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BARBITONE INDUCTION TIME, BODY TEMPERATURE AND THE ACTIONS OF RESERPINE ON MICE

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Abstract—Barbitone induction time in the mouse is linearly related to rectal temperature. Induction times, body weights and rectal temperatures have been measured at intervals during a subacute experiment in which mice received once daily intraperitoneal injections of 300 μg reserpine/kg on fourteen consecutive days. The reduced induction times occurring with cumulative doses smaller than 2·1 mg reserpine/kg may be attributed entirely to hypothermia. Cumulative doses larger than 2·1 mg/kg antagonized the barbiturate despite a persisting hypothermia. Body temperature and induction time are also reduced during the first 48 hr after a single intraperitoneal injection of 2 mg reserpine/kg, but only part of the potentiation is attributable to hypothermia.

INTRODUCTION

WE REPORTED earlier[1] that barbitone induction time in mice was reduced during the period of sedation after a single intraperitoneal injection of reserpine and that 2 days after this injection the animals' behaviour and barbitone induction times were normal. From 3 to 4 days after injection, however, the mice became hyper-excitable and their barbitone induction times were greater than normal. Because reserpine's effect on the rate of barbitone entry into the brain was one of consistent reduction, we suggested that the expected increase in barbitone induction time during the first 2 days was masked by an overwhelming reduction, possibly resulting from hypothermia.The experiments recorded here were intended to relate the effects of reserpine on body temperature with barbitone induction time in mice.

METHODS

Male fawn mice (GFF strain, from 18–24 g) were used in all the experiments. The induction time of barbitone hypnosis was measured by the method of Greig and Mayberry.[2] 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 in a room maintained at 24 °C, at which temperature the rectal temperatures of untreated mice remained constant. Groups of mice given barbitone only were included in each experiment involving measurements of induction time.

The body temperatures of mice were lowered by thoroughly wetting the fur with cold water and then drying it for 10 min in a stream of cool air. Slightly lower temperatures were obtained when cold 10% aqueous ethanol (1 ml per mouse) was used.

Temperatures were recorded by inserting a probe copper–constantin thermocouple into the rectum to a depth of from 2 to 2·5 cm. The potential difference across this thermocouple was balanced, through a variable resistance and a galvanometer, against part of that produced by two other thermocouples, one maintained at 26 °C and the other at 41 °C. With this apparatus stable readings were obtained within 15 sec of inserting the probe thermocouple into the rectum.

RESULTS

The rectal temperatures of chilled and normal mice were recorded immediately before the animals received single intravenous injections of barbitone and again at induction. All temperatures fell during the induction period, being 1·9 ± 0·23 °C (S.E.) lower at induction than before injection. This decrease was independent of both pre-injection temperature and duration of induction. The rectal temperatures of chilled mice not dosed with barbitone remained constant from 10 to 30 min after chilling. There was a highly significant correlation between barbitone induction time and pre-injection body temperature, the induction time decreasing linearly with decreasing body temperature between normal temperature (36–38 °C) and 27 °C. The equation relating induction time and body temperature (eighty-seven observations) was $Y = 1{\cdot}39X - 33{\cdot}35$, where Y = induction time (min) and X = body temperature (°C).

Although it was considered possible that some induction times might have been affected because of alcohol having been ingested by the mice licking their fur, no reduction in body temperature occurred in mice dosed orally with 10% aqueous ethanol (0·1 ml/20 g), and normal barbitone induction times were obtained in these mice 10 min after administering the alcohol.

One hour after a single intraperitoneal injection of reserpine (2 mg/kg), body temperature began to fall, and at 6 hr it was 3·9 °C less than normal (Table 1); within 24–48 hr of the injection it was again normal. No further changes were observed during the next 5 days.

TABLE 1. CHANGES IN BARBITONE INDUCTION TIME AND BODY TEMPERATURE PRODUCED BY A SINGLE INTRAPERITONEAL DOSE OF RESERPINE (2 mg/kg)

Hours after injecting reserpine	Group mean induction time* min ± S.E.		Group mean rectal temp.* °C ± S.E.	
	Control	Test	Control	Test
1	18·4 ± 0·32	9·3 ± 0·34	37·5 ± 0·20	37·0 ± 0·07
3	18·4 ± 0·32	6·7 ± 0·52	37·5 ± 0·20	35·4 ± 0·51
6	18·4 ± 0·32	4·7 ± 0·26	37·5 ± 0·20	33·6 ± 0·29
24	18·4 ± 0·22	12·4 ± 0·78	36·0 ± 0·21	34·7 ± 0·35
48	18·9 ± 0·38	17·4 ± 0·36	36·6 ± 0·15	35·8 ± 0·32

* Ten mice per group.

To minimize the fall in rectal temperature produced by a single large dose of reserpine, a suitable small intraperitoneal dose (300 μg/kg per day) was administered subacutely to a group of mice once daily on fourteen consecutive days. Body weights, rectal temperatures and barbitone induction times were measured on eleven occasions on different groups of twenty mice selected at random from the injected colony. The

times chosen were 3 hr after doses from 1 to 4 and from 7 to 11, and 3 hr and 3 days after the last dose. Similar measurements were made on groups of twenty control mice.

The results are given in Fig. 1. The rectal temperatures decreased only slightly during the first few days of the experiment, but after the seventh dose (cumulative

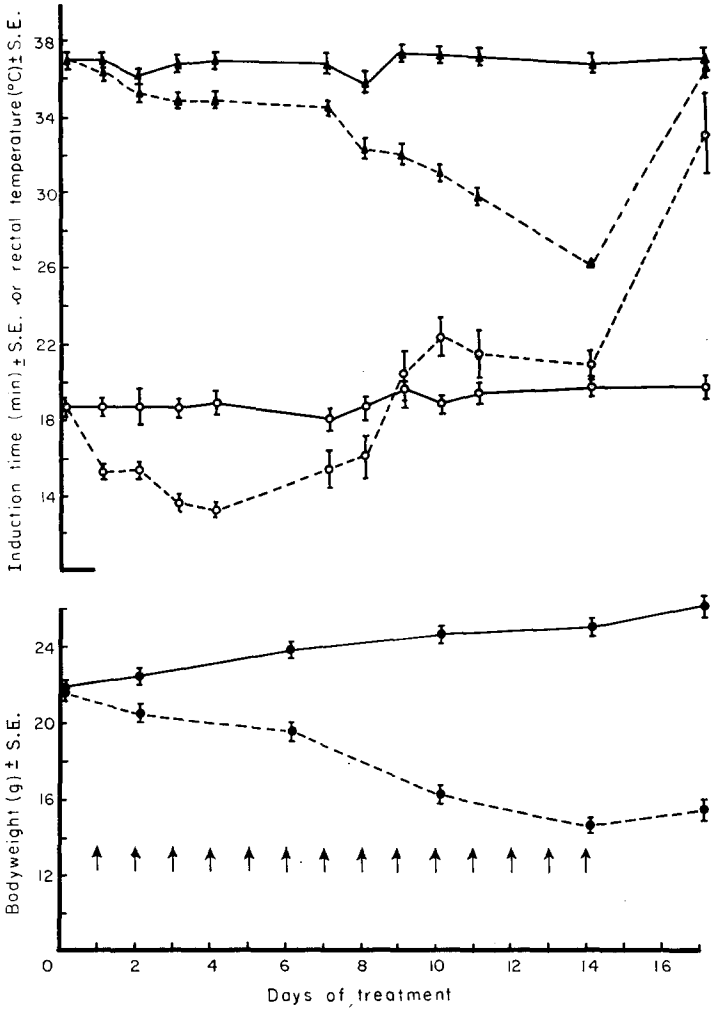

FIG. 1. Changes in barbitone induction time, bodyweight and rectal temperature produced by reserpine (300μ/kg.i.p.) Continuous line, control mice; broken line, reserpine-treated mice. ▲——▲ rectal temperature; ○——○ barbitone induction time; ●——● bodyweight.

dose 2·1 mg/kg) they fell steeply. However, 3 days after dosing ceased, when the mice had received a total dose of 4·2 mg/kg reserpine, the body temperatures had returned to normal. A similar effect on health was apparent. When the cumulative dose exceeded 1·8 mg/kg, the mice appeared to deteriorate rapidly, anorexia and diarrhoea appearing

with decreasing body weight and lowered rectal temperature. Towards the end of the dosing period all the mice were extremely weak and many died, but after dosing ceased the survivors improved rapidly in condition and slightly in body weight. Although body weight and rectal temperature continued to fall throughout the dosing period, barbitone induction time did not. Initially the induction time was reduced significantly ($P < 0{\cdot}001$), but on the ninth day no difference could be detected between the test and control groups. On the tenth day (cumulative dose = 3 mg/kg) the reserpine-treated animals had a significantly longer induction time than did the controls ($P < 0{\cdot}05$). Three days after dosing ceased, when the body temperatures were normal, the mice were highly excitable, and the barbitone induction time was nearly twice the control value.

A similar experiment, in which mice received once daily intraperitoneal injections of 100 μg reserpine/kg, was stopped on the fourteenth day, because body weight, body temperature and barbitone induction time were still completely unchanged.

Although in the subacute experiments we failed to reduce barbitone induction time without lowering body temperature, it was possible to deduce the extent to which falls in body temperature had affected the results from the relationship existing between body temperature and induction time. The induction times given in Fig. 1 are expressed in Fig. 2 as differences between the results obtained and those which

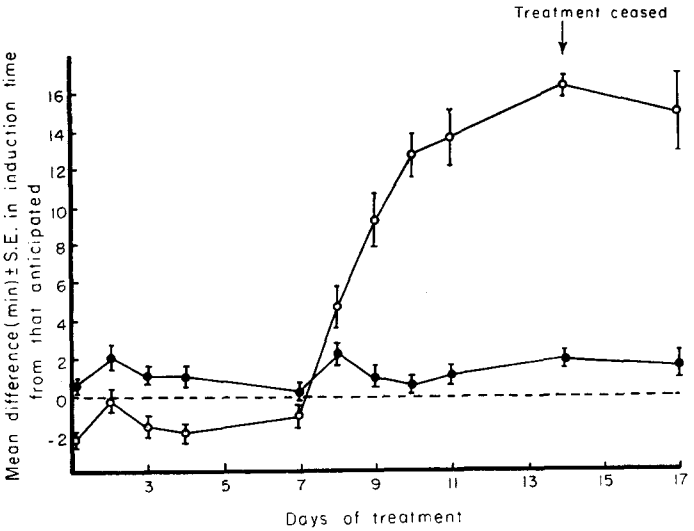

FIG. 2. Barbitone induction times given in Fig. 1 corrected for the effects of hypothermia, ●———● control mice; ⊙———⊙ reserpine-treated mice.

might have been expected from the body temperature. Throughout the experiment the induction times of the control groups were not very different from those calculated from their rectal temperatures. This finding applied also to the test animals during the first 7 days, but at cumulative doses greater than 2·1 mg/kg the differences between the recorded and the expected results became significant; at 4·2 mg/kg it was 16 min.

DISCUSSION

The experiments show that mouse body temperature falls after reserpine, whether the drug be administered as a single large dose or subacutely in much smaller doses. Whereas body temperature returned to normal within from 24 to 48 hr of the single dose, it continued to decline throughout the subacute experiment. In the latter body weight also was reduced, the mice becoming listless and suffering from diarrhoea and anorexia; in the later stages of the experiment many died. Despite their weakness, these mice were more resistant than normal mice to barbitone. Assessment of loss of righting reflex in these mice was not easy, and many mice were recorded as being anaesthetized when, in fact, they were probably too weak to turn over. Thus the induction times recorded in the later stages of the experiment were probably underestimated. Three days after dosing ceased the surviving mice were much healthier and became hyperexcitable. At this stage their rectal temperatures were within normal limits, but their induction times were approximately twice normal. In this experiment the increased resistance to barbitone occurred only after the cumulative dose of reserpine had reached 2·1 mg/kg. The reduced induction times occurring after cumulative doses smaller than 2·1 mg reserpine/kg may be attributed entirely to the falls in body temperature, since correcting for their effects increased the induction times to those of the controls (Fig. 2). Applying similar corrections to the induction times obtained during the first 2 days after a single intraperitoneal dose of 2 mg reserpine/kg did not increase them to the normal range. Thus the suggestion that "the expected increase in induction time during the first 2 days after a single intraperitoneal dose of 2 mg reserpine/kg has been masked by an overwhelming reduction possibly resulting from hypothermia" (Child, Sutherland and Tomich[1]) is not tenable. It appears that, on the contrary, the hypnotic action of barbitone during the first 2 days after a single dose of reserpine is potentiated by other mechanisms besides hypothermia, because the brain concentration of barbitone at induction is less than normal.[1] If small doses of reserpine are injected daily, no such potentiation occurs, the only effect being one of antagonism after a cumulative dose of 2·1 mg/kg.

It is not known to what extent dehydration[3] may have affected the results obtained in the subacute experiment or what changes occurred in brain amine or brain barbitone concentrations. These effects, and the mechanisms involved in the potentiation of barbitone by a single large dose of reserpine, are being investigated.

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