Br. J. Pharmac. (1974), 52, 519-526

MORPHINE ANALGESIA AND THE BULBOSPINAL NORADRENERGIC SYSTEM: INCREASE IN THE CONCENTRATION OF NORMETANEPHRINE IN THE SPINAL CORD OF THE RAT CAUSED BY ANALGESICS

H. SHIOMI & H. TAKAGI

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

1 Administration of an analgesic dose (10 mg/kg, s.c.) of morphine increased the concentration of a noradrenaline metabolite, normetanephrine (NM) in the spinal cord of normal rats. The time course of the change in the NM concentration corresponded approximately to that of the morphine analgesia. The concentration of noradrenaline was not affected.

2 A similar effect on the NM concentration was also observed after the administration of pentazocine (30 mg/kg, s.c.) and nalorphine (20 mg/kg, s.c.).

3 The NM increasing effect of morphine, pentazocine and nalorphine was found in the dorsal half of the spinal cord but not in the ventral half.

4 The increase in the concentration of NM induced by morphine, pentazocine or nalorphine was completely suppressed by naloxone (1 mg/kg, s.c.) given 5 min before the administration of these drugs.

5 When the spinal cord was transected at C1, the NM increasing effect of morphine disappeared, yet when the brain stem was transected at the inter-collicular level, the effect remained.

6 In morphine-tolerant rats, the concentration of NM in the spinal cord was almost the same as that observed in normal rats, but the increase in the concentration of NM in the spinal cord after the acute administration of morphine did not take place.

7 The NM concentration in the spinal cord of normal rats was not modified by aminopyrine (75 mg/kg, s.c.), chlorpromazine (10 mg/kg, s.c.), mephenesin (100 mg/kg, i.p.) or naloxone (25 mg/kg, s.c.).

8 The possible relation between morphine analgesia and the descending noradrenergic neurones in the spinal cord of rats is discussed.

Introduction

Effects of morphine on the concentration of biogenic monoamines in the brain have been extensively studied, but the results were controversial. Recently it has been shown that the changes in the concentration of metabolites of brain monoamines after a single dose of morphine were more pronounced than the changes in the concentrations of the monoamines. This suggests that the study of the metabolites provides a more reliable measure for nervous activity than the study of the monoamines themselves (Laverty & Sharman, 1965b; Fukui & Takagi, 1972).

The effect of morphine on the concentrations of biogenic monoamines or their metabolites in the spinal cord has so far received little attention (Bonnycastle, 1961).

Recently, Satoh & Takagi (1970a,b) reported that analgesic doses of morphine enhanced the ponto-bulbo spinal inhibitory influence on the spinal sensory transmission.

Dahlström & Fuxe (1965) have shown that bulbo-spinal noradrenaline (NA) and 5-hydroxytryptamine (5-HT) neurones, the cell bodies of which are situated in the medulla oblongata, distribute their nerve terminals in the ventral, dorsal and lateral horns of the grey matter in the spinal cord. These authors have described the existence of two pathways in the bulbo-spinal noradrenergic system of the rat. One fibre tract descends at the lateral margin of the ventral horn and the ventral part of the lateral funiculus, while the other descends in the lateral funiculus and terminates in the sympathetic lateral column and in the dorsal horn. Andén, Lundberg, Rosengren &

Vyklicky (1963) found that the released catecholamines in the spinal cord cause inhibition of the transmission of impulses from pain afferents to the ascending spinal pathway.

The metabolic pathways of NA in the brain have been well established (Rutledge & Jonason, 1967; Sharman, 1973). Several metabolites of NA are present in normal brain tissue. Häggendal (1963) described the presence of normetanephrine (NM), and 4-hydroxy-3-methoxyphenylethylene glycol and 3,4-dihydroxyphenylethylene glycol have also been identified and measured in the brain (Schanberg, Breese, Schildkraut, Gordon & Kopin, 1968; Sharman, 1969). Among these NA metabolites, NM appears to be produced mainly extraneuronally (Carlsson & Hillarp, 1962). The content of dopamine in the spinal cord is extremely low (Andén, 1965), suggesting that it does not play a major role in the spinal cord.

The present experiments were performed to determine whether analgesics, particularly morphine, accelerate the release of NA from descending noradrenergic fibres and thereby cause an increase of the NM concentration in the spinal cord and if such a change in NA metabolism is related to the analgesic action of morphine.

Methods

Male Wistar rats (180-220 g) were used. Except for certain procedures, the experiments were carried out at an ambient temperature of 23°C. They were decapitated under chloroform anaesthesia and immediately frozen in a mixture of CO_2 and ethanol (−70°C). The spinal cord was removed as soon as possible. Either the whole spinal cord (C1–S3) was used or it was blotted on filter paper, refrozen on solid CO_2 and cut into slices 5 mm thick. Each slice was divided by a cut through the central canal into a dorsal and a ventral half. The dorsal half consisted of the dorsal funiculi, the posterior parts of the lateral funiculi and the dorsal grays. The ventral half contained the ventral funiculi, the ventral parts of the lateral funiculi and the ventral greys. The dividing procedure was performed at 3°C. Two ventral halves or dorsal halves were pooled and extracted.

In order to study the site of action of morphine, the spinal cord was transected in some rats at C1. In others as inter-collicular section of the brain stem was performed under ether anaesthesia. After the rats had recovered from the anaesthesia (1.5 h after the end of inhalation), morphine (10 mg/kg, s.c.) was injected and the rats were decapitated 15 min later. The whole spinal cord was used in these experiments.

In order to induce tolerance to morphine, the drug was given at a dose of 10 mg/kg three times per day at intervals of 8 h for 5 days.

The analgesic effect was measured by chemical and mechanical stimuli. As a chemical stimulus, a retrograde injection of bradykinin into a carotid artery (BK method, Blane, 1967; Abe, Kaneko & Takagi, 1971) was used. As a mechanical stimulus, the pressure method described by Randall & Selitto (1957) was employed, except that the paws were not inflamed. The latter method was used in the experiments on morphine tolerance, since a graded change in threshold can be measured.

The extraction and fluorimetric determination of NM was carried out by the method of Anton & Sayre (1966). The mean recovery of NM was 69.5 ± 2.6% (mean ± s.e.). The fluorimetric determination of NA by the method of Laverty & Sharman (1965a) gave a mean recovery of 82 ± 3% (mean ± s.e.).

Materials used were morphine hydrochloride, pentazocine, nalorphine hydrochloride, naloxone hydrochloride, aminopyrine, chlorpromazine hydrochloride and mephenesin.

Except for mephenesin which was injected intraperitoneally, all drugs were given subcutaneously. The doses are given in terms of the salts.

Results

Effects of analgesics on the normetanephrine content of the spinal cord

The concentrations of NA and NM in the whole spinal cord of normal rats were 0.45 ± 0.02 μg/g (mean ± s.e.) and 0.033 ± 0.003 μg/g, respectively.

The time course of the effect of morphine (10 mg/kg) on the NM concentration in the whole spinal cord of the rat is shown in Figure 1. A rapid increase in the NM concentration in the whole spinal cord was observed after a single dose of morphine (10 mg/kg). An increase from 0.033 ± 0.003 μg/g to 0.064 ± 0.004 μg/g was obtained within 15 minutes. Thereafter the NM concentration declined slowly, but even after 90 min the concentration of NM was still significantly elevated.

Figure 2 shows the changes in the concentration of NM in the whole spinal cord 15 min after the administration of morphine at doses of 2.5, 5 and 10 mg/kg, respectively. In contrast, no increase of NM was observed in the brain stem even after the administration of a large dose (10 mg/kg) of morphine.

The concentration of NA in the whole spinal cord remained unchanged after the administration of morphine at an analgesic dose (2.5-10 mg/kg).

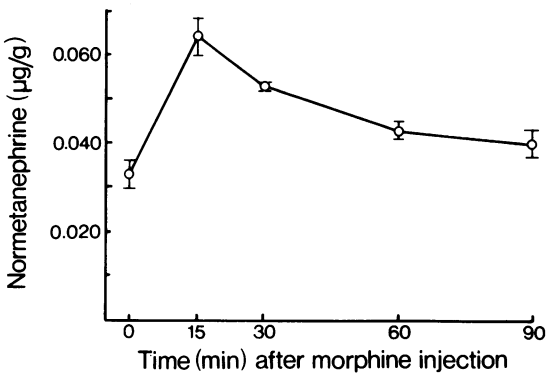


Fig. 1 Time course of the effect of morphine (10 mg/kg, s.c.) on the concentration of normetanephrine in the spinal cord of the rat. Values are the means for 6 or 8 experiments. Vertical bars indicate s.e. mean.

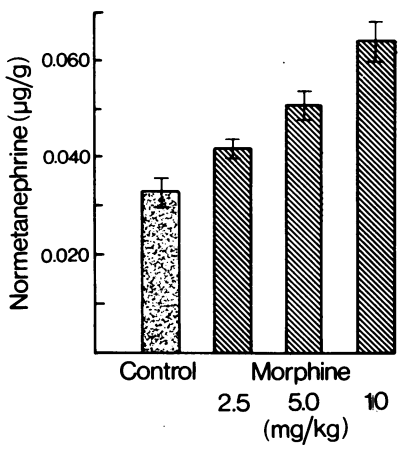


Fig. 2 Effects of various doses of morphine on the concentration of normetanephrine (NM) in the spinal cord of the rat. NM was estimated 15 min after the morphine injection. Columns show mean values from 6 to 8 experiments. Vertical bars indicate s.e. mean.

As shown in Fig. 3, two other centrally acting analgesics, pentazocine and nalorphine, which also have narcotic antagonist properties showed similar effects to morphine. A rapid increase in the concentration of NM in the whole spinal cord of rat was observed after a single administration of pentazocine (30 mg/kg) or nalorphine (20 mg/kg). The maximum increase was obtained within 15 minutes. The return to normal values was faster than after morphine.

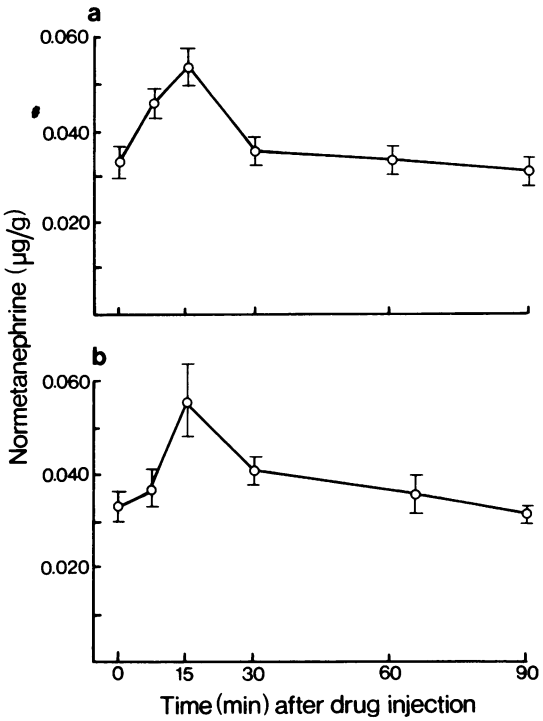


Fig. 3 Time course of the effects of (a) pentazocine (30 mg/kg, s.c.) and (b) nalorphine (20 mg/kg, s.c.) on the concentration of normetanephrine in the spinal cord of the rat. Values are the means from 6 experiments. Vertical bars indicate s.e. mean.

The maximum analgesic effect after the injection of morphine was reached within 30-60 min, and after pentazocine or nalorphine within 15-30 min; the effect then declined gradually. The duration of the analgesic effect of pentazocine or nalorphine was relatively short compared with that of morphine.

Aminopyrine (75 mg/kg) which had the maximum analgesic effect 60 min after administration had no effect on the concentration of NM in the whole spinal cord of rat. Chlorpromazine (10 mg/kg) and mephenesin (100 mg/kg), both of which had no analgesic effects, had no influence on the concentration of NM.

Effects of narcotic antagonists

The effects of naloxone on the increase in the concentration of NM induced by morphine, pentazocine and nalorphine are shown in Figure 4. A single dose of naloxone (1 mg/kg) did not influence the concentration of NM in the whole spinal cord of rat. However, the increase in the

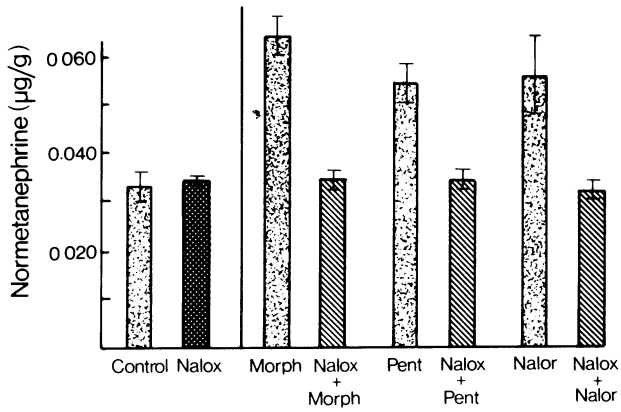
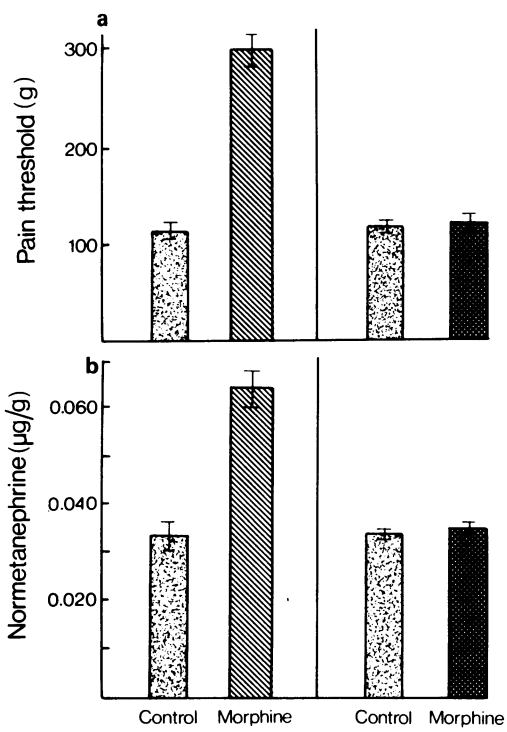


Fig. 4 Antagonism of naloxone to effects of analgesics on the concentration of normetanephrine in the spinal cord of the rat. Naloxone was injected 5 min before the administration of the analgesics and the rats were decapitated 15 min after this administration. Nalox, naloxone (1 mg/kg, s.c.); Morph, morphine (10 mg/kg, s.c.); Pent, pentazocine (30 mg/kg, s.c.); Nalor, nalorphine (20 mg/kg, s.c.). Columns show mean values from 6 experiments. Vertical bars indicate s.e. mean.

concentration of NM induced by morphine (10 mg/kg) was completely suppressed by naloxone given 5 min before morphine. The NM increasing effect of pentazocine (30 mg/kg) and of nalorphine (20 mg/kg) were also antagonized by a preceding injection of naloxone. Similarly, the analgesic effects of morphine, pentazocine and nalorphine, as estimated by the BK method, were completely antagonized by naloxone (1 mg/kg) given 5 min before the administration of these analgesics.



Effect of additional morphine on morphine-tolerant rats

In rats not tolerant to morphine (normal rats), the maximum increase in pain threshold after the administration of morphine (10 mg/kg) was 255% (from 116 ± 6 g in untreated rats to 296 ± 12 g). When morphine (10 mg/kg) was administered to rats for 5 days three times per day, tolerance to morphine developed on the 6th day as shown by the fact that this dose of morphine no longer produced an increase in pain threshold. At this time, the pain threshold of tolerant rats no longer differed from that of the non-tolerant rats (see Figure 5a).

Fig. 5 Effects of morphine (10 mg/kg, s.c.) on (a) the pain threshold and (b) the spinal concentration of normetanephrine (NM) in normal (left) and morphine-tolerant (right) rats. In normal rats the pain threshold and NM were measured 15 min after morphine administration. In morphine-tolerant rats, the pain threshold and NM content were measured 8 h after the last morphine injection on the 5th day in the controls. In experimental animals an additional dose of morphine was injected 8 h after the last 'habit forming' morphine injection and the pain threshold and NM content were measured 15 min later. Values are the means from 6 experiments. Vertical bars show s.e. mean.

Morphine-tolerant rats were divided into two groups. In the first group, the NM content of the whole spinal cord was measured without further treatment (controls). In the second group, it was measured 15 min after an additional injection of morphine (10 mg/kg) given 8 h after the last of the 'habit forming' morphine injections. In morphine-tolerant rats, the spinal concentration of NM did not differ from that of the non-tolerant control rats, but the NM increasing effect of morphine (10 mg/kg) completely disappeared (Figure 5b).

Regional effects of morphine on normetanephrine concentrations in the spinal cord

In normal rats, the concentrations of NA and NM in the ventral half of the spinal cord, including the ventral horn, were 0.37 ± 0.02 μg/g and 0.023 ± 0.002 μg/g, respectively and those in the dorsal half of the spinal cord, including the dorsal horn, were 0.42 ± 0.03 μg/g and 0.031 ± 0.001 μg/g, respectively.

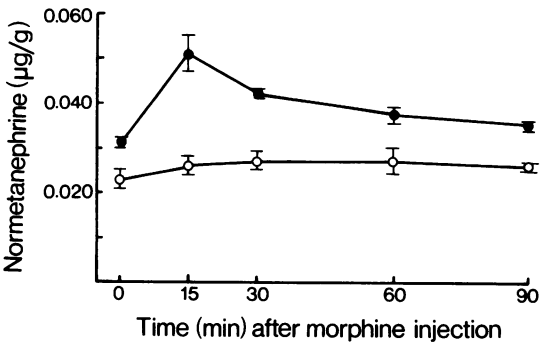


Fig. 6 Time course of the effect of morphine (10 mg/kg, s.c.) on the concentration of normetanephrine in the dorsal (•) and ventral half (○) of the spinal cord of rat. Values are the means from 6 experiments. Vertical bars indicate s.e. mean.

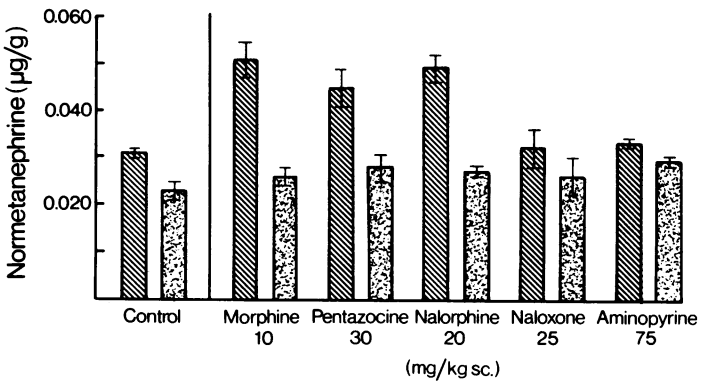


Fig. 7 Effects of various analgesics on the concentration of normetanephrine in dorsal (hatched columns) and ventral half (stippled columns) on the spinal cord of rat. All values measured 15 min after injection of analgesic. Values are the means from 6 experiments. Vertical bars indicate s.e. mean.

The time course of the effect of morphine (10 mg/kg) on the NM concentration in the ventral and dorsal half of spinal cord is shown in Figure 6. A rapid increase in the NM concentration in the dorsal half of the spinal cord was observed after a single dose of morphine (10 mg/kg) and a maximum increase (from 0.031 ± 0.001 μg/g to 0.052 ± 0.003 μg/g) was obtained within 15 min after which the concentration declined slowly. The time course paralleled that of the NM increase after morphine in the whole spinal cord and also that of analgesia. In contrast, in the ventral half of the spinal cord, morphine had no significant effect on NM concentration.

Figure 7 shows the effects of various analgesics on the concentration of NM in the ventral and dorsal half of the spinal cord. Pentazocine (30 mg/kg) or nalorphine (20 mg/kg) significantly increased the NM concentration in the dorsal half, but not in the ventral half. The maximum effects were reached at the same time as in the whole spinal cord. Neither naloxone (25 mg/kg) nor aminopyrine (75 mg/kg) had any influence on the concentration of NM in either the dorsal or the ventral half of the spinal cord.

Effect of transection of the neuroaxis on normetanephrine content

Inter-collicular transection The concentration of NM in the spinal cord of inter-collicularly

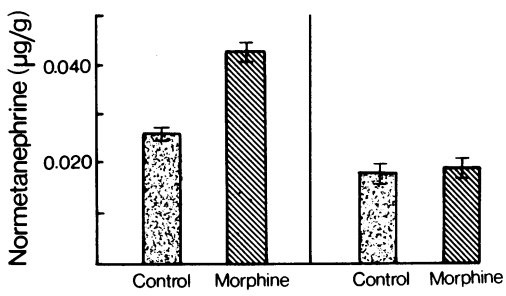


Fig. 8 Effects of transection of neuroaxis on the concentration of normetanephrine in the spinal cord of rat. Effect of a mid collicular transection (left) and of C1 level transection (right). Rats were killed 15 min after morphine administration (10 mg/kg, s.c.). Values are the means from 8 or 10 experiments. Vertical bars indicate s.e. mean.

transected control rats measured 1.75 h after transection was 0.026 ± 0.001 μg/g. This value increased to 0.043 ± 0.002 μg/g 15 min after an injection of morphine (10 mg/kg). Thus the NM increasing effect of morphine remained after intercollicular transection (Figure 8, left).

Spinal transection at C1 The concentration of NM in the whole spinal cord of rats transected at C1 was measured 1.75 h after transection. It was 0.018 ± 0.002 μg/g. This value remained unchanged after a subcutaneous injection of morphine (10 mg/kg) (Figure 8, right).

Discussion

The present results show that analgesic doses (2.5-10 mg/kg) of morphine increased the spinal concentration of NM in normal rats. The time course of this change corresponded roughly with that of the morphine analgesia. Furthermore, the NM increasing effect of morphine was dose-dependent. In contrast, morphine (10 mg/kg) had no influence on the concentration of NA in the spinal cord of normal rats.

The increase of the spinal concentration of NM produced by morphine in normal rats could be due to an increase in the biosynthesis and release of NA which in turn enhanced the formation of NM. This is supported by the evidence that morphine accelerates the biosynthesis of catecholamines in the brain (Clouet & Ratner, 1970; Smith, Sheldon, Bednarczyk & Villarreal, 1972; Fukui, Shiomi & Takagi, 1972).

The NM increasing effect of morphine disappeared after transection of the spinal cord at C1. It did not disappear after transection of the brain stem at the inter-collicular level. These results indicate that the primary site of action of morphine is in the lower brain stem where NA cell bodies are situated and that morphine causes an increase in the neuronal impulse flow of the noradrenergic bulbo-spinal pathway which in turn enhances the release of NA. Andén, Fuxe & Hökfelt (1966) have shown that after certain drugs, increase in the nervous impulse flow is essential for the depletion of the monoamines from the central nervous system.

This interpretation is consistent with the electrophysiological findings of Satoh & Takagi (1970a) that the lower brain stem (below the level of the inferior colliculus) plays a major role in the analgesic action of morphine on the pain afferent pathways of the spinal cord.

Recently, Wall (1967), Selzer & Spencer (1969) and Wagman & Price (1969) have demonstrated that lamina V cells of the dorsal horn of the spinal cord respond to the electrical or mechanical stimulation of the small myelinated afferents. They suggested an involvement of the lamina V cells in the pain mechanism. Moreover, Satoh, Nakamura & Takagi (1971) and Satoh, Doi & Takagi (1973) have shown that morphine inhibits the activities of the dorsal horn neurones induced by the intra-arterial injection of bradykinin in intact rabbits but not in spinal rabbits. Westman & Bowsher (1971) have demonstrated that the nerve cells in lamina V make synaptic contact with nerve terminals containing some dense-core vesicles which are probably noradrenergic terminals and may belong to the descending bulbo-spinal system described by Dahlström & Fuxe (1965).

In the present experiments, the NM increasing effect of morphine was seen in the dorsal half of the spinal cord but not in the ventral half. In addition, pentazocine (30 mg/kg) and nalorphine (20 mg/kg) which were classified as central analgesics by Lim, Guzman, Rodgers, Goto, Braun, Dickerson & Engle (1964) had a similar effect to morphine on the NM concentration in the dorsal half of the spinal cord, but naloxone (25 mg/kg), a pure morphine antagonist, and aminopyrine (75 mg/kg) had no effect on the NM in the dorsal or the ventral half.

These results indicate that central acting analgesics, such as morphine, pentazocine and nalorphine, act specifically on the bulbo-spinal noradrenergic neurones, the terminals of which are distributed in the dorsal horn, possibly in the lamina V cells. The NM increasing effect of morphine (10 mg/kg), pentazocine (30 mg/kg) and nalorphine (20 mg/kg) was prevented by the administration of naloxone (1 mg/kg). Naloxone antagonizes not only the analgesic effect of

morphine but also that of analgesics, which act as narcotic-antagonists such as pentazocine and nalorphine (Smits & Takemori, 1970).

Previously, it was shown that pretreatment with reserpine or tetrabenazine decreases morphine analgesia and that this effect can be reversed by the administration of DOPA (Sigg, Caprio & Schneider, 1958; Takagi, Takashima & Kimura, 1964; Takagi & Nakama, 1968). Hoffmeister (1968) reported that the effect of pentazocine was also antagonized by pretreatment with reserpine. Moreover, tetrabenazine antagonized the analgesic effect of morphine, pentazocine and nalorphine but did not antagonize that of aminopyrine (Shiomi, Murakami & Takagi, unpublished data). These studies together with the present results suggest that the NM increasing effects of pentazocine and nalorphine could also be mediated through the action of descending noradrenergic neurones.

Lim *et al.* (1964) have stated that all the non-narcotic antipyretic analgesics including aminopyrine had a peripheral blocking action at the 'pain' receptor and this was confirmed by Satoh *et al.* (1973). In the present experiments, aminopyrine and the neuroleptic drugs chlorpromazine and mephenesin had no influence on the content of NM in the spinal cord. Thus the NM increasing effect is considered to be specific for central acting analgesics.

The NM increasing effect of morphine disappeared when a single dose of morphine was given to morphine-tolerant rats. A similar tolerance phenomena has been observed on the enhancing effect of morphine on the biosynthesis and degradation of catecholamines (Smith *et al.*, 1972; Fukui & Takagi, 1972). These results support the hypothesis that catecholamines are involved in the depressant effects of morphine-induced analgesia (Takagi *et al.*, 1964). It is concluded that morphine and related central acting analgesics enhance the activity of the bulbo-spinal inhibitory noradrenergic system (Fig. 9), the terminal of which probably produces a postsynaptic inhibition at the dorsal horn neurone of the spinal cord (Satoh & Takagi, 1970b). A participation of 5-HT containing neurones on the analgesic effect of morphine was also suggested (e.g. Tenen, 1968; Vogt, 1974). Detailed studies of this problem are being carried out at present in our laboratory.

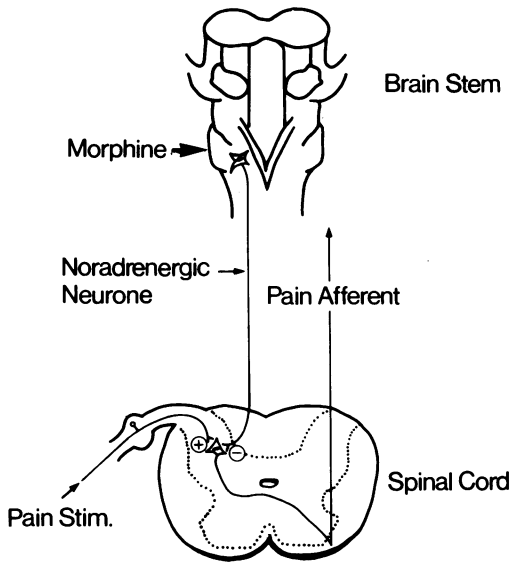


Fig. 9 Morphine and the bulbo-spinal noradrenergic system. See text.

Our thanks are due to M. Ohara, Kyoto University, for assistance with the manuscript.

References

ABE, T., KANEKO, T. & TAKAGI, H. (1971). Studies on the assessment of analgesic actions of narcotic antagonists with special reference to nociception induced by retrograde injection of bradykinin into the carotid artery of rats. *Folia Pharmacol. Jap.*, **67**, 9-14.

ANDÉN, N.-E. (1965). Distribution of monoamines and dihydroxyphenylalamine decarboxylase activity in the spinal cord. *Acta physiol. scand.*, **64**, 197-203.

ANDÉN, N.-E., FUXE, K. & HÖKFELT, T. (1966). The importance of the nervous impulse flow for the depletion of the monoamines from central nervous system by some drugs. *J. Pharm. Pharmac.*, **18**, 630-632.

ANDÉN, N.-E., LUNDBERG, A., ROSENGREN, E. & VYKLICKY, L. (1963). The effect of DOPA on the spinal cord. 1. Influence on transmission from primary afferents. *Acta. physiol. scand.*, **67**, 373-386.

ANTON, A.H. & SAYRE, D.F. (1966). Distribution of metanephrine and normetanephrine in various animals and their analysis in diverse biologic material. *J. Pharmac. exp. Ther.*, **153**, 15-29.

BLANE, G.F. (1967). Blockade of bradykinin-induced nociception in the rat as a test for analgesic drugs with particular reference to morphine antagonists. *J. Pharm. Pharmac.*, **19**, 367-373.

BONNYCASTLE, D.D. (1961). *The assessment of pain in man and animals*, ed. Keele, C.A. & Smith, R., p. 231. Edinburgh & London: Livingstone.

CARLSSON, A. & HILLARP, N.-A. (1962). Formation of phenolic acids in brain after administration of 3,4-dihydroxyphenylalamine. *Acta. physiol. scand.*, **55**, 95-100.

CLOUET, D.H. & RATNER, M. (1970). Catecholamine

biosynthesis in brains of rats treated with morphine. *Science, N.Y.*, **168**, 854-856.

DAHLSTRÖM, A. & FUXE, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbo-spinal neuron system. *Acta physiol. scand.*, **64**, Suppl. 247.

FUKUI, K., SHIOMI, H. & TAKAGI, H. (1972). Effect of morphine on tyrosine hydroxylase activity in mouse brain. *Eur. J. Pharmac.*, **19**, 123-125.

FUKUI, K. & TAKAGI, H. (1972). Effect of morphine on the central contents of metabolites of dopamine in normal and tolerant mice: its possible relation to analgesic action. *Br. J. Pharmac.*, **44**, 45-51.

HÄGGENDAL, J. (1963). The presence of 3-*O*-methylated noradrenaline (normetanephrine) in normal brain tissue. *Acta physiol. scand.*, **59**, 261-268.

HOFFMEISTER, F. (1968). On the possible relations between postganglionic adrenergic and cholinergic neurone blockade demonstrable in the peripheral autonomous nervous system and central analgesia. In *Pain*, ed. Soulairac, A., Cahn, J. & Charpentier, C., pp. 281-296. New York: Academic Press.

LAVERTY, R. & SHARMAN, D.F. (1965a). The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. *Br. J. Pharmac. Chemother.*, **24**, 538-548.

LAVERTY, R. & SHARMAN, D.F. (1965b). Modification by drugs of the metabolism of 3,4-dihydroxyphenylethylamine, noradrenaline and 5-hydroxy tryptamine in the brain. *Br. J. Pharmac. Chemother.*, **24**, 759-772.

LIM, R.K.S., GUZMAN, F., RODGERS, D.W., GOTO, K., BRAUN, C., DICKERSON, G.D. & ENGLE, R.J. (1964). Site of action of narcotic and non-narcotic analgesics determined by blocking bradykinin-evoked visceral pain. *Arch. int. Pharmacodyn.*, **152**, 25-58.

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

RUTLEDGE, C.O. & JONASON, J. (1967). Metabolic pathways of dopamine and norepinephrine in the rabbit brain *in vitro*. *J. Pharmac. exp. Ther.*, **157**, 493-502.

SATOH, M., DOI, T. & TAKAGI, H. (1973). Effects of narcotic and non-narcotic analgesics on dorsal horn unit activities in the spinal cord induced by intra-arterial injection of bradykinin. *Folia Pharmac. Jap.*, **69**, 79P.

SATOH, M., NAKAMURA, N. & TAKAGI, H. (1971). Effect of morphine on bradykinin-induced unitary discharges in the spinal cord of the rabbit. *Eur. J. Pharmac.*, **16**, 245-247.

SATOH, M. & TAKAGI, H. (1970a). Enhancement by morphine of the central descending inhibitory influence on spinal sensory transmission. *Eur. J. Pharmac.*, **14**, 60-65.

SATOH, M. & TAKAGI, H. (1970b). Effect of morphine on the pre- and postsynaptic inhibitions in the spinal cord. *Eur. J. Pharmac.*, **14**, 150-154.

SCHANBERG, S.M., BREESE, G.R., SCHILDKRAUT, J.J., GORDON, E.K. & KOPIN, I.J. (1968). 3-Methoxy-4-hydroxyphenylglycol sulfate in brain and cerebrospinal fluid. *Biochem. Pharmac.*, **17**, 2006-2008.

SELZER, M. & SPENCER, W.A. (1969). Convergence of visceral and cutaneous afferent pathways in the lumbar spinal cord. *Brain Res.*, **14** 331-348.

SHARMAN, D.F. (1969). Glycol metabolites of noradrenaline in brain tissue. *Br. J. Pharmac.*, **36**, 523-534.

SHARMAN, D.F. (1973). The catabolism of catecholamines. Recent studies. *Brit. med. Bull.*, **29**, 110-115.

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.

SMITH, C.B., SHELDON, M.I., BEDNARCZYK, J.H. & VILLARREAL, J.E. (1972). Morphine-induced increases in the incorporation of ^{14}C-tyrosine into ^{14}C-dopamine and ^{14}C-norepinephrine in the mouse brain: Antagonism by naloxone and tolerance. *J. Pharmac. exp. Ther.*, **180**, 547-557.

SMITS, S.E. & TAKEMORI, A.E. (1970). Quantitative studies on the antagonism by naloxone of some narcotic and narcotic-antagonist analgesics. *Br. J. Pharmac.*, **39**, 627-638.

TAKAGI, H. & NAKAMA, M. (1968). Studies on the mechanism of action of tetrabenazine as a morphine antagonist. II. A participation of catecholamine in the antagonism. *Japan. J. Pharmac.*, **18**, 54-58.

TAKAGI, H., TAKASHIMA, T. & KIMURA, K. (1964). Antagonism of the analgetic effect of morphine in mice by tetrabenazine and reserpine. *Arch. int. Pharmacodyn.*, **149**, 484-492.

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

VOGT, M. (1974). The effect of lowering the 5-hydroxytryptamine content of the rat spinal cord on analgesia produced by morphine. *J. Physiol., Lond.*, **236**, 483-498.

WALL, P.D. (1967). The laminar organization of dorsal horn and effects of descending impulses. *J. Physiol. Lond.*, **188**, 403-423.

WAGMAN, I.H. & PRICE, D.D. (1969). Responses of dorsal horn cells of *M. mulatta* to cutaneous and sural nerve A and C fiber stimuli. *J. Neurophysiol.*, **32**, 803-817.

WESTMAN, J. & BOWSHER, D. (1971). The fine structure of 'Non-specific' gray matter (Lamina V and VII) in the cat spinal cord. *Exp. Brain Res.*, **12**, 379-388.

(Received May 8, 1974)