Pharmacology Biochemistry & Behavior, Vol 24, pp 1083–1088, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Learned and Pharmacologically-Induced Tolerance to Ethanol and Cross-Tolerance to Morphine and Clonidine

HUGO A. JØRGENSEN,[1] OLE BERNT FASMER AND KJELL HOLE

Department of Physiology, University of Bergen, Norway

Received 14 February 1985

JØRGENSEN, H A , O B FASMER AND K HOLE *Learned and pharmacologically-induced tolerance to ethanol and cross-tolerance to morphine and clonidine*. PHARMACOL BIOCHEM BEHAV 24(4) 1083–1088, 1986 —The development of tolerance to the inhibitory effect of ethanol on the tail-flick reflex was studied in the spinal rat This preparation was used in order to avoid uncontrolled learning effects Tolerance due to intoxicated practice (learned tolerance) and tolerance due to mere ethanol exposure (pharmacologically-induced tolerance) were studied in separate experiments It was found that learned tolerance to ethanol also caused tolerance to morphine and clonidine, whereas pharmacologically-induced tolerance did not have the same effect The results challenge the concept of "behaviorally augmented tolerance" and suggest that learned and pharmacologically-induced tolerance involve different basal mechanisms in the CNS

Ethanol Morphine Clonidine Tolerance Cross-tolerance Learned Spinal reflex Tail-flick Spinal rats

TOLERANCE to the CNS effect of ethanol is mainly due to a reduced sensitivity to a given tissue concentration of the drug (functional tolerance) The factors of importance for development of this tolerance have been discussed in several studies. Some results [2] suggested that practice during intoxication (learning) was necessary for development of tolerance to the effect of ethanol on maze performance. More extensive studies [11, 14, 16] using maze and moving belt (treadmill) performance indicated, however, that the difference between tolerance development in animals given the opportunity to practice during intoxication and those exposed merely to the drug, was only one of rate. Furthermore, it was suggested that learned tolerance was not task-specific [15]. Tolerance acquired by intoxicated practice on the moving belt would also result in improved maze-performance during intoxication To emphasize that the acquisition rate was the only difference between more conventional pharmacologically-induced tolerance and learned tolerance, the latter was termed behaviorally augmented tolerance (BAT). In studies using morphine, a model of tolerance based on the principles of Pavlovian conditioning has been proposed [19,20]. Environmental cues repeatedly associated with the drug administration will eventually trigger mechanisms that counteract the drug effect and as a consequence, cue-dependent tolerance develops. These principles have been claimed also to be important in the development of tolerance to ethanol [1, 3, 6, 13, 17, 18].

The studies that provided support for the concept of BAT [14,16] have recently been replicated with addition of controls which were not included in the original experiments [24,25]. The results challenged the hypothesis that mere exposure to ethanol was sufficient to induce tolerance and suggested that tolerance is learned as a result of practice during intoxication. In our previous studies we have investigated the development of tolerance to ethanol-induced inhibition of the tail-flick reflex in intact and spinal rats [7,9] The reason for studying the effect of ethanol on the tail-flick reflex in spinal animals is that this preparation allows measuring the effect of a drug and the development of tolerance to it in a restricted part of the CNS. Based on an observation period of 9 days our experiments showed that tolerance developed only when the animals were tested repetitively under the influence of ethanol. Furthermore, the results indicated that Pavlovian conditioning was not likely to be involved.

In the present experiments we have studied how the results obtained with the tail-flick model are influenced by prolongation of the tolerance acquisition period and by increasing the dose of ethanol given in this period Cross-tolerance to morphine and clonidine was also studied. Spinally transected rats were used

METHOD

Animals

Sprague-Dawley rats (Møllegaard, Denmark) weighing 200–300 g at the beginning of the experiment were used. The rats were housed in pairs with free access to water The food was limited to 15 g of pellets per animal per day in order to reduce weight gain The light-phase lasted from 8:00 to 20:00

[1]Requests for reprints should be addressed to Hugo A Jørgensen, MD, Department of Physiology, Årstadveien 19, N-5000 Bergen, Norway

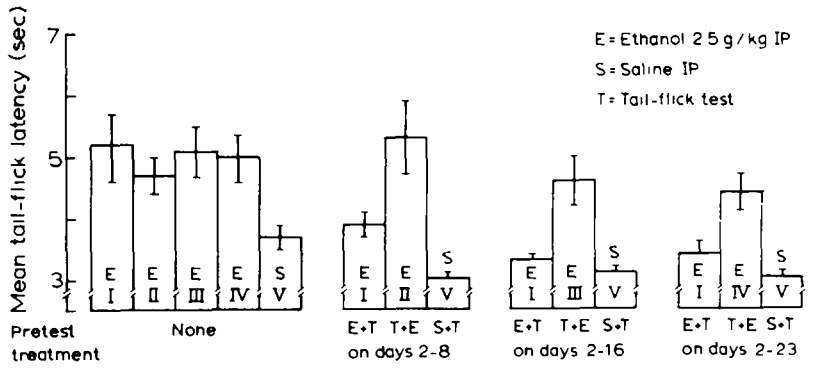

FIG 1 Experiment 1 Tail-flick latency in spinal rats, means±SE Five groups (n=11–15) Initially, the groups were tested 30 min after injections IP of ethanol 2 5 g/kg (I–IV) or saline (V) After treatment with ethanol followed by testing (I), ethanol after testing (II–III–IV) or saline followed by testing (V) for a varied length of time, as indicated below the columns, the groups were again tested after injections of ethanol (I–IV) or saline (V) These latencies were calculated as means of measurements on two consecutive days

hours. Ambient temperature was 22–23°C. All the experiments took place in the colony room between 12·00 and 15:00 hours

Surgery and Drugs

The rats were anesthetized with a combination of pentobarbital (40 mg/kg) and chloral hydrate (180 mg/kg) IP The spinal cord was transected at the level of TH_{9-10} as previously described [8]. Penicillin with protracted effect (Ditardopen, 250,000 IU) was given SC prior to surgery and on the second and the fourth postoperative day. The experiments started 2 weeks after surgery

During the experiments the rats were treated with ethanol 2 5 or 5 g/kg IP (21 or 42 ml/kg of a 15% (v/v) ethanol/isotonic saline solution). Controls received isotonic saline (21 ml/kg) In the cross-tolerance studies morphine hydrochloride 10 mg/kg and clonidine hydrochloride 0 4 mg/kg were also given These drugs were dissolved in isotonic saline and injected in a volume of 2 ml/kg SC

Test Procedure

The sensitivity of the spinal tail-flick reflex was measured with the tail-flick test [4] using an IITC INC. Mod 33 Analgesia-meter The heat stimulus was applied 1–2 cm from the tip of the tail and the intensity was adjusted to give a reaction time of 3–4 sec in spinal control animals The rats were adapted to the test procedure by daily testing during the 2 weeks period between surgery and the start of the experiments During the experiments the animals were in their home cages in the period between drug administration and tail-flick testing.

Determination of the blood alcohol concentration was performed on blood samples from the superior vena cava The samples were assayed enzymatically with test-combination No. 123960 from Boehringer Mannheim

Protocol

In the first experiment we studied the influence of intoxicated practice on development of tolerance during an observation period of 25 days The rats were randomly assigned to 5 groups (n=11–15/group). Four groups (I–IV) received daily injections of ethanol 2 5 g/kg IP One group (V) received the same volume of saline. The interval between injections and tail-flick testing was 30 min On day 1 the groups I–IV were treated with ethanol before testing Group V received saline before testing On days 2–25 group I continued the treatment with ethanol before testing Group II was treated with ethanol after testing on days 2–8 On day 9 and 10 it was switched over to ethanol before testing Group III was treated with ethanol after testing on days 2–16. On day 17 and 18 it was switched over to ethanol before testing Group IV was treated with ethanol after testing on days 2–23 and was switched over to ethanol before testing on day 24 and 25 Group V continued the treatment with saline before testing on days 2–25 (Fig. 1 illustrates the design) On day 26, group I and V both received ethanol 2 5 g/kg and 30 min later blood was sampled for blood alcohol determination

The second experiment was performed to study the effect of daily exposure to mere ethanol using a dose in the tolerance acquisition period that was markedly higher than the final test dose. Nineteen rats were injected on day 1 with ethanol 2.5 g/kg and the tail-flick latency tested 30 min later. Thereafter, the animals were randomized into 2 groups which received injections of ethanol 2.5 and 5 g/kg, respectively, on days 2–8 No testing was performed in this period On day 9 both groups were again tested after injections of ethanol 2 5 g/kg and samples for blood alcohol determination were collected immediately after the testing (Fig. 2 illustrates the design)

In the third experiment it was investigated if learned tolerance to ethanol was transferred to morphine and clonidine. The day before start of the experiment the rats were randomized into 7 groups (n=8–14/group) which were

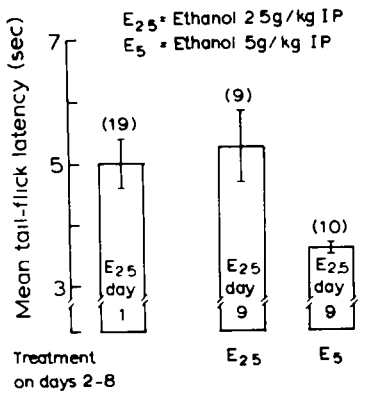

FIG 2 Experiment 2 Tail-flick latency in spinal rats, means±SE Nineteen rats On day 1, all animals were tested 30 min after injection IP of ethanol 2 5 g/kg Hereafter, the rats were randomized into two groups On days 2–8, these groups were daily injected with ethanol 2 5 kg/kg or 5 g/kg On day 9, both groups were again tested after ethanol 2 5 g/kg

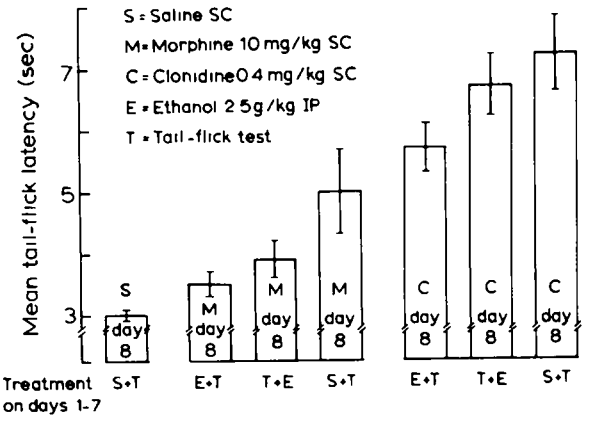

FIG 3 Experiment 3 Tail-flick latency in spinal rats, means±SE Seven groups (n=8–14) On the first 7 days, the groups were daily treated with injections IP of ethanol 2 5 g/kg followed by testing, ethanol after testing or saline followed by testing (as indicated below the columns) On day 8, morphine 10 mg/kg or clonidine 0 4 mg/kg was injected IP in groups representing the three different categories of treatment Saline was injected in a group treated with saline followed by testing The interval between injections and test was 45 min

tested 30 min after injections of ethanol 2.5 g/kg, to make sure that the groups initially reacted with the same sensitivity to ethanol. Then the groups were assigned to 3 different conditions of treatment. On days 1–7, 2 groups received daily injections of ethanol (2 5 g/kg) 30 min before testing, 2 groups received the same dose of ethanol 30 min after testing and 3 groups received saline 30 min before testing On day 8 injections of morphine (10 mg/kg) or clonidine (0 4 mg/kg) were given Each drug was given to 3 groups representing the different treatment conditions The remaining group pretreated with saline before test received saline injections (Fig. 3 illustrates the design). The interval between these injections and tail-flick testing was 45 min.

The fourth experiment was done in order to study if pharmacologically-induced tolerance to ethanol was transferred to morphine and clonidine. The day before start of the experiment the rats were randomized into 6 groups (n=9–12/group) which were tested 30 min after injections of ethanol 2.5 g/kg Then the groups were assigned to 3 different conditions of treatment On days 1–7, 2 groups received daily injections of ethanol 2.5 g/kg, 2 groups received ethanol 5 g/kg and 2 groups received saline. On day 8 injections of morphine or clonidine were given 45 min before tail-flick testing Each drug was given to 3 groups representing the different treatment conditions (Fig 4 illustrates the design)

All the experiments were conducted without knowledge of group designation.

For the statistical analysis one-factor ANOVA was used in experiments 1 and 2 Dunnett's test was used in experiments 3 and 4 The level of significance was set at 5%.

RESULTS

Figure 1 shows the results from the first experiment On the first day of the experiment there was no significant difference between the ethanol groups (I–IV), and all these groups had prolonged tail-flick latencies compared to the saline group (V). After treatment on days 2–8 of the groups I and II with ethanol before test (E+T) and ethanol after test (T+E), respectively, the two groups responded differently to the test dose of ethanol Group I showed significantly shorter tail-flick latencies both compared to group II, $F(1,23)=5.51$, $p<0\ 03$, and compared to its own level on day 1, $F(1,12)=6.42$, $p<0.03$, the latencies of group II (T+E) did not differ significantly from the latencies on day 1. Similar results were obtained after treatment on days 2–16 of group I (E+T) and Group III (T+E). Even when the treatment continued until day 24 for group I (E+T) and group IV (T+E) the two groups responded markedly different to the test dose of ethanol, $F(1,26)=9\ 52$, $p<0.005$ In the groups treated with ethanol after test, the tail-flick latencies tended to decrease with increased treatment period. This tendency, however, was not statistically significant. Group V (S+T) had significantly longer tail-flick latencies on the first day of the experiment compared to later test results. To improve the reliability, the tail-flick latencies registered were calculated as means of the measurements on two consecutive days following the pretest treatment period The blood alcohol concentration measured 30 min after injection of ethanol 2.5 g/kg on day 26 was the same (2.5±0.1 mg/ml, mean±SE) in group I (E+T for 25 days) and group V (S+T for 25 days).

Figure 2 shows the results from the second experiment. The group that received ethanol 5 g/kg without testing in the tolerance acquisition period (days 2–8) developed tolerance, showing significant shorter tail-flick latencies on day 9 both in comparison with its own level on day 1, $F(1,9)=16.45$, $p<0.003$, and in comparison with the group that received ethanol 2 5 g/kg in the same period, $F(1,17)=7.98$, $p=0.01$. The latter showed no reduction of the tail-flick latencies on day 9. The levels of blood alcohol in the samples taken immediately after testing on day 9 were not significantly different in the two groups (1.8±0.1 mg/ml and 1.9±0.1 mg/ml).

Figure 3 summarizes the results of the third experiment. Both morphine 10 mg/kg and clonidine 0 4 mg/kg caused an increase of the tail-flick latencies. The magnitude of the increase was dependent on the kind of pretreatment the

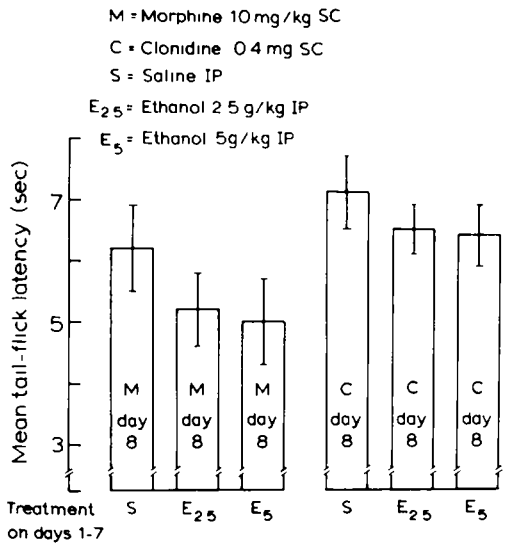

FIG 4 Experiment 4 Tail-flick latency in spinal rats, means±SE Six groups (9–12) On the first 7 days, the groups were daily treated with ethanol 2 5 g/kg, ethanol 5 g/kg or saline (as indicated below the columns) On day 8, morphine 10 mg/kg or clonidine 0 4 mg/kg was injected IP in groups representing the three different categories of treatment The interval between injections and test was 45 min

animals had been exposed to. The groups pretreated on days 1–7 with ethanol 2.5 g/kg before testing were significantly less sensitive to both morphine and clonidine than the groups pretreated with saline before testing, $t_{0\,975}(3\,36)=2\,32$, Cdiff=1 39 and $t_{0\,975}(3\,24)=2\,35$, Cdiff=1 33; Dunnett's test) Compared to the saline groups, the groups pretreated with ethanol after testing also tended to show a reduced sensitivity to both morphine and clonidine The tendency, however, was not significant

Figure 4 shows the results of the fourth experiment The groups pretreated with saline appeared to be the most sensitive to both morphine and clonidine. The groups pretreated with ethanol 2 5 g/kg and 5 g/kg showed a tendency to reduced sensitivity both to morphine and clonidine This tendency was not statistically significant and did not reflect the differences in the pretreatment doses of ethanol

On the day before start of both experiments 3 and 4, the groups had their tail-flick latencies tested 30 min after injections of ethanol 2.5 g/kg No statistically significant differences were observed between the groups in either experiment

DISCUSSION

The results in this study indicate that the factors of importance for development of tolerance to ethanol may depend on the experimental design When the animals in the first experiment, during a period of 25 days, received daily treatment with a fixed moderate dose of ethanol, repeated testing during intoxication seems to be the necessary factor for tolerance development The animals which received the same amount of ethanol without being tested under influence of ethanol developed only a slight, insignificant decrease in their sensitivity to ethanol. In this experiment the saline injected controls (group V) showed significantly different test results in the later part of the experiment when compared to the first day This has not previously been observed in similar experiments There is, however, no reason to believe that this difference will affect the conclusions drawn from the experiment In experiment 2, pharmacologically-induced tolerance developed during a 7 day period without any testing, if the daily treatment consisted of an ethanol dose markedly higher than the final test dose In this case the size of the dose given in the tolerance acquisition period was the decisive factor The measurements of blood alcohol concentration performed after termination of the experiments showed that differences in the exposure to ethanol during the tolerance acquisition periods did not cause differences in the level of blood alcohol 30 min after injection of the test doses of ethanol This indicates that the tolerance measured was functional

Morphine and clonidine, stimulating opiate receptors and alpha adrenoceptors respectively, caused a pronounced prolongation of the tail-flick latencies in control groups In experiment 3, daily testing during ethanol intoxication for a 7 day tolerance acquisition period significantly attenuated the response both to morphine and clonidine Pretreatment with ethanol after testing or ethanol only (even in double doses, experiment 4) did not have the same effect, although a tendency was observed The results in the present cross-tolerance experiments should be interpreted with caution due to the variance in the data However, both the morphine and the clonidine data point in the same direction and the results support the idea that cross-tolerance may occur if the drugs involved cause the same functional disturbances [10,12]

Nearly a decade ago, a series of experiments [11, 14, 16] were performed to study if learning, due to practice during intoxication, was as important for development of tolerance to ethanol as earlier suggested [2] It was found that development of tolerance to ethanol-induced impairment of maze and moving belt performance developed in full within 12 days in animals that daily performed the tasks while intoxicated, and within 20–24 days in animals which received the ethanol after practicing the tasks (to follow the time course of tolerance development the performance during intoxication was observed in all animals every fourth day) To emphasize that the exposure to mere ethanol was sufficient for development of tolerance and that practice during intoxication only accelerated the acquisition of tolerance and did not influence the final level, the phenomenon was designated "behavioral augmentation of tolerance" (BAT) [11,14] It was also found that tolerance acquired by intoxicated practice on the moving belt was not task-specific, but improved maze performance during intoxication as well [15] These findings further supported the hypothesis that BAT and more conventional pharmacologically-induced tolerance were based on the same underlying processes and indistinguishable from each other The fact that the group of animals that was supposed to be in a non-learning situation actually performed the task while intoxicated every fourth day (in connection with tolerance evaluation) was not considered to be a problem, since studies had shown that intoxicated practice every fourth day, with drug-free intervening days, did not cause tolerance development However, results from another study [5] suggested that practice on the moving belt during intoxication every fourth day, combined with ethanol after practice on the intervening 3 days, might be sufficient for learned tolerance to develop This suggestion was confirmed in recent reports [24,25] showing that daily exposure

to ethanol after practice did not cause tolerance to ethanol-induced impairment of moving belt performance during a 23 day period, whereas animals receiving ethanol before practice every fourth day and ethanol after practice on the intervening days reached the same level of tolerance, although delayed, as those receiving the ethanol before practice each day These results challenge the hypothesis that ethanol alone is sufficient to induce tolerance and stress the importance of learning in tolerance development. Furthermore, the difference in tolerance development, depending on whether the testing every fourth day was combined with drug-free intervals or intervals with a daily dose of ethanol after practice [16,25], suggests the possibility that practice important for tolerance development tested on the moving belt most probably also takes place during intoxication in the home cage. The transferance of BAT from the moving belt to the maze test [15] might be explained along the same line The lack of cross-learning which was found in the non-intoxicated state may not exclude that functions improved by intoxicated practice in one test can facilitate performance during intoxication in another test situation The learning effects can be difficult to avoid and a recent discussion [21,23] illustrates that it is still debated whether functional tolerance to ethanol is learned or not

The tail-flick reflex in spinal animals has no neural contact with the supraspinal part of the CNS and it is our experience that the reflex is elicited only by intended stimulation. For drugs inhibiting the tail-flick reflex, this model allows evaluation of drug effect and tolerance development in a restricted part of the spinal cord, not influenced by other parts of the CNS. Thus, the tail-flick latencies in the spinal animal do not seem to be influenced by uncontrolled associative learning or by other possibly confounding factors, such as sensitization of the skin (due to the thermal stimulation), procedural stress or changes in body temperature [7, 8, 9] By means of the tail-flick test in spinal rats we have found that both learned and non-learned tolerance to ethanol may occur. In the earlier reports discussing BAT [5, 14, 15, 16, 24, 25], the daily dose of ethanol given in the tolerance acquisition period usually did not exceed the final test dose. On this condition our results support the studies that challenge the concept of BAT [5, 24, 25] When a fixed daily dose of ethanol 2 5 g/kg was given during an observation period of 25 days, learning, in the form of intoxicated practice, seems to be necessary for tolerance development Whether this is true for longer observation periods than 25 days, we do not know The interference of non-functional tolerance may be a problem with longer observation periods Many studies have assumed the existence of pharmacologically-induced tolerance The interpretation of the results has, however, been hampered by the possibility of uncontrolled learning effects On the condition that the doses of ethanol given in the tolerance acquisition period are higher than the final test dose, our results support that tolerance can be induced pharmacologically

The learned tolerance to ethanol-induced impairment of the tail-flick reflex, and of other test-performances, seems to be caused by a learning process localized to the neuronal circuits involved in the task. The learning takes place when the circuits are repetitively activated during intoxication and will eventually cause a normalization of the impaired function. However, tolerance has been shown also to develop without task-specific stimulation. An attractive possibility is that if the exposure to ethanol in the tolerance acquisition period is strong enough (as in experiment 2), then the spontaneous neural activity during intoxication might provide a sufficient opportunity for the learned tolerance to develop. If that is the case, learned tolerance may, under certain circumstances, develop without any stimulus-enhanced activation of the neuronal circuits. According to this hypothesis the distinction between learned and pharmacologically-induced behavioral tolerance to ethanol will become meaningless as the meaning of the word "learning" becomes superfluous. The hypothesis seems compatible with most results concerning development of functional tolerance to ethanol. The observation that tolerance development to the effect of barbiturates on EEG is more pronounced in a behavioral state with high than with low neuronal activity [22] may support the hypothesis as well. However, our own experiments showed that learned tolerance to ethanol caused tolerance also to morphine and clonidine, whereas the same was not found to be true for pharmacologically-induced tolerance to ethanol These results challenge both earlier results [15] and the hypothesis discussed here, suggesting that different kinds of neural mechanisms are involved in learned and pharmacologically-induced tolerance.

In conclusion, our results suggest the existence of both learned and pharmacologically-induced tolerance to ethanol Furthermore, the results from the cross-tolerance studies suggest the possibility that the two kinds of tolerance may involve different kinds of basal mechanisms. These results are not in agreement with the concept of BAT. Further investigations are, however, needed in order to achieve more pervasive hypotheses in the field Especially, the suggestion of different basal mechanisms in learned and non-learned tolerance needs to be confirmed using different methods.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr B Srebro for helpful discussions We also thank Mrs Anita Kloster for excellent technical assistance The research was supported by the Norwegian Institute of Alcohol Research

REFERENCES

1 Cappell, H , C Roach and C X Poulos Pavlovian control of cross-tolerance between pentobarbital and ethanol *Psychopharmacology (Berlin)* **74**: 54–57, 1981

2 Chen, C S A study of the alcohol-tolerance effect and an introduction of a new behavioural technique *Psychopharmacologia* **12**: 433–440, 1968

3 Crowell, C R , R E Hinson and S Siegel The role of conditional drug responses in tolerance to the hypothermic effects of ethanol *Psychopharmacology (Berlin)* **73**: 51–54, 1981

4 D'Amour, F E and D L Smith A method for determining loss of pain sensation. *J Pharmacol Exp Ther* **72**: 74–79, 1941

5 Gallaher, E J The kinetics of alcohol tolerance in the rat the effects of practice Thesis, University of Washington, Seattle, 1976

6 Hinson, R E and S Siegel The contribution of pavlovian conditioning to ethanol tolerance and dependence In *Alcohol Tolerance, Dependence and Addiction*, edited by H. Rigter and J C Crappe Amsterdam Elsevier/North Holland Biomedical Press, 1980, pp 181–199

7 Jørgensen, H A and K Hole Learned tolerance to ethanol in the spinal cord *Pharmacol Biochem Behav* **20:** 789–792, 1984
8 Jørgensen, H A , O B Fasmer, O -G Berge, L Tveiten and K Hole Immobilization-induced analgesia Possible involvement of a non-opioid circulation substance *Pharmacol Biochem Behav* **20:** 289–292, 1984
9 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:** 293–295, 1985
10 Kalant, H Comparative aspects of tolerance to, and dependence on, alcohol, barbiturates and opiates In *Advances in Experimental Medicine and Biology*, vol 85B, edited by M M Gross New York Plenum Press, 1977, pp 169–186
11 Kalant, H , A E LeBlanc and R J Gibbins Tolerance to, and dependence on, some non-opiate psychotropic drugs *Pharmacol Rev* **23:** 135–191, 1971
12 Khanna, J M , A D Lê, H Kalant and A E LeBlanc Cross-tolerance between ethanol and morphine with respect to their hypothermic effects *Eur J Pharmacol* **59:** 145–149, 1979
13 Lê, A D , C X Poulos and H Cappell Conditioned tolerance to the hypothermic effect of ethyl alcohol *Science* **206:** 1109–1110, 1979
14 LeBlanc, A E , R J Gibbins and H Kalant Behavioral augmentation of tolerance to ethanol in the rat *Psychopharmacologia* **30:** 117–122, 1973
15 LeBlanc, A E , R J Gibbins and H Kalant Generalization of behaviorally augmented tolerance to ethanol and its relation to physical dependence *Psychopharmacologia* **44:** 241–246, 1975
16 LeBlanc, A E , H Kalant and R J Gibbins Acquisition and loss of behaviorally augmented tolerance to ethanol in the rat *Psychopharmacology (Berlin)* **48:** 153–158, 1976
17 Mansfield, J G and C L Cunningham Conditioning and extinction of tolerance to the hypothermic effect of ethanol in rats *J Comp Physiol Psychol* **94:** 962–969, 1980
18 Melchior, C L and B Tabakoff Modification of environmentally cued tolerance to ethanol in mice *J Pharmacol Exp Ther* **219:** 175–180, 1981
19 Siegel, S Evidence from rats that morphine tolerance is a learned response *J Comp Physiol Psychol* **89:** 498–506, 1975
20 Siegel, S Morphine tolerance acquisition as an associative process *J Exp Psychol (Anim Behav)* **3:** 1–13, 1977
21 Tabakoff, B , C L Melchior and P Hoffman Factors in ethanol tolerance *Science* **224:** 523–524, 1984
22 Wahlstrom, G Differences in tolerance to hexobarbital (enhexymalum NFN) after barbital (diemalum NFN) pretreatment during activity or rest *Acta Pharmacol Toxicol* **26:** 92–104, 1968
23 Wenger, J R and S C Woods Factors in ethanol tolerance *Science* **224:** 524, 1984
24 Wenger, J R , V Berlin and S C Woods Learned tolerance to the behaviorally disruptive effects of ethanol *Behav Neural Biol* **28:** 418–430, 1980
25 Wenger, J R , T M Tiffany, C Bombardier, K Nicholls and S C Woods Ethanol tolerance in the rat is learned *Science* **213:** 575–577, 1981