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The action of reserpine and α-methyl-*m*-tyrosine on the analgesic effect of morphine in rats and mice

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The actions of α-methyl-*m*-tyrosine (MMT) and reserpine upon the analgesic effect of morphine has been studied in rats and mice. In rats, reserpine antagonised the effect of morphine, while MMT did not cause any appreciable change of the effect of morphine, injected either 2 or 24 hr after MMT. Reserpine also produced its antagonistic action to morphine in rats which were previously (24 hr) treated with MMT. Both MMT and reserpine potentiated the effect of morphine in rats pretreated (24 hr previously) with iproniazid. In mice, both MMT and reserpine antagonised the effect of morphine. Reserpine failed to do so in MMT pretreated animals. The effect of morphine was restored 24 hr after the injection of MMT. It is suggested that the inhibitory action of reserpine upon the analgesic effect of morphine is due to the antagonistic action of brain 5-HT, which is activated after being released from its stores by reserpine. The pattern of reserpine—MMT—morphine interactions in rats probably differs from that in mice.

THE relationship between the actions of reserpine and morphine-like analgesics has been widely examined (Schneider, 1954; Raduoco-Thomas & Le Breton, 1957; Tripod & Gross, 1957; Sigg, Caprio & Schneider, 1958; Banić & Medaković, 1964) with the object of gaining a better understanding of the mechanisms of action of both reserpine and morphine-like analgesics and because there exists the possibility that the nature of pain itself may at least be partially explained by such studies.

The effects of reserpine (primarily sedation) have been explained by the ability of this drug to release the brain amines 5-hydroxytryptamine (5-HT), according to one concept (Brodie & Shore, 1957; Brodie & Costa, 1960) or noradrenaline according to the other (Carlsson, Lindquist & Magnusson, 1957; Kärki & Paasonen, 1959; Pletscher, Besendorf & Gey, 1959; Schaumann, 1958). Unfortunately, both views were based on indirect data. A closer approach to the problem was hindered by the fact that reserpine is equally active in releasing both 5-HT and noradrenaline.

Hess, Connamacher, Ozaki & Udenfriend (1961) have recently found that one amino-acid, α-methyl-*m*-tyrosine (MMT), releases noradrenaline from brain stores without appreciably depleting brain 5-HT stores. Thus MMT may be a "most effective means for obtaining experimental animals which are depleted of tissue noradrenaline, but still contain normal amounts of tissue 5-HT" (Hess & others, 1961).

Some controversy concerning the mechanism of reserpine action might also be due to species differences. Thus, while in rats reserpine antagonises the action of analgesics, both antagonism (Schneider, 1954; Schaumann, 1958) and potentiation (Tripod & Gross, 1957; Leme & Rocha e Silva, 1961) of the effects of morphine have been produced by reserpine in mice.

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The experiments now presented were made on rats and for comparison some were repeated on mice. Effects of MMT on the analgesic action of morphine and on the inhibitory action of reserpine on morphine analgesia were studied. The interaction between these drugs was also studied by means of a monoamine oxidase inhibitor (iproniazid).

The evidence suggests that the effect of reserpine on the analgesic action of morphine in rats is produced through brain 5-HT rather than through noradrenaline. This was less convincing in experiments on mice.

Methods

Rats, of both sexes, weighing approximately 180 g were used in groups of 10 animals. Each animal was placed in turn in a cylindrical cage, with the tail extending from the end of the cage. A beam of heat, from a 12 V 50 W bulb was focused on the tip of the tail of each rat, according to the method of D'Amour & Smith (1941), and the time until the heat induced movement of the tail was measured. To prevent the damage of the tail by heat stimulus in animals with total analgesia, the stimulation was applied for not longer than 15 sec. Drugs were injected intraperitoneally, except iproniazid, which was injected subcutaneously.

In experiments on mice, males weighing approximately 22 g were used. Analgesia was tested by the hot plate method of Woolfe & MacDonald (1944). A glass chamber was immersed into water, held at the thermostatically regulated temperature (53°). Each mouse in turn was placed into this chamber and the reaction time until the appearance of the paw-licking reflex was determined. Each group contained at least 15 animals. All drugs were injected intraperitoneally. To prevent paw tissue damage in mice with total analgesia, they were removed from the hot plate before this could occur. An arbitrary interval of double the control mean reaction time of each given group was selected for this purpose. The values were plotted on the graphs as percentages of this "cut-off time".

The results are presented graphically. But some of the animals did not react until the cut-off time had expired. They were given the reaction time of 15 sec in experiments in rats, and the maximum cut-off time in those in mice. Thus, the analgesic effect as plotted is not always exact and represents low values. This applies to curves showing the potentiation of the analgesic effect. It can be assumed therefore that the real difference between the control effect of morphine and the potentiated one is always larger than shown in the graphs.

The drugs used were morphine hydrochloride, reserpine (Serpasil, CIBA), iproniazid (Hoffmann–La Roche) and α-methyl-*m*-tyrosine.

Results

EFFECT OF RESERPINE ON THE ANALGESIC ACTION OF MORPHINE IN RATS

Reserpine abolishes the analgesic action of morphine in rats (Schneider, 1954; Schaumann, 1958; Banić & Medaković, 1964). This effect has been studied again, because it had to be compared with the effect of MMT on the action of morphine.

First, the analgesic action of morphine injected intraperitoneally (4 mg/kg) was established (Fig. 1). Then, it was found that, in accordance with Sigg & others (1958) and Schaumann (1959), reserpine, also injected intraperitoneally (1 mg/kg), did not affect the control mean reaction time of animals to the heat stimulus.

The effect of reserpine on the analgesic action of morphine, injected 3 hr later, is shown in Fig. 1. The analgesic action of morphine is

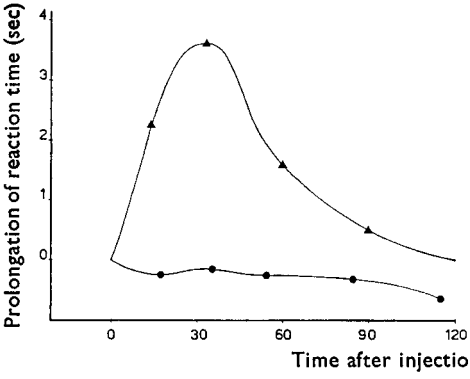
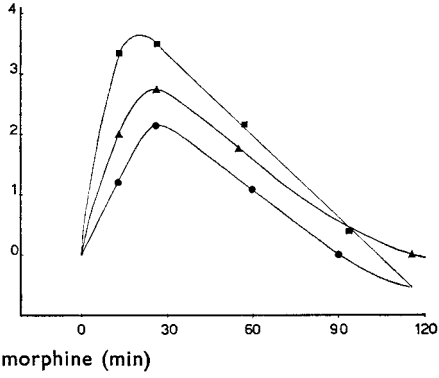


FIG. 1. Analgesic effect of morphine in control rats (–▲–) and the inhibition of this effect by the previous injection of reserpine (–●–).



Time after injection of morphine (min)

FIG. 2. The effect of pretreatment with MMT on the analgesic action of morphine in rats. Action of morphine in control animals (–▲–), in rats treated with MMT 3 hr (–■–) and 24 hr (–●–) respectively, before morphine.

abolished by the previous injection of reserpine. This finding is in accordance with the results of previous reports on the interaction between reserpine and morphine in rats (Schneider, 1954; Banić & Medaković, 1964).

EFFECT OF MMT ON THE ANALGESIC ACTION OF MORPHINE IN RATS

First the effect of morphine was determined in a group of animals. Similarly, it was established that MMT on intraperitoneal injection did not affect the control mean reaction time. However, contrary to reserpine, if MMT (400 mg/kg) is injected 90 min before morphine, the analgesic effect of the morphine was not inhibited (Fig. 2). Moreover, the effect of morphine seemed to be slightly potentiated by the previous injection of MMT. This potentiation was, nevertheless, very weak.

The effect of morphine was again assessed 24 hr after the injection of MMT and was found to be equal to the effect obtained in the first control experiment.

EFFECT OF RESERPINE ON THE ACTION OF MORPHINE AFTER MMT

The experiments above on the effects of reserpine and of MMT indicated a substantially different mode of action for these two drugs on the analgesic action of morphine. Since MMT, which releases only brain noradrenaline, was without effect on the action of morphine, it is reasonable

to suppose that the effect of reserpine, which releases 5-HT as well as noradrenaline, is due to its action on brain 5-HT stores. To obtain additional evidence, the effect of reserpine on the analgesic action of morphine was tested in animals which were pretreated with MMT, with aim of depleting their brains of noradrenaline.

The action of morphine (4 mg/kg) was determined first, then MMT (400 mg/kg) was injected. On the following day (21 hr later) the animals received reserpine (1 mg/kg i.p.) and 3 hr. later morphine (4 mg/kg). As Fig. 3 shows, the analgesic action of morphine was strongly inhibited.

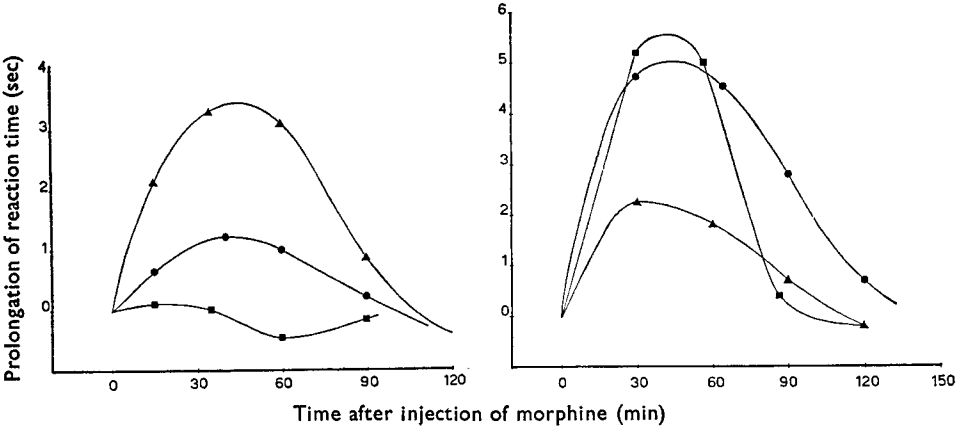


FIG. 3. The effect of reserpine on the analgesic action of morphine in rats pretreated with MMT. MMT (400 mg/kg) was injected once and reserpine twice, done 24 hr (–●–) and 48 hr (–■–) after MMT (always 3 hr before morphine). Control effect of morphine is indicated by (–▲–).

FIG. 4. The effect of reserpine (–■–) and MMT (–●–) on the analgesic action of morphine in rats, pretreated with iproniazid, 24 hr before morphine. Control effect of morphine is indicated by (–▲–).

The experiment was continued on the following day. Reserpine was reinjected (1 mg/kg), and 3 hr later the animals received morphine. The action of morphine was then completely abolished.

ACTION OF IPRONIAZID ON THE EFFECT OF RESERPINE AND MMT

Iproniazid may antagonise the effect of reserpine, and a reversal of the effect of reserpine may result in iproniazid-pretreated animals (Schaumann, 1958). The effects of iproniazid on the action of reserpine and MMT respectively were examined in relation to the analgesic action of morphine.

In the experiment shown in Fig. 4, iproniazid (100 mg/kg i.m.) was injected on the first day. On the next day MMT was injected first and morphine 2 hr later. As can be seen, the effect of morphine was strongly potentiated and prolonged. The experiment was continued, and iproniazid was re-injected on the same day. On the next day reserpine (1 mg/kg)

was injected and 2 hr after, morphine. The analgesic effect of morphine was potentiated and prolonged to the same extent as on the previous day after iproniazid and MMT (Fig. 4).

ACTION OF MMT AND RESERPINE ON THE EFFECT OF MORPHINE IN MICE

Our previous experiments (Banić & Medaković, 1964) suggested that the action of reserpine on the analgesic effect of morphine in mice might differ from that in rats. Therefore, some experiments were repeated in mice.

First, the action of MMT on the effect of morphine was studied, and compared with the action of reserpine on morphine analgesia. MMT (400 mg/kg) was injected 2 hr before morphine (10 mg/kg). As Fig. 5

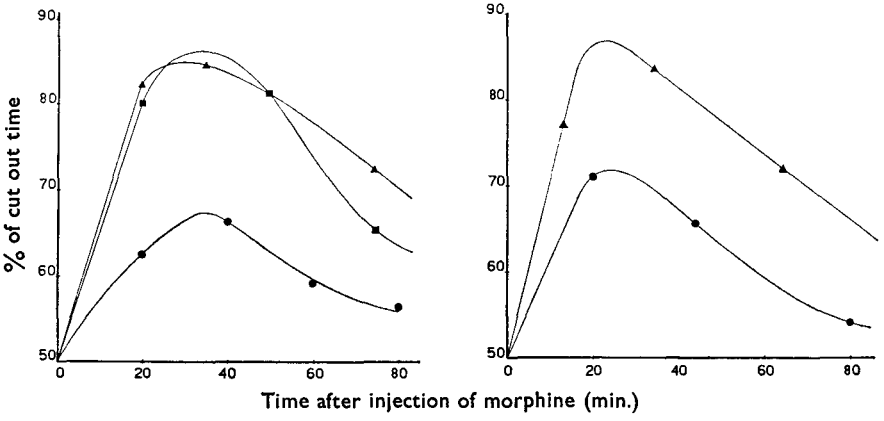


FIG. 5. The effect of MMT on the analgesic action of morphine in mice. The action of morphine in control animals (–▲–), in animals treated with MMT 3 hr (–●–) and 24 hr (–■–) before morphine.

FIG. 6. The effect of reserpine on the analgesic action of morphine in mice. The action of morphine in control animals (–▲–) and in those treated with reserpine 3 hr before morphine (–●–).

shows, the effect of morphine was significantly inhibited in MMT pretreated mice, as compared with the effect of morphine in untreated controls. The same effect on morphine analgesia was obtained with reserpine (Fig. 6). It is noteworthy that the effect of morphine was restored 24 hr after the treatment with MMT (Fig. 5).

In the second experiment mice first received MMT (400 mg/kg) and 24 hr later reserpine (2 mg/kg). This treatment was followed, 2 hr after reserpine, by the injection of morphine (10 mg/kg). Fig. 7 shows that reserpine did not antagonise the effect of morphine in mice which were pretreated with MMT on the previous day.

Discussion

At least three points can be discussed on the basis of the present results: (1) the evaluation of the concepts about the participation of noradrenaline and 5-HT respectively on the action of reserpine, (2) the

mode of action of brain amines on the analgesic effect of morphine, and (3) the mechanism of morphine analgesia.

Firstly, the effects of reserpine and of MMT on the analgesic action of morphine were studied in rats. According to the data presented, reserpine could affect the action of morphine in two ways: by direct action on the CNS or, indirectly, by its action on the stores of biologically active amines in the brain. Some actions of reserpine on the CNS have been explained by its direct action (Kobinger, 1958). However, the assumption that the effect of reserpine on morphine analgesia is produced indirectly, by its action in releasing the stores of biologically active brain amines, is favoured by the fact that those rauwolfia alkaloids which do not release these amines from the brain (e.g., rescinamine, serpentine, raubasine—Brodie, Shore & Pletscher, 1956) do not affect the analgesic effect of morphine (Schaumann, 1958). Therefore, the present results will be

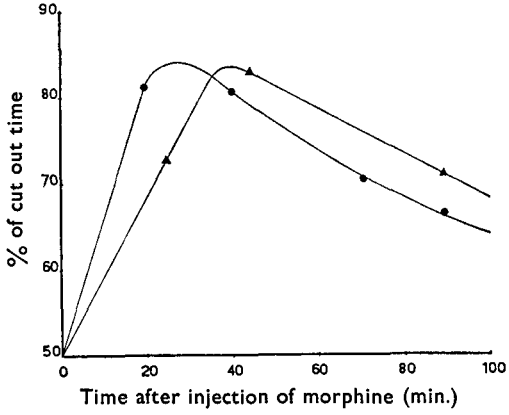


FIG. 7. The effect of reserpine on the analgesic action of morphine in mice treated with MMT (24 hr before morphine). The action of morphine in control animals (–●–) and in those pretreated with MMT and with reserpine (–▲–).

discussed on the assumption that the effect of reserpine is caused mainly indirectly, by its action on the amines, stored in the brain. The question now arises as to which of the actions, that on the stores of noradrenaline, or that on the stores of 5-HT, is responsible for the effect of reserpine.

Both MMT (Hess & others, 1961; Brodie & Costa, 1962) and reserpine (Holzbauer & Vogt, 1956) release noradrenaline from the stores in the brain. Consequently, if reserpine inhibits the action of morphine by acting on the stores of noradrenaline, MMT should produce a reserpine-like effect on morphine analgesia. However, we found the effects of these drugs to differ: while reserpine abolished the action of morphine, MMT did not do so. Hence, it is not likely that the inhibitory effect of reserpine was caused by its action on noradrenaline stores.

This assumption was further tested in the second experiment. First, the noradrenaline releaser MMT was injected and the effect of reserpine on morphine analgesia was examined in these animals on the following

day when reserpine also produced its inhibitory effect on the action of morphine in these animals. This fact favours the assumption that the action of reserpine on the stores of noradrenaline is not of primary importance for the inhibition of the effect of morphine. Reserpine releases 5-HT as well as noradrenaline and the action of reserpine on the stores of brain 5-HT has been repeatedly claimed to be responsible for the central effects of this drug (Brodie & Shore, 1957; Holtz, Balzer, Westermann & Wezler, 1957; Garratini Valzelli, 1958; Costa, Gessa, Kuntzmann & Brodie, 1962). Therefore, the fact that reserpine antagonised the action of morphine in rats pretreated with a high dose of MMT which depletes the brain stores of noradrenaline, but not those of 5-HT (Hess, 1961; Brodie & Costa, 1962), argues in favour of the assumption that the antagonism of reserpine to morphine was due to its releasing 5-HT. This agrees with the result on animals kept in a cold environment (Banić & Medaković, 1964), which suggested that reserpine acted on the analgesic effect of morphine through 5-HT rather than noradrenaline. It should be added that the relation between the actions of 5-HT and morphine has been much studied (Kosterlitz & Robinson, 1955, 1958; Gaddum & Picarelli, 1958; Medaković, 1957; 1958 b, c, d, 1959) and a highly specific antagonism between the actions of 5-HT and morphine has been found. Of special importance is the fact that the target tissue of this antagonism was always the nervous tissue, e.g., nervous elements in the isolated ileum of the guinea-pig. This is important since this isolated organ has been proposed and used as a suitable paradigm for studying central effects of analgesic drugs (Schaumann and others, 1952; Paton, 1957; Medaković, 1958). The reports on the antagonism between 5-HT and morphine on this isolated organ suggested that the competition between the two drugs takes place in the range of low concentrations (Medaković, 1958a). It seems reasonable to try to explain the antagonism between the actions of morphine and reserpine in the brain on a similar basis. This explanation is furthered by the concept of Brodie & his co-workers (1960, 1962), who explained the sedative effect of reserpine by its ability to release 5-HT from the pools onto the receptor sites in the brain. Hence, the hypothesis that the antagonistic action of reserpine to morphine is elicited by the presence of high concentrations of 5-HT at the receptor sites, and not because the brain stores of this amine are depleted by reserpine seems plausible. Our finding, that the second injection of reserpine in animals, which, after MMT, had already received a relatively small dose of reserpine on the preceding day (Fig. 3), still inhibited the action of morphine is in favour of the hypothesis. This second injection could be supposed to produce an additional effect by releasing those amounts of 5-HT which were not released by the first injection on the preceding day.

It may seem difficult to explain the findings obtained in experiments with iproniazid. As was shown, both MMT and reserpine potentiated the effect of morphine in iproniazid-pretreated animals. Hence, the effect of MMT was not qualitatively changed after iproniazid. However, the effect of reserpine was reversed. The same reversal by iproniazid of the

effect of reserpine was also obtained in experiments on animals which had received MMT 24 hr previously. As it can be assumed that brain stores in these animals were depleted of noradrenaline by MMT, the potentiating effect of reserpine must be ascribed to brain 5-HT. The assumption that 5-HT may exert two opposite effects, i.e., to antagonise and potentiate the action of morphine, may appear confusing. The inhibition of the brain monoamine oxidase by iproniazid should preserve brain 5-HT, and provoke its accumulation in high concentrations in the brain. Hence, one would now expect a more pronounced antagonism between reserpine and morphine than without iproniazid. The controversial finding that potentiation of the effect of morphine was obtained, instead of the expected deep inhibition, cannot be explained completely, but a feasible working hypothesis can be based on the fact that agents with high biological activity, for instance, acetylcholine, may cause opposite effects, depending on the actual concentrations. The same fact has already been established for 5-HT in experiments on the isolated guinea-pig ileum, where high concentrations of 5-HT antagonised the effect of smaller concentrations of the same drug (Rocha e Silva & Picarelli, 1953). It is noteworthy that a dual response can be obtained in rabbits given 5-hydroxytryptophan after a monoamine oxidase inhibitor; synchronisation is followed by desynchronisation of the brain EEG patterns (Costa, Pscheidt, Van Metter & Himwich, 1960). This finding prompted the hypothesis that monoamine oxidase inhibitors block 5-HT receptors through the presence of a large excess of 5-HT in the brain (Costa, Morpurgo & Revzin, 1961).

The experiments on mice showed that the mechanism of the action of reserpine on the effect of morphine might differ in this species from the action in rats. Contrary to the findings in rats, the previous injection of MMT antagonised the action of morphine, and reserpine failed to inhibit the action of morphine in mice which had received MMT 24 hr before reserpine.

The results of the present experiments in rats and mice do not seem to support the concept that the analgesic action of morphine is accomplished indirectly through its releasing brain noradrenaline stores. In rats, the analgesic action of morphine was not substantially changed by MMT pretreatment.

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