed that small doses of morphine injected into the aqueduct or fourth ventricle of rabbits inhibited the nociceptive response to electrical stimulation of the tooth pulp. They concluded that the analgesic effect was due to an action on the periaqueductal grey matter and on structures in the floor of the fourth ventricle. An action on the ventral surface of the brain stem was not envisaged although this site is readily reached by these injections, since the morphine passes into the subarachnoid space solely through the lateral recesses at the sides of the fourth ventricle and then at once reaches the ventral surface. Rabbits have no foramen of Magendie through which the cisterna magna could be reached. This foramen exists in primates only.

Satoh & Takagi (1971) stimulated the splanchnic nerves in cats and recorded the afferent volleys from the ventrolateral funiculi higher up in the spinal cord. These funiculi are thought to conduct visceral pain. The volleys were suppressed by intravenous injection of 4 mg of morphine and remained suppressed when the brain stem was transected at a level just caudal to the trapezoid bodies. But they were restored when the transection was made about 4 mm more caudally. The inhibitory effect of morphine on the afferent pain impulses must therefore have taken place within a 4 mm long block of brain stem, at least with the dose of morphine used, because in larger doses morphine acted on the spinal cord as well. This 4 mm long block did not include the aqueduct or the anterior half of the fourth ventricle which in the experiments of Herz *et al.* (1970) would have been reached by the injections into the aqueduct, but it included the posterior half.

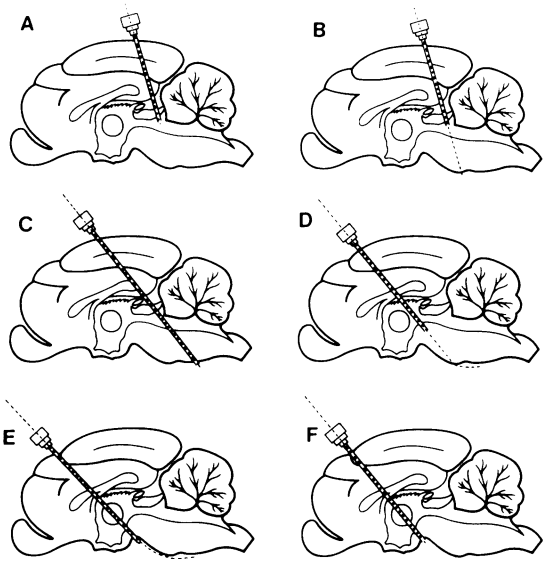

Figure 1 Diagrams of midsagittal sections of cat's brain with implanted Collison cannulae to illustrate the methods used for infusion of morphine into the fourth ventricle (A) or into the subarachnoid space beneath the ventral surface of the brain stem (B to F). The interrupted lines indicate the hollow needle inserted into and beyond the cannula shaft for the microinfusion of morphine. (For details see text.)

However, this part of the fourth ventricle is not usually reached by intraventricular injections since the lateral recesses through which the fluid passes into the subarachnoid space are situated in the middle of the ventricle. If morphine were to act at the same site in both series of experiments, the site would have to be on the ventral surface in a region caudal to the trapezoid bodies, a possibility not envisaged by either group of workers. This site is readily reached by intraventricular injections and forms the ventral surface of the 4 mm long block in the experiments of Satoh & Takagi (1971). It is also the site at which morphine apparently acts in the present experiments.

Some of the results have been presented to the Physiological Society (Dey & Feldberg, 1975b).

Methods

The experiments were done on cats of either sex weighing between 3 and 3.5 kg. For infusion of morphine into the fourth ventricle or into the subarachnoid space beneath the ventral surface of the brain stem either caudal to the pons or into the fossa interpeduncularis, a Collison cannula with a stilette inserted into its shaft was implanted in an aseptic operation under pentobarbitone sodium anaesthesia. After recovery from the operation the morphine was infused through these cannulae in a volume of 40 µl by a microinfusion pump which delivered this volume from a 100 µl syringe in 4 min 20 seconds. For the microinfusions the stilette was removed and a hollow stainless-steel needle (28 gauge) was inserted instead. The needle was connected to the syringe by a length of Polythene tubing, needle and tubing being filled with the solution to be infused. The general procedures of the operations as well as the infusions were those described previously (Dey *et al.*, 1975); details varied with the different implantations.

Infusion into the fourth ventricle

The Collison cannula was implanted in such a way that its tip lay in the ventricle near the end of the aqueduct. The method of implantation was the same as described by Feldberg & Gupta (1974) except that the cannula, which had a shaft 22 mm long was inserted at an angle of 28° instead of 20° directed posteriorly, insertion was in the mid line 7 mm anterior to the interaural line. The position of the cannular is shown in diagram A of Figure 1. The hollow stainless-steel needle was inserted through the entire length of the shaft and 1 mm beyond it.

Infusion into the subarachnoid space caudal to the pons

Four methods were used which are illustrated by diagrams B to E of Figure 1. Diagram B shows the method in which the Collison cannula was implanted into the fourth ventricle, but for the infusion the hollow stainless steel needle was inserted not only throughout the entire length of the shaft but also through the brain stem until the tip hit the bone. It was then withdrawn 1 mm before infusion began. The hollow needle was inserted under Althesin (2 mg/kg i.v.) anaesthesia lasting not longer than a few minutes. This short anaesthesia did not interfere with the subsequent testing for analgesia after infusion. Diagram C illustrates the method previously described by Dey *et al.* (1975) in which the Collison cannula with a shaft 37 mm long was implanted 1 mm lateral to the mid line and 13 mm anterior to the interaural line at an angle of 33° directed posteriorly so that the tip ended just beneath the ventral surface of the brain stem. Diagrams D and E illustrate two methods in which the hollow needle inserted into the cannula was allowed to glide 0.5 to 1 cm along the dura under the brain stem so that the tip lay in or a little beyond the region of the trapezoid bodies. The Collison cannulae with a shaft 28 mm long were implanted in the mid line in method D, 20 mm, and in method E, 23 mm, anterior to the interaural line at an angle of 40° directed posteriorly. In method D the tip of the

cannula ended within the brain stem and the hollow needle usually bent when, on being lowered (during short-lasting Althesin anaesthesia) it reached the bone; it then glided along the dura. This gliding was facilitated in method E by preparing the tip of the cannula so that on insertion, the part which would be nearest to the bone was deflected slightly upwards and extended 1 mm beyond the part facing the brain stem. Before the Collison cannula was fixed to the skull with acrylic cement, the gliding of the inserted hollow needle along the dura was tested because in method E, the tip of the cannula, or of the needle, sometimes hit the bone just on the sella turcica and then the needle did not glide along the dura. When this happened the cannula was withdrawn and reinserted 1 mm more caudally.

Infusion into the fossa interpeduncularis

The method described by Dey *et al.* (1975) was used. The shaft of the Collison cannula was 27 mm long and the position of the cannula is shown in diagram F of Figure 1.

At the end of the experiments, 40 µl of 0.8% bromophenol blue solution was infused through the Collison cannulae following the same procedure as for the morphine infusions. Fifteen minutes later, the cats were anaesthetized with intraperitoneal pentobarbitone sodium and after another few minutes the head was perfused from the aorta with 13% formalin solution. The brain was removed and with the naked eye the staining was observed of the dura, the walls of the ventricles and the surface of the brain stem. On infusion into the fourth ventricle there was no staining of the aqueduct or third ventricle, but deep staining of the anterior half of the floor of the fourth ventricle which contrasted sharply with the unstained posterior half. The lateral recesses and the dura beneath the brain stem were well stained; there was faint staining of its ventral surface; the basilar artery and its branches had usually taken up the dye and were lightly stained. With methods B, D and E, the dura beneath the brain stem was deeply stained, the staining extending from the sella turcica to the region of the medulla and sometimes of the cervical cord. The ventral surface of the brain stem and the basilar artery with its branches were faintly stained. There was no staining along the cannula tract in the brain stem or in the floor of the fourth ventricle. With method C the staining was either the same as described for methods B, D and E or, more usually, the dye had not freely entered the subarachnoid space and had deeply stained a region of varying size on the ventral surface around the tip of the cannula. In addition, the tract of the cannula either at its lower end or throughout its entire length was deeply stained, and sometimes the dye had even passed into the fourth ventricle and faintly stained the anterior half of its floor. There was no difference in the results obtained with morphine whether the tract and the floor of the fourth ventricle had become stained or not. Nevertheless the experiments in which the floor of the fourth ventricle became stained were not included in Table 1 which summarizes the results obtained on infusion of morphine into the subarachnoid space caudal to the pons. But the table includes two experiments (Nos. 9 and 12) in which the whole needle tract was found to be stained.

Injection into the cisterna magna

A Collison cannula was fixed to the back of the skull in an aseptic operation under pentobarbitone sodium anaesthesia so that the tip of the cannula shaft rested just above the atlanto-occipital membrane. For the injection, a hollow needle was lowered through the shaft piercing the membrane. The method has been described in detail elsewhere (Feldberg, Gupta, Milton & Wendlandt, 1973) and the position of the cannula is shown in the lower diagram of Figure 4. The morphine was injected in a volume of 0.1 ml and washed in by another 0.1 ml 0.9% w/v NaCl solution (saline). With this method of injection no morphine enters the cerebral ventricles which remain unstained when 0.8% bromophenol blue instead of morphine is similarly injected.

Testing for analgesia

The testing was carried out with the cat placed in a 50 × 50 cm and 53 cm high wire cage, the whole front of which could be opened. The tail pinch method of Russell & Tate (1975) was used in which a small diameter rod presses across the root of the tail. The method allows the application of graded pressure for a short time; the device works hydraulically and the hydraulic pressure is measured by a Bourdon gauge in kg/cm^2. Although this pressure is not identical with the force exerted on the tail it is proportional to it and can therefore be used, without conversion, for measurements of analgesia. In the present experiments pressure was maintained for 5 s, and with the special instrument used no response was produced by a pressure which varied in different experiments between 0.2 and 0.8 kg/cm^2 (mean 0.4 kg/cm^2). When a response was obtained by applying stronger pressure it was classified as either weak, medium or strong.

A weak response which need not always be one to pain, consisted of slight tension in the animal, usually in the hindquarters, or of lifting of the hindquarters, or of the cat slowly getting up and slowly moving about, or of turning the head toward the pincher, staring at it, but not trying to bite it, or of holding the pincher with a forepaw without attempting to remove it. Sometimes the cat got up quickly for a moment at the beginning of the pressure, but then relaxed; this, too, was classified as a weak response.

A strong response suggested intense pain. It consisted of the cat jumping up, occasionally somer-

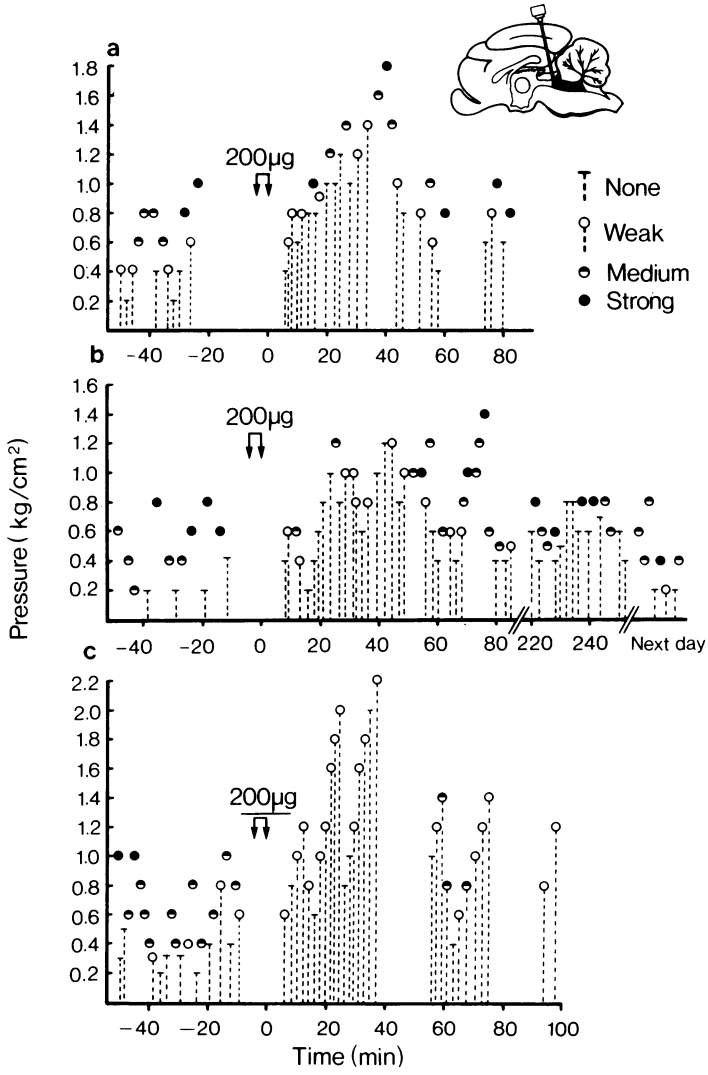

Figure 2 Responses in three cats to pressure exerted for 5 s on the root of the tail before and after infusion of 200 μg of morphine into the fourth ventricle. In this and Figures 3 and 4, the ordinates give the pressure in kg/cm² measured by a Bourdon gauge in the device used which is not identical, but proportional to that exerted on the tail (see Methods section).

saulting and/or of strong arching and vigorous struggling, of strong biting of the pincher or the attached tubing, and continued biting after the pressure was removed, and sometimes of miaowing or hissing and salivation as well.

A medium response suggested definite pain. It consisted of strong arching as the main or sole reaction, or of quickly getting up and rapidly moving about, or of some struggling with or without an attempt to remove the pincher, or of mild biting of the pincher or attached tubing, but with little or no struggling, or of a single miaow. Sometimes it was difficult to decide between a medium and a strong, or between a medium and a weak response.

In the course of several experiments a medium and a strong response became associated, particularly after morphine administration, with tachypnoea, mydriasis and the appearance or intensification of shivering which outlasted the 5 s period of increased pressure exerted on the tail for a minute or two. The morphine used was the sulphate and all values given in the text refer to the salt.

Results

Infusion into the fourth ventricle and into the subarachnoid space beneath the ventral surface of the brain stem

Fourth ventricle. Infusion of 200 μg of morphine into the fourth ventricle produced strong analgesia. The effect was not the result of absorption of morphine into the blood stream, because an intravenous injection of double the dose did not produce analgesia. The analgesia produced on infusion into the fourth ventricle began to develop within 5 to 10 min and became maximal within 15 to 40 min after the infusion, but varied in intensity and duration from experiment to experiment. This is illustrated by the three experiments of Figure 2.

The top record (Figure 2a) is from an experiment in which the analgesic effect was maximal after about 30 min, when the pressure exerted on the root of the tail could be increased to as much as 1.4 kg/cm^2 without producing more than a weak reaction. Before the infusion this happened when the pressure was not greater than 0.4 kg/cm^2. The middle record (b) is from an experiment in which some remnant of the analgesic effect was present 4 h after the infusion, and the bottom record (c) illustrates an experiment in which the analgesia produced was particularly strong so that a pressure of 2.2 kg could be applied without producing more than a weak response. This experiment illustrates another feature often encountered during morphine analgesia; a weak response was elicited whether the pressure applied was only 0.8 kg/cm^2 or as much as 2.2 kg/cm^2. Before a morphine infusion the difference in pressure required to change a weak to a medium or a medium to a strong response was relatively small, such a steep gradient often no longer pertained during the morphine analgesia.

Another feature of the morphine analgesia not evident from the scoring used was the delay of the reaction. Before the morphine infusion, a medium or strong reaction as well as a weak reaction occurred usually immediately the pressure was raised, whereas during the morphine analgesia the cat often did not react immediately, but only during the last second or two of the 5 s period of increased pressure, or at the very end of this period, or even a second or two later.

In two experiments in which 100 μg of morphine were infused the pressure which would produce not more than a mild response was, before the infusion, 0.2 kg/cm^2 in the one and 0.6 kg/cm^2 in the other experiment; in both it increased after the infusion to 1 kg/cm^2. And in three experiments in which 50 μg were infused this pressure increased from 0.4 kg/cm^2 in all three experiments before, to 0.6, 0.8 and 1.2 kg/cm^2 respectively after the infusion. Smaller doses than 50 μg were not tested, but the infusion of saline (40 μl) had no analgesic effect.

Subarachnoid space beneath the brain stem. Infusion of 200 μg of morphine into the subarachnoid space caudal to the pons produced strong analgesia, and the results obtained with any of the four methods which are illustrated in the diagrams B, C, D and E, of Figure 1 were essentially the same. Table 1 summarizes the results of 13 experiments, and for four of them (Nos 1, 7, 9 and 11) the development and disappearance of the analgesia is illustrated in Figure 3.

The analgesia produced on the infusion of morphine

Table 1 Analgesia produced in 13 cats on infusion of 200 μg morphine into subarachnoid space caudal of pons

	Threshold pressure (kg/cm²) on tail which produces no, or weak response		*Time (min) after morphine infusion*		
Cat. no.	*Before morphine*	*After morphine*	*Beginning of analgesia*	*Maximal analgesia*	*Method used (see Figure 1)*
1	0.3	0.8	1	7	D
2	0.3	1.0	1	17	E
3	0.3	1.0	6	33	D
4	0.4	1.0	1	6	E
5	0.2	1.2	6	16	C
6	0.4	1.2	3	9	B
7	0.4	1.4	6	30	C
8	0.6	1.4	3	10	C
9	0.3	1.6	5	28	C
10	0.4	1.6	2	19	E
11	0.6	2.0	3	12	E
12	0.8	2.2	6	25	C
13	0.8	2.6	5	16	D

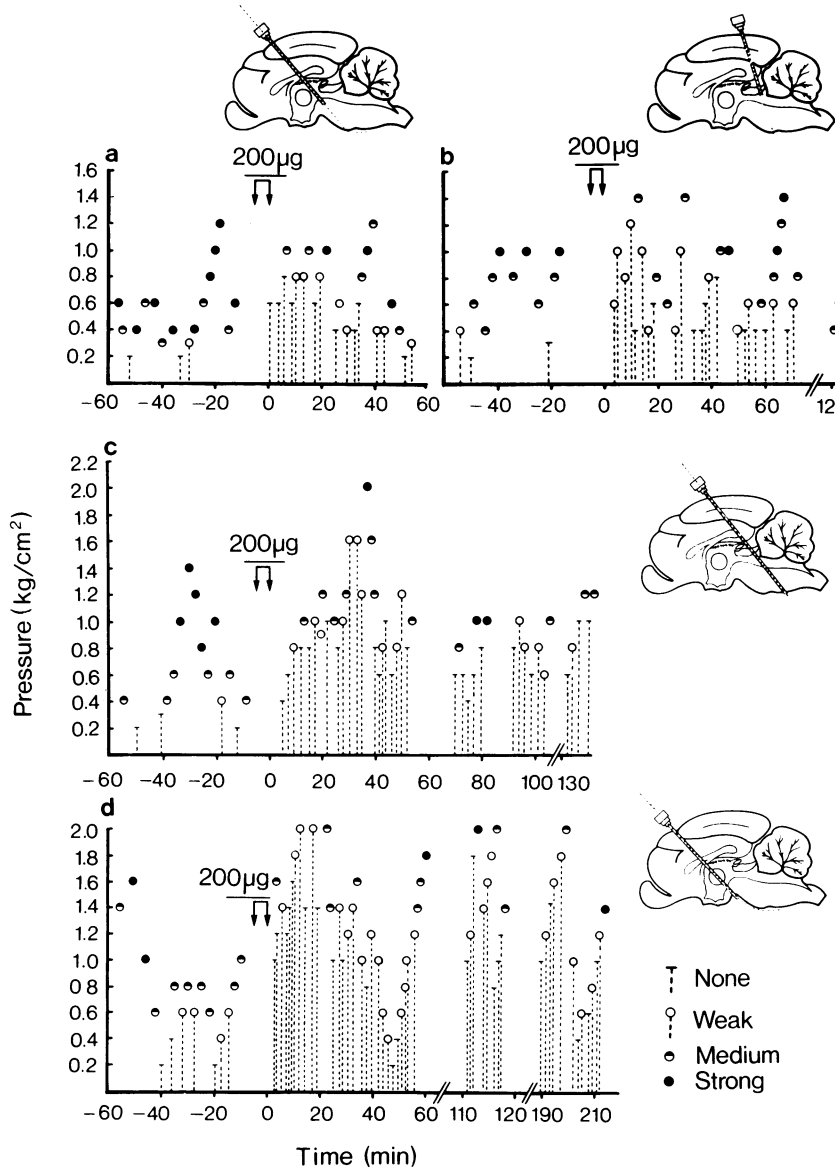

Figure 3 Responses in four cats to pressure exerted for 5 s on the root of the tail before and after infusion of 200 μg of morphine into the subarachnoid space beneath the ventral surface of the brain stem using the methods D and B (top records, a and b), C (middle record, c), and E (bottom record, d) of Figure 1.

caudal to the pons often began earlier and developed more rapidly than on its infusion into the fourth ventricle. On infusion of 200 μg into the fourth ventricle no definite signs of analgesia were obtained when the tail pinch test was made earlier than 5 min after the infusion, but this happened in 7 out of the 13 experiments of Table 1, and in three of them signs of analgesia were already present 1 min after the infusion. Further, on infusion into the fourth ventricle, it took 20 to 40 min for the analgesia to develop fully whereas in 6 of the 13 experiments of Table 1 it took less than 15, and in three, even less than 10 minutes.

In experiment Nos 5 and 12 of Table 1 in which method C was used, the infusion of bromophenol blue at the end of the experiment resulted in deep staining of the needle tract within the brain stem. The infused

morphine probably followed the same distribution. Nevertheless, an action on structures along the needle tract can be excluded even as a contributory factor in the morphine analgesia, because in two experiments (not included in the Table) in which method C was used the tip of the infusion needle had not pierced the ventral surface, but had stopped about 1 mm short of it, no analgesia developed after the morphine infusion. On subsequent bromophenol infusion, it was found that the dye had not entered the subarachnoid space but had deeply stained the whole needle tract in the brain stem and at its lower end around the tip of the cannula a cavity had formed which was deeply stained as well.

Figure 3 illustrates four experiments of Table 1 in which the infusion of 200 μg of morphine into the subarachnoid space caudal to the pons produced analgesia of different degree and duration. For each experiment a different method of infusion (either B, C, D or E of Figure 1) was used. In the experiment of Figure 3a, analgesia had disappeared within 50 min; in that of Figure 3b it was maximal within 9 min, and had not fully disappeared within 70 min; in the experiments c and d analgesia persisted even longer, for over 2 h in c and for over 3.5 h in d. These two experiments illustrate another feature encountered in that analgesia after having diminished, sometimes increased again.

In one experiment in which method E was used, the needle had pierced the dura and glided subdurally in the region of the trapezoid bodies. No analgesia developed on infusion of 200 and 400 μg morphine. In two experiments, one of which is illustrated in Figure 4a, the infusion of 200 μg of morphine was into the fossa interpeduncularis (method F of Figure 1). No analgesia developed.

Other effects. During or shortly after the infusions of 200 μg of morphine into the fourth ventricle or into the subarachnoid space caudal to the pons, the vessels of the pinna dilated and the ears became red and hot. This skin vasodilatation persisted for up to 3 hours. Another long lasting effect was midriasis. On infusion into the fourth ventricle it occurred within a few minutes, on infusion caudal to the pons 15 to 60 min after the infusion. Other effects not regularly observed were salivation, retching, vomiting, shivering and in one experiment on infusion caudal to the pons, tachypnoea and panting.

The behavioural changes brought about by the infusions into the fourth ventricle and caudal to the pons consisted of an initial period of quietness with or without signs of stupor and catalepsy followed by a characteristic state of hyperexcitability. In addition, the cats became affectionate and playful.

Either during or shortly after the infusions, the cats became quiet. A previously lively cat no longer moved about, but assumed a low sitting position. Some of the cats became stuporous. Slight cataleptic features were observed in one cat on infusion into the fourth ventricle and pronounced catalepsy lasting for over 2 h developed in two cats (Nos 11 and 13 of Table 1) on infusion caudal to the pons. The two cats could be put in abnormal positions which they retained without struggling for 20 to 30 s, for instance, when they were placed in an erect posture with the forepaws on the rung of an inverted stool. Nevertheless when the forepaws dropped from the rung the cats moved away in a well co-ordinated manner showing no signs of ataxia.

Before the state of hyperexcitability developed the cats were sitting upright with a startled look: the eyes wide open, pupils dilated, no blinking and the ears held sharply erect. This condition in which the cats were staring vacantly, lasted between a few minutes and over half an hour, and the the cats began to make, every few seconds, abrupt side-to-side movements of the head, and less frequently, rapid, short-lasting circling movements which apparently occurred spontaneously. But they could be triggered off by sudden noises and were provoked each time the cats were touched. Yet the cats were not frightened. In between the circling movements they resumed the upright sitting position with a wide-eyed vacant stare. The abrupt side-to-side movements of the head and the short-lasting rapid circling movements continued usually for over 2 hours. On infusion into the fourth ventricle the condition of hyperexcitability developed only rarely, but the preceding state in which the cats sat upright with a wide-eyed vacant stare was always present. Between the state of quietness and hyperexcitability, or when the hyperexcitability was wearing off, or in both conditions, the cats were affectionate and playful.

Injections into the cisterna magna

Morphine produced analgesia on injection into the cisterna magna but the doses required were larger than on infusion into the fourth ventricle or on infusion into the subarachnoid space caudal to the pons. However, the injections were made in a larger volume, in 0.1 ml washed in by another 0.1 ml as compared to 40 μl the volume used for the infusions of morphine.

With 400 μg of morphine injected into the cisterna there was just an indication of analgesia, but with 800 μg a strong and long-lasting analgesia ensued, as illustrated by the experiment shown in Figure 4b. In this experiment the tail pinch test could not be applied during the first 10 min after the injection because of frequent bouts of scratching movements which morphine is known to produce on injection into the cisterna and into the cerebral ventricles. The scratching movements are attributed to an action on structures near the dorso-lateral surface of the upper cervical cord (Mehes, 1939, Königstein, 1939; Banerjee, Feldberg & Lotti, 1968). After the first 10 min the bouts of scratching movements occurred

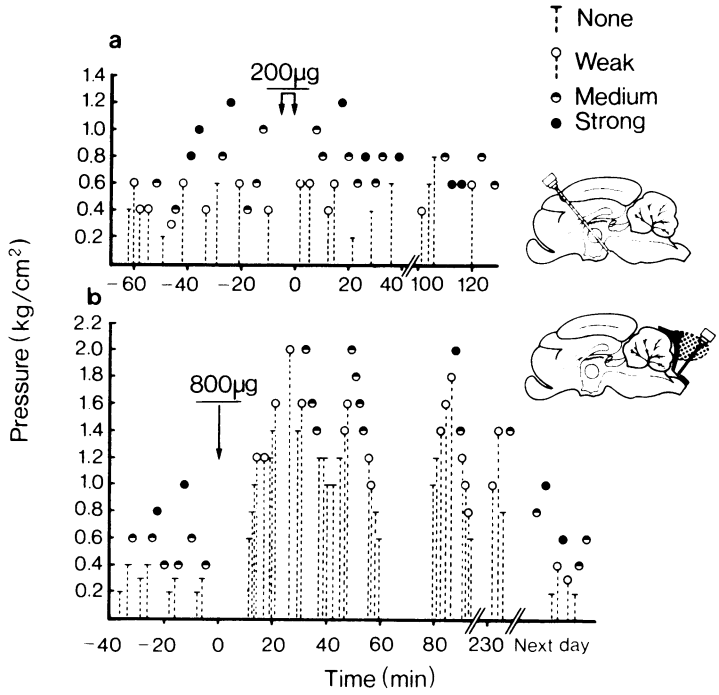

Figure 4 Responses in two cats to pressure exerted for 5 s on the root of the tail. (a) Before and after infusion of 200 µg of morphine into the fossa interpeduncularis using method F of Figure 1. (b) Before and after injection of 800 µg of morphine into the cisterna magna.

less frequently allowing tests to be made in between, and after another 10 to 20 min they occurred at long intervals and in between the cats became quiet so that there was scarcely any interference with the tests. At this stage the pupils were dilated and the ears were hot and red. During the initial stage of frequent bouts of scratching movements there was tachypnoea and sometimes there were short periods of panting. In one experiment in which bouts of scratching movements were less violent and occurred less frequently during the first 10 min after the injection, the tail pinch test was applied every minute from the third minute after the injection; the first signs of analgesia occurred in the eleventh minute.

From results obtained on injection of bromophenol blue into the cisterna magna it is evident that even during the violent bouts of scratching substances injected into the cisterna do not enter the cerebral ventricles. Bromophenol blue injected into the cisterna magna also produces violent bouts of scratching movements (Feldberg & Fleischhauer, 1960). At the end of the experiment shown in Figure 4b, 0.2 ml of a 0.8% bromophenol blue was injected into the cisterna. After a few minutes the bouts of scratching movements became so violent that the cat was anaesthetized, 10 min after the injection, with intraperitoneal pentobarbitone sodium. The scratching movements continued and the cat was killed after another 10 minutes. Post mortem, no dye was found in the cerebral ventricles; there was no staining of the floor of the fourth ventricle, of the walls of the aqueduct, third and lateral ventricles. But the dorsal and ventral surface of the brain stem and cervical cord were deeply stained, and the dye had spread along the cerebellum and cerebral cortices and part of their surfaces were well stained. A similar staining had previously been observed with smaller amounts of bromophenol blue injected into the cisterna magna of anaesthetized rabbits (Banerjee, Burks, Feldberg & Goodrich, 1968).

Discussion

The present experiments on cats show that morphine produces analgesia when introduced into the liquor space in doses too small to be effective on intravenous injection. The effect could therefore not have been produced after absorption into the blood stream. Instead, the morphine must have acted on structures reached from the liquor space.

The analgesia occurred not only on infusion of

morphine into the fourth ventricle, but also on its infusion or injection into the subarachnoid space, either into the space beneath the brain stem caudal to the pons, or into the cisterna magna. In both circumstances, the structures acted upon must have been reached from the subarachnoid space since the morphine would not have entered the cerebral ventricles. The fact that somewhat larger doses were required to produce analgesia on injection into the cisterna than on infusion beneath the brain stem, suggests an action on structures near its ventral surface because on the way to this surface morphine injected into the cisterna becomes more diluted with c.s.f. than when infused directly beneath it. This surface would also readily be reached, without much dilution, by morphine infused into the fourth ventricle, because of the communication through the lateral recesses with the subarachnoid space.

The ventral surface of the brain stem would therefore appear to be the site at which morphine acts in all three circumstances when producing analgesia. It is possible that morphine infused into the fourth ventricle acts on an additional site in the floor of this ventricle although no evidence to support the existence of such a site was obtained by comparing the times it took for the first signs of analgesia to appear and for full analgesia to develop. These times were often shorter on infusion into the space beneath the ventral surface than on infusion into the fourth ventricle.

The situation is different when morphine is injected into a lateral or into the third ventricle when it may act not only on the ventral surface of the brain stem but also on the grey matter of the wall of the third ventricle and on the periaqueductal grey since microinjections of morphine into these regions were found to produce analgesia (Tsou & Tang, 1962; Jacquet & Lajtha, 1973; Pert & Yaksh, 1974). In fact, it was recently found that on infusion of morphine into the third ventricle, the analgesia produced was more pronounced than on infusion into the fourth ventricle or into the subarachnoid space caudal to the pons (Dashwood & Feldberg, unpublished experiments). With the methods used in the present experiments, however, the third ventricle and aqueduct are not reached by the morphine.

What then are the structures on or near the ventral surface of the brain stem on which morphine may act when producing analgesia? One mechanism by which morphine is thought to produce analgesia is by activating a descending inhibitory pathway which originates in the raphe nuclei, is tryptaminergic, projects to the spinal cord and there exerts an inhibitory action on the small-diameter afferent fibres conveying pain impulses. There is abundant evidence for this mechanism of action. Morphine analgesia is reduced or abolished by substances which selectively deplete tryptaminergic neurones of their 5-hydroxytryptamine (5-HT), like *p*-chlorophenylalanine (Tenen, 1968; Fenessy & Lee, 1970; Samanin, Gomulka & Valzelli, 1970; Görlitz & Frey, 1972; Vogt, 1974) and 5,6-dihydroxytryptamine (Genovese, Zonta & Mantegazza, 1973; Diaz, Ellison & Masuoka, 1974; Vogt, 1974). Furthermore, electrical stimulation of the raphe nuclei and of many points of the periaqueductal grey in their vicinity elicit analgesia which is blocked by 5-HT depletion produced by *p*-chlorophenylalanine (Reynolds, 1969; Akil & Mayer, 1972; Mayer & Liebeskind, 1974; Mayer, Wolfle, Akil, Carder & Liebeskind, 1974; Akil & Liebeskind, 1975; Proudfit & Anderson, 1975), whereas lesions of the raphe nuclei which lead to 5-HT depletion block morphine analgesia (Redjemi, Oliveras, Guilbaud & Besson, 1974; Proudfit & Anderson, 1975).

It is doubtful that substances injected into the cerebral ventricles reach the raphe nuclei. Otherwise a substance which depletes the 5-HT stores of the brain should also exert this effect on the 5-HT of the raphe nuclei when injected intraventricularly. Yet, 5,6-dihydroxytryptamine given into a lateral ventricle while reducing the 5-HT of the spinal cord by nearly 90% had scarcely any effect on the mid brain 5-HT (Baumgarten, Evetts, Holman, Iversen, Vogt & Wilson, 1972; Vogt, 1974). This was in contrast to the effect of *p*-chlorophenylalanine given systematically. It depleted not only the 5-HT of the spinal cord, but also that of the midbrain (Vogt, 1974). A convincing explanation of this difference in the effect of the tryptamine derivative would be that it does not reach the raphe nuclei on intraventricular injection, but reached its descending tryptaminergic fibres on their way to the spinal cord. The site reached and acted upon by the 5,6-dihydroxytryptamine would then appear to be at the ventral surface of the brain stem lateral to the pyramids, because these descending tryptaminergic fibres run to the ventral surface of the medulla and form a narrow distinct band lateral to each pyramid. This was shown by Nobin, Baumgarten, Björkland, Lachenmayer & Stenevi (1973). An additional observation favouring this view was made by Vogt (1974). In her rats a permanent brownish discolouration indicating the spread of 5,6-dihydroxytryptamine was found following its injection into a lateral ventricle 'on the surface of the third ventricle, the aqueduct and the anterior part of the fourth ventricle' but not on its posterior part which is evidence that it had passed through the lateral recesses into the subarachnoid space. From these various findings it would appear that 5,6-dihydroxytryptamine has the ability to deplete 5-HT stores when acting on fibres of tryptaminergic neurones.

Morphine, too, would not reach the raphe nuclei when infused into the fourth ventricle, or into the subarachnoid space beneath the brain stem, or injected into the cisterna magna. But in all three circumstances, it would reach the ventral surface of the brain stem. Therefore if the morphine analgesia obtained in the present experiments is due to activation of the tryptaminergic descending inhibitory

pathway, the possibility has to be considered that it, too, acts not on synapses or cells, but on nerve fibres belonging to tryptaminergic neurones. This possibility would be compatible with all observations made in favour of the theory that morphine analgesia involves activation of a descending inhibitory tryptaminergic pathway arising from the raphe nuclei.

If activation of a descending inhibitory tryptaminergic pathway is also responsible for the analgesia produced by morphine applied systemically, and all the evidence with *p*-chlorophenylalanine and with lesions of the nucleus raphe magnus for the role of such a descending inhibitory pathway in morphine analgesia was obtained with the morphine applied systemically, then its action under this condition may be on the raphe nuclei themselves. The finding that 5,6-dihydroxytryptamine merely reduced the analgesia of morphine given subcutaneously but did not abolish it is readily explained on the assumption that not all descending fibres from this raphe nucleus were reached and affected by the 5,6-dihydroxytryptamine injected intraventricularly with the result that the spinal cord 5-HT was greatly reduced but not fully depleted as after systemic application of *p*-chlorophenylalanine. As pointed out by Vogt (1974), activation of the remaining intact tryptaminergic fibres may have been sufficient to produce analgesia.

But even on systemic application, the analgesic effect of morphine may in part, at least, be due to an action not on the raphe nuclei themselves, but on their descending tryptaminergic fibres. For instance, in the experiments of Satoh & Takagi (1971) described in the introduction, intravenous morphine (4 mg/kg) acted caudal to the trapezoid bodies within a 4 mm long block of brain stem tissue which at most included but the very distal remnants of raphe nuclei. An action on them may be sufficient to account for the inhibitory effect morphine produced in these experiments on afferent pain impulses. This possibility cannot be excluded. But there is the other possibility of an action on the descending tryptaminergic fibres as they emerge on the ventral surface in this region of the brain stem, which is penetrated here by numerous small arteries and thus provided with an abundant blood supply.

The concept that morphine, like 5,6-dihydroxytryptamine, acts on nerve fibres may apply only to fibres of tryptaminergic neurones, because of their peculiar property of becoming activated for a long time by morphine. Non-myelinated fibres of non-tryptaminergic neurones may not share this morphine sensitivity. For instance, Kosterlitz & Wallis (1964) found no effect of morphine on the C fibres of peripheral nerves, but they looked mainly for an inhibitory effect. If sensitivity to morphine is peculiar to fibres of tryptaminergic neurones, other effects of morphine, apart from analgesia, may well be accounted for by a synaptic action which may even apply to the analgesia produced by large doses of morphine on the spinal cord. In the experiments of Satoh & Takagi (1971), cervical cord transsection did not prevent large doses of morphine (8 to 10 mg/kg intravenously) from abolishing the afferent volleys in the cord produced on stimulation of the splanchnic nerves. The descending tryptaminergic fibres in the cord were not previously put out of action in these experiments, either by lesion of the raphe nucleus magnus, by *p*-chlorophenylalanine or by 5,6-dihydroxytryptamine. But if the effect was not dependent on the integrity of the descending tryptaminergic fibres in the cord, it could result from a synaptic action of morphine in the spinal cord.

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