Pharmacology Biochemistry and Behavior, Vol. 44, pp. 981–983, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

BRIEF COMMUNICATION

Development of Rapid Tolerance to Pentobarbital and Cross-Tolerance to Ethanol on a Motor Performance Test With Intoxicated Practice

MARCELA BITRÁN[1] AND HAROLD KALANT[2]

Department of Pharmacology, University of Toronto, Toronto M5S 1A8, Canada and Addiction Research Foundation of Ontario, Toronto M5S 2S1, Canada

Received 8 October 1992

BITRÁN, M. AND H. KALANT. *Development of rapid tolerance to pentobarbital and cross-tolerance to ethanol on a motor performance test.* PHARMACOL BIOCHEM BEHAV 44(4) 981–983, 1993.—Male Wistar rats given a single moderate dose (1.7 mg/kg, IP) of pentobarbital (PB), followed by six trials on the moving belt apparatus during the next hour, showed tolerance to the motor-impairing effects of a second dose of 17 mg/kg given 24 h later. A control group that received saline before the first test showed the usual initial sensitivity when tested with PB 24 h later. Three weeks later, the first group showed cross-tolerance to the effects of ethanol (1.7 g/kg, IP) on the same test, while the second group did not. These findings support the suggestion that rapid tolerance is closely similar to chronic tolerance and that the contribution of intoxicated practice results in a long-lasting component that applies to cross-tolerance to ethanol on the same test.

Pentobarbital Ethanol Motor impairment Rat Rapid tolerance Cross-tolerance

TOLERANCE to ethanol and other centrally acting drugs has been shown to occur within three different time frames, commonly designated as *acute* (within a single session) (15), *rapid* (demonstrable in a second drug exposure 8–24 h after the first) (3), and *chronic* (developing during repeated drug exposures over days or weeks) (7). A growing body of evidence suggests that these three forms of tolerance are produced either by the same mechanism or by mechanisms that, if different, are nevertheless modulated in the same ways by the same factors. For example, all three are facilitated by the opportunity to practice the tested task while under the effect of the drug (1, 5,8,13), both rapid and chronic tolerance (and possibly acute) are prevented by inhibitors of cerebral protein synthesis such as cycloheximide (18) or anisomycin (2), and both show asymmetry of cross-tolerance between ethanol (EtOH) and pentobarbital (PB) (6,9,10). Indeed, it has been proposed that rapid tolerance is an economical model that can be used to study the effects of various factors and that predicts accurately how they will influence the development of chronic tolerance (9).

Chronic tolerance shows a considerable degree of test specificity, which has been interpreted as evidence that the stimulus to the development of tolerance is not the mere presence of the drug but the degree of functional impairment it produces (7). The previous demonstrations of asymmetry of rapid cross-tolerance between EtOH and PB employed tests that involve little learning, viz., drug-induced hypothermia, loss of righting reflex, and loss of resistance to sliding on an inclined plane (9). The present article extends the comparison by using a more complex sensorimotor performance test (the moving belt test) that involves a considerable degree of learning before the drug effect can be tested (17). When chronic tolerance on this test is produced by a paradigm that involves learning under the influence of a drug, such as intoxicated practice or drug-cued associative learning, the tolerance and cross-tolerance are much longer lasting than when they are produced by a nonlearning paradigm (12). In the present study, we therefore tested cross-tolerance 3 weeks after production of rapid tolerance in this learning-dependent model

[1] Current address: Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Universidad Católica de Chile, Santiago, Chile.
[2] To whom requests for reprints should be addressed.

for comparison with previous findings in the absence of such learning.

METHOD

Subjects

Two groups of male Wistar rats (n = 12 per group), weighing about 150 g when purchased (Charles River, Montréal, Canada), were individually housed in an environmentally controlled room at 21–23°C and 40% relative humidity, with lighting on from 0700–1900 h. Water and standard Purina rat chow were available ad lib.

Moving Belt Test

Training period. In this test, rats are trained to walk on a motor-driven metal mesh belt that moves continuously over a shock grid (17). When the rat puts one or more paws off the belt, it receives mild electric foot-shock and a cumulative timer is activated to record the total time off belt during a 2-min trial. Rats were trained to a criterion of 99% correct performance (i.e., not more than 1.2 s off the belt during any 2-min trial). Training sessions began within the first week after arrival of animals in the vivarium.

Test sessions. The motor impairment was measured for each rat in six 2-min trials starting at 7, 17, 27, 37, 47, and 57 min after an IP injection of either saline, PB (17 mg/kg as a 1.7% w/v solution in saline), or EtOH (1.7 g/kg as a 17% w/v solution in saline). The time off the belt was recorded in each trial within the session; in almost all cases, the maximum score after drug injection was seen on either the first or second trial, whereas saline-injected animals never fell below the performance criterion.

Experimental design. Each rat in group 1 (S-P-E) was given an IP injection of saline on day 1, of PB on day 2 (24 h after the first injection), and of EtOH on day 16. Each rat in group 2 (P-P-E) received injections of PB on days 1 and 2 and EtOH on day 16. All injections were of the same volume, 1 ml/100 g bw. Each rat was then tested as described above, on each test day. Immediately after completion of the rat's final trial of the session, a 50- or 100-μl blood sample was collected from the cut tip of the tail into a glass capillary tube for analysis of E or PB, respectively, by gas chromatographic methods described previously (11,14).

Statistical Analyses

Statistical comparisons between the test results in the two treatment groups, or in the P-P-E group on days 1 and 2, were carried out by general linear model analyses of variance (ANOVAs) using the NCSS program for IBM-PC. Comparisons of the blood alcohol or PB concentrations (BACs, BPCs) were carried out by Student's t-test for unpaired data.

RESULTS

Effect of Pentobarbital on Days 1 and 2

Administration of a single PB injection on day 1 produced the expected pattern of intoxication in the P-P-E group, which showed peak impairment on the 7-min test, and a steady decline in effect in all the following trials (Fig. 1). The S-P-E group, which received saline on day 1, showed no impairment on any of the trials. On day 2, after the same dose of PB the P-P-E group was significantly less impaired than it had been on day 1 and less than the S-P-E group on day 2 (Fig. 1). The results of all three tests under PB were subjected to a two-way ANOVA (treatments, trials), which showed highly significant

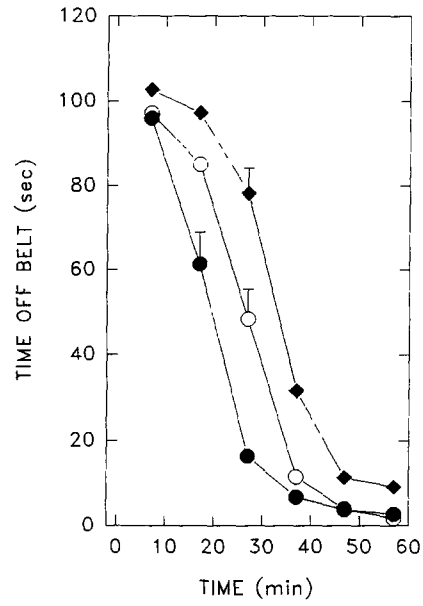

FIG. 1. Development of rapid tolerance to pentobarbital in group P-P-E. Each animal was injected with pentobarbital, 17 mg/kg IP, and then tested on the moving belt apparatus in six 2-min trials beginning 7, 17, 27, 37, 47, and 57 min after injection. ○, P-P-E group on day 1; ●, P-P-E group on day 2 (24 h after the first test); ♦, S-P-E group on day 2 (first test under pentobarbital). Each curve differed significantly from the other two; see the text for statistics.

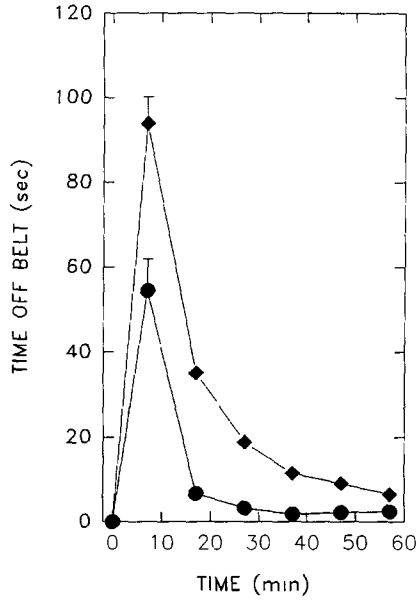

FIG. 2. Cross-tolerance to ethanol in rats that had developed rapid tolerance to pentobarbital. Each animal was injected with ethanol, 1.7 g/kg IP, and tested on the moving belt apparatus as in Fig. 1, 2 weeks after the day-2 tests shown in Fig. 1. No drug treatment was given in the intervening period. ♦, S-P-E group; ●, P-P-E group. The curves were significantly different; see the text for statistics.

main effects of treatment, $F(2, 192) = 57.12$, $p < 0.0001$, and trials, $F(5,192) = 313.19$, $p < 0.0001$, as well as a significant treatment × trials interaction term, $F(10, 192) = 8.62$, $p < 0.0001$. The latter indicated that the loss of drug effect across trials proceeded with different time courses in the three groups, that is, most rapidly in the P-P-E group on day 2. Duncan's multiple-range test confirmed that all three curves in Fig. 2 were significantly different from each other at the $p < 0.05$ level. This difference was not due to more rapid elimination of PB on day 2 because the mean BPCs on days 1 and 2 were 0.446 ± 0.02 and 0.482 ± 0.03 μg/100 ml, respectively ($t = 1.000$, $p > 0.30$).

Day-16 Results

The test results when both groups were exposed to EtOH on day 16 showed a marked intergroup difference (Fig. 2). The P-P-E group was clearly cross-tolerant to EtOH while the S-P-E group showed the usual effect of the 1.7-g/kg dose in naive rats. ANOVA revealed highly significant main effects of group, $F(1, 21) = 42.93$, $p < 0.0001$, trial, $F(5, 110) = 134.26$, $p < 0.0001$, and group × trial interaction, $F(5, 110) = 9.94$, $p < 0.0001$. This difference between groups could not be attributed to differences in BAC because the respective values for groups S-P-E and P-P-E were 233.3 ± 7.6 and 235.3 ± 3.4 mg/dl, respectively ($p > 0.90$).

DISCUSSION

Administration of a single moderate dose of PB (17 mg/kg), followed by six trials on the moving belt test during the next hour, resulted in rapid tolerance to PB in a second test 24 h later. This confirms the earlier report by Khanna et al. (9) using different tests of drug effect. Of specific interest is the fact that the present paradigm has been shown to involve a major component of learning through task practice under the influence of the drug because six trials after drug administration produced rapid tolerance while two trials after the same dose did not (1).

As in the previous study (9), rapid tolerance to PB resulted in cross-tolerance to EtOH. In that work, however, the PB and EtOH tests were carried out on successive days so that the finding of cross-tolerance to EtOH did not illuminate the duration of the phenomenon. In the present study, the cross-tolerance had been produced by two successive PB exposures 2 weeks earlier, and no further drug-linked practice had occurred. Therefore, the cross-tolerance observed 2 weeks later indicated a long-lasting phenomenon, consistent with the long duration of learning-augmented tolerance and different from the short duration of tolerance produced in nonlearning paradigms (12). The lack of cross-tolerance to EtOH in rats that received a single PB test session 2 weeks earlier is consistent with the observation that even repeated test sessions under drug, if separated by intervals of 4 days or longer, do not produce tolerance (16).

The reason for the previously reported absence of cross-tolerance to PB in animals made tolerant to EtOH, in both rapid and chronic paradigms (4,9), despite the clear evidence of cross-tolerance to EtOH in rats made tolerant to PB in both models [(10) and the present work], is not yet evident. Cross-tolerance to PB has been shown when tolerance to EtOH was produced in a model involving associative learning that conditionally linked environmental cues to drug presentation (4,19). Whether different mechanisms of tolerance are involved, or merely different methods of triggering the same adaptive processes, remains to be clarified.

ACKNOWLEDGEMENTS

The authors are indebted to Girish Shah for assistance with the statistical analyses and to Lonnie Currin for help in training animals. The views expressed in this article are those of the authors and do not necessarily represent the policy of the Addiction Research Foundation.

REFERENCES

1. Bitrán, M.; Kalant, H. Learning factor in rapid tolerance to ethanol-induced motor impairment. Pharmacol. Biochem. Behav. 39: 917-922; 1991.
2. Bitrán, M.; Kalant, H. Effect of anisomycin on the development of rapid tolerance to ethanol-induced motor impairment. Pharmacol. Biochem. Behav. (in press).
3. Crabbe, J. C.; Rigter, H.; Uijlen, J.; Strijbos, C. Rapid development of tolerance to the hypothermic effect of ethanol in mice. J. Pharmacol. Exp. Ther. 208:128-133; 1979.
4. El-Ghundi, M.; Kalant, H.; Lê, A. D.; Khanna, J. M. The contribution of environmental cues to cross-tolerance between ethanol and pentobarbital. Psychopharmacology (Berl.) 97:194-201, 1989.
5. Gallaher, E. J.; Loomis, T. A. The rapid onset of ethanol tolerance in Wistar rats following intensive practice on the moving-belt task. Toxicol. Appl. Pharmacol. 48:415-424; 1979.
6. Gougos, A.; Khanna, J. M.; Lê, A. D.; Kalant, H. Tolerance to ethanol and cross-tolerance to pentobarbital and barbital. Pharmacol. Biochem. Behav. 24:801-807; 1986.
7. Kalant, H. Tolerance, learning, and neurochemical adaptation. Can. J. Physiol. Pharmacol. 63:1075-1079; 1985.
8. Kalant, H.; LeBlanc, A. E.; Gibbins, R. J. Tolerance to, and dependence on, some nonopiate psychotropic drugs. Pharmacol. Rev. 23:135-191; 1971.
9. Khanna, J. M.; Kalant, H.; Shah, G.; Weiner, J. Rapid tolerance as an index of acute tolerance. Pharmacol. Biochem. Behav. 38: 427-432; 1991.
10. Khanna, J. M.; Lê, A. D.; Gougos, A.; Kalant, H. Effect of chronic pentobarbital treatment on the development of cross-tolerance to ethanol and barbital. Pharmacol. Biochem. Behav. 31:179-186; 1988.
11. Khanna, J. M.; Lê, A. D.; Kalant, H.; Kim, C. Differential sensitivity to ethanol, pentobarbital and barbital in spontaneously hypertensive (SH) and normotensive Wistar Kyoto (WK) rats. Psychopharmacology (Berl.) 86:296-301; 1985.
12. Lê, A. D.; Kalant, H. Learning as a factor in ethanol tolerance. In: Erinoff, L., ed. Neurobiology of drug abuse: Learning and memory. Washington, DC: DHHS; 1991:193-207.
13. Lê, A. D.; Kalant, H. Influence of intoxicated practice on the development of acute tolerance to the motor impairment effect of ethanol. Psychopharmacology (Berl.) 106:572-576; 1992.
14. LeBlanc, A. E. Microdetermination of alcohol in blood by gas-liquid chromatography. Can. J. Physiol. Pharmacol. 46:665-667; 1968.
15. LeBlanc, A. E.; Kalant, H.; Gibbins, R. J. Acute tolerance to ethanol in the rat. Psychopharmacologia 41:43-46; 1975.
16. LeBlanc, A. E.; Kalant, H.; Gibbins, R. J. Acquisition and loss of behaviorally augmented tolerance to ethanol in the rat. Psychopharmacology (Berl.) 48:153-158; 1976.
17. LeBlanc, A. E.; Kalant, H.; Gibbins, R. J.; Berman, N. D. Acquisition and loss of tolerance to ethanol by the rat. J. Pharmacol. Exp. Ther. 168:244-250; 1969.
18. LeBlanc, A. E.; Matsunaga, M.; Kalant, H. Effects of frontal polar cortical ablation and cycloheximide on ethanol tolerance in rats. Pharmacol. Biochem. Behav. 4:175-179; 1976.
19. Melchior, C. L.; Tabakoff, B. Features of environment-dependent tolerance to ethanol. Psychopharmacology (Berl.) 87: 94-100; 1985.