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Conditional Shifts in Thermic Responses to Sequentially Paired Drugs and the "Conditional Hyperactivity" Hypothesis

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TAUKULIS, H. K. *Conditional shifts in thermic responses to sequentially paired drugs and the "conditional hyperactivity" hypothesis.* PHARMACOL BIOCHEM BEHAV **25**(1) 83–87, 1986.—Previous experiments have demonstrated that upward shifts in a rat's thermic response to certain drugs may be observed when these drugs have been paired on several occasions with agents that induce hypothermia. A "conditional hyperactivity" hypothesis suggests that these upward shifts may simply reflect drug elicited increases in body movements which translate into higher temperatures. The present experiment explored this hypothesis. Atropine sulfate (10 mg/kg) was paired with sodium pentobarbital (40 mg/kg) on multiple occasions and several tests were conducted with both drugs. This treatment yielded a conditional hyperthermic response to atropine, but the drug was not found to elicit an increase in gross motor movements. Of greatest interest was the finding that the atropine, when injected 30 min prior to a hypnotic dose of pentobarbital (80 mg/kg), attenuated the hypothermia normally induced by this barbiturate while leaving the duration of hypnosis unaffected. This upward thermic shift cannot be accounted for by the "conditional hyperactivity" hypothesis because the animals were immobile while under pentobarbital's influence. These findings suggest that autonomic events, as yet unspecified, may underlie certain conditional temperature increases.

Pentobarbital Atropine sulfate Drug-drug associations Conditional thermic effects Hyperthermia Conditional hyperactivity

THE sequential pairing of two drugs, when repeated on several occasions, will yield conditional changes in a laboratory rat's response to one or both of the drugs (see [6,10] for reviews). Upward shifts in rectal temperature, for example, have been observed with various drug combinations. When pentobarbital (PB) has been paired with lithium chloride (LiCl), the rat's normal hypothermic response to PB is attenuated [9]; and atropine sulfate (AS) will elicit a conditional hyperthermia subsequent to multiple pairings of this antimuscarinic agent with chlorpromazine hydrochloride (CPZ) or ethanol (ETH) [11].

A possible explanation for these thermic shifts is that the cue drug (the first drug in the drug-drug sequence) elicits a conditional increase in motor activity. That is, the anticipation of LiCl, CPZ, or ETH generated by the cue drug may elicit a state of hyperarousal and hyperactivity which translates into higher body temperature. It is also possible that such increased locomotion is goal-directed behavior reflecting the organism's attempt to compensate for the unconditional hypothermia which LiCl, CPZ, and ETH all induce.

The present experiment was primarily designed to test this "conditional hyperactivity" hypothesis. Rats were given repeated pairings of atropine sulfate (the cue drug) with a dose of pentobarbital (40 mg/kg) that would produce pronounced sedation and hypothermia. At varying intervals, tests involving one or both of the drugs were performed. In Phase 1, the effect of AS alone on rectal temperature was examined. In Phase 2, the effect of AS on pentobarbital-induced hypothermia and sleep time was tested. Here the rats' responses to hypnotic doses of pentobarbital were compared under two conditions: with and without an AS injection administered 30 min prior to the barbiturate. In Phase 3, the animals were injected with AS alone and placed into a chamber designed to monitor their activity levels. The conditional hyperactivity hypothesis would predict AS-elicited increases in temperature and activity level in Phases 1 and 3, but no conditional thermic shifts in Phase 2 due to the immobilization of the animals by the pentobarbital.

METHOD

Subjects

Thirty-six male Long-Evans rats were used as subjects. They were obtained from Charles River Canada at a weight range of 75–100 g and raised in the laboratory until they attained a weight range of 270–320 g. Each animal was housed in a stainless steel cage on a colony rack in a room maintained at 23–24°C with a photoperiodic cycle of 10 hr light to 14 hr darkness. Rat chow (Purina) was available at all times, but water bottles were removed for the duration of the light portion of each cycle as a matter of routine. Removal of

the bottles served a practical purpose: it facilitated retrieval of the animals from their cages and reduced water spillage onto the bedding material on days when the animals were handled repeatedly.

Apparatus

Rectal temperatures were measured with a Cole-Parmer thermistor thermometer (Model No. 8522-10 or 8110-20) and a YSI temperature probe (Yellow Springs Instruments Model No. 423). For measurements of sleep duration, the animals were placed into a plastic box (30×20×13 cm, l×w×h) fitted with a cardboard "V" trough. For activity monitoring, a disk of flexible polypropylene (1.5 mm thickness) was affixed to a speaker (20.0 cm in diameter), and a polypropylene cylinder (21.5 cm in diameter, 23.7 cm high) was mounted atop the plastic disk. Deflections of the disk, and hence the speaker membrane, produced voltage shifts that were monitored via a strip-chart recorder (Houston Instruments, Model No. B5117-5I).

Drugs

Two drugs were employed: sodium pentobarbital (*Somnotol*, MTC Pharmaceuticals) and atropine sulfate (Sigma Chemical Company). The pentobarbital was diluted with normal saline to a concentration of 20 mg/ml. Atropine sulfate was dissolved in normal saline to a concentration of 5 mg/ml. It was always injected at a dose of 10 mg/kg. All injections were IP.

Procedure

All animals were weighed on each day of the experiment. This was done at least 60 min prior to any scheduled temperature reading.

Because handling of rats will cause unconditional shifts in body temperature, care was taken to give the animals as much experience with the injection and temperature-taking procedures as possible. On each of five days before drug treatment sessions were begun, every rat was removed from its home cage and weighed. One temperature reading was then taken. The experimenter (wearing a leather glove) placed the subject on a table and gently restrained it by holding it at the base of the tail. The thermistor probe was inserted into the rectum to a depth of 6 cm and held there until the digital display stabilized (defined as a 10-sec period of no change). Following this procedure, the probe was removed, the animal was injected with physiological saline (2 ml/kg), and it was then returned to its home cage. Approximately 60 min later, the thermometric reading (but not the saline injection) was repeated. A similar procedure was carried out on several occasions during the entire course of the experiment at a frequency of once every six days as indicated below.

Phase 1. The thirty-six rats were assigned to three groups (n=12 per group) on the basis of body weights so that the mean weights and standard deviations were similar. During a thirty-day treatment period, Group AS-PB received 10 pairings of atropine sulfate with sodium pentobarbital (40 mg/kg). The two injections were spaced 30 min apart on each occasion. Group PB-AS received the identical exposure to the two drugs but in reverse order: pentobarbital preceded atropine sulfate by 30 min in each case. Group SAL-SAL received two equivalent-by-volume (2 ml/kg) saline injections; these animals experienced neither of the two drugs except during test sessions as described below. During each 72-hr interval between these treatment sessions, all animals were given a pair of saline injections (2 ml/kg). On every second occasion, these saline injections were preceded and followed by an insertion of the thermistor probe; a temperature reading was taken using the procedure described above. These saline-saline treatments were deemed necessary in order to reduce the predictive validity of the injection cues so that the atropine would be the only perfectly reliable predictor of the pentobarbital state in Group AS-PB. They also served to increase the animals' familiarity with the temperature-reading procedure, thereby reducing the probability of handling-induced hyperthermia.

In the three-day period following the tenth drug pairing, the thermic response of each rat to (a) a placebo injection of physiological saline and (b) an injection of atropine sulfate was assessed. For the first two days rectal temperature readings were obtained immediately prior to an injection of saline and at the following intervals thereafter: 30, 60, 120, and 180 min. On the third day, this procedure was repeated, except that atropine was substituted for saline.

Phase 2. The second phase of this experiment began with a continuation of the drug-pairing sessions carried out in the initial portion of Phase 1. Five more AS-PB, PB-AS, or SAL-SAL pairings were administered to the appropriate groups every third day over a fifteen-day period, and one saline-saline pairing was administered between each pair of drug-treatment sessions.

In a five-day period following the last drug pairing, the response of each rat to a hypnotic dose of pentobarbital was determined on two occasions: once following an injection of atropine, and once following an injection of saline. Half the animals of each group received the AS-PB test first followed several days later by the SAL-PB test, while the other half of each group experienced the tests in reverse order. On its test day, an animal was removed from its home cage and its temperature was taken. It was then injected with atropine (or saline) and returned to its cage. Thirty minutes later, a hypnotic dose of pentobarbital (80 mg/kg) was administered. When the animal was fully immobile, it was removed from its home cage and placed on its back in a V-shaped trough. A thermistor probe was inserted and the probe's lead was taped to the base of the animal's tail. Temperature readings were obtained at 60 min following the pentobarbital injection and also at the point at which the animal regained its righting reflex. Sleep time was recorded, as defined by the interval between the loss and return of the righting reflex.

Phase 3. For this phase of the experiment, Group SAL-SAL was discarded. Groups AS-PB and PB-AS were given six more drug-pairing sessions according to the schedule and procedure described in Phase 1. Within four days after the last pairing, each animal was injected with physiological saline and, 60 min later, was placed into an activity chamber for 10 min. Four days later this procedure was repeated, except that the animals were injected with atropine 60 min prior to the activity measure.

RESULTS

Phase 1

The mean temperature shifts across time, relative to a pre-injection baseline, obtained for each of the three groups for both the atropine test day and the immediately preceding saline test day are displayed in Table 1. While the saline injection appeared to have no differential effects on the groups, the atropine injection elicited a mild hyperthermia in Group AS-PB. Two-way (Groups × Time) analyses of

TABLE 1

MEAN SHIFTS IN RECTAL TEMPERATURE IN RESPONSE TO SALINE OR ATROPINE SULFATE

Group	Minutes 30	60	120	180
	After Saline Injection			
AS-PB	0.22(± 0.09)	0.08(± 0.12)	−0.56(± 0.16)	−0.88(± 0.14)
PB-AS	0.17(± 0.15)	0.09(± 0.19)	−0.76(± 0.19)	−0.98(± 0.18)
SAL-SAL	0.14(± 0.06)	0.03(± 0.07)	−0.62(± 0.14)	−0.97(± 0.16)
	After Atropine Injection			
AS-PB	0.39(± 0.14)	0.58(± 0.20)	0.46(± 0.23)	0.11(± 0.25)
PB-AS	−0.23(± 0.12)	0.00(± 0.12)	−0.16(± 0.21)	−0.60(± 0.22)
SAL-SAL	−0.12(± 0.11)	−0.16(± 0.14)	−0.41(± 0.14)	−0.72(± 0.13)

Each value in the table represents a mean temperature change (± S.E.M.), in degrees Celsius, from a pre-injection baseline. No statistically significant differences among groups were obtained in response to the saline injection. Following the atropine injection, Group AS-PB exhibited a significant hyperthermia relative to both Groups PB-AS and SAL-SAL; the latter two groups did not differ from one another.

TABLE 2

MEAN CHANGES IN RECTAL TEMPERATURE PRODUCED BY A HYPNOTIC DOSE OF PENTOBARBITAL (PB) FOLLOWING A SALINE (SAL) OR ATROPINE SULFATE (AS) INJECTION

Group	Test with SAL plus PB	Test with AS plus PB
	Temperature Shift at 60 Min after Pentobarbital	
AS-PB	−2.48(± 0.15)	−1.63(± 0.23)*
PB-AS	−2.53(± 0.16)	−2.52(± 0.25)
SAL-SAL	−2.58(± 0.21)	−2.38(± 0.24)
	Temperature Shift at Return of Righting Reflex	
AS-PB	−2.33(± 0.17)	−1.48(± 0.26)*
PB-AS	−2.31(± 0.14)	−2.49(± 0.25)
SAL-SAL	−2.42(± 0.18)	−2.42(± 0.23)

Each value in the table represents a mean temperature change (± S.E.M.), in degrees Celsius, from a pre-injection baseline. The pentobarbital dose employed here was 80 mg/kg. *Group AS-PB differed significantly from each of the other two groups under these conditions.

TABLE 3

MEAN DURATION OF PENTOBARBITAL-INDUCED HYPNOSIS FOLLOWING A SALINE (SAL) OR AN ATROPINE SULFATE (AS) INJECTION

Group	After SAL plus PB	After AS plus PB
	Minutes to Return of Righting Reflex (± S.E.M.)	
AS-PB	171.3(± 8.8)	167.5(± 10.4)
PB-AS	199.6(± 18.2)	194.7(± 16.6)
SAL-SAL	181.5(± 9.2)	177.5(± 11.4)

Pentobarbital dose: 80 mg/kg. No significant differences between or within groups were obtained.

covariance with baseline temperature as the covariate revealed no significant temperature differences on the saline day (all p's>0.05), but significant effects on the atropine day. Both the Groups factor, $F(2,32)=7.12$, $p<0.01$, and the Time factor, $F(3,99)=20.20$, $p<0.001$, reached significance, with no Groups × Time interaction, $F(6,99)=1.07$. Pairwise comparisons yielded significant differences between Groups AS-PB and PB-AS, $F(1,21)=6.27$, $p<0.02$, and between Groups AS-PB and SAL-SAL, $F(1,21)=11.55$, $p<0.01$, but not between Groups PB-AS and SAL-SAL, $F<1$.

The mean pre-injection (baseline) temperature for each of Groups AS-PB, PB-AS and SAL-SAL was 37.9°C on the saline test day; and on the atropine test day, the pre-injection means were 37.6, 37.7, and 37.7°C, respectively.

Phase 2

The rectal temperatures obtained during the pentobarbital hypnosis induced during this phase are displayed in Table 2. Mean shifts in rectal temperature from a pre-drug baseline are presented for both the saline-plus-pentobarbital and atropine-plus-pentobarbital conditions. Separate analyses of covariance were performed on the temperatures obtained at 60 min post-pentobarbital and at the return of the righting reflex. In each case, the pre-drug baseline served as the covariate. In the SAL-plus-PB condition, the small differences between groups were not statistically significant, with $F(2,32)<1$ in each case. In the AS-plus-PB test, however, similar analyses indicated significant differences at both 60 min, $F(2,32)=3.83$, $p<0.05$, and at the return of the righting reflex, $F(2,32)=5.21$, $p<0.02$. Pairwise comparisons indicated that Group AS-PB differed from each of the other two groups ($p<0.05$), but Groups PB-AS and SAL-SAL did not differ from one another.

Groups AS-PB, PB-AS, and SAL-SAL exhibited mean baseline temperatures of 38.4, 38.4, and 38.3°C, respectively, prior to the saline injection on the saline-plus-

pentobarbital day and mean baseline temperatures of 38.1, 38.2, and 38.2°C, respectively, prior to the atropine injection on the atropine-plus-pentobarbital day.

The mean duration of pentobarbital-induced hypnosis for each of the three groups is shown in Table 3. These were compared using a 3×2 (Groups × Test Condition) analysis of variance with the Test Condition treated as a repeated measure. No significant differences emerged: Groups Factor, $F(2,33)=1.31$, $p>0.05$, and Test Condition Factor, $F<1$.

Phase 3

One subject from Group AS-PB died before this test could be performed, reducing the size of this group to 11. As a measure of activity, gross body movements were counted. These were defined as movements that caused recorder-pen deflections of 2.5 cm or more. This criterion was chosen as a result of pilot studies with the activity monitoring apparatus. Pen deflections smaller than 2.5 cm reflected head movements, minor paw displacements, and rhythmic trunk movements related to breathing. Larger pen deflections indicated major body shifts, both horizontal and vertical. The counts obtained in this way were compared both between and within groups (atropine vs. saline conditions). The following means (±S.E.M.) were obtained: Group AS-PB, 136±12.6 (saline condition) and 119±10.8 (atropine condition); Group PB-AS, 107±15.2 (saline condition) and 110±15.6 (atropine condition). A 2×2 (Groups × Test Condition) analysis of variance with the Test Condition treated as a repeated measure yielded no significant differences, $F<1$ in all cases.

DISCUSSION

The conditional hyperthermia in response to atropine observed in Phase 1 of this experiment parallels similar reports of upward thermic shifts elicited by cue drugs that have repeatedly preceded hypothermic agents [9, 10, 11]. Here, too, the conditional hyperactivity explanation might have been applicable; but the findings of Phases 2 and 3 militated against it. In Phase 2, all rats were administered a hypnotic dose of pentobarbital, preceded either by a saline injection or an injection of atropine sulfate. Although the animals were immobile in both instances, the atropine had the effect of attenuating the pentobarbital-induced hypothermia in those rats for which the drug had previously signalled 40 mg/kg of the barbiturate (Group AS-PB). The conditional hyperactivity hypothesis cannot account for this phenomenon; and it is further called into question by the failure to detect an increase in gross body movements in response to atropine in Phase 3.

The Phase 2 results are interesting precisely because the attenuation of hypothermia occurred while the animals were anesthetized. This suggests that the atropine-pentobarbital pairings administered to Group AS-PB resulted in a conditional autonomic response of some kind. One possibility to be considered is that the atropine cue may have triggered the release of those endogenous peptides that are known to bring about hyperthermia when injected into the preoptic-anterior hypothalamic region of the brain, an area known to function as a "central thermostat" [2]. A number of studies have shown that β-endorphin plays a role in stress-induced increases in rectal temperature through its activity in the hypothalamus and that its effect can be blocked by administration of the narcotic antagonists naloxone and naltrexone [1, 2, 4, 5, 8]. If the combined effects of atropine and pentobarbital are sufficiently stressful to induce such endorphin activity, then atropine alone may, with repeated atropine-pentobarbital pairings, come to elicit the release of this peptide. But one would expect to see this "conditional stress response" to atropine in Group AS-PB only. Although both Groups AS-PB and PB-AS presumably experienced equivalent amounts of drug-induced stress during the treatment sessions, only in Group AS-PB did the atropine acquire a cue function, the consequences of which became apparent during testing in Phases 1 and 2.

It is also possible that this postulated endorphin release may not be stress-related, but instead may serve a thermoregulatory function. That is, the animal is cued by the atropine to anticipate pentobarbital hypothermia and its body activates mechanisms to counteract it. Yet a third possibility is that the rise in rectal temperature may be a by-product of some other process that does not directly involve endorphins, perhaps one that elicits a general increase in metabolic activity and its concomitant heat production.

The fact that the atropine cue altered the thermic effect but not the hypnotic effect of pentobarbital is a point worth noting because it suggests that the two effects are not directly associated. That is, it is likely that the physiological events underlying these phenomena are quite distinct. The possibility has been considered that, had the atropine been paired with 80 mg/kg (the hypnotic dose) rather than 40 mg/kg during the first portion of Phase 1, then a reduction of sleep time might have been detected during the atropine-plus-pentobarbital test session. However, the 80 mg/kg dose was not used throughout because it was feared that a pentobarbital-induced anesthesia might interfere with the learning or memory of an atropine-pentobarbital association [3]. This might have precluded the development of a conditional thermic effect, the phenomenon of primary interest in this experiment.

Although atropine sulfate appeared to have no effect upon gross motor movements in Phase 3, the results of this portion of the experiment should be interpreted cautiously. The negative outcome encourages the rejection of the conditional hyperactivity hypothesis, of course; but it is possible that a different activity-monitoring apparatus might have detected subtle behavioral changes that were missed in the present study. It is known that the type of apparatus employed in studies of spontaneous motor activity can dramatically influence the nature of the response obtained [7].

While conditional hyperactivity was not implicated as the mediator of atropine-induced hyperthermia in this instance, it is unwarranted to conclude that it does not play a role in all instances of conditional hyperthermia to a cue drug. In earlier studies [11] with atropine and chlorpromazine, for example, the thermic shift induced by atropine may have been behaviorally mediated. A comparison of various drug combinations in experiments in which animals are tested in sensitive activity-monitoring devices could prove instructive.

The study of learned associations between drug states and the consequences of these associations is in its infancy. As Taukulis [10] and Revusky [6] have pointed out, the phenomenon seems to extend to a broad variety of psychoactive drug combinations, and the potential implications and applications of the phenomenon are gradually becoming apparent. The present experiment adds yet another drug combination to the list of associable agents, but this point by itself is relatively trivial. Drug combinations must be selected with a specific goal in mind. Atropine was chosen here because of

its proven value as a cue drug [10,11], and pentobarbital was selected because the problem to be addressed required that the cued drug have hypnotic properties. The broad significance of this experiment, beyond the fact that it refutes the "conditional hyperactivity" hypothesis of hyperthermia, is not that it provides yet another example of a drug-drug association, but that such an association can have consequences even in an unconscious organism.

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