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INFLUENCE OF METHYLDOPA ON CENTRAL EFFECTS OF RESERPINE

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Methyldopa potentiated hypnosis due to hexobarbitone in mice, as did reserpine, chlorpromazine and 5-hydroxytryptamine. Methyldopa antagonized the increase by reserpine of sleep due to hexobarbitone, but enhanced the potentiation by chlorpromazine and 5-hydroxytryptamine of hypnosis due to hexobarbitone. The sedative effect of reserpine in mice and the emetic effect in pigeons were also antagonized by methyldopa. However, the effects of reserpine on convulsions due to leptazol and in causing ptosis were not antagonized by methyldopa. It is suggested that some effects of reserpine (potentiation of hexobarbitone-sleeping time, sedation and emesis) are unrelated to changes in brain amine levels and that methyldopa, with its weak reserpine-like actions, is an antagonist to reserpine.

We have previously reported that, in cats treated with methyldopa, reserpine failed to produce the usual reduced sensitivity to the cardiovascular actions of tyramine, although it depleted cardiac noradrenaline (Varma & Benfey, 1963). In these studies it was observed that the behavioural effects of reserpine in cats treated with methyldopa were much less pronounced, although the miosis was unaffected.

The studies described here were carried out to investigate the influence of methyldopa on some central effects of reserpine. It was hoped that the results would not only give additional information on the interactions of methyldopa and reserpine but also throw some light on the mode of the central actions of reserpine.

METHODS

Male albino mice weighing 18 to 25 g were used. Food but not water was withdrawn the night before the experiment. Drugs were injected intraperitoneally unless otherwise stated. Controls, run with each experimental group, received an equal volume of 0.9% saline in place of the drug. Throughout the study methyldopa was used in a dose of 100 mg/kg of body weight. This dose was selected after pilot experiments with lower and higher amounts.

Sleeping time due to hexobarbitone in mice. This was taken as the time between loss and return of the righting reflex after the intraperitoneal injection of hexobarbitone sodium (100 mg/kg), as described by Salmoiraghi, Sollero & Page (1956). To observe actions on the sleeping time, methyldopa was injected 4 hr, reserpine (5 and 10 mg/kg) 1 hr, chlorpromazine (2.5 mg/kg) 30 min, and 5-hydroxytryptamine (20 mg/kg) 10 min, before hexobarbitone.

Sedation and ptosis due to reserpine in mice. The method was similar to that of Vernier, Hanson & Stone (1962). The degree of sedation was obtained from the time required by the

animals to walk to the margin of a circular wire mesh of 12 cm radius after being placed in the centre. Methyldopa was injected 4 hr before reserpine. The time taken by the control animals to walk the specified distance varied between 2.3 and 7.5 sec. If an animal did not walk the distance in 3 min, as occurred after injection of reserpine, the observation was terminated and 3 min was taken as the result.

Action of reserpine on convulsions due to leptazol in mice. Chen, Ensor & Bohner (1954), using the method of Orloff, Williams & Pfeiffer (1949) for producing convulsions by intravenous infusion of leptazol, demonstrated that reserpine hastened the onset of persistent convulsions by leptazol. We have used the method of Orloff *et al.* (1949) with minor modifications. In these experiments the weight of the animals ranged between 18 and 20 g, leptazol (7.5 mg/ml.) was infused into the tail vein at 0.206 ml./min, and methyldopa was injected 6 hr and reserpine (5 mg./kg) 5 hr before the infusion. The times of onset of persistent convulsions, of the tonic extensor phase and of death were noted.

Emesis induced by reserpine in pigeons. The method of Earl, Winters & Schneider (1955) was used. Each bird, weighing between 300 and 400 g, was used for only one observation. The dose of reserpine (0.5 mg/kg) produced vomiting in all the pigeons (Gupta & Dhawan, 1960). Methyldopa (100 mg/kg), which caused vomiting in five of ten animals, was injected in one group 16 hr and in the other 4 hr before reserpine. A control group was injected with 0.9% saline. The injections were made into the pectoral muscle. The birds were observed for 4 hr after the reserpine injection and the time of onset of vomiting (regurgitation of food) was noted.

Drugs. Methyldopa (Aldomet; Merck, Sharpe & Dohme), reserpine (Serpasil; Ciba), chlorpromazine hydrochloride (Largactil; Poulenc), 5-hydroxytryptamine creatinine sulphate hydrate (Calbiochem), hexobarbitone sodium (Evipal Sodium; Winthrop) and leptazol (Metrazol; Bell-Craig) were used. The doses of chlorpromazine and 5-hydroxytryptamine are expressed as the salts.

RESULTS

Effects of methyldopa on sleeping time due to hexobarbitone. Methyldopa injected 4 hr earlier significantly prolonged the sleep due to hexobarbitone (Table 1).

TABLE 1

EFFECTS OF METHYLDOPA ON SLEEPING TIME DUE TO HEXOBARBITONE IN MICE

Values are means with standard errors. The numbers of experiments are in parentheses. * Differs significantly, $P<0.05$, and ** $P<0.01$, from the corresponding values in the control group (no methyldopa). Methyldopa was injected 4 hr, reserpine 1 hr, chlorpromazine 30 min, and 5-hydroxytryptamine 10 min, before hexobarbitone

	Sleeping time (min) due to hexobarbitone after				
Treatment	No other drug	Reserpine 5 mg/kg	Reserpine 10 mg/kg	5-Hydroxytryptamine, 20 mg/kg	Chlorpromazine, 2·5 mg/kg
Saline (control)	27±2·4 (36)	74±6·6 (20)	89±3·8 (20)	58±3·7 (16)	90±7·0 (15)
Methyldopa (100 mg/kg)	*37±3·1 (36)	*56±4·6 (26)	**54±4·3 (26)	**74±4·0 (16)	**141±9·2 (15)

Reserpine prolonged the sleep due to hexobarbitone more than did methyldopa, but its effect was significantly inhibited by methyldopa.

5-Hydroxytryptamine significantly prolonged sleep. When 5-hydroxytryptamine was administered to mice treated with methyldopa, there was a further significant

prolongation of sleep. Similarly, the prolongation of sleep due to hexobarbitone by chlorpromazine was significantly enhanced in the mice treated with methyldopa.

Thus methyldopa antagonized reserpine on sleeping time due to hexobarbitone and enhanced the effects of 5-hydroxytryptamine and chlorpromazine.

Effects of methyldopa on sedation and ptosis due to reserpine. Methyldopa did not cause sedation measurable by the method, but it reduced the sedation due to reserpine (Table 2).

The same mice were used to record the degree of ptosis; methyldopa did not inhibit the ptosis induced by reserpine.

TABLE 2

EFFECTS OF METHYLDOPA ON SEDATION INDUCED BY RESERPINE IN MICE

Sedation was assessed as the prolongation of the time required to walk a fixed distance. Values are means and standard errors. * Differs significantly, $P<0.05$, and ** at $P<0.01$, from the corresponding values in the control group (no methyldopa). Methyldopa (100 mg/kg) was injected 4 hr before reserpine

Treatment	No. of animals	Time (sec) required to walk a fixed distance		
		No reserpine	Time after reserpine	
			150 min	210 min
Reserpine (0·6 mg/kg)	24	1·2±0·4	38±13	57±16
Methyldopa and reserpine (0·6 mg/kg)	10	1·0±0·1	*7·8±6·6	**2·5±0·9
Reserpine (1·3 mg/kg)	22	2·2±0·7	106± 16	130± 16
Methyldopa and reserpine (1·3 mg/kg)	10	1·7±0·2	**14±4·3	**41± 28
Reserpine (2·5 mg/kg)	18	0·7±0·3	154± 13	167±7·6
Methyldopa and reserpine (2·5 mg/kg)	10	2·7±0·9	75±26	*108± 23

Effects of methyldopa on inhibition of convulsions due to leptazol by reserpine. Reserpine significantly reduced the times of onset of persistent convulsions, of the tonic extensor phase and of death during leptazol infusion. Methyldopa had little effect on the convulsions due to leptazol and did not antagonize reserpine (Table 3).

TABLE 3

EFFECTS OF METHYLDOPA AND RESERPINE ON CONVULSIONS DUE TO LEPTAZOL IN MICE

Values are means and standard errors. **Differs significantly, $P<0.01$, from the values of the control group. Methyldopa (100 mg/kg) was injected 6 hr and reserpine (5 mg/kg) 5 hr before leptazol

Treatment	No. of animals	Time (sec) after start of leptazol infusion of			Tonic extension (% of mice)
		Persistent convulsions	Tonic extension	Death	
Saline (control)	23	73±3·7	96±4·3	113±6·2	87
Methyldopa	22	72±5·2	122±18	139±12	50
Reserpine	11	**31±1·3	**34±1·3	**43±4·8	100
Methyldopa and reserpine	11	**37±2·4	**44±4·2	**53±3·7	82

Effects of methyldopa on emesis induced by reserpine in pigeons. Reserpine caused vomiting in each of the twenty-two control pigeons (Table 4). Methyldopa produced vomiting in five of ten animals. When these birds were injected with reserpine 4 hr later, none of them showed emesis. Of the twenty pigeons injected with methyldopa 16 hr earlier, only eleven vomited after reserpine. The onset of vomiting after reserpine was significantly delayed in these animals.

TABLE 4

EFFECT OF METHYLDOPA ON EMESIS INDUCED BY RESERPINE IN PIGEONS

Values are means and standard errors. **Differs significantly, $P<0{\cdot}01$, from the values of the control group. The dose of methyldopa was 100 mg/kg and that of reserpine 0·5 mg/kg

		Emesis in pigeons	
Treatment	No. of animals	Incidence (%)	Time of onset (min)
Saline and reserpine	22	100	54±2·8
Methyldopa	10	50	45±6·5
Methyldopa and, 4 hr later, reserpine	10	0	—
Methyldopa and, 16 hr later, reserpine	20	55	**94±8·3

DISCUSSION

Methyldopa antagonized some effects of reserpine (prolongation of sleep due to hexobarbitone in mice and sedation in mice and emesis in pigeons due to reserpine) but did not influence others (ptosis and potentiation of convulsions due to leptazol in mice). These results indicate that not all the studied effects of reserpine are produced by the same mechanism.

Brodie, Shore, Silver & Pulver (1955) and Shore, Silver & Brodie (1955) suggested that the potentiation of the hypnotic effect of hexobarbitone by reserpine, chlorpromazine and 5-hydroxytryptamine is due to an increase in the sensitivity of the central nervous system to the hypnotic agent and does not depend on a change of the rate of metabolic transformation of the barbiturate. The observation that methyldopa antagonized the prolongation by reserpine of sleep due to hexobarbitone, but not that produced by 5-hydroxytryptamine or chlorpromazine, suggests that reserpine acts differently from 5-hydroxytryptamine and chlorpromazine. Methyldopa itself prolonged the duration of hypnosis due to hexobarbitone and is reported to have a sedative effect (Smith, 1960). It is therefore unlikely that methyldopa antagonized the potentiation by reserpine of hypnosis due to hexobarbitone by virtue of a central stimulant effect. The observations that methyldopa antagonized the effect of reserpine on sleeping time and that the effects of methyldopa and 5-hydroxytryptamine were additive are not consistent with the concept of Brodie & Shore (1957) that sedation due to reserpine is mediated by 5-hydroxytryptamine.

Smith (1960) showed that the subcutaneous injection of 100 mg/kg of methyldopa into mice reduced the 5-hydroxytryptamine in the brain by about 30% and that this reduction was maximal 1 hr after treatment. Carlsson & Lindquist (1962) found that the intraperitoneal injection of 400 mg/kg of methyldopa leads to a

brief decrease in 5-hydroxytryptamine and dopamine levels in the mouse brain and a prolonged and more marked decrease in noradrenaline content. As methyldopa mimics reserpine in prolonging sleep due to hexobarbitone, one may think that the drug acts by depletion of brain amines. However, if this were so one would expect methyldopa to potentiate the effects of reserpine rather than to decrease them. Methyldopa behaves like reserpine also in producing emesis in pigeons, but it antagonizes the emetic response to reserpine.

We have previously found (Varma & Benfey, 1963) that, in cats treated with methyldopa, reserpine does not reduce the sensitivity of the cardiovascular actions of tyramine, although it reduces cardiac noradrenaline and abolishes the effects of sympathetic nervous stimulation on the heart. We interpreted this result to mean that there is a direct antagonism between methyldopa and reserpine which is unrelated to changes in tissue catechol amine levels. Our present results suggest that some of the central actions of reserpine (prolongation of sleep, sedation and emesis) are also direct and not related to changes in brain amine concentrations. It appears that methyldopa, with its weak reserpine-like actions, is a direct antagonist of reserpine.

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