Pharmacology Biochemistry & Behavior, Vol. 36, pp. 933–936. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Dose Effects on Heart Rate Conditioning When Pentobarbital Is the CS and Amphetamine Is the US

SAM REVUSKY AND STEVE REILLY

Department of Psychology, Memorial University, St. John's, Newfoundland, Canada A1B 3X9

Received 29 March 1989

REVUSKY, S. AND S. REILLY. *Dose effects on heart rate conditioning when pentobarbital is the CS and amphetamine is the US.* PHARMACOL BIOCHEM BEHAV 36(4) 933–936, 1990.—If sodium pentobarbital is injected into rats 30 min prior to *d*-amphetamine sulphate on four or five occasions, there is a learned effect of pentobarbital on heart rate. The conditioned response is a higher heart rate than found in rats with a control history of exposure to the same drugs. In Experiment 1, when the pentobarbital dose was 32 mg/kg throughout, this effect was obtained with amphetamine doses of 2, 4, 8, or 16 mg/kg. In Experiment 2, when the amphetamine dose was 12 mg/kg throughout, pentobarbital doses of 16 and 32 mg/kg yielded conditioning, while 8 mg/kg yielded equivocal results.

Classical conditioning Heart rate Pentobarbital *d*-Amphetamine

WHEN two drugs are injected in sequence and the procedure is interpreted as classical conditioning, the first drug is considered a conditioned stimulus (CS) and the second drug is considered an unconditioned stimulus (US). Conditioning is demonstrated by development of a conditioned response (CR) to the CS that can be attributed to the CS-US pairing (2).

There are a number of methods of demonstrating drug-drug conditioning (1, 4, 6, 11, 12). The method used here is to inject pentobarbital (the CS drug) into rats 30 min prior to the injection of *d*-amphetamine (the US drug). HR conditioning usually occurs after 2–4 pentobarbital-amphetamine pairings. The CR to the pentobarbital CS is an increased heart rate (HR) that begins about 10 min after the pentobarbital injection and lasts about an hour (6). Such rapid conditioning is suggestive of an innate predisposition toward such conditioning (3) that enables the animal to better regulate its internal environment by anticipating the US (4). Presumably, the CS corresponds to naturally occurring internal signals, the US corresponds to naturally occurring aftereffects, and the CR contributes toward homeostatic regulation in the natural life of the animal.

In our earlier work, we had used high doses of each drug, 32 mg/kg of sodium pentobarbital and 12–24 mg/kg of *d*-amphetamine sulfate, because we thought this might maximize the likelihood of successful results. In another drug-drug conditioning procedure, Avfail, high doses of both the CS and US drugs seemed necessary (5). In the Avfail procedure, pairing the CS drug with the US drug endows the CS drug with the capacity to interfere with the conditioning of taste aversions produced either by the US drug (1), by some other drug (7), or by the CS drug itself (5). In our initial experimentation, it would have been foolhardy to suppose that HR conditioning is obtainable through lower US doses than is Avfail. However, HR conditioning was easily obtained with high doses of each drug (6) and here we tried to determine if such high doses really were necessary. In Experiment 1, the pentobarbital dose was 32 mg/kg for all rats and the dose of *d*-amphetamine sulphate was varied from 2 to 16 mg/kg for different experimental groups. Experiment 2 was similar except that the amphetamine dose was held constant at 12 mg/kg, while the pentobarbital doses were 8, 16, or 32 mg/kg for different groups.

In each experiment, the controls were subjected to both the pentobarbital CS and the amphetamine US, but the amphetamine was not injected until the following day, a procedure that prevents conditioning (6) and equates prior exposure to both the CS drug and the US drug. Furthermore, both experimental and control groups were equally under the pharmacological influence of recently injected pentobarbital during the test of conditioning. However, although these pharmacological effects were taken into account in the experimental design, it is noteworthy that, for our purposes, pentobarbital has no important effect on HR. The pentobarbital CS produces some rise in HR on an initial exposure, but after 4 or 5 exposures, it does not increase HR among unconditioned controls during the period when the CR occurs (6).

METHOD

Subjects and Materials

The naive male Sprague-Dawley rats were housed in individual stainless steel cages with unlimited access to dry Purina chow. All experimentation was conducted in the animal housing room, which was illuminated 24 hours per day. During the experiments, the rats received water on a schedule of two days of free access followed by two days without water. Just prior to initiation of this regimen, the weight range of the rats was 187–210 g. On the day

before Trial 5 (the first test apparatus session), electrodes (safety pins) were implanted subcutaneously on the left shoulder and right flank for HR recording.

The pentobarbital used was Somnotol brand. The *d*-amphetamine sulfate was a powder donated by Smith, Kline and Bechman of Canada. All injections were diluted in saline to equal 1 ml/kg.

Apparatus

Each of eight identical test chambers consisted of an aluminum shell (12.2 cm high and 19.1 cm diameter) that contained a rigid plastic liner into which the rat was placed. In the centre of the steel mesh lid was a swivel that permitted the animal to move freely while its electrodes were connected by alligator clips to the HR monitoring equipment. Further details, including a photograph of a test apparatus, have been published elsewhere (6).

The output from the electrodes was amplified bipolarly and read by means of a Labmaster board and Labpac software (Scientific Solutions, Inc.) for 1.2 sec at 1-msec intervals. In each 1.2-sec sampling period, data was obtained from all 8 rats and placed into an array in the memory of an AT type computer (Tatung). Overall control was by a program in Microsoft QuickBasic that determined peak amplitudes and translated them into a rate. This HR determination was crosschecked through the program for various conceivable artifacts. It also could be overridden by the operator on the basis of a graph of amplified outputs over time that was displayed on the video monitor for each rat. When an HR was rejected, there was a second sampling period to determine the HR once again. We know of only one bias: a double heart beat (extrasystole), which occurred very rarely, was rejected by the QuickBasic program as an error.

Experiment 1

Each of 4 experimental groups contained 20 rats and each of the 4 control groups contained 10 rats. Each pair of experimental and control groups was assigned to a different dose of *d*-amphetamine: 2, 4, 8, or 16 mg/kg. One experimental rat (8 mg/kg) died before Trial 5 and one control rat (16 mg/kg) died after completing Trial 5.

There were 4 conditioning trials in the home cage prior to 2 test trials in the HR monitoring apparatus. With rare exceptions, animals were run in squads of 8. Each squad contained 0–2 animals from each of the 8 groups and groups were balanced for assignment to each of the 8 experimental chambers. HRs were sampled at 3-min intervals during Trials 5 and 6.

Trials were administered four days apart while the rats were 16–20 hours water deprived. During the conditioning trials all rats were injected IP with the CS drug (32 mg/kg of sodium pentobarbital) and then, 30 min later, the experimental animals were injected IM with the appropriate dose of *d*-amphetamine; the control animals were injected with the amphetamine on the following day.

Trials 5 and 6 were the test trials and were conducted in the test chambers. The CS drug was injected following an 18-min acclimatization period and the animals remained in their chambers for a further 48 min. For the experimental animals the US drug was administered when the rats were removed from the chambers on completion of the trial; the control animals received their amphetamine, as in Trials 1–4, on the following day. Trial 6 was identical in all respects to Trial 5 except that amphetamine was omitted.

Experiment 2

The *d*-amphetamine dose was held constant at 12 mg/kg with experimental and control groups subjected to different doses of pentobarbital: 8, 16, or 32 mg/kg. The procedure was identical to that of Experiment 1 except that the control subjects received an injection (1 ml/kg) of normal saline at the time the experimental animals were injected with the US drug and vice versa. There were 20 rats per group except for 18 rats in the 32 mg/kg experimental group and 27 rats in the 32 mg/kg control group. One rat in the 16 mg/kg control group died during the conditioning stage.

Inferential Statistics

Neither experiment yielded a reliable difference among the various control groups and hence the control data were pooled within each experiment. Conditioning was considered to have occurred if, after the pentobarbital injection, an experimental group exhibited reliably higher HRs than the pooled controls. There was no important danger of experimentwise error due to multiple comparisons between groups because there were adequate constraints from prior findings and the logic of the experiment. But the many HR measurements at different 3-min intervals taken for each group were a source of concern. Hence, we used *t*-tests based on mean HR during a criterion period beginning 24 min after the pentobarbital injections and ending 48 min afterward. As explained elsewhere (6), this is a reasonable criterion and more conservative than the usual repeated measure analysis, which is flawed for the present application. Given statistical significance according to this overall criterion, the CR was considered to be apparent during all successive 3-min determinations in which the CR yielded $p<0.10$, two-tailed, provided at least one of these determinations was in the criterion period. For instance, if the overall criterion was met and all individual *t*-test results from 6 min after pentobarbital injection until 48 min after injection yielded $p<0.10$, two tails, the duration of the CR was from 6 until 48 min after the pentobarbital injection. We also provided for statistical evaluation of unanticipated effects but none emerged.

RESULTS

The present CR is defined entirely in terms of differences from controls rather, than is usual, at least partly in terms of a change from a baseline. Hence it is appropriate to first consider the control data for Experiment 1 (Fig. 1). In both Trials 5 and 6, the control curves indicate that during the first 18 min after the rat is placed in the test chamber, prior to any injection, its HR decreases. This decrease is a recovery from a transient rise in HR rate that occurred when the rat was moved from its home cage and placed in the test chamber. The handling involved in the pentobarbital injection procedure also produces a transient rise in HR followed by a recovery. This rise is not mainly due to the pentobarbital because a similar rise in HR occurs when saline is injected (6). There is also an overall reduction in HR among the controls from Trial 5 to Trial 6 that occurs because the rats are becoming increasingly more accustomed to the test chamber and the associated handling procedures (6).

It was decided, a priori, that Trial 6 was a better test of conditioning than Trial 5 because disruptive effects due to novelty of the test apparatus ought to have dissipated. In Experiment 1, each of the four doses of *d*-amphetamine produced conditioning on Trial 6 relative to the pooled controls during our overall criterion period (beginning 24 min after pentobarbital injection) at $p<0.005$ (Fig. 1, right side). The controls subjected to different amphetamine doses did not differ reliably among themselves. On each determination beginning 27 min after the pentobarbital injection, each experimental group exhibited reliably higher HRs than the pooled controls. In the case of the 16 mg/kg amphetamine group, significance was present on each determination beginning 6 min

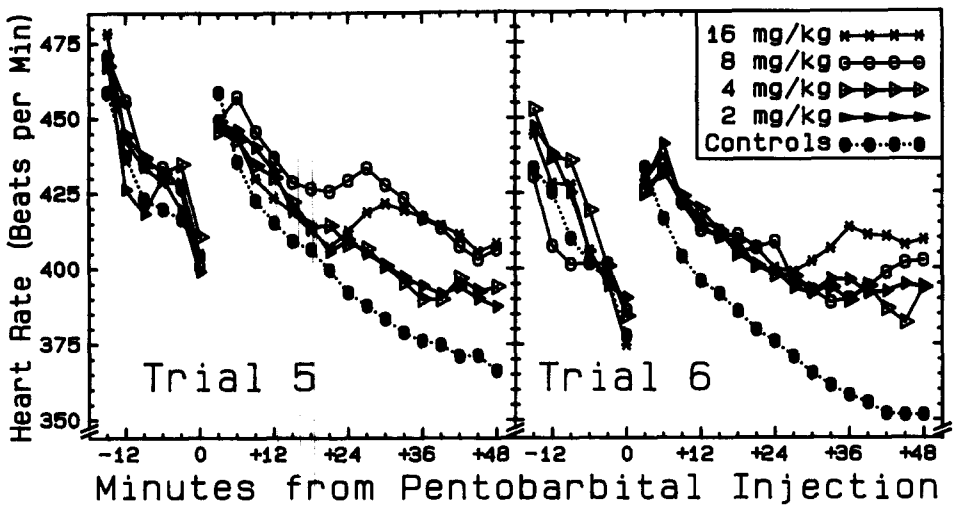

FIG. 1. Effects on heart rate conditioning when different doses of *d*-amphetamine were used as the US drug and 32 mg/kg of sodium pentobarbital was the CS drug.

after the pentobarbital. To test for a difference among the doses during the criterion period on Trial 6, we looked for a trend as a function of the logarithm of the dose and found none. Although the CR was evident sooner after pentobarbital injection in the 16 mg/kg experimental group than in the other experimental groups, there is no statistical evidence that this is due to a nonchance difference among the experimental groups.

On Trial 5 of Experiment 1 (Fig. 1, left side), which followed 4 CS-US pairings, the 8 and 16 mg/kg amphetamine doses yielded significant overall conditioning, but the 2 and 4 mg/kg doses yielded statistically marginal conditioning ($p<0.10$, two tails). The trend for increased HR with the log of the amphetamine dose yielded $p<0.05$ on a one-tailed basis. Due to the negative results for this measure on Trial 6, this cannot be considered convincing evidence for a dose effect.

The control data for Experiment 2 (Fig. 2) were very similar to those for Experiment 1 (Fig. 1) and the conditioning produced by the 32 mg/kg CS dose of pentobarbital on each of Trials 5 and 6 was similar to that produced when the same pentobarbital dose was used with the higher amphetamine doses of Experiment 1. On each of Trials 5 and 6, this conditioning was unequivocal for 32 mg/kg pentobarbital ($ps<0.001$ as compared to the pooled controls, which did not differ among themselves). All successive determinations yielded significant results from 15 min after pentobarbital injection on Trial 5 and from 12 min after injection on Trial 6. This pattern of results is exactly what would be expected on the basis of the results of Experiment 1.

The new information from Experiment 2 was that conditioning was inferior or nonexistent with pentobarbital doses below 32 mg/kg (Fig. 2). Contrary to our a priori expectations, the conditioning for these CS doses seemed better on Trial 5 than on Trial 6. The 8 mg/kg and 16 mg/kg pentobarbital groups exhibited significant conditioning on Trial 5 ($ps<0.05$) but not on Trial 6, although the 16 mg/kg pentobarbital group yielded $p<0.10$, two-tailed, on Trial 6. On Trial 5, there were no overall statistically significant differences among the three experimental groups

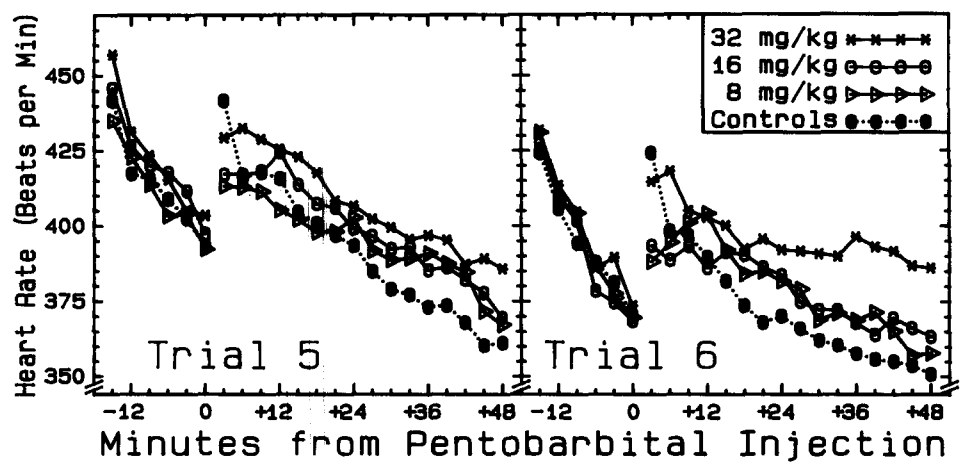

FIG. 2. Effects on heart rate conditioning of different doses of sodium pentobarbital as the CS drug when the US drug was 12 mg/kg of *d*-amphetamine throughout.

in HR during the criterion period. But on Trial 6, which was a selected a priori as the criterion trial, this difference had $p<0.02$ and the 8 mg/kg and 16 mg/kg groups each had lower HRs than the 32 mg/kg group during the criterion period at $p<0.02$. Because the CR produced by the pentobarbital-amphetamine combination is a heightened HR and because the Trial 5 result for 16 mg/kg pentobarbital was significant, there is enough evidence to allow a one-tailed interpretation that 16 mg/kg pentobarbital produced conditioning on Trial 6. The 8 mg/kg pentobarbital dose group did not meet the primary statistical requisite for conditioning, a higher HR during the criterion period than among the controls, but it was not statistically distinguishable from the 16 mg/kg pentobarbital group which did exhibit conditioning. Thus, we are not sure whether the 8 mg/kg pentobarbital dose produced conditioning. The possibility cannot be excluded that additional trials might have produced clearer conditioning for the 8 mg/kg dose, but we doubt it because earlier work (6) shows, if anything, weakening of the observable HR effect with additional trials.

DISCUSSION

Unfortunately, we understand little of the specific mechanisms underlying drug-drug conditioning of HR. In still unpublished work, we have varied the US, and obtained very similar conditioning with nicotine, but not with atropine, lithium, caffeine, or footshock (9). This shows that the US is not generalized stress or stimulation, but we are unable to come up with any more specific explanation accounts for this pattern of results. This forces us into a very empirical discussion of these results.

In both experiments, there were four conditioning trials in the home cage prior to two test trials in the HR monitoring apparatus. Drug-drug conditioning, at least with the procedure used here, is not context specific; that is, pairings of the two drugs while the rat is in its home cage produce conditioning that is at least as pronounced as that resulting from pairings of the two drug injections in the HR monitoring apparatus (8). A contrary earlier report (6) was due to misinterpretation of certain results. The procedure of conditioning the rats in the home cage is very efficient because one simply administers the paired injections without the extra labor of keeping the rats in the test apparatus. It also makes it certain that external cues from the test apparatus are not part of the CS complex. However, it does not yield acquisition data, which is not easily recorded in the home cage. In earlier experiments in which all trials were administered in the HR monitoring apparatus (6,8), we sometimes observed evidence for conditioning on the third or fourth test trial, but never earlier.

As in earlier work, drug-drug conditioning occurred after very few conditioning trials. But, in contrast to earlier work based on high doses, conditioning was demonstrated at doses that correspond closely by behavioral criteria to doses in frequent use among humans. Amphetamine doses of up to 5 mg/kg are effective rewards for rats (10) and such doses are probably similar in effect to those used recreationally by humans. By our informal observations, the 8 mg/kg pentobarbital did not cause the rats to go to sleep, the 32 mg/kg dose caused all the rats to go to sleep within 5–10 min, while the 16 mg/kg dose had variable effects. Hence, the 16 mg/kg pentobarbital dose has a sedative effect probably weaker than that of barbiturate doses used by humans for sedation.

Drugs are frequently used in combination both clinically and recreationally. We believe that the principles demonstrated here are applicable to CRs other than a change in HR and to a variety of drug-drug combinations and can cause changes in the therapeutic effects of drugs. That the present results are not unique is clear from the effects of pairing diazepam with chlorpromazine (13). Diazepam's efficacy as a muscle relaxant is reduced while its capacity to reduce anniety is increased. Thus, different clinical effects of diazepam are changed differently by the very same pairings. Hence, it is very likely that still other therapeutic effects of drugs can become enhanced or degraded due to drug-drug conditioning.

ACKNOWLEDGEMENTS

This study was supported by a grant from the National Sciences and Engineering Research Council of Canada. Sharon Molloy and Michele Blake expertly administered the procedures. We thank Smith, Kline and French, Ltd., of Canada for donating the amphetamine.

REFERENCES

1. Lett, B. T. Pavlovian drug-sickness pairings result in the conditioning of an antisickness response. Behav. Neurosci. 97:779–784; 1983.
2. Pavlov, I. P. Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. In: Anrep, G. V. (trans.). London: Oxford Unversity Press; 1927. (Reprint, New York: Dover.)
3. Revusky, S. Associative predispositions. In: Marler, P.; Terrace, H., eds. Dahlem Workshop on the Biology of Learning. Berlin: Springer Verlag; 1984:447–460.
4. Revusky, S. Drug interactions measured through taste aversion procedures with an emphasis on medical applications. Ann. NY Acad. Sci. 443:250–271; 1985.
5. Revusky, S.; Coombes, S.; Pohl, R. W. Pharmacological generality of the Avfail effect. Behav. Neural Biol. 34:240–260; 1982.
6. Revusky, S.; Davey, V.; Zagorski, M. Heart rate conditioning with pentobarbital as a conditioned stimulus and amphetamine as an unconditioned stimulus. Behav. Neurosci. 103:296–307; 1989.
7. Revusky, S.; Harding, R. K. Pairing pentobarbital with one toxin causes it to attenuate taste aversions produced by a different toxin: Implications for conditioned antisickness theory. Behav. Neurosci. 100:685–694; 1986.
8. Revusky, S.; Reilly, S. When pentobarbital is the CS and amphetamine is the US, conditioning depends upon the type of CR. Behav. Neurosci.; in press.
9. Revusky, S.; Reilly, S. Drug-drug heart rate conditioning in rats: Nicotine is an effective US. Manuscript in preparation.
10. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Res. 253:185–193; 1982.
11. Taukulis, H. K. Attenuation of pentobarbital-elicited hypothermia in rats with a history of pentobarbital-LiCl pairings. Pharmacol. Biochem. Behav. 17:695–697; 1982.
12. Taukulis, H. K. Conditional hyperthermia in response to atropine associated with a hypothermic drug. Psychopharmacology (Berlin) 90:327–331; 1986.
13. Taukulis, H. K.; Brake, L. D. Therapeutic and hypothermic properties of diazepam altered by a diazepam-chlorpromazine association. Pharmacol. Biochem. Behav. 34:1–6; 1989.