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Learned Tolerance to Ethanol in a Spinal Reflex Separated From Supraspinal Control

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JØRGENSEN, H. A., O.-G. BERGE AND K. HOLE. *Learned tolerance to ethanol in a spinal reflex separated from supraspinal control.* PHARMACOL BIOCHEM BEHAV 22(2) 293–295, 1985.—We have recently reported that ethanol-induced inhibition of the tail flick reflex in intact and spinal rats is diminshed during an eight day period if the animals are tested daily under the influence of ethanol. Ethanol only, or testing before ethanol administration, is not followed by tolerance. In the present study we used the tail flick testing of spinal rats to investigate the effect on tolerance development of repetitively triggering the tail flick reflex during intoxication, and of just placing the intoxicated animals in the test apparatus. We also investigated if damage to the tail tissue, due to repetitive prolonged test exposure, would facilitate the reflex and thereby reduce the inhibitory effect of ethanol. The results indicated that triggering of the reflex in the presence of ethanol was necessary for the tolerance to develop. Facilitation of the tail flick reflex, due to damage of the tail tissue, was not revealed. Thus the tolerance observed seems to be caused by an adaption to ethanol learned by structures involved in the tail flick reflex.

Ethanol Tolerance Learning Spinal reflex Tail flick Spinal rats

THE CNS sensitivity to ethanol is reduced by exposure to the drug. The mechanisms responsible for this development of tolerance are unknown but learning seems to be of importance. In a recent study we investigated the development of tolerance to ethanol-induced inhibition of the tail flick reflex in intact and spinal rats [6]. The results indicated that the ethanol-induced reflex inhibition was diminished only if the animals were tested repetitively under the influence of ethanol. Ethanol only, or testing before ethanol administration, did not result in tolerance. It was also noteworthy, that the acquired tolerance was not attenuated by a change in test environment. A likely explanation of the findings is that a test-dependent learning process is taking place in the spinal cord resulting in an adaptation to the effect of ethanol. An alternative possibility, however, is that the tolerance observed is caused by a facilitation of the reflex due to prolonged exposure to heat stimuli [15]. The group that developed tolerance in our previous study was the only one repetitively tested in the intoxicated state and therefore more heavily exposed to the heat stimulus on the tail than the other groups. Another factor possibly influencing the results is the general activation of the animal from being subjected to the test situation. In the present experiments we studied whether development of tolerance to ethanol-induced inhibition of the tail-flick reflex is dependent on the repetitive triggering of the reflex in the presence of ethanol or if it is sufficient for development of tolerance just to place the intoxicated animals in the test apparatus without releasing the reflex. We also investigated whether damage to the tail tissue, due to repetitive prolonged exposure to heat stimuli, induces facilitation of the reflex and thereby reduces the inhibitory effect of ethanol by mechanisms unrelated to tolerance to the drug. Only spinal rats were used. As this is an extension of a previous study [6] with the same basic experimental procedures, some of the control groups were not included in the present report.

METHOD

Animals

The subjects were male Spraque-Dawley rats (Møllegaard, Denmark), weighing 200–300 g. The rats were housed in pairs with free access to water. To reduce the weight gain, food was limited to 15 g pellets per animal per day. The light-phase lasted from 8:00 to 20:00 hours. Ambient temperature was 22–23°C. All experiments took place between 12:00 and 15:00 hours.

Surgery and Drugs

The rats were anaesthetised with a combination of pentobarbital (40 mg/kg) and chloral hydrate (180 mg/kg) IP. The transection of the spinal cord was performed at the level of Th_{9-10} according to a procedure previously described [7]. The experiments started 2 weeks after surgery.

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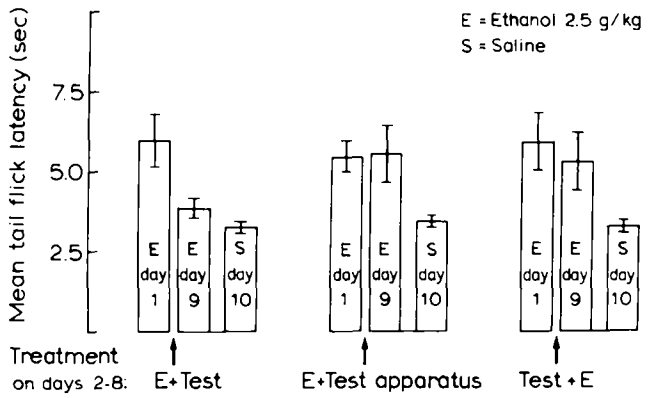

FIG. 1. Tail flick latency of spinal rats, mean±SE. On days 1 and 9, all three groups (n=9–10/group) received injections of ethanol 2.5 g/kg IP. On days 2–8 the groups received different treatments as indicated below the columns. On day 10 basal levels were obtained after saline injections. The interval between injections and the tail flick test was 30 min.

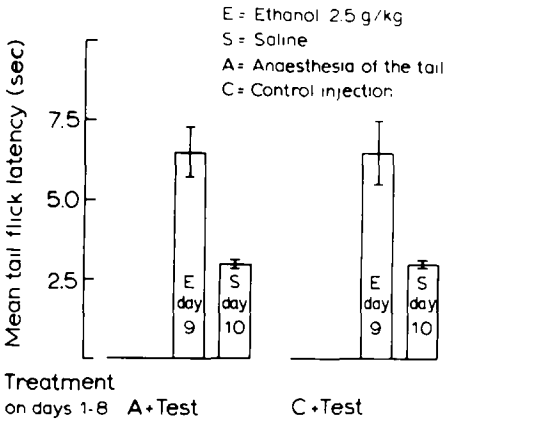

FIG. 2. Tail flick latency of spinal rats, mean±SE. Two groups (n=10/group) received ethanol 2.5 g/kg IP on day 9 and saline on day 10. Before being tested on days 1–8, the groups received a local anaesthetic either blocking the nerves at the base of the tail or as a control injection SC in the back. The interval between injections and the tail flick test was 30 min.

During the experiments, rats were injected with ethanol 2.5 g/kg IP (21 ml/kg of a 15% (v/v) ethanol/isotonic saline solution) or the same volume of isotonic saline. Rats treated with local anaesthesia were injected subcutaneously with 1 ml of Marcain adrenalin (Astra, Bupivacain hydrochloride 5 mg/ml and adrenalin 5 μg/ml).

Test Procedure

The spinal reflex sensitivity was measured as tail flick latency using an IITC INC. Mod. 33 Analgesia-meter. Radiant heat was focused on the tip of the tail and the beam intensity was adjusted to give a reaction time of 2.7–3.5 sec in spinal control animals. To limit damage to the tissue the beam was switched off after 10 sec (cut off time). All animals were daily handled and adapted to the test procedure during the 2 weeks period between surgery and the start of the experiment.

Protocol

The aim of the first experiment was to study the effect of specific activation of the reflex and general activation of the animal on development of tolerance. Spinal rats were randomly assigned to 3 groups (n=9–10/group). On days 1 and 9, the groups received ethanol 2.5 g/kg IP. The tail flick testing was performed 30 min after the injection. On days 2–8 the groups were daily injected with ethanol 2.5 g/kg followed either by testing or by being placed in the test situation for 10 sec (without heat stimulation of the tail), or the ethanol injection was given 30 min after the testing (Fig. 1 illustrates the design). On day 10 all the animals were injected with saline followed by testing.

In the second experiment we investigated if damage to the tail tissue, induced by thermal stimulation, would facilitate the reflex and reduce the inhibitory effect of ethanol.

Spinal rats were randomized into 2 groups (n=10/group). On days 1–8, one group received daily Marcain adrenalin injections in the base of the tail, giving moderate local anaesthesia of the tail. The other group (controls) received the same volume of the anaesthetic subcutaneously in the back. All animals were tested 30 min after the injection. On day 9 both groups received ethanol 2.5 g/kg followed by testing. On day 10 both groups received saline followed by testing.

In both experiments the animals were placed in their home cages in the period between injection and testing. The experiments took place in the colony room and the observer had no information about the treatment of the groups.

The statistical analysis was performed by means of one-factor ANOVA. The level of significance was set at 5%.

RESULTS

The results in the first experiment are shown in Fig. 1. Ethanol 2.5 g/kg caused an increase of the tail flick latency. The group that was tested 30 min after ethanol administration (E+Test) on days 2–8, developed tolerance measured as a significantly shorter tail flick latency on day 9 than on day 1, $F(1,9)=5.48$, $p<0.05$. The group that was placed in the test apparatus after ethanol administration without receiving the tail stimulus (E+Test apparatus) and the group that was tested before the injection of ethanol (Test+E) had approximately similar prolonged tail flick latencies on days 1 and 9. To test for differences in basal levels, all three groups were, on day 10, injected with saline followed by testing. The results of the groups were not significantly different.

In the second experiment one group (A+Test) received nerve block anaesthesia of the tail before being tested on days 1–8. The tail flick latencies in this period ranged from 4.2 to 5.3 sec. The tail flick latencies in the control group (C+Test) on days 1–8, ranged from 2.5 to 3 sec, which is comparable to tail flick latencies obtained in untreated animals. Thus the group with local anaesthesia of the tail had a heavier exposure to the heat stimulus than the control group. This was also reflected by a difference in the appearance of the tip of the tail in the two groups. In the group daily exposed to prolonged heat stimuli, the tip of the tail became red with slight oedema and sometimes with ulcerations. No tissue changes were observed in the group with normal tail flick latencies. On day 9 both groups were tested after injections of ethanol and on day 10 after saline. No difference between groups was observed in tail flick latencies (Fig. 2).

DISCUSSION

The experiments showed that ethanol 2.5 g /kg IP had an inhibitory effect, measured as prolonged tail flick latency, on the tail flick reflex in spinal rats. Daily activation of the reflex in the presence of ethanol for 8 days was sufficient for the development of tolerance to the inhibitory effect of the drug. Daily exposure to the test apparatus (without triggering of the reflex) or to testing before ethanol administration was not followed by tolerance. Furthermore, it was shown that although changes in the tail tissue were induced by repetitively testing the animals in the presence of a drug that prevented normal withdrawal of the tail, these changes did not seem to influence the tail flick latencies obtained after either ethanol or saline administration. This seems to exclude an "unspecific" facilitation of the reflex as the cause of the observed development of tolerance.

The Pavlovian conditioning model of tolerance is proposed by several [2, 4, 5, 8, 10, 12, 13] who have reported an association between the tolerance and the environment of drug administration. Other types of learning have also been suggested [1, 3, 14]. In a recent study [6] we presented results indicating that learned tolerance may also develop in the spinal cord. We concluded that tolerance to ethanol-induced inhibition of the tail flick reflex in rats could not be explained by the Pavlovian conditioning model as it was not contingent upon a distinct environment. In accordance with earlier findings in intact animals [3, 9, 11, 14] we found that the development of tolerance in the spinal cord seemed to require that the function that was tested was activated in intoxicated state. The present results support and extend the previous findings. The adaptation to the ethanol-induced inhibition of the tail flick reflex seems to be a learning process localized to the neuronal circuits involved in the reflex and the learning takes place when the circuits are repetitively activated during intoxication. The exact nature of this learning process can not be determined on the basis of the present result.

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