Br. J. Pharmac. Chemother. (1968), **33**, 544–551.

TEMPERATURE EFFECTS AND CATALEPSY PRODUCED BY MORPHINE INJECTED INTO THE CEREBRAL VENTRICLES OF RABBITS

BY

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(*Received March* 30, 1968)

It was recently shown that morphine, like 5-hydroxytryptamine (5-HT), produces hypothermia in rats, but hyperthermia in cats, when acting on the anterior hypothalamus (Lotti, Lomax & George, 1965 ; Banerjee, Feldberg & Lotti, 1968). Because morphine depletes the monoamines of the brain the possibility has been discussed that 5-HT may be the mediator of its temperature effects in these species. In the present experiments morphine was injected into the cerebral ventricles of rabbits to see if in this species, too, its effect on temperature resembles that of 5-HT similarly applied.

In the course of the experiments, an effect of morphine was obtained which so far seems to have been observed with this alkaloid in rats only (Reynolds & Randall, 1957)—that is, catalepsy. It developed not only when the morphine was given by the intraventricular route but also following its injection into the cisterna magna.

METHODS

New Zealand white or Dutch rabbits of both sexes weighing 1.7–3 kg were used. A Collison cannula was aseptically implanted under pentobarbitone sodium anaesthesia into the left lateral cerebral ventricle. The point of insertion was 7 mm lateral to the midpoint of the sagittal suture as described by Hasselblatt & Sproull (1961). An interval of at least 3 days was allowed between implantation of the cannula and the injection of drugs. They were injected in a volume of 0.1 ml. followed by 0.05 ml. of 0.9% NaCl solution.

For injections into the cisterna magna the rabbit was restrained by hand, the neck was flexed and a 23 gauge needle with stilette was pushed through the skin and muscle in the midline just posterior to the lower margin of the occipital bone until the tip of the needle was felt to penetrate the dura. If the tip had entered the cisterna, clear cerebrospinal fluid (c.s.f.) welled up in the hub of the needle when the stilette was removed. A 1 ml. glass syringe filled with either 0.9% NaCl or the morphine solution was then attached to the needle and a volume of 0.1 ml. fluid was allowed to flow in by gravity. If no c.s.f. appeared in the needle hub or if the fluid was tinged with blood, no injections were made. Little discomfort seemed to be caused either by insertion of the needle or by the injection, for the rabbit made no attempt to struggle.

In order to see which areas of the brain are reached by solutions injected in this way, 0.1 ml. of a 0.2% solution of bromophenol blue, prepared as described by Feldberg & Fleischhauer (1960a), was injected into the cisterna magna of unanaesthetized rabbits. Within a few minutes this produced vigorous scratching movements which result from an action on structures near the dorso-lateral surface of the upper cervical cord, because bromophenol blue applied to this region is known to

elicit these movements in cats (Feldberg & Fleischhauer, 1960b). Twenty minutes after the injection the rabbits were killed under pentobarbitone sodium anaesthesia by opening the thorax and clamping off the heart. The brain was then fixed by perfusing the head through the cannulated thoracic aorta first with 0.9% NaCl solution, then with 30% formalin. The perfusion fluid was allowed to flow out from the cut jugular veins. After removal of the brain and cervical cord the regions stained by the dye were observed with the naked eye.

Rectal temperature was measured by a thermistor probe (Yellow Springs) inserted about 10 cm into the rectum and held in position by adhesive tape affixed to the tube of the probe and gently wrapped around the base of the tail. The temperature was monitored continuously by a Kent multi-channel recorder. The figures reproduced in this paper are plotted from the tracings obtained in this way.

Drugs used were morphine sulphate and nalorphine hydrobromide (Lethidrone, Burroughs Wellcome & Co.). The doses refer to the salts. The morphine was dissolved in pyrogen-free 0.9% NaCl solution and for the injections pyrogen-free syringes and needles were used.

RESULTS

Injections into the cerebral ventricles

Intraventricular injections of morphine 25–100 μg produced changes in rectal temperature, in the tone of the ear vessels and in respiration; in addition, they induced a condition of catalepsy or "catatonic stupor" followed by rigidity and hyper-reflexia. None of these effects was produced when the same or even larger doses of morphine (250 or 500 μg) were injected intraperitoneally.

Following an intraventricular injection of 25 μg morphine, temperature rose gradually 1° to 1.5° C during the subsequent 3–4 hr. Curve A of Fig. 1 illustrates this response. In one experiment the rise was preceded by a small fall. This was the common result obtained with 100 μg when an initial fall of 0.2° to 0.3° C during the first half hour preceded a rise of up to 1.5° C during subsequent hours (curve B). In some experiments, with 100 μg, the fall was more pronounced and longer lasting (curve C); in one it was absent and the response was a gradual rise similar to that usually seen after 25 μg. An intraventricular injection of 250 or 500 μg morphine resulted in a steep rise in temperature which remained elevated for several hours; but the rise was associated with excitement and increased muscular activity sometimes leading to convulsions. A response to 250 μg is illustrated by curve D.

During the initial fall in temperature the ear vessels were dilated but they often remained dilated during the subsequent rise; they also dilated following the injection of 25 μg, which produced only a rise in temperature.

The main effect on respiration was bradypnoea preceded, during the initial fall in temperature, by a period of rapid shallow respiration. The bradypnoea developed gradually over 1.5 hr; when the rate fell to 20/min or less, respiration sometimes became intermittent, a period of apnoea being followed by 4 or 5 rapid respirations. During this stage cyanosis was observed in the ears, eyes and nose.

The most striking effect was a condition of catalepsy which appeared within 1.5 hr, became fully developed within another hour and persisted for several hours. When fully developed the rabbit lay on its belly with its head resting on the floor and could be put into abnormal positions without resisting. Catalepsy was recognized by the following tests.

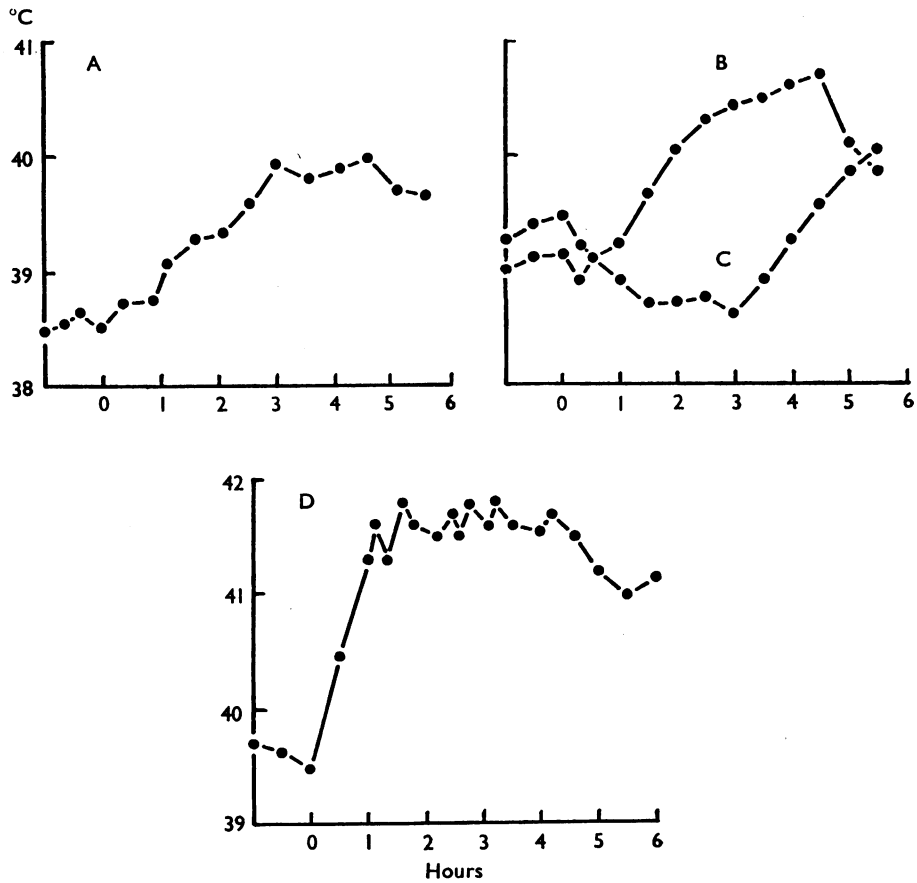

Fig. 1. Records of rectal temperature obtained from unanaesthetized rabbits. At zero time injections of morphine sulphate through an indwelling Collison cannula in the left lateral cerebral ventricle, A, 25 μg; B and C, 100 μg; and D, 250 μg.

(1) A foreleg when abducted or extended could be held in this abnormal position by the experimenter without the rabbit making any effort to resist. When the limb was released the rabbit did not return it to a more normal position for many seconds, or even minutes.

(2) A foreleg when brought round behind the rabbit's back so that it rested across the spine remained in this position for some time after the limb was released.

(3) The rabbit when placed in a nearly erect position with its forepaws over the rung of an inverted stool maintained this posture with its head held erect, staring ahead with open eyes until its forepaws were taken off the rung 5–10 min later. This condition is illustrated in Fig. 2. Sometimes the rabbit gradually lost its hold on the rung and sagged to the ground.

(4) The rabbit when slowly turned onto its back did not resist and, with all four legs half extended, remained in this position without support. When it suddenly flopped over,

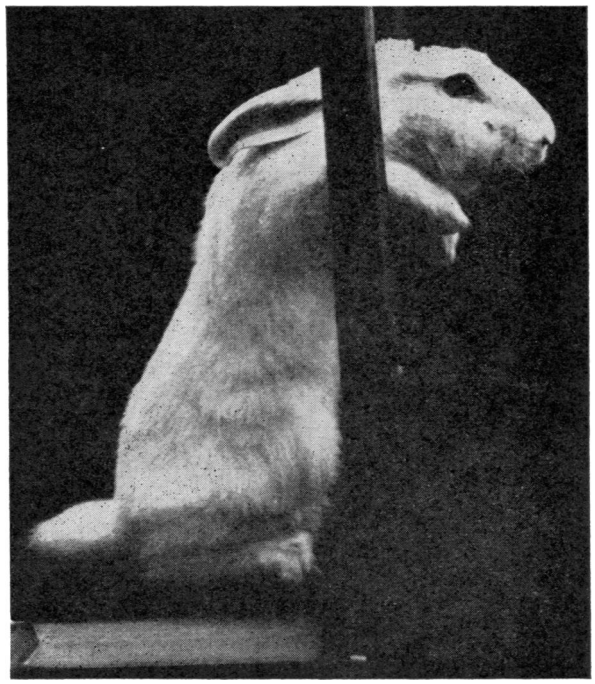

Fig. 2. Catalepsy produced by an injection of 100 μg morphine sulphate through an indwelling Collison cannula in the left lateral cerebral ventricle.

it did so in a well co-ordinated manner ; that is, its movements were not impaired. This was also evident when the rabbit made some movements after being handled. Most of the time the rabbit lay on its belly with its head on the floor, but it would give a startle response to sudden noises or to being touched on the back.

With fully developed catalepsy, which occurred regularly after 100 μg morphine, usually after 25 μg and once after 10 μg, all four tests were positive. Otherwise only tests 1 and 2 were positive, and with test 3 the rabbit maintained the nearly erect position for a short time, less than 1 or 0.5 min.

A few hours after the injection of morphine (100 μg) strong rigidity of the limbs was encountered on handling the animal, and there was pronounced hyper-reflexia in response to tapping the spine or the limbs. In addition, the rate of respiration approached 10/min and cyanosis deepened. On intraventricular injection of 100 μg nalorphine, the bradypnoea, cyanosis, catalepsy, rigidity and hyper-reflexia were abolished. Respiration improved within a minute and its rate increased to over 100/min within 3 min while cyanosis disappeared. Catalepsy, rigidity and hyper-reflexia passed off more slowly.

Injections into the cisterna magna

Catalepsy, rigidity, hyper-reflexia and slowing of respiration occurred also on intracisternal injection of morphine (100 μg). Catalepsy began earlier (after about 40 min)

than after an intraventricular injection, and the rigidity and hyper-reflexia, which were a late sign when the morphine was applied by the intraventricular route, developed almost simultaneously with catalepsy. Within a few minutes of an intracisternal injection of 100 μg of nalorphine, or of an intravenous injection of 1 mg, catalepsy, rigidity, hyper-reflexia and bradypnoea disappeared.

Following the intracisternal injection of morphine 25 μg there were signs of catalepsy although it did not fully develop ; there was some rigidity but no hyper-reflexia. When the rabbit was placed in a nearly erect position with its forepaws over a horizontal bar 24 cm above the ground, the animal did not place its hind feet flat on the ground but rested on its tarsal bones with the feet flexed so that they pointed upwards. The rabbit usually did not maintain this position, but when the test was repeated with the horizontal bar only 12 cm above the ground, it remained in this less erect posture for several minutes.

With 250 μg given intracisternally, full catalepsy with rigidity developed within 15 to 30 min, but there were no convulsions. The animals appeared to be hyperexcitable and were easily startled. When placed in a nearly erect position with the forepaws over a horizontal bar, and having maintained this posture for several minutes, they sometimes, as if startled, leaped over the bar and then hid in a corner of the cage.

When bromophenol blue instead of morphine was injected intracisternally it was found that the dye had not entered the cerebral ventricles. There was no staining of the floor of the fourth ventricle or of the walls of the aqueduct, third and lateral ventricles. The hypothalamus, caudate nucleus and hippocampus, which become deeply stained when bromophenol blue is injected into the cerebral ventricles, showed no trace of staining. On the other hand, the dye had spread over wide regions in the subarachnoid space. Not only the medulla oblongata and cervical cord but also the entire ventral surface of the brain stem was found to be stained, and the staining was particularly deep in the mid-brain region bordering the interpeduncular cisterna. The bromophenol blue had passed up to the anterior end of the subarachnoid space and had stained the olfactory bulbs. The bromophenol blue had also reached the dorsal surface of the brain stem and stained the corpora quadrigemina. The cerebellum was stained on its anterior surface and around the lower end of the vermis. The cerebrum was stained on its ventral surface in the piriform and olfactory lobe, on the anterior poles of the frontal cortices above the olfactory tracts and in the regions above the corpora quadrigemina.

DISCUSSION

The changes in body temperature obtained with intraventricular injections of morphine depended on the dose given. The steep long-lasting rise obtained with the largest doses is probably not, or not entirely, due to an action on the anterior hypothalamus because it was associated with excitement, muscular activity and sometimes with convulsions, effects which by themselves would raise body temperature. At present, it is also not possible to decide whether the gradual rise in temperature obtained with the smaller doses of morphine results from its action on the anterior hypothalamus or from increased muscle tone preceding the rigidity. The fact that the vasodilatation in the ears often persisted during the rise suggests that the hyperthermic response results not from an action in the temperature regulating centres but from the increased muscle tone. If this

were the case, morphine would have only a weak and irregular hypothermic effect when acting on the anterior hypothalamus of rabbits. Its hypothalamic temperature effects would then resemble those of 5-HT not only in cats and rats, but in rabbits as well. The similarity could mean that morphine either has a direct 5-HT-like effect on the anterior hypothalamus in these three species, or that it acts indirectly through the release of 5-HT.

On the other hand, if the rise results from an action on the anterior hypothalamus then, on the assumption that the monoamines are the mediators of the temperature responses, morphine would release not only 5-HT but noradrenaline as well. This explanation differs from that suggested for the temperature effects produced in rats and cats by morphine given by the intraventricular route or injected directly into the anterior hypothalamus. In these two species the temperature effects could be accounted for by release of 5-HT alone, because when applied in this way morphine, like 5-HT, lowers temperature in rats and raises it in cats. In rats noradrenaline and adrenaline also lower temperature when acting on the anterior hypothalamus, so in this species, as in rabbits, release of both 5-HT and catecholamines could account for the temperature response to morphine. In cats there was no sign of release of catecholamines as far as the temperature response was concerned, although reduction of hypothalamic noradrenaline and adrenaline occurs also in cats and was in fact first described in this species (Vogt, 1954).

With reserpine, which also depletes the brain of its monoamines, a fall preceded the rise in temperature when it was injected into the cerebral ventricles of cats, and this fall could convincingly be explained by release of noradrenaline, because on repeated daily injection it was no longer produced by the second or third reserpine injection, as the brain monoamines became depleted (Banerjee, Burks, Feldberg & Goodrich, 1968). With morphine this initial fall was not obtained in cats, therefore the monoamines may not be the mediators of its temperature responses and we would then have to assume that the anterior hypothalamus responds differently in different species not only to these amines but to morphine as well.

Catalepsy, which is the most prominent early feature of morphine action in rats, had hitherto not been obtained with morphine in other species. The present experiments, however, show that morphine produces this effect also in rabbits, not when administered systemically, but when injected either into the cerebral ventricles or into the cisterna magna.

We may safely conclude that morphine injected intracisternally does not reach the cerebral ventricles because it was found that the ventricular walls remained unstained when a dye, bromophenol blue, was injected into the cisterna. On the other hand, morphine injected into the cerebral ventricles will readily pass into the subarachnoid space through the foramina of Luschka in the lateral recesses of the fourth ventricle. The site of morphine action for catalepsy must therefore be one that can be reached from the subarachnoid space, or from this space as well as from the ventricular cavities.

From the staining of the brain following an intracisternal injection of bromophenol blue we may also conclude that morphine similarly injected will spread from the cisterna magna around the cervical cord, medulla oblongata and mesencephalon, and that it will pass along the ventral surface of the brain up to the olfactory bulbs. Theoretically the

catalepsy might thus be due to activation or paralysis of superficial structures in any of these parts reached by the intracisternal injection. The structures most likely to be affected, however, seem to be those reached from the interpeduncular cisterna, structures located in the most ventral parts of the mesencephalon and in the region of transition between mesencephalon and diencephalon, because small electrolytic lesions in these regions at the base of the brain stem produce catalepsy. In their studies on cats, Ingram, Barris & Ranson (1936) obtained catalepsy of varying duration following damage " to some structure or structures in the neighbourhood of the mamillary bodies, such as the posterior hypothalamic nuclei, the supramamillary area, the lateral hypothalamic area and the region just caudal to the mamillary bodies." Some of these regions could also be reached from the third ventricle, so that morphine injected into the cerebral ventricles may produce catalepsy by penetrating the brain from both its inner and outer surface, that is, from the ventral part of the third ventricle and from the interpeduncular cisterna. In this connection it is interesting to note that in rats Lotti *et al.* (1965) sometimes observed catalepsy after injections of morphine into the hypothalamus. The catalepsy seen in cats with small doses of bulbocapnine injected into the cerebral ventricles (Feldberg & Sherwood, 1955) has to be accounted for in the same way as the catalepsy produced by intraventricular morphine.

For the rigidity and probably also for the hyper-reflexia resulting from morphine injected into the cerebral ventricles or cisterna magna, the same site or sites appear to be implicated as for catalepsy, because Ingram *et al.* (1936) observed rigidity as well as catalepsy in their lesion experiments. On the other hand, sites reached only from the ventricular cavities appear to be implicated in the convulsions produced by the larger doses of morphine injected intraventricularly, because no convulsions were obtained with the intracisternal injection. Lotti *et al.* (1965) obtained convulsions sometimes on injections of morphine into the hypothalamus of rats.

The finding that slowing of respiration was more pronounced on intraventricular than on intracisternal injection of morphine favours the view that morphine affects respiration partly through an action on neurones in the floor of the fourth ventricle or in the hypothalamus, as Lotti *et al.* (1965) observed depression of respiration in rats when they injected morphine into this part of the brain, but partly also through an action on the chemosensitive area at the ventrolateral surface of the medulla oblongata from where procaine, for instance, produces respiratory depression when acting from the cerebrospinal fluid spaces (Loeschcke & Koepchen, 1958; Mitchell, Loeschcke, Massion & Severinghaus, 1963; Haranath, Naseem & Sitaramayya, 1965).

SUMMARY

1. In unanaesthetized rabbits, morphine sulphate was injected through a chronically implanted cannula into the left lateral ventricle and temperature was recorded.

2. The injection of 25 μg resulted in a gradual rise in temperature, of 100 μg in a gradual rise preceded by a fall, and of 250 or 500 μg in a steep rise associated with excitement and increased muscular activity sometimes leading to convulsions.

3. The injections of 25 to 100 μg produced in addition pronounced catalepsy followed by hyper-reflexia, rigidity and slowing of respiration.

4. All the effects of the morphine were abolished by the injection of 100 μg of nalorphine into the cerebral ventricles.

5. Catalepsy, hyper-reflexia, rigidity and slowing of respiration were also obtained when 100 μg morphine sulphate was injected into the cisterna magna. The effects were again abolished by nalorphine injected either into the cisterna magna (100 μg) or intravenously (1 mg).

6. The site of action of morphine when producing these effects is discussed.

U. B. is a Commonwealth Medical Fellow; T. F. B. is supported by U.S.A. Public Health Service Fellowship No. 1-F2-GM-31,186-01 from the National Institute of General Medical Sciences, and C. A. G. is supported by U.S.A. Public Health Service Fellowship No. 1-F2-NB-32, 251-01 NSRB, from the National Institute of Neurological Diseases and Blindness.

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