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Learned Tolerance to Ethanol in the Spinal Cord

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JØRGENSEN, H. A. AND K. HOLE. *Learned tolerance to ethanol in the spinal cord.* PHARMACOL BIOCHEM BEHAV **20**(5) 789–792, 1984.—Learning has been claimed to be of major importance in the development of tolerance to ethanol. In the present study we investigated the influence of learning on tolerance to ethanol-induced inhibition of a spinal reflex (tail-flick response) in intact and spinal rats. On day 1 and 9, groups of rats were injected with either ethanol 2.5 g/kg IP or saline 30 min prior to tail-flick testing. On days 2–8 the groups were treated differently in order to reveal the importance of the drug alone, the test alone and the combination of the two on development of tolerance. On day 10, the rats rendered tolerant in the home room were transferred to a new test room to be tested. Both in intact and spinal rats development of tolerance was observed only if the animals were repetitively tested while intoxicated. Tolerance acquired in the home room was not attenuated by transfer to a new environment. Results in the spinal rats suggested that adaptive mechanisms leading to tolerance may also be located in the spinal cord. The tolerance observed may be regarded as learned from practice while intoxicated.

Ethanol Tolerance Learning Spinal reflex Tail flick Spinal rats

DEVELOPMENT of tolerance to the effect of ethanol on the CNS implies that adaptive mechanisms gradually diminish the effect exerted by a given dose of ethanol. Changes in drug absorption, distribution, excretion and metabolism (dispositional tolerance) may contribute to tolerance. However, changes in the CNS sensitivity to the drug are generally more important [16]. Several recent studies in animals, in which the ethanol was given during a period of days or a few weeks, have attempted to explain the acquired tolerance partly or totally in terms of learning. The results from most of the studies indicate that classical conditioning of adaptive mechanisms is involved [3, 5, 7, 8, 9, 10], but also other types of learning have been suggested [1, 4, 14, 15].

These studies examined development of tolerance to the effect of ethanol on complex behaviour (narcosis, thermoregulation, maze and treadmill performance). To test the pervasiveness of the learning effect in tolerance it seems of interest also to study tolerance to ethanol in a simple behavioural model which can be studied both with and without neuronal contact with the brain. In the present study we investigated whether learning influences acquisition of tolerance to ethanol-induced inhibition of a spinal reflex. Both intact and spinally transected rats were used.

METHOD

Animals

Subjects were male Sprague-Dawley rats (Møllegaard, Denmark), weighing 200–300 g at the beginning of the experiment. The rats were housed in pairs with free access to water. To stabilize body weight during the period of experiments, 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 from 1200 to 1500 hours.

Surgery and Drugs

Rats assigned to spinal groups were anaesthetised with pentobarbital (40 mg/kg) and chloral hydrate (180 mg/kg) IP. The transection of the spinal cord was performed in the following way: the level of Th_{9-10} was ensured by palpation and proc. spinosi and laminae were exposed through an incision. One lamina corresponding to Th_9 or Th_{10} was removed with a dental burr and the spinal cord was cut with fine scissors. The blood was carefully removed by suction before closure with sutures. Penicillin with protracted effect (Ditardopen, 250000 IU) was given SC prior to surgery and on the second and the fourth postoperative day. The experiments began 14 days after surgery.

During the experiments the rats were treated 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.

Test Procedure

The spinal reflex sensitivity was measured with the tail-flick test [6] using an IITC INC. Mod. 33 Analgesia-meter. Radiant heat was focused 1–2 cm from the tip of the tail and beam intensity was adjusted to give a reaction time of 4–5 sec in intact control animals. To limit damage to the tissue, the beam was switched off after 10 sec (cut off time). As previously reported [2] the spinalization by itself shortened the tail-flick latencies by approximately 25%. All animals were daily handled and adapted to the tail-flick test procedure for 2 weeks before the start of the experiment.

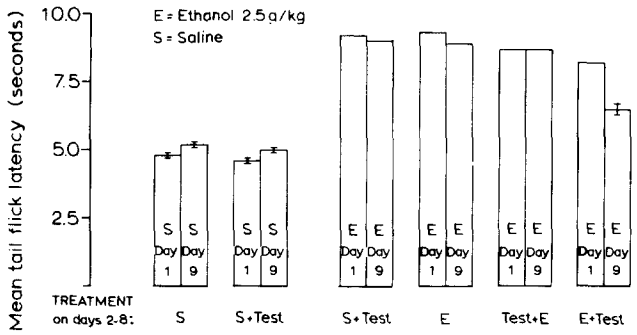

FIG. Tail-flick latency of intact rats, mean±SE (absence of standard error indicates several cut off values in the data). Six groups (n=12 per group). On day 1 and 9, four groups received injections of ethanol 2.5 g/kg IP and two groups received saline. On days 2–8 the groups received different treatments as indicated below the columns. Interval between injection and tail-flick test was 30 min.

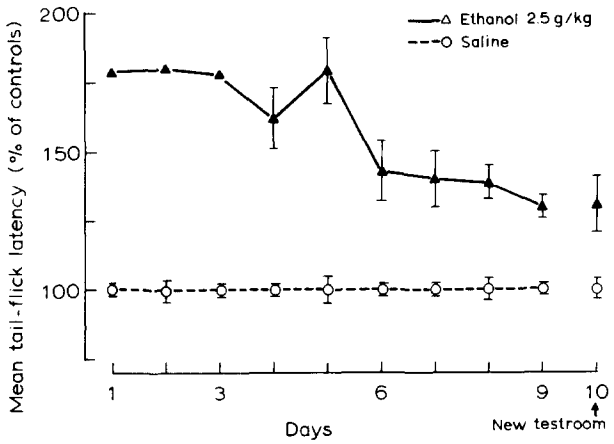

FIG. 2. Time course of tolerance development in tail-flick latency of intact rats (n=12 per group) treated with daily injections of ethanol 2.5 g/kg IP followed by tail-flick testing 30 minutes later. Test values are expressed as percentage of saline controls (n=12 per group). Mean±SE (absence of standard errors indicates several cut off values in the data). The testing took place in the home room on days 1–9, and in a new test room on day 10.

Protocol

In the first experiment intact rats were randomly assigned to six groups (n=12 per group). On day 1 and 9, four groups received ethanol 2.5 g/kg IP and the two remaining groups received the same volume of saline (Fig. 1 illustrates the design). The tail-flick testing was performed on all groups 30 min after the injection. On days 2–8 the four ethanol groups (ethanol on day 1 and 9) were daily injected with either saline followed by testing, ethanol without testing, ethanol followed by testing or ethanol after testing. The two saline groups (saline on day 1 and 9) received on days 2–8 saline without testing and saline followed by testing respectively. During the 9 day period the animals were kept in their home room.

On day 10, the two groups which throughout the experiment consistently received ethanol followed by testing and saline followed by testing, were transferred to a new test room with distinctly different visual, auditory and olfactory stimuli. In the new test room they were again injected and tested as before.

In the second experiment five groups (n=9–12 per group) of spinal rats were used. All groups and procedures were as in the first experiment with the exception that on day 1 and 9 only one group received saline, this group received saline followed by testing on days 2–8.

To avoid bias when testing on the critical day 9, the observer was not aware of the group designation.

Statistics

One-factor ANOVA was used, except in the first experiment where the ethanol groups were evaluated by means of a two-tailed Wilcoxon matched-pairs signed-rank test (due to cut off values in the data). The significance level was set at 5%.

RESULTS

Intact Animals

Figure 1 summarizes the results obtained on day 1 and 9 in the first experiment. On both days, the tail-flick latencies in the four groups receiving ethanol 30 min prior to testing were significantly prolonged compared to the saline controls. Only one group developed tolerance to ethanol during the experiment, showing significantly shorter tail-flick latencies on day 9 than on day 1 ($N=12$, $T=11.5$, $p<0.05$). This group received ethanol followed by testing on days 2–8. The other ethanol groups had identically prolonged tail-flick latencies when tested on day 1 and 9. The mere exposure to ethanol in the period between day 1 and 9 did not cause development of tolerance, neither did repeated testing alone shorten the tail-flick latencies.

Figure 2 shows the time course of tolerance development in the group of intact rats which consistently received ethanol followed by testing throughout the 9 day experiment, as well as their performance in a new test room on day 10. The tail-flick latencies are expressed as percentage of the controls (saline followed by testing). On day 10, when the animals were injected and tested in a new test room, the tail-flick latencies in both the ethanol and the control group were slightly but not significantly increased. However, as it is shown in Fig. 2, the acquired level of tolerance was not influenced, $F(1,11)=1.81$, $p>0.05$.

Spinal Animals

Figure 3 summarizes the results obtained in the spinal rats on day 1 and 9. The results are essentially the same as in the intact animals. Only one group developed tolerance between day 1 and 9, $F(1,10)=9.43$, $p<0.01$, this group was tested during the influence of ethanol on days 2–8. The tendency seen in the group that was repetitively tested before ethanol injections was not reproduced in a later experiment. The tolerant spinal group was also tested in a new test room on day 10. No attenuation of the tolerance was seen (data not shown).

In order to examine for differences between groups in basal tail-flick latencies, acquired as a result of unequal test exposure, all animals were injected with saline prior to testing on day 11. No differences were found between groups.

DISCUSSION

The present study showed that ethanol 2.5 g/kg IP inhibited spinal reflex activity, measured as the tail-flick latency,

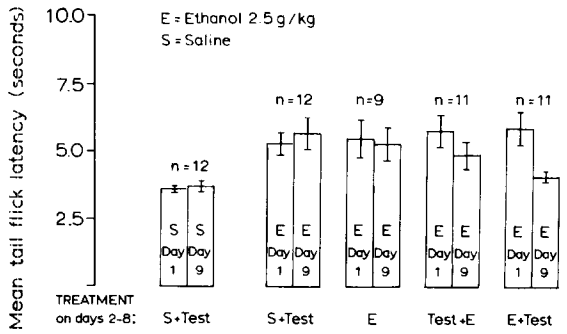

FIG. 3. Tail-flick latency in spinal rats, mean±SE. Five groups (n=9–12 per group). On day 1 and 9, four groups received injections of ethanol 2.5 g/kg IP and one group received saline. On days 2–8 the groups received different treatments as indicated below the columns. Interval between injection and tail-flick test was 30 min.

in both intact and spinal rats. It was also shown that development of tolerance due to daily injections of ethanol for 9 days occurs only under certain conditions. The results in both intact and spinal rats indicate that tolerance is not the consequence of mere exposure to ethanol. The test procedure alone did not influence the tail-flick response, and change in the peripheral receptor sensitivity, due to heat-induced damage of the tissue, was not observed in any of the groups. The development of tolerance in this paradigm seems to require that the animals are repetitively tested in the intoxicated state. Thus, the tolerance may be regarded as learned from practice while intoxicated.

The concept of learned tolerance to ethanol has been proposed in several earlier reports [1, 3, 4, 5, 7, 8, 9, 14, 15]. These reports however, seem to imply that the brain participates in the adaptation leading to tolerance. The present results in spinal rats indicate that adaptive mechanisms may also be localized in the lower spinal cord or in peripheral receptors.

The study does not exclude that the brain influences the isolated lower spinal cord or the peripheral receptors via the systemic circulation. A possible influence however, is not likely to be important for the learned tolerance, since the spinal transection prevents information from the spinal reflex to reach the brain.

In studies concerning tolerance to morphine, it was postulated that learning by association was of major importance [11,12]. The tolerance was dependent on environmental cues that became associated with drug administration during the tolerance acquisition phase. Later, this concept was claimed also to apply to ethanol-induced tolerance [3, 5, 7, 8, 9, 10]. Although conditioning of adaptive mechanisms counteracting the ethanol effects is evidently possible, it is, in our opinion, not proved to be necessary for acquisition of tolerance. In this study we did not attempt to create distinct stimuli in relation to testing, and we found no association between environment and tolerance either in intact or spinal animals, which is in agreement with results on intact rats reported by others [4, 14, 15]. It is also difficult to understand how possible internal stimuli associated with the drug administration could be tied to tolerance, as long as the drug alone did not call adaptive mechanisms into action.

The results in the present study indicate that daily exposure to ethanol 2.5 g/kg for 9 days is sufficient to cause tolerance to ethanol-induced inhibition of a spinal reflex. The acquisition of tolerance is limited to conditions where the neuronal circuits involved in the reflex are activated in the presence of ethanol, which is in agreement with results from electrophysiological studies in an abdominal ganglia preparation from Aplysia [13]. Furthermore, our results indicate that the adaptive mechanisms leading to tolerance may be located outside the brain, in this case probably in the spinal cord. Although the results do not easily fit in with classical conditioning [11,12], it seems reasonable to explain the results in terms of learning.

Further studies are necessary to investigate how results in this paradigm are influenced by increasing the dose of ethanol and by prolongating the observation period.

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