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SOME EFFECTS OF RESERPINE ON BARBITONE ANAESTHESIA IN MICE

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Abstract—Barbitone induction time has been measured at various intervals after pretreating mice with reserpine: special attention was paid to the rate of penetration of the barbitone into the brain. The period of sedation resulting from intraperitoneal administration of reserpine coincides with a period in which barbitone induction time is greatly reduced. Two days after reserpine the mice are no longer sedated, and barbitone induction time is normal. From 3 to 4 days after reserpine the animals are hyperexcitable, and this phase coincides with one in which barbitone induction time is greatly increased. The mice become normal for the second time from 5 to 6 days after reserpine. It is shown that the increased induction time results from a slower rate of penetration of barbitone into the brain.

INTRODUCTION

BEILER, *et al.*[1] have reported that certain enzymes reduce the induction time of barbitone in mice, but do not influence the duration of hypnosis by hexobarbitone. They concluded that the enzymes increased the permeability of the blood–brain barrier to barbitone, thus hastening the onset of hypnosis. We have investigated the effects of other substances, especially those with pronounced central action, on the induction time of barbitone and its rate of penetration into the brain, and have shown that a reduction in this time does not necessarily imply an increase in rate of penetration of barbitone into the brain.[2] During these experiments it was observed that the initial potentiation (reduced induction time) produced by reserpine reverted to antagonism (increased induction time) a few days later. Previously we had noticed a similar reversal in the effect of reserpine on the duration of hexobarbitone-induced hypnosis.[3] It was observed also that mice were easily excited after recovering from the sedation induced by reserpine. The experiments described below were carried out in an attempt to correlate post-reserpine hyperexcitability with the reduction observed in barbitone activity.

METHODS

Male fawn mice (GFF strain, from 16 to 22 g) were used in all the experiments.

The induction time of barbitone hypnosis was measured by the method of Greig and Mayberry.[4] Groups of mice were injected intravenously with sodium barbitone (5·5 mg in 0·4 ml/20 g), each injection taking from 20 to 30 sec. The interval between the injection and the loss of righting reflex was recorded as the induction time. The experiments were conducted at room temperature (approximately 19 °C).

Groups of mice given barbitone only were included in every experiment involving measurements of induction time.

Barbitone concentrations in the brain were determined by Butler's method.[5] Groups of four mice were injected intravenously with sodium barbitone (5·5 mg in 0·4 ml/20 g) and then killed after 2, 5, 10, 15, 20, 25, 30 or 35 min. The brains from each group were weighed together and assayed for total barbitone.

TABLE 1. CHANGES IN BARBITONE INDUCTION TIME PRODUCED BY RESERPINE OR CHLORPROMAZINE

Time after injecting drug	Group* mean induction time (min ± s.e.)			
	After reserpine (2 mg/kg i.p.)		After chlorpromazine (2 mg/kg i.p.)	
	Test	Control	Test	Control
1 hr	8·0±0·4	19·5±1·0	11·3±0·6	20·0±0·9
2 hr	4·9±0·2	19·5±1·0	not tested	
3 hr	3·2±0·3	19·5±1·0	10·7±0·8	20·0±0·9
6 hr	3·8±0·4	20·0±1·0	15·9±0·3	20·0±0·9
1 day	12·9±1·3	19·2±0·6	18·9±0·7	21·1±0·6
2 days	20·3±0·7	18·3±0·7	19·4±0·8	18·6±0·5
3 days	34·5±1·2	17·7±0·5	21·2±0·7	19·0±0·6
4 days	31·3±2·8	18·1±1·3	20·3±0·9	20·4±0·8
5 days	22·6±0·9	18·3±0·9	20·8±0·5	20·5±0·6
6 days	20·2±0·8	19·0±0·4	21·0±0·7	20·1±0·7
7 days	19·0±1·1	18·0±1·3	21·0±0·8	21·0±0·7

* Ten animals per group.

TABLE 2. BRAIN BARBITONE CONCENTRATIONS AFTER PRETREATMENT WITH RESERPINE

Time after intraperitoneal reserpine (2 mg/kg)	Brain barbitone concentration (moles × 10^{-6}/g wet tissue)*								Brain barbitone concentration at induction (moles × 10^{-6}/g wet tissue)
	Time in minutes after intravenous injection of sodium barbital (275 mg/kg)								
	2	5	10	15	20	25	30	35	
1 hr	0·38	0·59	0·74	0·86	0·93	1·03	1·06	1·06	0·69
2 hr	0·35	0·57	0·82	0·84	0·93	1·02	1·00	1·07	0·58
1 day	0·37	0·54	0·86	1·03	1·06	1·12	1·07	1·12	1·00
2 days	0·38	0·61	0·79	0·95	1·04	1·14	1·00	1·07	1·08
3 days	0·55	0·64	0·82	0·97	1·02	1·04	1·07	1·12	1·08
4 days	0·48	0·65	1·00	1·03	1·08	1·15	1·12	1·18	1·16
5 days	0·49	0·64	0·91	1·00	1·08	1·14	1·12	1·20	1·13
6 days	0·51	0·71	0·93	1·09	1·17	1·14	1·24	1·26	1·18
7 days	0·49	0·68	0·97	1·04	1·10	1·07	1·12	1·12	1·09
Controls, untreated	0·52	0·71	0·89	1·05	1·17	1·17	1·24	1·18	1·12

* Each value is the mean of two results.

RESULTS

The changes in barbital induction time produced by pretreating the mice with reserpine (2 mg/kg intraperitoneally) are given in Table 1, and the changes in the rate of penetration of barbitone into the brain in Table 2.

Induction time was shortest from 3 to 6 hr after administering reserpine. It returned to normal after approximately 2 days, but during the third and fourth day the induction time was markedly increased. Finally, after about 6 days, the induction time returned to normal for the second time.

The rate of penetration of barbitone into the brain was reduced by pretreatment with reserpine, and during the first day the barbitone concentration in the brain at the onset of hypnosis was appreciably less than in control animals. The curves for barbitone concentration in the brain plotted against time for the control mice and for those receiving reserpine three days before the barbitone are shown in Fig. 1. The points

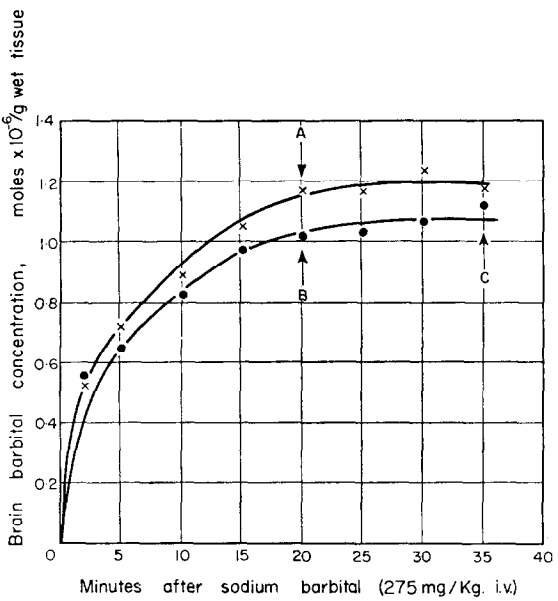

FIG. 1. The effect of reserpine on the rate of penetration of sodium barbitone into mouse brain: ×——× barbital alone; ●——● barbital 3 days after intraperitoneal reserpine 2 mg/kg.

marked *A*, *B* and *C* represent the barbitone concentrations in control mice at induction (*A*), in reserpinized mice at the induction time of normal mice (*B*) and in reserpinized mice at induction (*C*). We wished to know whether the concentrations at *A*, *B* and *C* were in fact significantly different from one another; hence a further eighty mice received an intraperitoneal injection of reserpine (2 mg/kg) and sodium barbitone intravenously 3 days later. Ten groups of four mice were killed 20 min after the barbitone (*B*) and the other ten groups 35 min after (*C*). Ten groups of four mice that had not received reserpine were killed 20 min after intravenous barbitone (*A*) and the concentrations of barbitone in their brains were measured. The results are given in Table 3, together with a Student *t* test for significance of difference between the groups. It will be seen that the brain barbitone concentration at *B* differs significantly from those at both *A* and *C* ($P < 0{\cdot}01$), whereas the latter two values do not differ from one another ($P < 0{\cdot}2$). Thus the increase in induction time 3 days after reserpine is simply a reflection of the reduced rate of entry of barbitone into the brain, the concentration at induction remaining unaltered.

The results of the above experiments prompted us to study the effects of chlorpromazine under similar conditions. Chlorpromazine (2 mg/kg intraperitoneally) reduced barbitone induction time, the reduction being greatest from 1 to 3 hr after dosing. The induction time returned to normal within 2 days and remained unchanged during the next 5 days (Table 1).

TABLE 3. BRAIN BARBITONE CONCENTRATIONS AT POINTS A, B AND C IN FIG. 1

Brain barbitone concentrations (moles $\times 10^{-6}$/g wet tissue)		
Controls, no reserpine	Tests, 3 days after reserpine (2 mg/kg i.p.)	
Point *A* 20 min after sodium barbitone i.v.	Point *B* 20 min after sodium barbitone i.v.	Point *C* 35 min after sodium barbitone i.v.
1·12	1·04	1·11
1·09	1·01	1·13
1·18	1·04	1·20
1·06	1·06	1·13
0·98	1·04	1·11
1·06	0·97	1·11
1·10	1·03	1·10
1·10	1·06	1·20
1·16	0·98	1·15
1·16	1·05	1·06
1·10±0·02*	1·03±0·01*	1·13±0·01*

* Standard error of mean.
Student '*t*' between *A* and *B* = 3·44 ($P < 0·01$).
Student '*t*' between *A* and *C* = 1·25 ($P > 0·2$).
Student '*t*' between *B* and *C* = 5·98 ($P < 0·001$).

DISCUSSION

The experiments with reserpine described above show that the period of sedation and barbitone potentiation (reduced induction time) gives place to one of excitation and barbitone antagonism (prolonged induction time). The finding of excitation agrees basically with that of Taeschler and Cerletti,[6] who showed that the stimulant effects of lysergic acid diethylamide in mice are potentiated by pretreatment with reserpine, and that of Frommel and Gold,[7] who reported that guinea-pigs are excitable and difficult to handle a few days after receiving the total alkaloids of Rauwolfia.

It is interesting to recall the suggestion of Lessin and Parkes,[8] that the sedative and pentobarbitone-potentiating actions of reserpine in mice kept at room temperature are related to the drug's hypothermic action. More recently, these workers[9] have shown that the duration of reserpine's sedative and hypothermic actions differs from that for its facilitation of leptazole convulsions, the time-course of the latter following more closely that of the reduction in the concentration of brain 5-hydroxytryptamine.

Our own experiments show that the action of reserpine on the rate of entry of barbitone into the brain is one of consistent reduction, the time course also following closely that of the reduction of mouse brain 5-HT.[10] It would have therefore been reasonable to expect that the increased induction time experienced 3 and 4 days after

reserpine should also occur during the first 2 days. Since this is not so, we believe that the action of barbitone is impeded by reserpine during the first 2 days, but this inhibition is masked by an overwhelming potentiation, possibly resulting from hypothermia.

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