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The Sedative and Lethal Actions of Reserpine in Mice, as Modified by 5-Hydroxytryptophan, 3,4-Dihydroxyphenylalanine, Methylphenidate, and Nikethamide in Cold and Warm Environments

By

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It has been demonstrated that reserpine does not decrease the spontaneous motility of mice if the body temperature is not decreased (LESSIN & PARKES 1957). GARATTINI & VALZELLI (1958) reported that reserpine administered after a cold stress of many hours caused no sedation and had no effect on the 5-hydroxytryptamine (5HT) content of rat brain. At an environmental temperature of 37° reserpine did not increase barbiturate sleeping time and had no consistent effect on the amount of 5HT in the brain BRODIE *et al.* (1960) reached almost the same results in their cold experiments (+4°); they also noted a greater fall in the norepinephrine than in the 5HT content of rat brain.

CARLSSON *et al.* (1957) reported that 3,4-dihydroxyphenylalanine (DOPA) alone, and to an even greater extent when used with 5-hydroxytryptophan (5HTP), abolished the sedative effect of reserpine in mice at room temperature. DOPA also diminished the toxicity of reserpine in mice exposed to cold (NEČINA & KREJČI 1961).

The purpose of our work has been to investigate the effect of DOPA, 5HTP and some stimulants of the central nervous system on the sedative and lethal action of reserpine in mice at cold and warm environmental temperatures.

Materials and Methods.

Male white mice weighing 17–25 g were kept at room temperature (23°) before the experiments. During the experiment they were placed in plastic cages, five in each, at an environmental temperature of +9° or +37°.

Reserpine (CIBA), dissolved in acetic acid, dl-5-hydroxytryptophan (Hoffmann La Roche), dl-3,4-dihydroxyphenylalanine (Hoffmann La Roche), methylphenidate

hydrochloride (Ritalin ®, CIBA) and nikethamide (Corditon ®, Medica) in aqueous solution were injected intraperitoneally.

The reserpine dose was 2 mg/kg. It was given either as a single dose, or one mg per day on two consecutive days. With the animals kept at a cold or a warm temperature, some groups received 200 mg/kg DOPA or 5HTP, separately or together, three times, at intervals or 3–4 hours. Some groups received 50 mg/kg nikethamide or 25 mg/kg methylphenidate hydrochloride in the same way.

Results

A. *Cold experiments.* The temperature of +9° (+6° to +11°) was used because all the control mice survived it for 24 hours. At lower temperatures (0° and +4°) most of the mice died in some hours.

The main results are seen in figure 1. After reserpine, administered as a single dose of 2 mg/kg a few hours before or after beginning the exposure to cold, all mice died within 24 hours. About half of them died in 6 hours.

The same results were also observed when 1 mg/kg of reserpine was administered twice, the second dose at the beginning of cold exposure. If the last dose was given 24 hours previously, all the mice survived 6 hours and some 24 hours. The sedative action was lowest in this group.

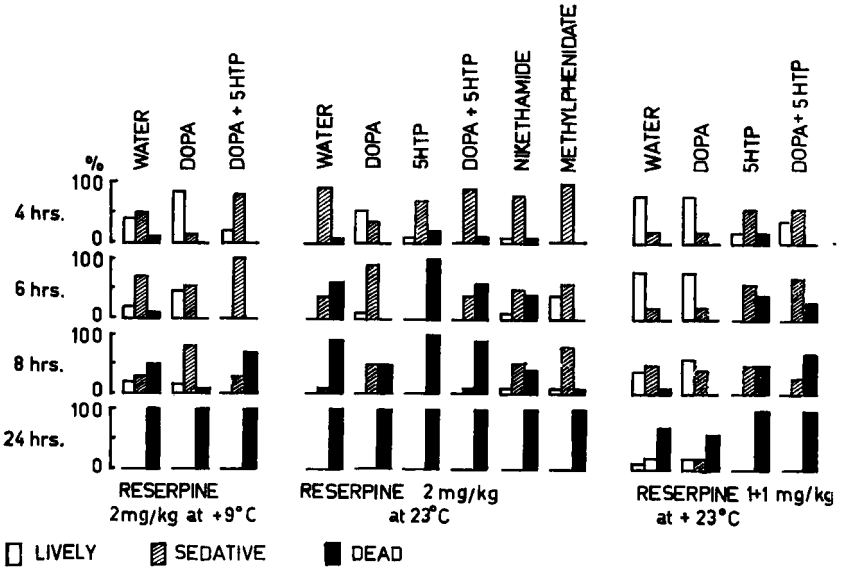

Figure 1. Sedative and lethal actions of reserpine to mice in cold environment (+9°), modified by various drugs. Reserpine 2 (mg/kg) was injected as a single dose, 2½ hrs. after(left) or before(centre) beginning exposure to cold, or in two doses, 48 and 24 hrs. before exposure(right). 5HTP(200mg/kg), DOPA(200 mg/kg), methylphenidate(25 mg/kg), nikethamide(50 mg/kg) or water was administered at 0.4, and 8 hrs. after putting the mice into the cold environment. The first dose was given just after the reserpine when this was injected in cold. Ten mice in each group.

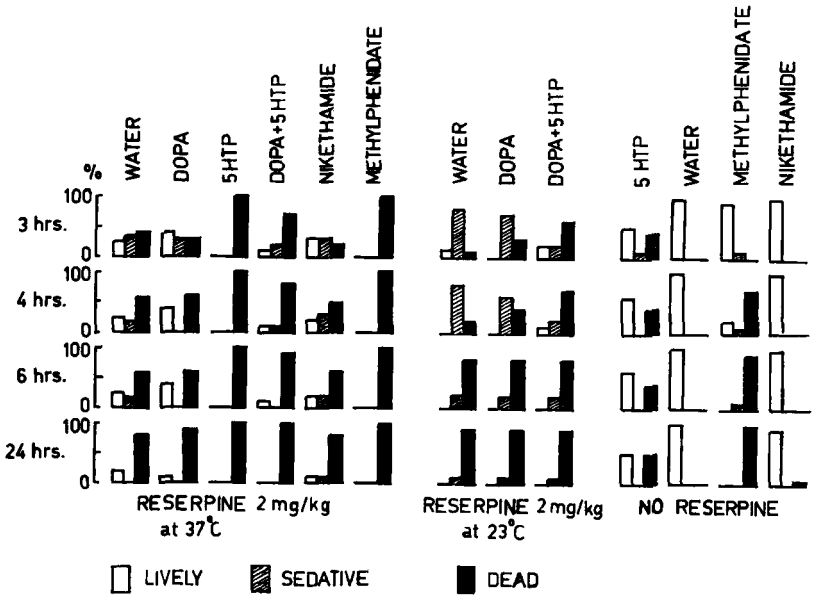

Figure 2. Sedative and lethal actions of reserpine to mice in warm (37°) environment, modified by some drugs. Reserpine (2 mg/kg) was administered 1½ hrs. after(left) or 1½ hrs. before(centre) beginning exposure to heat. The mice received 5HTP(200 mg/kg, DOPA (200 mg/kg), methylphenidate (25 mg/kg), nikethamide (50 mg/kg) or water 1½, 3 and 6 hrs. after being put into the warm environment. Ten mice in each group.

The mice receiving reserpine 2 mg/kg at room temperature were slightly more sedated than those receiving the drug in the cold. Some mice received reserpine just at the time of being placed in the cold and showed the same effects as the previously cold-stressed animals.

5HTP increased the sedation and mortality of the reserpinized mice.

DOPA at the doses used did not alter the 24-hour mortality. In those groups in which reserpine-induced sedation and mortality were apparent in 4–8 hours, these effects were antagonized by DOPA. Smaller doses of this amino acid (30–100 mg/kg) had the same effect when administered repeatly at the same intervals. When given along with DOPA, 5HTP prevented the stimulating action of DOPA.

Nikethamide and *methylphenidate* were administered to the groups of mice receiving reserpine 2 hours before the cold exposure. They reduced the sedative action and mortality due to reserpine. Methylphenidate was the best antagonist to reserpine.

B. *Heat experiments* (figure 2). All the control mice were alive after exposure to the environmental temperature of 37° for 24 hours. Most of the reserpinized mice died under these conditions. Convulsions were common, and the sedative action was never very pronounced. Slight sedation was more common in the groups receiving reserpine at 23° before

beginning heat exposure than in those receiving it at 37°. The survival time of the latter was somewhat shorter than that of the former animals.

5HTP shortened further the survival time of the reserpinized animals. This effect was more marked at 37° than at 9°. When 5HTP was administered alone at 37°, it was lethal to half of the mice.

DOPA alone has no clear effect on reserpine mortality at 37°, but it partly antagonized the effect of 5HTP in reserpinized mice.

Methylphenidate stimulated the spontaneous motility and markedly decreased the survival time of both normal and reserpinized mice. *Nikethamide* was almost inactive at this temperature.

At a higher surrounding temperature (+39°) the toxicity of reserpine, as well as that of 5HTP, increased further, but normal control mice remained lively. Methylphenidate first increased motor activity and then soon caused the death of the reserpinized mice, as at 37° environmental temperature. Nikethamide had no effect on the survival time.

Discussion

The mortality of the reserpinized mice was high in both cold and warm environments. GARATTINI & VALZELLI (1958) and BRODIE *et al.* (1960 found no notable sedation in rats if reserpine was injected in the cold after a 4 hours exposure to cold. Most of our mice, however, were sedated in cold experiments. It is possible that mice are so little resistant to cold stress that the "sedation" is, at least in part, due to exhaustion, as emphasized by ERSPAMER (1961).

The less marked reactions in those mice receiving reserpine 24 hours before exposure to cold suggest that these effects are due to some other effects of reserpine rather than to the liberation of amines in the brain (SHEPPARD & ZIMMERMAN 1959). This view is supported by the fact that nikethamide and methylphenidate also diminished the effects of reserpine in the cold, although they have not been shown to cause depletion of the brain amines. It is possible that DOPA, nikethamide and methylphenidate in our cold experiments acted as non-specific stimulants that increased motor activity. They would then have increased heat production and antagonized the hypothermia caused by the paralysis of thermoregulation. Possibly, then these drugs did not inhibit the action of reserpine in the heat experiments and the toxicity of reserpine was even enhanced by methylphenidate, the best antagonist in the cold and the strongest motor stimulant in the heat.

Besides the central actions of reserpine, the depletion of catecholamines of the adrenal glands and other peripheral tissues may modify thermoregulation (HOFFMAN 1959). KRONEBERG & SCHÜMANN (1960) have de-

monstrated a much more pronounced depletion of adrenaline from the adrenal medulla after reserpine in the cold than at the normal room temperature.

Although reserpine toxicity in the heat environment was suprisingly high, sedative action was absent. So the increased "free" 5HT (BRODIE 1957) need not be responsible for the lethal action of reserpine, although BRODIE *et al.* (1960) considered this to be associated with the tranquillizing action of reserpine. Because 5HT reduces the body temperature (FASTIER *et al.* 1957), the increased reserpine toxicity after 5HTP in the cold and the heat experiments may have been due to superadded depressing of thermoregulation caused by reserpine and 5HT. This possibility is supported by the observation (KÄRJÄ *et al.* 1961) that in mice at room temperature 5HTP can, depending on the dose, decrease or increase the body temperature. SHEMANO & NICKERSON (1959) reported that at environmental temperatures below 30° 5HT reduces the body temperature of the rat, but increases it at temperatures above 30°.

As mentioned before, CARLSSON *et al.* (1957) showed that DOPA and 5HTP together effectively antagonized the action of reserpine at room temperature. In our experiments performed at higher and lower temperatures the reserpine antagonizing effect of DOPA was inhibited by 5HTP.

It should be noted that 5HTP and DOPA have been observed to compete for the same decarboxylating enzyme (WESTERMANN *et al.* 1958; YUWILER *et al.* 1959). Further, an antagonism between 5HT and norepinephrine *in vivo* has been reported (GORDON *et al.* 1958).

Summary

The effect of 5-hydroxytryptophan (5HTP), 3,4-dihydroxyphenylalanine (DOPA), nikethamide and methylphenidate on the sedative and lethal actions of reserpine has been studied in mice kept at cold (+9°) or warm (+37° and 39°) environmental temperatures.

In the cold, a half of the reserpinized mice died in eight hours and all of them in 24 hours. The sedative action in the cold was almost the same when reserpine was administered in the cold or before beginning the cold experiment. If reserpine was given in two doses and the cold experiment was begun 24 hours after the second, the animals survived a little longer and showed less sedation. DOPA, nikethamide, and methylphenidate decreased and 5HTP increased the sedative and lethal actions of reserpine; DOPA partly antagonized this action of 5HTP.

In the warm environment most of the reserpinized mice died in 24 hours. Convulsions were common, sedation was slight and infrequent. Without reserpine 5HTP was lethal to half the mice. As in the cold, it greatly in-

creased the toxicity of reserpine. This effect of 5HTP was partly antagonized by DOPA; when given alone DOPA had no effect on the toxicity of reserpine.

Methylphenidate alone was very toxic in the heat. It also greatly enhanced the lethality of reserpine, whereas nikethamide was inactive.

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