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Effect of morphine on the cerebral contents of metabolites of dopamine in normal and tolerant mice: its possible relation to analgesic action

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Summary

1. The administration of an analgesic dose (10 mg/kg, s.c.) of morphine increased the concentrations of the dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the brains of normal mice, and the time course of the change in the DOPAC concentration corresponded approximately to that of morphine analgesia. The increase in the concentration of the DOPAC induced by morphine (20 mg/kg, s.c.) was completely suppressed by nalorphine (2 mg/kg, s.c.) given 5 min after the morphine administration.

2. In morphine-tolerant mice the concentrations of DOPAC and HVA in the brain did not differ from those observed in normal mice, and the increase in the concentrations of DOPAC and HVA in brain after the acute administration of morphine no longer occurred.

3. Nalorphine (2 mg/kg) given alone did not cause any change in brain DOPAC and HVA concentrations in normal mice.

4. The morphine-induced increase in DOPAC and HVA concentrations in the brain are discussed in the light of the hypothesis that dopamine might participate not only in the extrapyramidal motor system but also in the sensory mechanisms of the brain.

Introduction

The relation between morphine analgesia and the concentration of noradrenaline (NA) in the animal brain has been extensively studied (Gunne, 1959; Maynert & Klingman, 1962; Sloan, Brooks, Eisenman & Martin, 1963; Clouet, 1968). However, the effect of morphine on the content of dopamine (DA) or its metabolites in the brain has been little studied. Laverty & Sharman (1965) have reported that in the cat large doses of morphine (30–50 mg/kg) increase the homovanillic acid concentration (HVA) in the caudate nucleus without affecting the concentration of dopamine. Takagi & Nakama (1966) have shown that morphine (20 mg/kg) causes a slight decrease in the concentration of DA in mouse brain which is followed by a reduction in the concentration of NA. Gunne, Jonsson & Fuxe (1969) showed that the acute administration of morphine (20 mg/kg) caused an accelerated depletion of brain DA after inhibition of catecholamine synthesis in rat.

Recently, the metabolic pathways of DA in the brain have been well established (Hornykiewitz 1966; Rutledge & Jonason, 1967). DA is oxidatively deaminated by

monoamine oxidase (MAO) to 3,4-dihydroxyphenylacetic acid (DOPAC). More recently, Roffler-Tarlov, Sharman & Tegerdine (1971) showed that DOPAC is not normally metabolized by catechol-*O*-methyl transferase (COMT) to HVA in the mouse striatum. DA can also be *O*-methylated by COMT to methoxytyramine which is further metabolized by MAO to HVA.

The present experiments were performed to determine whether analgesic doses of morphine affect the DA metabolism and if so, is the effect related to the development of tolerance.

Methods

Experiments were carried out with male mice weighing 13–15 g. Morphine was injected subcutaneously and the analgesic effect was measured by a tail-pinch method with a 500 g pressure clip as described by Takagi, Inukai & Nakama (1966). Complete analgesia was taken as a positive response. In each experiment ten mice were used.

In order to make mice tolerant to morphine, a dose of 20 mg/kg daily, was administered to each mouse for 8 days.

After measuring the analgesic effect of morphine, the mice were killed by decapitation and their brains (minus the cerebellum) were quickly removed, blotted on a filter paper and weighed. Five brains were pooled and then homogenized in 0·4 N perchloric acid. The extraction and fluorimetric determination of DOPAC and HVA were carried out according to the methods described by Werdinius (1967) and Andén, Roos & Werdinius (1963), respectively.

Materials used were morphine hydochloride and nalorphine hydrochloride. Drug doses are given in terms of the salt.

Results

Effect of morphine in non-tolerant mice

The cerebral concentrations of DOPAC and HVA in normal mice were $0{\cdot}30 \pm 0{\cdot}02$ μg/g (mean $\pm$ S.E.) and $0{\cdot}32 \pm 0{\cdot}02$ μg/g, respectively.

The time courses of the effects of different doses of morphine on the DOPAC and HVA concentrations in brains of non-tolerant (normal) mice are shown in Fig. 1.

The maximum analgesic effects after administration of morphine at doses of 5, 10 and 20 mg/kg were 40, 80 and 100% of the mice tested respectively and these were obtained 15–30 min after the injections and then gradually declined.

A rapid increase in the DOPAC concentration in brain was observed after a single administration of an analgesic dose (5–20 mg/kg) of morphine and the maximum increase (about 57% increase after a dose of 10 mg/kg) was obtained within 15 min and the concentration then declined slowly (Fig. 1). On the other hand, the rate of the increase in the concentration of HVA in the brain was slower than that of DOPAC and its maximum increase (about 22% increase after a dose of 10 mg/kg) was observed 30 min after the morphine injection and then the concentration of HVA slowly declined (Fig. 1).

The increase in the concentration of DOPAC induced by the morphine administration (20 mg/kg) was completely suppressed by nalorphine (2 mg/kg) given 5 min after the morphine administration. The effect of nalorphine on the increase in the concentration of HVA induced by the morphine administration was not investigated.

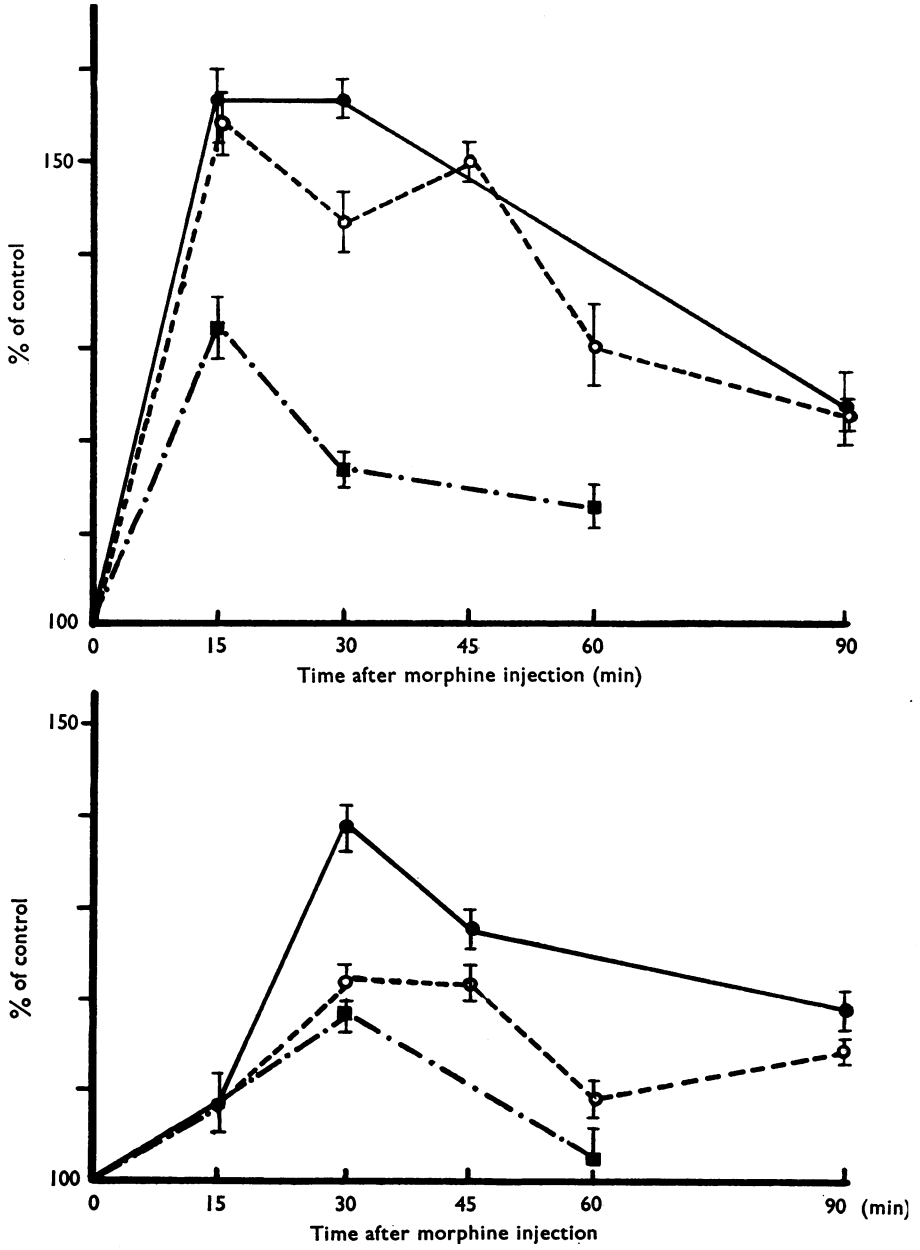

FIG. 1. Time courses of the effects of different doses of morphine on the concentrations of DOPAC (upper) and HVA (lower) in mouse brain. Values are the mean ±S.E. from four or five experiments. (●——●), 20 mg/kg; (○- - -○), 10 mg/kg; (■- — -■), 5 mg/kg.

Effect of morphine in tolerant mice

When morphine (20 mg/kg) was repeatedly administered to mice for 8 days, tolerance could be demonstrated on the ninth day in 40% of the mice by the fact that this dose of morphine no longer produced analgesia. The tolerant mice were selected and divided into two groups: DOPAC and HVA were estimated in the brains of mice from one group without further treatment and in the brains of the second group 30 min after a dose of morphine (20 mg/kg). In morphine-tolerant mice the cerebral concentrations of DOPAC and HVA did not differ from those

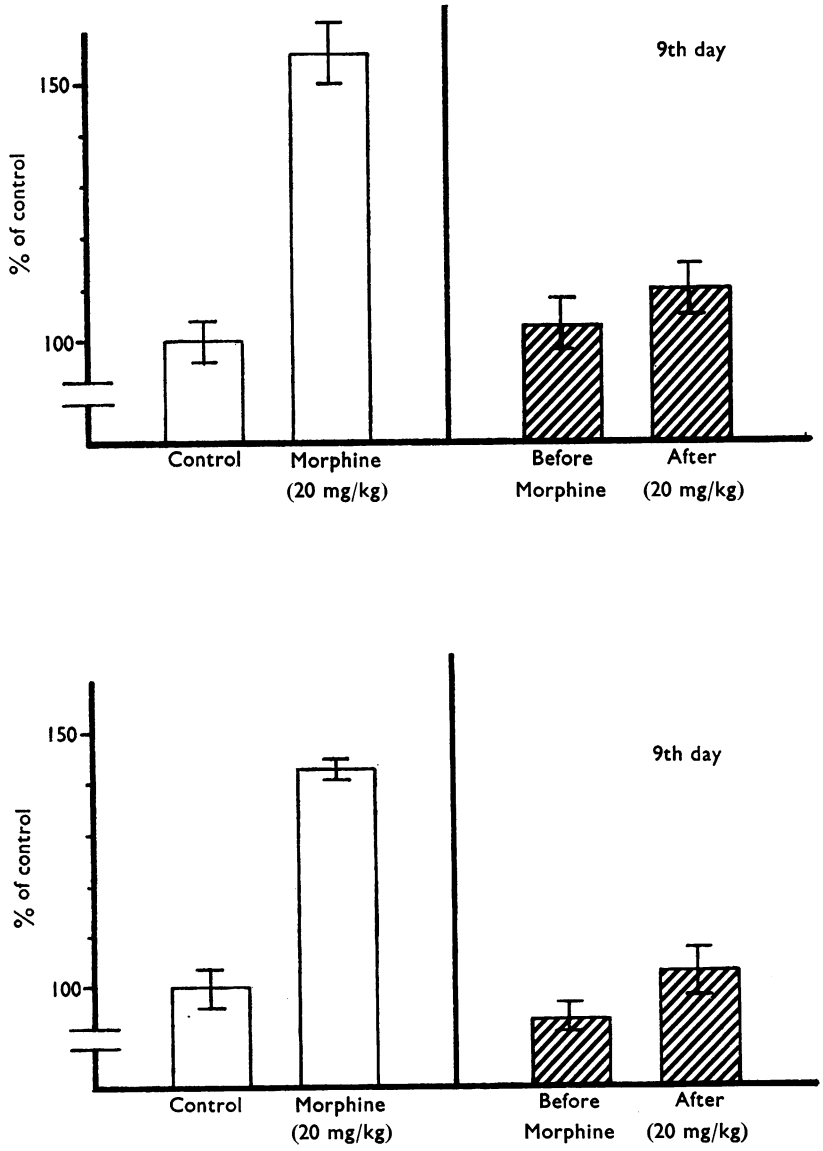

FIG. 2. Effects of morphine (20 mg/kg) on the concentrations of DOPAC (upper) and HVA (lower) in non-tolerant (normal) (left) and tolerant (right) mice. DOPAC and HVA were measured 30 min after morphine injection. Values are the mean ± S.E. from four or five experiments.

of the non-tolerant groups but the increase in DOPAC and HVA after the acute administration of morphine (20 mg/kg) was greatly reduced (Fig. 2).

Effect of nalorphine in non-tolerant mice

Nalorphine (2 mg/kg) did not cause any significant changes in the concentrations of DOPAC and HVA in the brains of non-tolerant mice.

Discussion

These results show that an analgesic dose (10 mg/kg) of morphine increased the cerebral concentrations of DOPAC and HVA in normal mice and the time course of the increased DOPAC concentration and the morphine analgesia approximate to each other. In addition, the increase in the cerebral concentration of DOPAC by morphine was prevented by nalorphine. Morphine (10 mg/kg) causes only a slight decrease in the concentrations of NA and DA in the brains of normal mice (Takagi & Nakama, 1966).

The mechanism of the increase in the cerebral concentrations of DOPAC and HVA produced by morphine in normal mice might be attributed to several factors. Morphine could increase the biosynthesis of DA leading to an enhanced formation of DOPAC and HVA. The reuptake of DA into storage forms might be impeded by morphine, thus increasing the amount of free amines available for metabolic degradation. Morphine might also inhibit the removal of DOPAC and HVA from brain. Since Clouet & Ratner (1970), Smith, Villarreal, Bednarczyk & Sheldon (1970) and Fukui & Takagi (unpublished observation) reported that morphine accelerated the *in vivo* biosynthesis of catecholamines from ^{14}C-tyrosine, the first possibility seems to be most probable. However, the other possibilities cannot be ruled out.

Clinical and experimental findings indicate that the nigro-neostriatal dopaminergic pathway is involved in extrapyramidal motor control (Hornykiewicz, 1966). It is also reported that the electrical stimulation of the substantia nigra releases DA and HVA into the cerebral ventricle (Portig & Vogt, 1969). The present results may be interpreted as the result of an action of morphine on the extrapyramidal motor system, causing the cataleptic symptoms such as the stiff erection of the tail. However, there is some evidence suggesting that dopamine may serve as an inhibitory neurotransmitter not only in the corpus striatum (Hornykiewicz, 1966) but also in other central structures such as the cerebral cortex (Krnjevic & Phillis, 1963) and the cuneate and gracilis nuclei of the dorsal column (Steiner & Meyer, 1966).

Moreover, the existence of a descending inhibitory pathway from the caudate nucleus to the sensory afferent system has been demonstrated: the electrical stimulation of the caudate nucleus inhibits the evoked potentials recorded from the midbrain reticular formation or the intralaminar complex (nucleus centre median) of the thalamus during the electrical stimulation of a cutaneous nerve (Krauthamer, 1963; Albe-Fessard, 1968). In addition it has been demonstrated that there are some descending inhibitory mechanisms from various higher centres acting on spinal sensory transmission (Hagbarth & Kerr, 1954; Urabe, Tsubokawa, Sakurai & Seki, 1965). Also, analgesic doses of morphine enhance the central, descending, inhibitory influence on spinal transmission and the postsynaptic inhibition of the monosynaptic spinal reflex in cats (Satoh & Takagi, 1970a, b).

These studies together with the present results suggest that DA stimulates the descending inhibitory system directly or that DA might have an inhibitory action on the inhibitory neurone which depresses the descending inhibitory mechanism, and that the analgesic action of morphine may be mediated through DA action. To some extent this suggestion seems to be supported by the observations that pretreatment of mice with reserpine or tetrabenazine decreases morphine analgesia and that this effect can be reversed by administration of DOPA (Schneider, 1954; Sigg, Caprio & Schneider, 1958; Takagi, Takashima & Kimura, 1964; Takagi & Nakama, 1968). However, the possibility that other monoamines including NA may also participate in the sensory mechanism cannot be ruled out.

In the present experiments, there was no significant increase in DOPAC and HVA concentrations after a single injection of morphine in morphine-tolerant mice. These results are compatible with a previous finding that pretreatment with tetrabenazine suppressed the development of tolerance to morphine and this was reversed by DOPA injection (Takagi & Kuriki, 1969). These results are also in favour of the hypothesis that DA participates in the mechanism of action of morphine.

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REFERENCES

ALBE-FESSARD, D. (1968). Central nervous mechanism involved in pain and analgesia. In: *Proceedings of the Third International Pharmacology Meeting*, vol. **9**. *Pharmacology of Pain*, ed. Lim, Armstrong & Pardo, pp. 131–168. Oxford: Pergamon Press.

ANDÉN, N. E., ROOS, B. E. & WERDINIUS, B. (1963). On the occurrence of homovanillic acid in brain and cerebrospinal fluid and its determination by a fluorometric method. *Life Sci.*, **7**, 448–458.

CLOUET, D. H. (1968). Biochemical responses to narcotic drugs in the nervous system and in other tissues. *Int. Rev. Neurobiol.*, **11**, 99–128. New York: Academic Press.

CLOUET, D. H. & RATNER, M. (1970). Catecholamine biosynthesis in brains of rats treated with morphine. *Science, N.Y.*, **168**, 854–856.

GUNNE, L. M. (1959). Noradrenaline and adrenaline in the rat brain during acute and chronic morphine administration and during withdrawal. *Nature, Lond.*, **184**, 1950–1951.

GUNNE, L. M., JONSSON, J. & FUXE, K. (1969). Effects of morphine intoxication on brain catecholamine neurons. *Eur. J. Pharmac.*, **5**, 338–342.

HAGBARTH, K. E. & KERR, D. I. R. (1954). Central influences on spinal afferent conduction. *J. Neurophysiol.*, **17**, 295–307.

HORNYKIEWICZ, O. (1966). Dopamine (3-hydroxytyramine) and brain function. *Pharmac. Rev.*, **18**, 925–964.

KRAUTHAMER, G. M. (1963). Inhibition of evoked potentials by striatal stimulation and its blockage by strychnine. *Science, N.Y.*, **142**, 1175–1176.

KRNJEVIC, K. & PHILLIS, J. W. (1963). Actions of certain amines on cerebral cortical neurones. *Br. J. Pharmac. Chemother.*, **20**, 471–490.

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

MAYNERT, E. W. & KLINGMAN, G. I. (1962). Tolerance to morphine. I. Effect on catecholamines in the brain and adrenal glands. *J. Pharmac. exp. Ther.*, **135**, 285–295.

PORTIG, P. G. & VOGT, M. (1969). Release into the cerebral ventricles of substances with possible transmitter function in the caudate nucleus. *J. Physiol., Lond.*, **204**, 687–715.

ROFFLER-TARLOV, S., SHARMAN, D. F. & TEGERDINE, P. (1971). 3,4-Dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid in the mouse striatum: a reflection of intra- and extraneuronal metabolism of dopamine? *Br. J. Pharmac.*, **42**, 343–351.

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

SATOH, M. & TAKAGI, H. (1970a). Enhancement by morphine on 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.

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.

SLOAN, J. W., BROOKS, J. W., EISENMAN, A. & MARTIN, W. R. (1963). The effect of addiction to and abstinence from morphine on rat tissue catecholamine and serotonin levels. *Psychopharmacology*, **4**, 261–270.

SMITH, C. B., VILLARREAL, J. E., BEDNARCZYK, J. H. & SHELDON, M. I. (1970). Tolerance to morphine-induced increase in ^{14}C-catecholamine synthesis in mouse brain. *Science, N.Y.*, **170**, 1106–1108.

STEINER, F. A. & MEYER, M. (1966). Actions of L-glutamate, acetylcholine and dopamine on single neurones in the nuclei cuneatus and gracilis of the cat. *Experientia*, **22**, 58–59.

TAKAGI, H., INUKAI, T. & NAKAMA, M. (1966). A modification of Haffner's method for testing analgesics. *Jap. J. Pharmac.*, **16**, 287–294.

TAKAGI, H. & KURIKI, H. (1969). Suppressive effect of tetrabenazine on the development of tolerance to morphine and its reversal by dopa. *Int. J. Neuropharmac.*, **8**, 195–196.

TAKAGI, H. & NAKAMA, M. (1966). Effect of morphine and nalorphine on the content of dopamine in mouse brain. *Jap. J. Pharmac.*, **16**, 483–484.

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. *Jap. 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. *Archs int. Pharmacodyn. Thèr.*, **149**, 484–492.

URABE, M., TSUBOKAWA, T., SAKURAI, H. & SEKI, M. (1965). Relation of brain stem activity to the evoked potential in the spinal cord following the single volley of the splanchnic nerve. *Folia Psychiat. Neurol. Japon.*, **19**, 49–67.

WERDINIUS, B. (1967). Prenylamine and brain monoamine metabolism. *Acta pharmac. tox.*, **25**, 1–8.

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