STUDIES ON THE MECHANISM OF ACTION OF TETRABENAZINE AS A MORPHINE ANTAGONIST

II. A PARTICIPATION OF CATECHOLAMINE IN THE ANTAGONISM*

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Earlier observations in this laboratory have demonstrated that tetrabenazine, a brain monoamine releaser, antagonizes morphine analgesia in mice (1) and that it antagonizes the depressive effects of morphine on the several afferent pathways in the central nervous system in cat (2).

More recently it has also been demonstrated that the change of brain noradrenaline (NA) plays more important role than that of serotonin (5-HT) in the antagonism of morphine analgesia by tetrabenazine or reserpine (3) and that morphine decreases the content of dopamine (DA) as well as that of NA in the brain (4).

The purpose of this study was to investigate the mechanism of tetrabenazine antagonism of morphine analgesia in relation to DA and NA content in the brain.

METHODS

Experiments were conducted with male mice (*dd*-strain) weighing 13 to 15 g. Analgesic action of morphine was measured by the tail-pinch method with 500 g pressure clip described by the authors (5). In each experiment 10 mice were used. Mice were killed by decapitation, and their whole brain were quickly removed, rinsed in ice cold isotonic saline, blotted on a filter paper and weighed. Five brains were pooled and homogenized in 0.4 N perchloric acid. DA and NA were assayed fluorimetrically according to the method described by Bertler *et al.* (6) and by Carlsson and Waldeck (7), respectively. 5-HT was assayed according to Bertler *et al.* (6). Drugs used were: morphine hydrochloride, tetrabenazine methanesulphonate and *dl*-3,4-dihydroxyphenylalanine (DOPA).

All drugs were administered subcutaneously except DOPA, which was given intraperitoneally.

RESULTS

1. Effect of tetrabenazine on the DA, NA and 5-HT content in mouse brain

After subcutaneous administration of 40 mg/kg of tetrabenazine, a marked sedation

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was observed within 30 to 40 minutes and lasted for several hours. The DA content in brain decreased within 40 minutes to about 30 per cent of its normal value and remained at this level for 2 hours (Fig. 1) and then gradually increased (not shown in figure). The NA content decreased also within 40 minutes and its maximum effect, 80 per cent decrease of its normal value, was obtained in 1.5 hours after injection and remained at this level for 2 hours (Fig. 1).

The effect of 40 mg/kg of tetrabenazine on the brain 5-HT content was shown in Fig. 1. 5-HT content decreased to about 50 per cent of its normal value in 1.5 hours after drug administration.

2. Effect of DOPA on the content of DA and NA in the tetrabenazine-pretreated mice

If DOPA (100 mg/kg) was given into mice 1.5 hours after administration of tetrabenazine 40 mg/kg, DA content in brain

T: Tetrab. 40mg/kg s.c.

FIG. 1. Effect of subcutaneous injection of tetrabenazine (40 mg/kg) on the contents of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in mice brain.

T: Tetrab. 40mg/kg s.c.

D: DOPA 100mg/kg i.p. (1.5h after T)

FIG. 2. Effect of DOPA on the contents of brain DA and NA in tetrabenazine-pretreated mice.

Light line: Changes of DA and NA levels after subcutaneous injection of tetrabenazine 40 mg/kg.

Heavy line: Changes of DA and NA levels after intraperitoneal injection of DOPA (100 mg/kg) in tetrabenazine-pretreated mice.

Abscissa: Time after injection of tetrabenazine.

Ordinate: Changes of DA and NA.

increased rapidly to normal value within 30 minutes after DOPA and then decreased rapidly to 60 per cent of its normal value (Fig. 2). In contrast, NA content after injection of DOPA increased to about 50 per cent of normal value and remained at this level for 30 minutes (Fig. 2).

3. Effect of DOPA on the analgesic effect of morphine in the tetrabenazine-pretreated mice

Pretreatment of mice with 40 mg/kg of tetrabenazine markedly suppressed the analgesic effect of 20 mg/kg of morphine (Fig. 3).

If DOPA (100 mg/kg) was given 30 minutes before morphine (20 mg/kg) into mice which were pretreated with 40 mg/kg of tetrabenazine, the analgesic effect of morphine markedly recovered, indicating the administration of DOPA suppressed the tetrabenazine antagonism of morphine analgesia (Fig. 3). In a case of administration of 200 mg/kg of DOPA similar tendency as that observed in administration of 100 mg/kg of DOPA was observed (Fig. 3).

Effect of 20 mg/kg of morphine alone on the brain DA and NA levels of normal mice were, as previously reported (4), a 32 per cent decrease in DA and a 23 per cent decrease in NA at their maximum (Fig. 3).

FIG. 3. Effect of DOPA on tetrabenazine-induced antagonism of morphine analgesia and brain monoamine changes in mice. Left ordinate: Per cent change of brain DA and NA levels. Right ordinate: Per cent change of analgesic effect of morphine (20 mg/kg).

DISCUSSION

Present experiments demonstrated that tetrabenazine decreased markedly the DA and NA content in the mouse brain, while it induced a slight reduction of 5-HT, confirming the work of Pletscher *et al.* (14) and Quinn *et al.* (15).

In order to investigate whether tetrabenazine antagonism of morphine analgesia is related to the change of DA and/or NA levels in the brain, the influence of administration of DOPA on the morphine analgesia in tetrabenazine-pretreated mice was determined. Thirty minutes after administration of 100 mg/kg of DOPA on the tetrabenazine-pretreat-

ed mice, the DA and NA levels in the brain returned to the normal value and about 50 per cent of the normal value, respectively and in such mice a considerable recovery of morphine analgesia was observed.

The releasing effect of tetrabenazine (40 mg/kg) on the 5-HT was relatively weak, about 50 per cent decrease in maximum. This is compatible with a previous paper (3) reported that the role of 5-HT in the antagonism of morphine analgesia by tetrabenazine or reserpine is less important than that of NA.

Considering these findings, tetrabenazine antagonism of morphine analgesia may be attributed to a decreasing action of brain DA as well as NA.

There is some controversy concerning the mechanism of antagonism of morphine analgesia by reserpine and other monoamine releasers.

Rudzik and Mennear (8) have concluded that the antagonism of morphine analgesia by reserpine is due to some intrinsic property of reserpine other than its effect on brain amines, since neither α -methyl-DOPA nor α -methyl-*m*-tyrosine (α -MMT), brain monoamine depleting agents, significantly elevated ED50 value of morphine.

On the other hand Medaković and Banić (9) have suggested that the inhibitory action of reserpine on the morphine analgesia is due to the releasing action of brain 5-HT since α -MMT which releases NA from brain stores without an appreciably depleting brain 5-HT stores, antagonized the effect of morphine in mice. In addition they have reported that in rats α -MMT did not cause any appreciable change of the analgesic effect of morphine and action of α -MMT differs from that observed in mice.

These discussions based mainly on their observations concerning the effect of α -MMT or α -methyl-DOPA on morphine analgesia. However, it is of important to note that α -MMT is decarboxylated to α -methylmetatyramine which is subsequently β -hydroxylated to metaraminol at the NA storage sites and the drop in amine levels is mainly due to displacement by metaraminol (10, 11).

It is also shown that after injections of α -methyl-DOPA the lost NA has been roughly replaced by α -methyl NA (12, 13). From these findings Andén (11) has concluded that the drop in NA is mainly due to displacement by the amine analogues formed. Moreover the displacement of NA by metaraminol in the central nervous system does not result in any impairment of transmission (11, 16), suggesting a possibility that metaraminol which is a weak sympathomimetic amine acts as substitute for noradrenergic transmitter. These results show that the use of α -MMT or α -methyl-DOPA does not provide an adequate basis to analyse the action of reserpine or tetrabenazine.

SUMMARY

1. The mechanism of antagonism of morphine by tetrabenazine was investigated in mice.
2. A single injection of tetrabenazine 40 mg/kg s.c. into mice caused a rapid reduction of DA and NA. Both amines decreased within 1 hour 25–30 per cent and 20 per cent of their normal values, respectively and remained at these levels for about 2 hours.

3. If DOPA was given 30 minutes before morphine into mice which had received tetrabenazine 1.5 hours earlier, DA and NA content in brain recovered to normal level and to about 50 per cent of normal value, respectively. At that time, morphine analgesia was considerably recovered.

4. The mechanism of the antagonism of analgesic action of morphine by tetrabenazine was discussed in relation to the depleting effect by tetrabenazine of DA and NA.

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