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Antinociceptive activity of morphine after injection of biogenic amines in the cerebral ventricles of the conscious rat

C. G. SPARKES AND P. S. J. SPENCER*

Pharmacology Laboratories, Department of Pharmacy, The University of Aston, Birmingham B4 7ET

Summary

1. A simple cannula and a cannula guide for making injections into the cerebral ventricles of conscious rats are described.

2. Intraventricular injections of 5-hydroxytryptamine (5-HT) or of noradrenaline (NA) were without effect on the nociceptive threshold of rats.

3. Intraventricular injection of 5-HT potentiated the antinociceptive effect of morphine. Reserpine pretreatment antagonized the antinociceptive effect of morphine ; this effect was reversed by intraventricular injection of 5-HT.

4. Intraventricular injection of NA attenuated the antinociceptive action of morphine but was without effect on the inhibition by reserpine of the antinociceptive effect of morphine.

5. Subcutaneous injection or slow intravenous infusion of either 5-HT or NA (up to 300 μg/rat) were without significant effect on the antinociceptive effects of morphine.

6. Intraperitoneal administration of dopa increased the nociceptive threshold above normal, but reduced the antinociceptive effect of morphine. Intraventricular injection of either dopa or dopamine had no antinociceptive effect but inhibited that of morphine.

7. It is suggested that the antinociceptive effect of morphine may depend on the balance between the concentrations of 5-HT and NA in the brain.

Introduction

Although morphine has been used therapeutically for many years, relatively little is known about its mode of action in the brain. There is considerable evidence that the central pharmacological effects of morphine can be interpreted as an interaction with one or more central transmitters, but this evidence is often confusing and brain acetylcholine, catecholamines and indoalkylamines have all been implicated (Slaughter, 1950 ; Vogt, 1954 ; Sigg, Caprio & Schneider, 1958 ; Medakovic & Banic, 1964 ; Maynert, 1967 ; Radouco-Thomas, Singh, Garcin & Radouco-Thomas, 1967 ; Harris, Dewey, Howes, Kennedy & Pars, 1969). The reasons for the contradictory results can be attributed to several factors, including the inadequacy of analgesic testing methods in men and animals, marked qualitative and quantitative

* Present address: Welsh School of Pharmacy, UWIST, Cardiff.

differences in drug effects in different animal species, and the presence of interfering peripheral effects for both morphine and various agents used to alter central neurotransmitter concentrations.

The purpose of our investigation was to alter central transmitter concentrations by the injection of transmitters into the cerebral ventricles and to study the effects of these altered concentrations on the antinociceptive action of morphine. In this way, a high local concentration of transmitter in the brain could be achieved without having significant amounts of these agents in the peripheral circulation. This method also avoided the use of subcutaneous or intravenous injections to alter central transmitter concentrations, for most agents have peripheral actions too. The results were reported in brief to the meeting of the British Pharmacological Society (Sparkes & Spencer, 1969) and at the International Symposium on Pain held at Rotachegern, Munich, in October 1969.

Methods

Male Wistar rats weighing 250–300 g were housed in individual cages in a room maintained at a constant temperature of 21° ±1·0° C, and allowed food and water *ad libitum.*

Method of intraventricular injection

The method was a modification of that used by Hayden, Johnson & Maickel (1966). A cannula guide of 20 gauge stainless steel tubing was embedded in a Perspex block, 7 mm × 6 mm × 6·35 mm deep, so that the steel tubing protruded from the underside of the block by 4 mm. A stilette of 26 gauge stainless wire was placed down the lumen of the guide before implantation and left in position at all times, only being removed for the injections (Fig. 1).

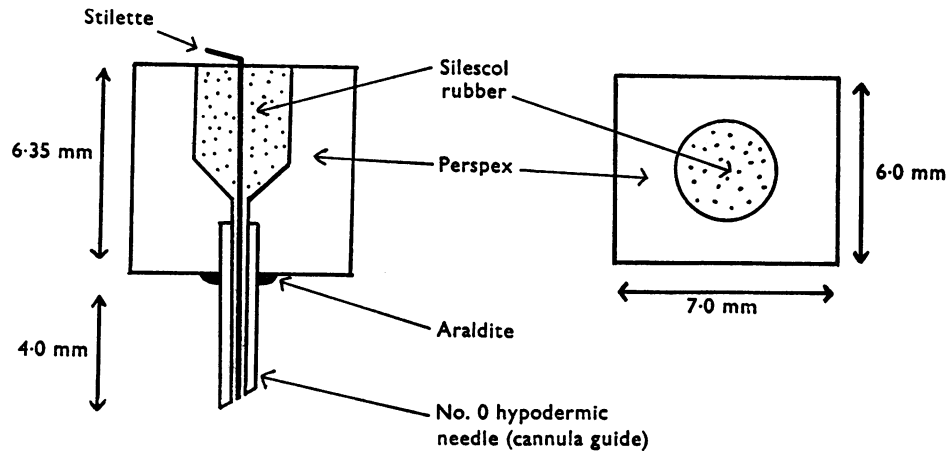

FIG. 1. Vertical and plan views of the cannula guide implanted into the lateral ventricles of rats.

The Perspex block was fixed to the skull of the rat with stainless steel screws and dental acrylic cement, the procedure of implantation being carried out under general anaesthesia induced by a mixture of halothane, nitrous oxide and oxygen. The guide was placed on the skull so that the tubing penetrated the skull at a point 2·5 mm lateral and 0·9 mm caudal to the bregma. Animals were used not sooner than 24 h and up to 18 days after recovery from the implantation operation.

The cannula through which the injections were made was a modified 26 gauge hypodermic needle 11·3 mm long, which was passed down the guide into the left lateral ventricle. All injections were made in volumes of 10 μl. After use, the rats were killed, their brains removed, and the positions of cannula guides verified by serial sectioning in the coronal plane.

Evaluation of analgesic activity

The noxious stimulus was produced by applying progressively increasing pressure to the rat's hind foot, according to the method described by Randall & Selitto (1957). The original method had been developed for the evaluation of non-narcotic analgesics on the inflamed foot. Since in our experiments morphine-like agents were used, it was not necessary to inflame the foot. A steadily increasing pressure was applied to the dorsal surface of one hindpaw, using a commercially available apparatus (Arnold R. Horwell Ltd., London). As the pressure increased, the stimulus reached a critical point when the animal commenced to remove or attempt to remove the paw from the stimulating apparatus. The load (g) applied at this end-point was recorded. Experience showed that loads in excess of 500 g caused damage to the paw and, therefore, this load was never exceeded.

All results were expressed as the 'analgesic index', I, as described by Cox, Ginsburg & Osman (1968). Thus, I is given by the formula:

$$I=\Delta_{obs}/\Delta_{max},$$

where $\Delta_{obs}=P_{obs}-P_i$; $\Delta_{max}=P_{max}-P_i$ (P_i, P_{obs} and P_{max} were respectively: the initial threshold pressure, the threshold pressure after treatment, and 500 g.

Meaningful statistical analysis cannot be carried out on groups in which one or more animals have an analgesic index of 1·0.

Intravenous infusions in conscious animals

Intravenous infusions were made via polyethylene cannulae placed in the jugular vein of animals anaesthetized with a mixture of halothane, nitrous oxide and oxygen. The free end of the cannula was brought out through the skin at the back of the neck. Infusions were made at a rate of 1 ml/h from a continuous infusion apparatus, not less than 24 h and up to 3 weeks after implantation of the intravenous cannula.

Drugs

Morphine hydrochloride, (−)-noradrenaline (NA) bitartrate, 5-hydroxytryptamine (5-HT) hydrochloride, DL-dopa and dopamine hydrochloride were dissolved in sterile apyrogenic 0·9% sodium chloride solution (saline). Reserpine was in a stabilized aqueous solution. Tetrabenazine was dissolved in three drops of lactic acid and 0·8 ml of ethanol and made up to volume with distilled water, the pH being adjusted to 4·5 with sodium bicarbonate. All doses refer to the base.

Results

Nociceptive sensitivity of the conscious rat after intraventricular and subcutaneous injections of 5-hydroxytryptamine and noradrenaline

5-Hydroxytryptamine was injected into the ventricles of conscious rats in doses ranging from 1 to 50 μg. Within this dose range there was no elevation of the nociceptive threshold. The spontaneous activity of the animals was considerably reduced with the higher doses (15–50 μg) and some abnormal behaviour noted, such as rearing to a defensive attitude and remaining in this position for some time. Injections of NA in doses ranging from 5 to 80 μg also had no significant effect upon the nociceptive threshold. After doses of 40–80 μg, however, the animals appeared to be in a hyperalgesic state, since vocalization and avoidance activity were easily provoked by pinching the tail or by similar manipulations. No quantitative measure of this hyperalgesia could be made by the test used.

These agents were also administered peripherally in order to compare their central and peripheral effects. Subcutaneous injections of 5-HT (5–50 μg) had no effect on the nociceptive threshold and had very little effect on the behaviour of the animals. Intravenous infusions of 5-HT at rates of 5-50 μg/h over a period of 1 h were also without significant effects. Similarly, subcutaneous injections and intravenous infusions of NA (20–80 μg) were without detectable effect on behaviour.

Subcutaneous injections of large doses of 5-HT (1–2 mg/kg or approx. 300 μg/rat) produced central nervous depression but no significant change in the nociceptive threshold. Similar doses of NA were near the lethal dose but did not produce any significant changes in the nociceptive threshold.

Antinociceptive activity of morphine and its modification by biogenic amines

Intraventricular injection of 5-HT and NA

Preliminary experiments indicated that the time to the peak antinociceptive effect of submaximal doses of subcutaneously injected morphine was 30–60 minutes. Morphine (8 mg/kg) increased the analgesic index to a value greater than 0·5 but not normally greater than 0·75.

In subsequent experiments with NA and 5-HT, groups of five animals were used. The nociceptive threshold was measured before injection and the animals given morphine (8·0 mg/kg) subcutaneously. Thirty minutes later, the nociceptive threshold was again measured, followed by an intraventricular injection of either 5-HT (5 μg) or NA (20 μg). The nociceptive thresholds were measured at regular intervals thereafter. Control animals received saline intraventricularly 30 min after the injection of morphine.

The effects of intraventricular injections of 5-HT on the morphine response are shown in Fig. 2. Usually, the response to morphine disappeared between 2·5 and 3 h after injection. Injection of 5-HT at 30 min potentiated but did not prolong the antinociceptive activity. In other experiments injection of 2·5 μg of 5-HT caused a smaller but still significant potentiation.

Intraventricular injection of NA (20 μg), on the other hand, caused the antinociceptive effect of morphine to be completely inhibited (Fig. 3). Injection of 10 μg NA initially suppressed the morphine effect, which then recovered until after

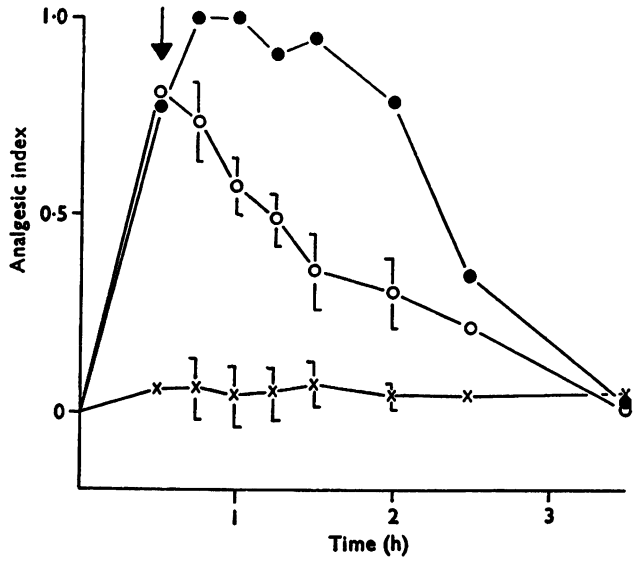

FIG. 2. Effect of intraventricular injection of 5-HT on the antinociceptive effect of morphine. Vertical bars indicate standard error of the mean response of groups of five rats. At time 0, two groups (●, ○) received 8·0 mg/kg morphine subcutaneously and one group (×) 1·0 ml/kg saline subcutaneously. Thirty minutes later, at arrow, 5·0 μg of 5-HT (●) or saline (○, ×) was injected intraventricularly.

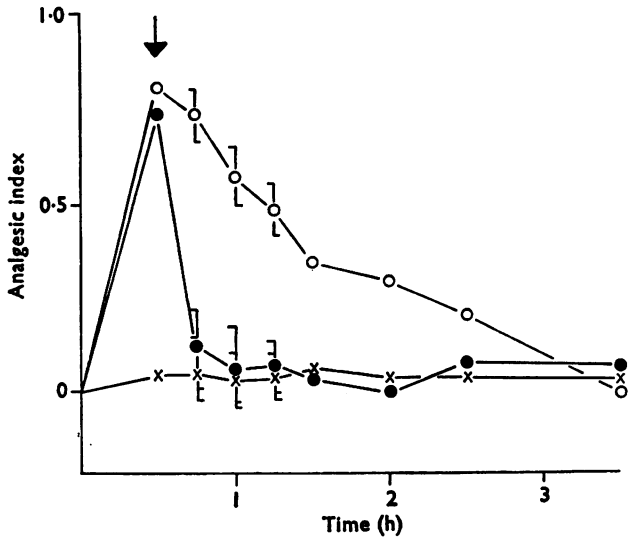

FIG. 3. Effect of intraventricular injection of NA on the antinociceptive effect of morphine. Vertical bars indicate standard error of the mean response of groups of five rats. At time 0, two groups (●, ○) received 8 mg/kg morphine subcutaneously and one group (×) 1·0 ml/kg saline subcutaneously. Thirty minutes later, at arrow, 20·0 μg NA (●) or saline (○, ×) was injected intraventricularly.

2 h it was not significantly different from that seen in the groups given morphine but no intraventricular noradrenaline.

It was considered possible that these effects may have been due to actions of the amines on peripheral structures following their leakage from the central nervous system. Therefore, NA (20–80 μg) or 5-HT (5–50 μg) was either injected subcutaneously or infused intravenously over a period of 1 h into morphine treated rats. At these dose levels there were no significant effects on the antinociceptive action of morphine.

Subcutaneous injections of high doses of 5-HT or NA (1–2 mg/kg: approx. 300 μg/rat) were without significant effect on the antinociceptive effects of morphine.

Peripheral administration of DL-*dopa*

Radouco-Thomas *et al.* (1967) have shown that dopa given intraperitoneally to guinea-pigs increases the nociceptive threshold. This effect is only observed in rabbits if the catechol-*O*-methyltransferase inhibitor pyrogallol is present (Munoz & Paeile, 1967), when the peripheral administration of dopa causes increases in brain concentrations of noradrenaline. It might be anticipated therefore that, in the rat, subcutaneous injection of dopa would inhibit the antinociceptive effect of morphine but would have no effect of its own on the nociceptive threshold.

The results of such an experiment are shown in Fig. 4. Four groups of five rats were used: two groups received 300 mg/kg of dopa intraperitoneally and the other two groups 5 ml/kg of saline. Forty-five minutes later the groups were injected subcutaneously with morphine (8 mg/kg) or with saline.

The usual antinociceptive effect of morphine was seen in animals pretreated with saline. There was a significant elevation of the nociceptive threshold in the animals treated with dopa but not given morphine. Yet, when dopa was administered before

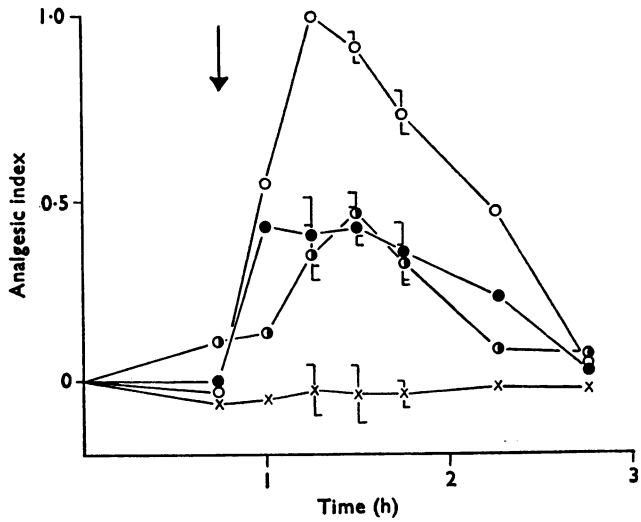

FIG. 4. Effect of intraperitoneal injection of dopa on the nociceptive threshold and the antinociceptive effect of morphine. Vertical bars indicate standard error of the mean response of groups of five rats. At time 0, groups 1 and 2 received dopa (300 mg/kg) and groups 3 and 4 saline (5 ml/kg). Forty-five minutes later, at arrow, groups 1 (—●—) and 3 (—○—) received morphine (8 mg/kg s.c.); groups 2 (—◑—) and 4 (—×—) saline (1 ml/kg s.c.).

morphine, the morphine effect was reduced to a level that was not significantly different from that seen in animals receiving dopa alone. To decide whether these effects of dopa on the nociceptive threshold were central or peripheral, dopa was next injected directly into the cerebral ventricles of rats.

Intraventricular administration of dopa

Preliminary experiments indicated that dopa alone (5–40 μg), injected into the ventricles of conscious rats, had no effect on the nociceptive threshold. When dopa was injected into the ventricles 30 min after morphine (8 mg/kg s.c.) little if any effect was seen. However, if dopa was injected at the same time as morphine, a significant inhibition of the antinociceptive effect of morphine was seen (Fig. 5). Thus, although dopa depresses the antinociceptive effect of morphine when injected into the cerebral ventricles, this antagonism does not appear as readily as with NA. This difference may be explained either by different diffusional characteristics of NA and dopa, causing a delay in dopa reaching the active site, or by the need for dopa to be converted to dopamine or NA. The dose of dopa used (40 μg) did not have any significant antinociceptive effect of its own.

Experiments were also conducted with dopamine and very similar results were obtained; when 20 μg of dopamine was injected into the cerebral ventricles of rats at time 0, along with 8 mg/kg of morphine subcutaneously, the antinociceptive effects of morphine were reduced by 30% at 75 min ($P=<0{\cdot}02$).

Effect of reserpine pretreatment on the antinociceptive activity of morphine

Reserpine, which depletes tissues of both catecholamines and 5-HT, antagonizes the antinociceptive properties of morphine in rats and mice (Schneider, 1954; Schaumann, 1958; Sigg *et al.*, 1958; Medakovic & Banic, 1964; Takagi, Takashima

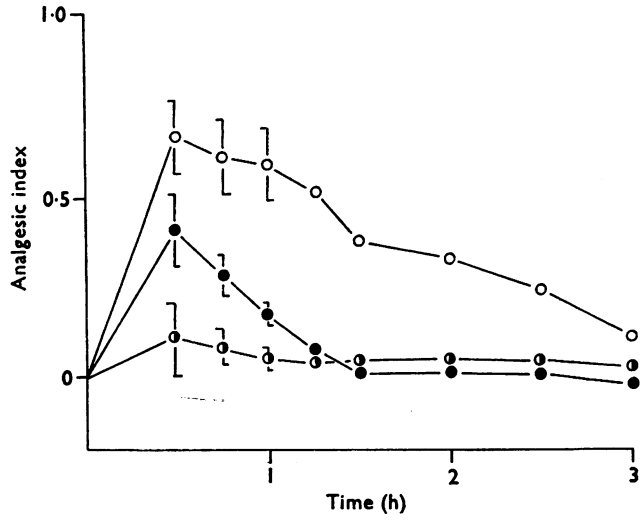

FIG. 5. Effect of intraventricular injection of dopa on the antinociceptive effect of morphine. Vertical bars indicate standard error of the mean response of groups of five rats. All injections were given simultaneously at time 0: (—○—) received morphine (8·0 mg/kg s.c.+ saline i.vent.); (—●—) received morphine s.c.+dopa (40 μg i.vent.); and (—◑—) was given saline (1 ml/kg s.c.+dopa 40 μg i.vent.).

& Kimura, 1964 ; Rudzik & Mennear, 1965 ; Contreras & Tamayo, 1967 ; Ross & Ashford, 1967 ; Verri, Graeff & Corrado, 1968). In contrast, there have been reports of a potentiation of the antinociceptive effect of morphine in mice (Tripod & Gross, 1957 ; Garcia Leme & Rocha e Silva, 1961). Attenuation of the antinociceptive effect of morphine has also been reported for tetrabenazine pretreatment (Takagi *et al.*, 1964). In view of these results it was of interest to study the effects of intraventricular injections of NA and 5-HT on the antinociceptive effects of morphine in rats pretreated with reserpine.

Medakovic & Banic (1964) reported that, in rats, the antinociceptive effect of morphine could be abolished by reserpine (1 mg/kg) given 3 h before morphine (4 mg/kg). Our preliminary experiments indicated that 3 h after injection of 5 mg/kg of reserpine, there was an increase in the antinociceptive effect of morphine. Therefore, experiments were designed to determine the time course of the effect of reserpine on the antinociceptive effect of morphine. Ten groups of five rats were injected with 5 mg/kg of reserpine intraperitoneally. From 3 to 90 h after this injection the nociceptive threshold was determined and morphine (8 mg/kg) injected subcutaneously. The nociceptive threshold was then tested again 1 h after the morphine injection (Fig. 6).

There was some potentiation of the antinociceptive effect of morphine at 3 h followed by a progressive reduction in the antinociceptive effect until a maximum inhibition was reached at 15 h after the injection of reserpine. This inhibition continued for about 6 h when there was a slow recovery until after 5 days the response to morphine was restored.

The results of these experiments indicate that the effect of reserpine, potentiation or attenuation of the antinociceptive effect of morphine, may be dependent on the doses used and the time interval after injection of reserpine. Subsequently all experiments with reserpine were carried out 16–20 h after the administration of reserpine (5 mg/kg i.p.).

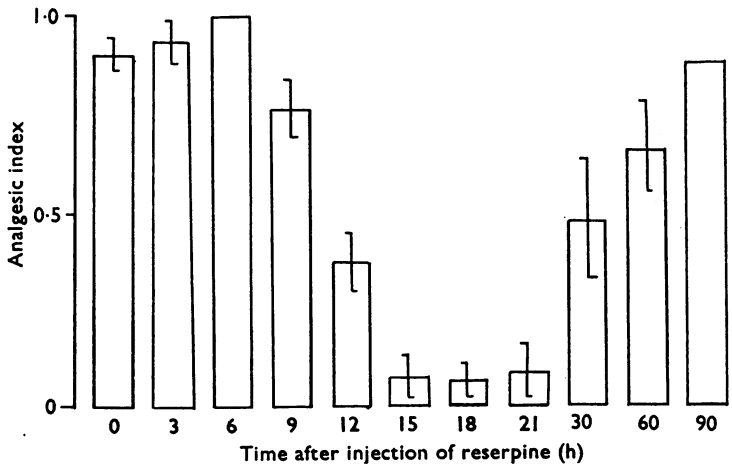

FIG. 6. Antinociceptive effect of subcutaneous injections of morphine (8 mg/kg) at various times after intraperitoneal injection of reserpine (5 mg/kg). One hour before the time indicated, the nociceptive threshold (P_i) was determined, the rats were then given morphine and the threshold (P_{obs}) determined again 1 h later. Each column represents the mean obtained from five rats, and the vertical bars the S.E. of the means.

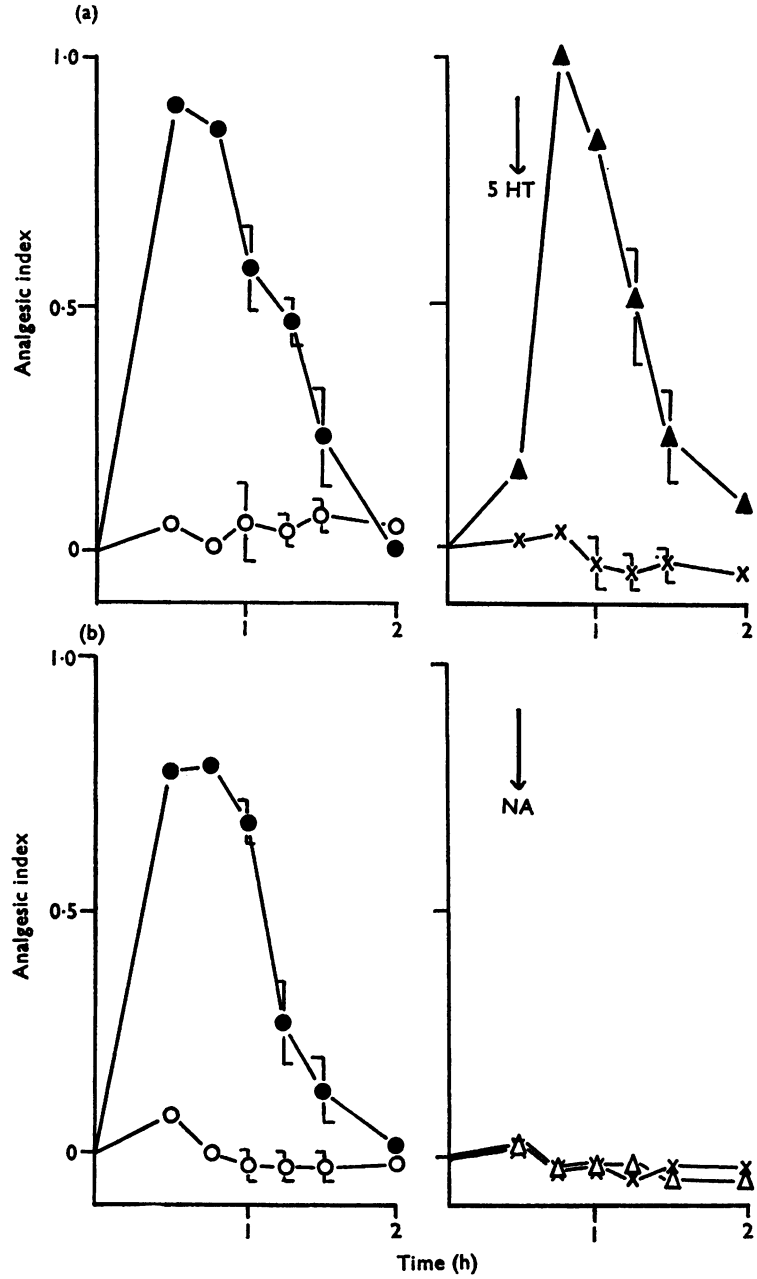

FIG. 7. Effects of intraventricular injections of 5-HT or NA on the antinociceptive activity of morphine in reserpinized animals. Vertical bars indicate standard error of the means obtained from five rats. (a), The left-hand side illustrates the effect of morphine (8 mg/kg s.c.), given at 0 h to rats injected intraperitoneally 16 h previously with reserpine (5 mg/kg) (—○—), or saline (2 ml/kg) (—●—). On the right-hand side, all rats were injected with reserpine; 30 min after morphine (—▲—) or saline (—×—), all animals were given 5-HT (5 μg i.vent.). (b), The left-hand side illustrates the effects as in (a) in a different group of rats. On the right-hand side, all animals were injected with reserpine; 30 min after morphine (—△—) or saline (—×—), all animals were given NA (20 μg i.vent.).

Effects of intraventricular injections of 5-HT or NA in reserpinized animals with or without morphine

Groups of five animals were pretreated with reserpine (5 mg/kg) intraperitoneally at −16 hours. This was followed by 8 mg/kg of morphine, subcutaneously, at 0 h, after first determining the basal nociceptive threshold ; 30 min after the morphine injection, the nociceptive threshold was determined again and the animals were given intraventricular injections of 5 μg 5-HT, 20 μg NA or saline. Nociceptive thresholds were then determined at regular intervals for 3 h (Fig. 7a, b).

On the left hand side of each figure the normal response to morphine is seen ; this response is inhibited by reserpine pretreatment. On the right hand side of Fig. 7a, the effect of 5-HT given to reserpinized animals in the presence and absence of morphine is seen. In the absence of morphine, 5-HT had no effect on the nociceptive threshold of reserpinized animals, but in the presence of morphine it restored the antinociceptive effect of morphine. Likewise, the right-hand side of Fig. 7b illustrates the effect of intraventricular injections of NA in reserpinized rats. NA did not restore the antinociceptive activity of morphine.

In similar experiments tetrabenazine (30 mg/kg i.p.) was given 4 h before the subcutaneous injection of morphine (8 mg/kg) and essentially similar results were obtained ; 5-HT but not NA restored the antinociceptive effect suppressed by the pretreatment with tetrabenazine.

Control experiments were performed in which the two amines were either injected subcutaneously or infused intravenously. As in earlier experiments they were without effect on the nociceptive threshold.

Discussion

Many previous studies have attempted to link the mechanism of the antinociceptive action of morphine with one or more endogenous substances. It has been suggested that 5-HT plays a mediator role in morphine analgesia (Medakovic & Banic, 1964 ; Nicak, 1965 ; Schaumann, 1958 ; Tenen, 1968 ; Verri *et al.*, 1968) but the results have been variable. Medakovic & Banic (1964) suggested that 5-HT antagonizes the analgesic effect of morphine directly, whereas Herold & Cahn (1968) and Saarnivaara (1969a) suggested that 5-HT may be the mediator of pain and thereby antagonize morphine. In contrast, other authors have suggested that increases in 5-HT concentrations in the brain potentiate the analgesic effects of morphine (Sigg *et al.*, 1958 ; Nicak, 1965 ; Tenen, 1968). The results presented here would support this latter hypothesis since intraventricular injections of 5-HT do not have any marked antinociceptive effect themselves, but potentiate the antinociceptive effect of morphine. In our experiments, this effect of 5-HT was seen only after intraventricular but not after subcutaneous injection or intravenous infusion. On the other hand Saarnivaara (1969a) found that peripherally administered 5-HT potentiated the effect of morphine in rabbits and concluded that this was due to a peripheral action. It seems possible that this peripheral mechanism exists in the rabbit but not in the rat, although Saarnivaara made no attempt to show that the effects she observed were wholly due to a peripheral action of 5-HT.

The sympathomimetic amines have been implicated frequently in the mechanisms of morphine analgesia, but with variable results. Some authors have reported that

NA and related substances have analgesic activity of their own in mice (Colville & Chaplin, 1964), dogs (Leimdorfer & Metzner, 1949), guinea-pigs (Radouco-Thomas, Radouco-Thomas & Le Breton, 1957), cats (Rothballer, 1959), rabbits (Saarnivaara, 1969b) and mice (Handley & Spencer, 1969). Other workers have suggested that catecholamines have no antinociceptive effect of their own in mice (Milosevic, 1955) or rabbits (Tsou & Jang, 1964), yet others have reported that they potentiate the antinociceptive effects of morphine in mice (Sigg *et al.*, 1958 ; Nott, 1968), rats (Contreras & Tamayo, 1966) and rabbits (Verri *et al.*, 1968 ; Saarnivaara, 1969b). There is one report that peripherally administered NA reduces the antinociceptive activity of morphine in mice (Milosevic, 1955). Our results indicate that neither intraventricular nor intravenous or subcutaneous injections of NA have significant effects on the nociceptive threshold of rats, but that intraventricular injections of catecholamines do attenuate the antinociceptive effects of morphine in rats.

Reserpine blocks the incorporation of NA, dopamine and 5-HT into neuronal stores, thus leading to a disappearance of these amines from both central and peripheral tissues (cf. Anden, 1968). Our results, showing that intraventricular injections of 5-HT restore the antinociceptive effect of morphine after its inhibition by reserpine, suggest that the antimorphine effect of reserpine is mediated by the depletion of the central stores of 5-HT. This finding is supported by that of Tenen (1968) who found that *p*-chlorophenylalanine, which depresses synthesis of 5-HT, also reduces the antinociceptive effects of morphine in rats. In contrast Saarnivaara (1969a) found that, in rabbits, *p*-chlorophenylalanine potentiates the effect of morphine. Thus once again different effects are seen in rats and rabbits.

Our results suggest that, in the rat, the threshold to nociceptive stimuli and its alteration by morphine are in some way dependent on a dynamic balance between the concentrations of NA and 5-HT in the brain, with 5-HT promoting and NA antagonizing the antinociceptive effects of morphine. The evidence suggests that these interactions are mediated primarily at the central level.

REFERENCES

ANDEN, N. (1968). Effect of reserpine and other drugs on the monoamine metabolism with special reference to the CNS. *Annls. Med. exp. Biol. Fenn.*, **16**, 361–366.

COLVILLE, K. I. & CHAPLIN, E. (1964). Sympathomimetics as analgetics. Effects of methoxamine, methamphetamine, metaraminol and norepinephrine. *Life Sci.*, **3**, 315–322.

CONTRERAS, E. & TAMAYO, L. (1966). Effects of drugs acting in relation to sympathetic functions on the analgesic action of morphine. *Archs int. Pharmacodyn. Thér.*, **160**, 312–320.

CONTRERAS, E. & TAMAYO, L. (1967). Influence of changes in brain 5-hydroxytryptamine on morphine analgesia. *Arch. Biol. Med. exper.*, **4**, 69–71.

COX, B. M., GINSBURG, M. & OSMAN, O. H. (1968). Acute tolerance to narcotic analgesic drugs in rats. *Br. J. Pharmac. Chemother.*, **33**, 245–256.

GARCIA LEME, J. & ROCHE E SILVA, M. (1961). Analgesic action of chlorpromazine and reserpine in relation to that of morphine. *J. Pharm. Pharmac.*, **13**, 734–742.

HANDLEY, S. L. & SPENCER, P. S. J. (1969). Analgesic activity after intracerebral injection in the mouse. *Br. J. Pharmac.*, **35**, 361*P*.

HARRIS, L. S., DEWEY, W. L., HOWES, J. F., KENNEDY, J. S. & PARS, H. (1969). Narcotic-antagonist analgesics: Interactions with cholinergic systems. *J. Pharmac. exp. Ther.*, **169**, 17–22.

HAYDEN, J. F., JOHNSON, L. R. & MAICKEL, R. P. (1966). Construction and implantation of a permanent cannula for making injections into the lateral ventricle of the rat brain. *Life Sci.*, **5**, 1509–1515.

HEROLD, M. & CAHN, J. (1968). The possible role of serotonin in pain. In: *Pain*, ed. Soulairac, A., Cahn, J. & Charpentier, J., pp. 251–280. London & New York: Academic Press.

LEIMDORFER, A. & METZNER, W. R. (1949). Analgesia and anaesthesia induced by epinephrine. *Am. J. Physiol.*, **157**, 116–121.

MAYNERT, E. W. (1967). Analgesic drugs and brain neurotransmitters. *Arch. Biol. Med. exper.*, **4**, 36–41.

MEDAKOVIC, M. & BANIC, B. (1964). The actions of reserpine and alpha-methyl-*m*-tyrosine on the analgesic effect of morphine in rats. *J. Pharm. Pharmac.*, **16**, 198–206.

MILOSEVIC, M. P. (1955). Effect of adrenaline on the analgesic response of mice to morphine and related drugs. *Archs int. Pharmacodyn. Thér.*, **104**, 50–56.

MUNOZ, C. & PAEILE, C. (1967). Changes in morphine analgesia induced by drugs which modify catecholamine content of the brain. *Arch. Biol. Med. exper.*, **4**, 63–68.

NICAK, A. (1965). The influence of serotonin and amphetamine on the analgesic effect of morphine after reserpine premedication in rats and mice. *Med. exp. (Basel)*, **13**, 43–48.

NOTT, M. W. (1968). Potentiation of morphine analgesia by cocaine in mice. *Eur. J. Pharmac.*, **5**, 93–99.

RADOUCO-THOMAS, S., RADOUCO-THOMAS, C. & LE BRETON, E. (1957). Action de la noradrénaline et de la réserpine sur l'analgesie expérimentale. *Arch. exp. Path. Pharmak.*, **232**, 279–281.

RADOUCO-THOMAS, S., SINGH, P., GARCIN, F. & RADOUCO-THOMAS, C. (1967). Relationship between experimental analgesia and brain monoamines: catecholamines and 5-hydroxytryptamine. *Arch. Biol. Med. exper.*, **4**, 42–62.

RANDALL, L. O. & SELITTO, J. J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Archs int. Pharmacodyn. Thér.*, **111**, 409–419.

ROSS, J. W. & ASHFORD, A. (1967). The effect of reserpine and α-methyl-dopa on the analgesic action of morphine in the mouse. *J. Pharm. Pharmac.*, **19**, 709–713.

ROTHBALLER, A. B. (1959). The effects of catecholamines in the central nervous system. *Pharmac. Rev.*, **11**, 494–547.

RUDZIK, A. D. & MENNEAR, J. H. (1965). Antagonism of analgesics by amine depleting agents. *J. Pharm. Pharmac.*, **17**, 326–615.

SAARNIVAARA, L. (1969a). Effect of 5-hydroxytryptamine on morphine analgesia in rabbits. *Ann. Med. exp. Fenn.*, **47**, 113–123.

SAARNIVAARA, L. (1969b). Analgesic activity of some sympathetic drugs and their effect on morphine analgesia in rabbits. *Ann. Med. exp. Fenn.*, **47**, 180–190.

SCHAUMANN, W. (1958). Beeinflussung der analgetischen Wirkung des Morphins durch Reserpin. *Arch. exp. Path. Pharmak.*, **235**, 1–9.

SCHNEIDER, J. A. (1954). Reserpine antagonism of morphine analgesia in mice. *Proc. Soc. exp. Biol. Med.*, **87**, 614–615.

SIGG, E. B., CAPRIO, G. & SCHNEIDER, J. A. (1958). Synergism of amines and antagonism of reserpine to morphine analgesia. *Proc. Soc. exp. Biol. Med.*, **97**, 97–100.

SLAUGHTER, D. (1950). Neostigmine and opiate analgesia. *Archs int. Pharmacodyn. Thér.*, **83**, 143–148.

SPARKES, C. G. & SPENCER, P. S. J. (1969). Modification of morphine analgesia in the rat by biogenic amines administered intraventricularly. *Br. J. Pharmac.*, **35**, 362–363*P*.

TAGAKI, H., TAKASHIMA, T. & KIMURA, K. (1964). Antagonism of morphine in mice by tetrabenazine and reserpine. *Archs int. Pharmacodyn. Thér.*, **149**, 484–492.

TENEN, S. S. (1968). Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine a serotonin depletor. *Psychopharmacologia (Berl.)*, **12**, 278–285.

TRIPOD, J. & GROSS, F. (1957). Different effects of central depressant drugs on the analgetic and central stimulating effects of morphine. *Helv. physiol. pharmac. Acta*, **15**, 105–115.

TSOU, K. & JANG, C. S. (1964). Studies on the site of analgesic action of morphine by intracerebra microinjection. *Scientia Sinica.*, **13**, 1099–1109.

VERRI, R. A., GRAEFF, F. G. & CORRADO, A. P. (1968). Effect of reserpine and alpha-methyl-tyrosine on morphine analgesia. *Int. J. Neuropharmac.*, **7**, 283–292.

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.

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