Pharmacology Biochemistry and Behavior, Vol. 45, pp. 225–228, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

RAPID COMMUNICATION

Effect of Anisomycin on the Development of Rapid Tolerance to Ethanol-Induced Motor Impairment

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Received 6 October 1992

BITRÁN, M. AND H. KALANT. *Effect of anisomycin on the development of rapid tolerance to ethanol-induced motor impairment.* PHARMACOL BIOCHEM BEHAV **45**(1) 225–228, 1993.—Male Wistar rats given a single moderate dose (1.7 g/kg, IP) of ethanol (EtOH), followed by six trials on the moving belt apparatus during the next hour, showed functional tolerance to the motor-impairing effects of a second dose given 24 h later if the first EtOH was preceded and followed by an injection of saline. The same EtOH dose and intoxicated practice did not produce tolerance if the saline injections were replaced by two doses of anisomycin (60 mg/kg each, SC) 15 min before and 105 min after the first dose of EtOH. This finding suggests that rapid tolerance, like chronic tolerance, requires de novo synthesis of protein during a short period immediately related to the test experience.

Ethanol Motor impairment Rapid tolerance Rat Anisomycin

TOLERANCE to ethanol (EtOH) and other centrally acting drugs has been shown to occur in three different time frames, referred to as *acute* (within a single session) (13), *rapid* (demonstrable in a second drug exposure 8–24 h after first) (3), and *chronic* (developing during repeated drug exposures over days or weeks) (7). The relationship among these three forms of tolerance is still the subject of much investigation, but it is known that all three forms can be facilitated by the opportunity to practice the tested task while under the effect of the drug (1,6,9,12). It is also known that chronic tolerance, like learning, is impaired by the action of inhibitors of cerebral protein synthesis, such as cycloheximide, during the time of the drug exposure (15). It therefore seemed useful to test whether rapid tolerance is similarly dependent upon de novo protein synthesis.

In the present study, another inhibitor of protein synthesis, anisomycin, has been tested for its effects on the development of rapid tolerance to EtOH-induced motor impairment in the rat. Anisomycin has the advantage that its duration of action as an inhibitor of protein synthesis is much shorter than that of cycloheximide; a dose that produces 80% inhibition of cerebral protein synthesis lasts for only about 2 h in the mouse (5). Therefore, by using anisomycin it is possible to define more precisely the time period within which protein synthesis must occur to permit rapid tolerance to develop.

METHOD

Subjects

Four groups of male Wistar rats (n = 15 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 the moving belt test (MBT), rats are trained to walk on a motor-driven metal mesh belt that moves

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continuously over a shock grid (14). When a rat puts one or more paws off the belt, it receives a 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 belt during any 2-min trial). Training sessions began within the first week after arrival of animals in the vivarium.

Test sessions. 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 EtOH (1.7 g/kg as a 17% w/v solution in saline). The time off belt was recorded in each trial within the session; in almost all cases, the maximum score was seen on either the first or second trial. Upon completion of the last trial in each session, 50 μl blood was taken from the cut tip of the tail for gas chromatographic determination of blood ethanol concentration (11). The experimenter conducting the trials was kept blind with respect to the treatments rats had received.

Experimental design. On day 1, each rat was given an SC injection of either anisomycin (60 mg/kg dissolved in 0.1 ml saline) or an equal volume of saline, followed 15 min later by the IP dose of ethanol (1.7 g/kg) or an equal dose of saline. Each rat was then tested as described above, and a second SC injection, the same as the first, was given 105 min after the IP injection. The four groups differed with respect to the treatments they received: group 1, saline SC + saline IP (S-S); group 2, anisomycin SC + saline IP (A-S); group 3, saline SC + EtOH IP (S-E); and group 4, anisomycin SC + EtOH IP (A-E).

On day 2, all four groups received only the 1.7-g/kg dose of EtOH IP and were tested on the moving belt apparatus as described above. On day 3, 24 h after the preceding test, the A-E group was retested under the same dose of EtOH alone.

Statistical Analyses

On each day, one or more rats failed to show any appreciable alcohol effect on performance in the MBT and were found to have much lower blood alcohol concentrations (BACs) than other rats of the corresponding groups. This was taken as evidence of unsatisfactory injections, the EtOH probably having been injected into the intestine or intramuscularly rather than IP. Therefore, the results for any rat that failed to show at least 30 s maximum impairment score were excluded from the analysis.

Statistical comparisons among the test results in the different treatment groups, or in the A-E group on the three different days, were carried out by general linear model analyses of variance (ANOVAs), using the NCSS program for the IBM-PC, followed by posthoc comparisons of specific groups, when appropriate. Comparisons of the BACs were carried out either by Student's *t*-test for unpaired data in two-group comparisons or by one-way ANOVA for three or more groups.

RESULTS

Effect of Anisomycin on Day 1

Administration of a single EtOH injection produced the expected pattern of intoxication in the S-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 A-E group showed practically the same peak effect, but the subsequent decrease in impairment scores was slower than in the S-E group (Fig. 1). A two-way ANOVA (treatments, trials) showed highly significant main effects of treatment, $F(1, 162) = 9.22$, $p < 0.0028$, and trials, $F(5, 162) = 46.44$, $p < 0.0001$, but a nonsignificant treatment × trials interaction term, $F(5, 162) = 1.05$, $p > 0.39$. The two saline control groups (S-S and A-S) did not show any impairment, that is, they did not exceed the training criterion for time off belt.

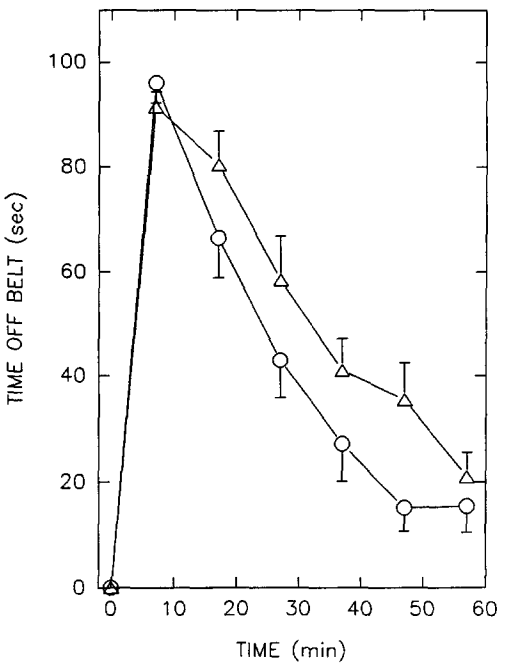

FIG. 1. Time course of ethanol (EtOH)-induced motor impairment on day 1 in rats injected SC with saline (○) or anisomycin, 60 mg/kg in saline (△), 15 min before IP administration of EtOH (1.7 g/kg). Each rat was tested on the moving belt apparatus at 7, 17, 27, 37, 47, and 57 min after the EtOH. Vertical bars represent largest SEM for any trial within each group.

BACs in the two EtOH-treated groups were slightly but significantly different: S-E, 221 ± 4 mg/dl; A-E, 211 ± 3 mg/dl, $t(28) = 2.152$, $p < 0.05$. However, the more noteworthy finding was that the anisomycin group, despite these slightly lower BACs, had the higher impairment scores at all times after 7 min, as noted above.

Day 2 Tests

The test results in the four groups, all receiving the same dose of EtOH without any pretreatment, are shown in Fig. 2. ANOVA again showed highly significant main effects of treatments (i.e., the day 1 treatment groups), $F(3, 294) = 28.16$, $p < 0.0001$, and trials, $F(5, 294) = 103.75$, $p < 0.0001$, but a nonsignificant treatments × trials interaction, $F(15, 294) = 1.62$, $p > 0.05$, indicated that the general time course of decline in error score was similar in the different groups. Duncan's multiple-range test confirmed that the impairment scores for the S-E group were significantly different from those of all other groups at the $p < 0.05$ level, but the three remaining groups did not differ significantly among themselves.

BACs in groups 1–4 at the end of the tests were 221.6 ± 4.7, 215.3 ± 3.5, 201 ± 2.5, and 205.1 ± 3.0 mg/dl, respectively. One-way ANOVA indicated a significant difference among groups, $F(3, 49) = 7.58$, $p < 0.0003$. *A posteriori* comparisons by Duncan's multiple-range test showed that

groups S-S and A-S (controls receiving saline instead of EtOH on day 1) had significantly higher levels than groups S-E and A-E (treated EtOH with on day 1), but those that had received anisomycin did not differ from those receiving saline instead.

Day 3 Results

The test results for the A-E group, retested with EtOH alone on day 3, are shown in Fig. 3 together with the results for the same group on days 1 and 2. A two-way ANOVA (days, trials) showed significant main effects of days, $F(2, 234) = 85.89$, $p < 0.0001$, and trials, $F(5, 234) = 65.01$, $p < 0.0001$, as well as a significant days × trials interaction, $F(10, 234) = 3.75$, $p < 0.0001$. The latter result indicated that the time course of results across the six trials differed on the 3 days in which the same group was tested. Posthoc pairwise comparisons by Scheffé's test showed that each day's results differed from those of the other two days at the $p < 0.05$ level. The mean BACs on the three days were: day 1, 210.9 ± 2.6; day 2, 205.1 ± 2.9; and day 3, 210.0 ± 2.9 mg/dl. ANOVA confirmed that these were not significantly different, $F(2, 39) = 1.24, p > 0.29$.

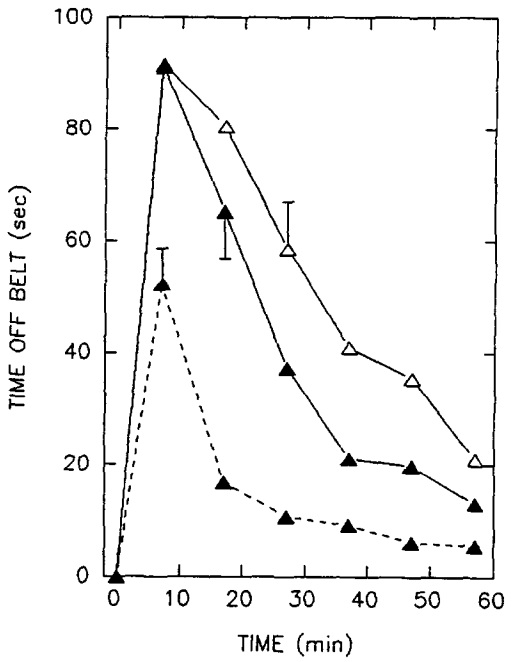

FIG. 3. Time course of ethanol (EtOH)-induced motor impairment in the anisomycin + EtOH group on days 1 (△), 2 (-▲-), and 3 (--▲--). On all 3 days, animals received EtOH (1.7 g/kg, IP) and were tested at the times shown. However, no anisomycin was given on days 2 and 3.

DISCUSSION

Administration of anisomycin 15 min before injection of EtOH on day 1 caused a small but significant increase in the degree of motor impairment produced by the EtOH. This does not appear to be due to a pharmacokinetic effect because the BAC was actually a little lower in the anisomycin group than in controls. The reason for the lower BAC is not apparent. Conceivably, it might reflect slower absorption of the injected EtOH from the peritoneal cavity or faster metabolic elimination. In the present context, however, the significant finding is that the anisomycin enhancement of the EtOH-induced motor impairment appears to be due to a central pharmacodynamic effect. The fact that peak impairment at 7 min after EtOH injection was unaffected by anisomycin, but that the subsequent decline in EtOH effect was slower in the A-E than the S-E group, suggests that anisomycin was inhibiting the development of acute (within-session) tolerance. This point is unproven, however, and other results with cycloheximide and a different method of measuring acute tolerance have suggested that acute tolerance is not dependent upon new protein synthesis (J. M. Khanna et al., unpublished).

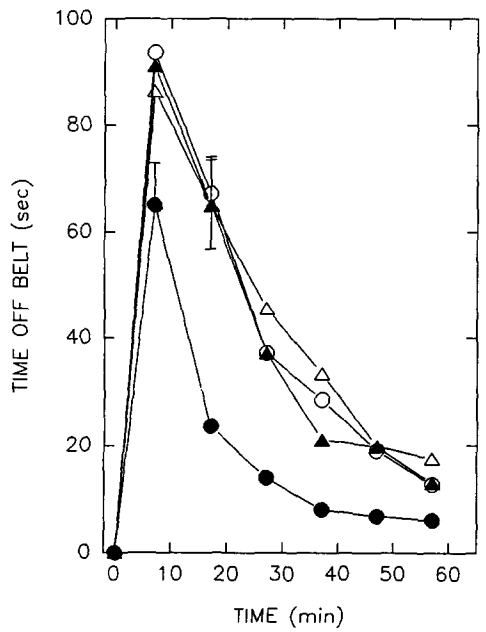

FIG. 2. Time course of ethanol (EtOH)-induced motor impairment (day 2) in groups of rats treated 24 h earlier with saline + saline (○), saline + EtOH (●), anisomycin + saline (△), and anisomycin + EtOH (▲). For the day 2 tests, all groups received EtOH alone (1.7 g/kg, IP) before being tested on the moving belt apparatus as described in Fig. 1 legend.

Other studies (21) suggested that factors that increase the acute effect of EtOH should thereby increase the stimulus to the development of tolerance. In the present instance, however, this was not the case; anisomycin on day 1 prevented the appearance of rapid tolerance to EtOH on day 2. This conclusion must be derived from the comparison of groups S-E and A-E on day 2, rather than of the group A-E results on days 1 and 2, because the anisomycin potentiation of the EtOH effect on day 1 prevents meaningful comparison with the day 2 effect in the absence of anisomycin.

The absence of tolerance in group A-E on day 2 could not have been due to any continuing action of anisomycin because the EtOH impairment curve was virtually identical to that seen in the S-E group on day 1, that is, there was no sign of continuing potentiation of the EtOH effect. One would not expect any continuing effect of anisomycin because the dosage used here has been shown to maintain effective inhibition of cerebral protein synthesis for only about 4–6 h (5). It is therefore unlikely that the anisomycin given on day 1 was preventing the *expression* of tolerance on day 2. Rather, it appears to

have inhibited the *acquisition* of tolerance. In contrast, the dose of EtOH on day 2, acting in the absence of anisomycin, was able to produce rapid tolerance seen in the test on day 3.

We recently reported that learning probably plays a role in the development of both rapid tolerance (1) and acute tolerance (9) to the effects of EtOH on the moving belt test. The two doses of anisomycin on day 1, 15 min before and 105 min after the EtOH, would have prevented protein synthesis for a period of about 4 h bracketing the animal's experience of EtOH-induced impairment on the test. This probably represents the critical period in which consolidation of the learned component of tolerance must take place. Unfortunately, the present results do not prove this conclusively because repeated EtOH experiences at intervals of less than 24 h after anisomycin were not explored. However, Pavlovian conditioning of stimuli from the EtOH test environment can also contribute to the development of tolerance (4,10,17), and Nelson and Alkon found a similar critical period of 2–24 h for the consolidation of synaptic changes underlying the acquisition of conditioned responses in *Hermissenda* (18,19). Recent studies in this laboratory examined the effects of two other agents that potentiate the acute effects of EtOH, viz., vasopressin and (+)MK-801, a selective blocker of the NMDA type of glutamate receptor. These had opposite effects on the appearance of rapid tolerance to EtOH on day 2. Vasopressin, acting through central V_1 receptors, produced a marked and long-lasting tolerance after one dose of EtOH given on the same occasion as the vasopressin (22). In contrast, (+)MK-801 prevented the appearance of rapid tolerance to EtOH (8). Blockade of the NMDA receptor by D,L-amino-phosphonovalerate has been shown to prevent the activation of protein kinase C in long-term potentiation (16), and the NMDA receptor-linked channel blocker (+)MK-801 presumably does the same. In contrast, vasopressin activates protein kinase C in pituitary cells (2), and protein kinase C has been suggested as the critical factor in the synaptic changes associated with Pavlovian conditioning (20). It is therefore possible that the action by which anisomycin inhibits rapid tolerance to EtOH may also be an inhibition of the synthesis of protein kinase C during the period immediately after the EtOH test on day 1. It should be possible to test this hypothesis more directly.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. Pierre Dessain, Vice President and Medical Director of Pfizer Canada Inc., for a generous gift of anisomycin; to John Mihic for valuable assistance in performing 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.

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