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Attenuation of Pentobarbital-Elicited Hypothermia in Rats with a History of Pentobarbital-LiCl Pairings[1]

HARALD K. TAUKULIS

Sir Wilfred Grenfell College, Memorial University of Newfoundland
Corner Brook, Newfoundland, Canada A2H 6P9

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TAUKULIS, H. K. *Attenuation of pentobarbital-elicited hypothermia in rats with a history of pentobarbital-LiCl pairings.* PHARMAC. BIOCHEM. BEHAV. 17(4) 695–697, 1982.—Rats were given five separate pairings (sequential IP injections) of pentobarbital and lithium chloride, both hypothermia-inducing agents. When the animals were subsequently tested with a single injection of pentobarbital alone, they exhibited an attenuated hypothermia relative to controls that had either (a) received pentobarbital-LiCl pairings spaced twenty-four hours apart, or (b) received only placebo injections of normal saline. This phenomenon provides further evidence that rats can learn an association between drug states and may help to explain why pentobarbital-LiCl pairings tend to eliminate pentobarbital's capacity to produce a conditioned flavor aversion.

Pentobarbital Lithium chloride Hypothermia Drug associations Conditioned drug effects

A SUB-ANESTHETIC dose of sodium pentobarbital will produce a mild flavor aversion in a rat if the animal has consumed a novel-tasting solution shortly before the drug injection. However, no aversion will result if the animal has had a history of repeated exposure to pentobarbital in which the drug has always been closely followed by a toxic injection of lithium chloride (LiCl) [4, 5, 6].

This "aversion failure" might be explained in the following way. In the course of repeated pentobarbital-LiCl pairings, the rat forms an association between the two drug states. Pentobarbital, because it signals that LiCl is imminent, gradually comes to elicit a conditioned compensatory response. That is, it triggers a set of physiological phenomena that serve to counteract the immediate effects of the LiCl. At the same time, these physiological phenomena also reduce the usual effects of pentobarbital, thereby negating this drug's capacity to produce a conditioned flavor aversion [3].

This hypothetical explanation for the aversion failure effect is made plausible by the numerous demonstrations of conditioned compensatory responses in other contexts [10]. It has often been found that, when environmental cues are repeatedly paired with a drug state, these cues may come to trigger physiological responses that are opposite in direction to the unconditioned effects of the drug [7, 8, 9, 10]. For example, environmental cues present prior to repeated morphine administration in rats will elicit a hypothermia that seems to compensate for the hyperthermia induced by the drug itself [1,9]. The inverse of this phenomenon has been demonstrated with ethanol: environmental cues can trigger a conditioned hyperthermia in anticipation of the hypothermic effect of the drug [2].

Before a "conditioned compensatory response" explanation for the aversion failure effect can be seriously considered, it is necessary to demonstrate that pentobarbital-LiCl pairings do, in fact, alter a rat's physiological response to pentobarbital. This was the purpose of the present experiment. Both pentobarbital and LiCl produce an unconditioned hypothermia as one of their effects, and hence it seemed reasonable to select core temperature as an index of modified reactivity to pentobarbital. Specifically, it was predicted that rats with a history of pentobarbital-LiCl pairings will exhibit an attenuated hypothermia in response to a subsequent test dose of pentobarbital administered alone.

METHOD

Subjects

Twenty-four male Long-Evans rats weighing 250–300 g at the start of the experiment were used as subjects. They were housed in individual, translucent, polypropylene cages (Hazleton HP 301) with wire tops. The cages were kept in a room maintained at 23–24°C with a photoperiodic cycle of 10 hours light to 14 hours darkness. Rat chow (Purina) was available at all times, but water intake was restricted. All animals were maintained on a series of 96-hr drinking cycles in which they were given free access to demineralized water during Hours 1–48 and were totally deprived of water during Hours 49–96.

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Apparatus

Rectal temperatures were measured with a Digi-Sense Thermistor Thermometer (Cole-Parmer No. C-8522-10) and a YSI temperature probe (Yellow Springs Instruments Model No. 423).

Drugs

Two drugs were employed: sodium pentobarbital (Somnotol, MTC Pharmaceuticals) and lithium chloride (Fisher Scientific Company). The pentobarbital was diluted with normal saline to a concentration of 10 mg/ml. The lithium chloride was dissolved in distilled water to create a 0.4 Molar concentration.

Procedure

During the first of the 96-hr drinking cycles (Cycle 1), the rats were familiarized with the experimental procedure. During Hours 66 and 90 of the cycle, they were injected (IP) with 2.0 ml of a normal saline solution. Rectal temperatures were taken immediately prior to and at 60 and 120 min after each of these injections. For each temperature reading throughout the experiment, the thermistor probe was inserted into the rectum to a depth of 6 cm while the animal was loosely held by the experimenter. At 30 sec after insertion, the temperature was recorded.

During each of the subsequent five 96-hr cycles (Cycles 2–6), rats in Group PB-LiCl (n=8) were injected (IP) with 20 mg/kg of pentobarbital during Hour 91. Thirty minutes later, they received an IP injection of lithium chloride (10 ml/kg of the 0.4 Molar solution). Rectal temperature readings were taken at 30 min prior to the pentobarbital injection and at 60, 120, 180, and 240 min after the lithium chloride injection.

Two control groups were included in the experiment. Group PB-24-LiCl (n=8) received the same doses of pentobarbital and lithium chloride as those administered to Group PB-LiCl except that a 24-hr interval separated the two injections. That is, pentobarbital was administered during Hour 67 of each cycle and LiCl during Hour 91. Rectal temperatures were taken 30 min prior to and at 120 and 180 min after the pentobarbital injection, as well as 60 min prior to and at 60, 120, 180, and 240 min after the lithium chloride injection. (For the sake of symmetry, rectal temperatures were also taken from rats in the other two groups at times coinciding with the pre- and post-pentobarbital readings of Group PB-24-LiCl.) Group SAL (n=8) was treated exactly like Group PB-LiCl except that equivalent-by-volume injections of normal saline were substituted for both pentobarbital and LiCl. Thus, these animals received no drugs during this portion of the experiment.

The test of the rats' thermic responses to pentobarbital alone was performed during a final 96-hr cycle (Cycle 7). During Hour 91, animals in all three groups were given a single IP pentobarbital injection (20 mg/kg). Rectal temperatures were taken 30 min prior to this injection and at 30-min intervals following the injection to a termination at 240 min.

RESULTS

Figure 1 illustrates the results of the pentobarbital-alone test performed during Cycle 7. All three groups exhibited equivalent temperature losses in the first 30 min after pentobarbital administration. Thereafter, the groups diverged. While the two control groups continued to show decreases for another hour, rectal temperatures in Group PB-LiCl stabilized and then began to increase. An overall 3×8 (Groups × Time) ANOVA yielded significance for both the Groups factor, $F(2,21)=3.64$, $p<0.05$, and the Time factor, $F(7,147)=32.23$, $p<0.001$. Of greatest interest, however, was the Groups × Time interaction, $F(14,147)=3.89$, $p<0.001$, which indicated that the differences among groups varied as a function of the post-pentobarbital interval. Since a separate ANOVA indicated that Groups PB-24-LiCl and SAL did not differ (F's<1 for both the Groups factor and the Groups × Time interaction), these groups were combined for statistical purposes. A comparison of these groups with Group PB-LiCl at each post-pentobarbital interval yielded p's<0.01 at 60, 90, 120, and 150 min and p's>0.05 at 30, 180, 210, and 240 min. This analysis indicated that, as predicted, Group PB-LiCl had exhibited an attenuated hypothermia relative to the control groups.

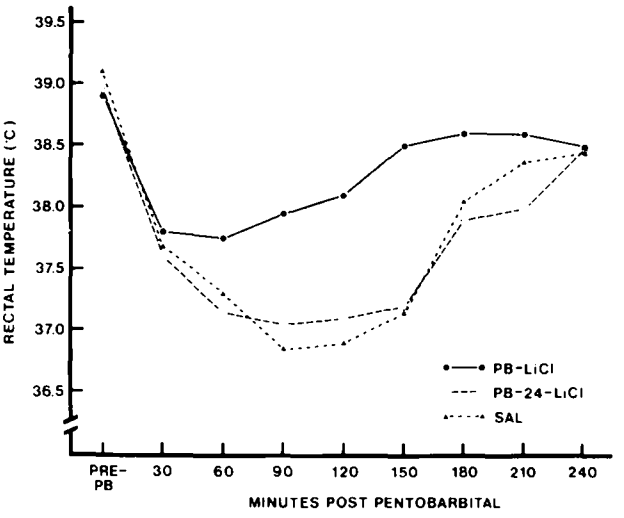

FIG. 1. Mean rectal temperatures obtained 30 min prior to (PRE-PB) and at 30-min intervals after an IP injection of pentobarbital administered during Cycle 7 of the experiment.

It was originally anticipated that some attenuation of hypothermia would be detected prior to the Cycle 7 pentobarbital test, during the pentobarbital-LiCl pairing phase of the experiment. That is, as the animals in Group PB-LiCl learned the drug association during Cycles 2–6, it was expected that the pentobarbital might come to elicit a compensatory response which would reduce the degree of unconditioned hypothermia produced by the two drugs combined. Such an effect was not detected, however, as can be seen in Table 1. The numbers in this table represent temperature changes (relative to a pre-drug baseline) in response to either pentobarbital plus LiCl (Group PB-LiCl), LiCl alone (Group PB-24-LiCl), or normal saline (Group SAL). These readings were taken during hours 92–95 of Cycles 2–6. It is apparent that the pentobarbital-LiCl combination produced a relatively greater hypothermia than did LiCl alone, and that the temperature loss was always most pronounced at 60 min after LiCl administration, lessening gradually thereafter. This overall pattern did not change significantly across cycles. When Cycles 2 and 6 were compared in a 2×2×4 (Groups × Cycles × Time) ANOVA, it was found that the difference in temperature loss between Groups PB-LiCl and PB-24-LiCl did not diminish: both the Groups × Cycles and

TABLE 1

MEAN CHANGES IN RECTAL TEMPERATURE PRODUCED BY PENTOBARBITAL PLUS LICL (GROUP PB-LICL), BY LICL ALONE (GROUP PB-24-LICL), OR BY NORMAL SALINE (GROUP SAL)

		Minutes Post LiCl (or Saline)			
Group	Cycle	60	120	180	240
	2	−3.45	−2.82	−2.07	−1.16
	3	−3.08	−3.02	−1.97	−0.99
PB-LiCl	4	−3.24	2.72	−2.02	−1.13
	5	−2.83	−2.27	1.28	−0.78
	6	3.27	−2.61	1.42	−0.82
	2	−2.67	1.97	−1.72	−1.04
	3	−2.49	2.15	−1.39	0.75
PB-24-LiCl	4	−2.28	2.20	−1.57	0.67
	5	−2.04	1.95	−1.04	0.77
	6	2.28	−2.10	1.36	−0.71
	2	−0.56	−0.31	−0.62	−0.28
	3	−0.15	−0.27	0.24	−0.06
SAL	4	−0.07	−0.27	0.33	−0.16
	5	+0.48	+0.27	+0.60	+0.25
	6	+0.03	+0.18	+0.38	+0.39

Each value in the table represents a mean temperature change, in degrees Celsius, from a pre-injection baseline.

the Groups × Cycles × Time interactions had p's>0.05. Thus, any conditioned response that pentobarbital may have come to elicit by Cycle 6 was not sufficiently potent to significantly alter the hypothermia produced by pentobarbital and LiCl in combination.

DISCUSSION

The attenuated hypothermia observed in Group PB-LiCl during Cycle 7 can be ascribed to an association between pentobarbital and lithium chloride learned by the rats during the drug-pairing phase of the experiment. It did not merely reflect the development of an unconditioned tolerance to pentobarbital's hypothermia-inducing property. Group PB-24-LiCl received exactly the same number of pentobarbital injections as did Group PB-LiCl, and yet these animals exhibited as pronounced a hypothermia as that shown by the drug-naive group, Group SAL.

It is clear from this experiment that a history of pentobarbital-LiCl pairings can bring about a change in at least one of the physiological effects induced by pentobarbital. However, it is not yet certain that this phenomenon can be explained in terms of a "conditioned compensatory response." The experiment failed to detect a gradually diminishing hypothermia in response to the pentobarbital-LiCl combination experienced by Group PB-LiCl in Cycles 2–6—an effect that should have been observed if pentobarbital, acting as a conditioned stimulus, had indeed come to trigger a set of physiological phenomena that would compete with lithium's unconditioned effects. Perhaps no attenuated hypothermia was noted here because only five drug pairings were administered. This number may have been insufficient to ensure the conditioning of an anticipatory response powerful enough to affect the substantial temperature loss elicited by pentobarbital and lithium together, even though it was sufficiently strong to counter the effect of pentobarbital alone. This explanation can be tested by simply increasing the number of pairings. It should be mentioned that only five pentobarbital-LiCl pairings were administered in the present experiment because it is known that this number is adequate to produce the "aversion failure" phenomenon reported recently [4, 5, 6].

Despite this drawback, the outcome of the test phase (Cycle 7) of the experiment nonetheless lends some credence to the suggestion that the aversion failure effect may be attributable to a conditioned alteration of the rats' physiological response to pentobarbital which, in turn, reduces that drug's capacity to produce a flavor aversion. This explanation is, of course, speculative since it remains to be shown that temperature changes are indices of physiological events that are in some way linked to the flavor aversion process.

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