Pharmacology Biochemistry and Behavior, Vol. 3, pp. 201–204. ANKHO International Inc., 1975. Printed in the U.S.A.

Metrazol Impairs Conditioned Aversion Produced by LiCl: A Time Dependent Effect

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(Received 31 October 1973)

MILLNER, J. R. AND T. PALFAI. *Metrazol impairs conditioned aversion produced by LiCl: A time dependent effect.* PHARMAC. BIOCHEM. BEHAV. 3(2) 201–204, 1975. – The effects of 40 mg/kg Metrazol on a conditioned saccharin aversion produced by LiCl were studied in two experiments. In Experiment 1, it was found that Metrazol administered 10 min before or after LiCl did not impair conditioned aversion to saccharin. In Experiment 2, Metrazol was given 2 min before, 0 or 3 min after the administration of LiCl. Under these conditions, impairment did occur. It was concluded that Metrazol may impair conditioned taste aversion in a time-dependent manner. The present findings are discussed in terms of their relationship to ECS as an interfering agent and retroactive and proactive effects on the CS and/or the UCS.

Amnesia Conditioned aversion Metrazol Lithium chloride

IN a conditioned taste aversion paradigm, a novel taste, e.g. saccharin, serves as a conditioned stimulus (CS). When the CS is followed by toxicosis, the unconditioned stimulus (UCS), subsequent intake of the novel taste solution is suppressed [4, 9, 16]. Toxicosis has been induced by x-irradiation [17], apomorphine [1], lithium chloride [9,11], and various other agents [2, 3, 5].

There are at least two aspects of this paradigm that make it particularly interesting for the study of memory storage processes. First, often a single CS-UCS pairing is sufficient to produce considerable suppression; second, the CS-UCS interval may be extended up to 12 hours [17], and still result in learning. Such an extended CS-UCS interval permits an investigation of the effects of neurological traumas induced by electroconvulsive shock (ECS) or Metrazol (Met) on both the CS, as well as a retroactive effect on the CS-UCS pairing.

Recently, several studies focused on the effects of ECS on learned taste aversions induced by LiCl [7, 8, 10]. Kral [6] reported that ECS interfered with learned taste aversion regardless of the time of administration, as long as it occurred during the CS-UCS interval. In a subsequent report [8], however, it was found that retroactive impairment only occurred when the LiCl injection was immediately followed by ECS. Partial impairment was observed if the ECS was administered 2.5 min after the LiCl, whereas no impairment was seen if ECS followed LiCl by 5, 10, or 15 min. Since Kral and Beggerly [8] observed that the rat "first begins to show signs of toxicosis within approximately 10 min of LiCl injection", the reported impairment may have been the result of a time-dependent interference with the CS trace, a proactive effect on the US trace, and/or interference with the development of toxicosis.

Ahlers and Best [1], on the other hand, reported that Metrazol-induced seizures will interfere not only with the association of the CS trace with the UCS, but will impair retroactively an established CS-UCS association. Among several procedural differences in the latter study [1], apomorphine toxicosis was used as the UCS. The use of this drug as the toxic agent necessitated two CS-UCS pairings to produce reliable suppression. In contrast, with LiCl a single pairing results in suppression. Thus, the difference in reinforcement magnitude shown to be an important variable in other paradigms [12, 13, 15], may have been responsible for the difference in findings.

Considering this possibility, the present study investigated the effect of Metrazol induced seizures on saccharin aversion produced by LiCl during and following CS-UCS association.

EXPERIMENT 1

In the Ahlers and Best study [1], Metrazol was administered either 15 min before or 120 min after the apomorphine injection. In both instances, Metrazol interfered with or impaired conditioned aversion. In the present study, therefore, it was hypothesized that Metrazol given 10 min

[1] This research was supported by MH-23225-01 from the Department of Health, Education, and Welfare, Public Health Service, to the second author.

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following the UCS should produce similar impairment if LiCl were used.

Method

Animals. Forty-six naive male hooded rats (300–350 g) from the Syracuse University colony were used. They were housed in wire-mesh cages in a temperature (72°) and humidity (50%) controlled room with a 12-hour light–dark cycle. Purina lab chow was available ad lib throughout the experiment, in the home cage. Food was never present in the test box.

Apparatus. Drinking was measured in a Plexiglas box with a grid floor (test box). An elliptical opening (1 in. long, 0.5 in. wide) in the front panel of the box allowed access to an outside fluid source. This consisted of a 30 cc disposable syringe modified to hold a metal drinking spout which could only be contacted by the animal's tongue. The test apparatus was housed in a lighted (40 W) refrigerator shell to attenuate external sound; the shell was equipped with an air-exhaust system and a Plexiglas viewing window.

Procedure. During the initial 5 days of the experiment, ad lib food and water intakes were recorded in the home cages. Following this period, the animals were placed on a 23 1/2 hour water deprivation schedule for the remainder of the experiment. Each animal received water for 30 min per day for five days: 15 min in the test box after which the animal was immediately returned to its home cage, and 15 min in the home cage 45 min after the experimental session.

On Day 6 (training day), all groups had access to a 0.2% sodium saccharin (Merck) solution in the test box for 15 min. Following the session the animals were taken to their home cages. Ten minutes later, each animal received an intraperitoneal (I.P.) injection of either LiCl, Met or distilled water (DW). A second injection was administered 10 min after the first.

The animals were divided into 6 experimental groups (N = 6 each) and one control group (N = 10). The experimental groups received one of the following combinations of injections: LiCl-Met, Met-LiCl, LiCl-DW, DW-LiCl, Met-DW, and DW-Met. The control group received DW-DW. The dose for LiCl (Mallinckrodt) was 125 mg/kg (concentration – 125 mg/cc), and for Met (Knoll Pharmaceuticals) it was 40 mg/kg (concentration – 100 mg/cc). The DW injection was 1 cc/kg body weight.

On Day 7, all animals were given a 15 min water test in the test box. Animals that did not drink an amount equal to (within 1 cc), or greater than their baseline water intakes were given an additional water test on Day 8. Animals which continued to suppress water intake, indicating generalized suppression, were discarded. Two animals did not meet this criterion, and were discarded.

Day 8 was saccharin test day; all animals had access to the saccharin solution in the test cage for 15 min. The dependent measure was saccharin intake; aversion was expressed in terms of suppression ratios (SR):

$$SR = \frac{\text{Day 8 saccharin intake}}{\text{Day 6 saccharin intake + Day 8 saccharin intake}}$$

An SR of 0.500 was taken to indicate no suppression; SR's greater or less than 0.500 were considered facilitation and suppression, respectively.

Results and Discussion.

Seizures were observed in 68% of the animals given Metrazol, within 2 min of injection. All animals given LiCl showed signs of toxicosis: ataxia approximately within 3 min following injection.

Mean SR's and standard deviations are shown in Fig. 1.

An overall analysis of variance indicated significant differences between the groups, $F(6,39) = 11.62$, $p<0.01$, Z scores were then computed by subtracting each group SR mean from the theoretical SR mean (0.500). This procedure allowed the assessment of either suppression or facilitation within each group. All groups given LiCl suppressed saccharin intake significantly on Day 8 (LiCl-DW: $Z = 2.42$; DW-LiCl: $Z = 2.41$; LiCl-Met: $Z = 2.54$; Met-LiCl: $Z = 3.09$; all p's < 0.01). These data indicate that Metrazol given 10 min before or after a LiCl injection did not impair the acquisition or the retention of a conditioned aversion. Other comparisons were not significant (DW-MET: $Z = 0.69$; Met-DW: $Z = 0.79$; p's>0.10), which suggests that Metrazol given 10 or 20 min following a saccharin solution does not itself produce conditioned aversion.

EXPERIMENT 2

The results of Experiment 1 are different from the findings of Ahlers & Best [1], who reported impairment of conditioned taste aversion if Metrazol was given 15 min before or 120 min after apomorphine injection. A possible explanation of this difference may be in our use of LiCl as the UCS, LiCl being a more potent toxic agent. Since the nature of the reinforcement has been reported to affect the interferability gradient [12, 13, 15] in other tasks, it is possible that with shorter intervals Metrazol may produce impairment of LiCl induced conditioned aversion, as has been shown with ECS [8,10]. Similarities between the effects of ECS and Metrazol seizures have been pointed out elsewhere [13,14], consequently, the present experiment investigated the possible time-dependent effect of Metrazol on LiCl induced conditioned aversion.

Method

Procedure. Twenty-four naive male rats of the same description as in Experiment 1 were used. The apparatus and procedures were the same as used previously with 3 experimental groups (N = 6 each) and one control group (N = 6). All experimental groups received a LiCl injection 10 min after the 15 min saccharin training session on Day 6. One of the experimental groups (Met-2 min LiCl) received Metrazol 2 min before LiCl while the other two groups (LiCl-Met-0 min; LiCl-Met-3 min) received Metrazol immediately or 3 min after the LiCl injection. The control group received two DW injections 10 min after the training session.

Since the behavioral pharmacological effects of Metrazol appear within approximately 2 min and those of LiCl within 3 min of injection, one may suggest that any effect of Metrazol in the LiCl-Met-0 min group may be confounded in that the interaction of the drugs may prevent the animal from fully experiencing toxicosis. While controlling for this possibility is not feasible, two additional control groups were given I.P. injections of Nembutal (60 mg/kg in place of Met. These groups were LiCl-Nem-0 min and DW-Nem-0 min (N = 6 each.)

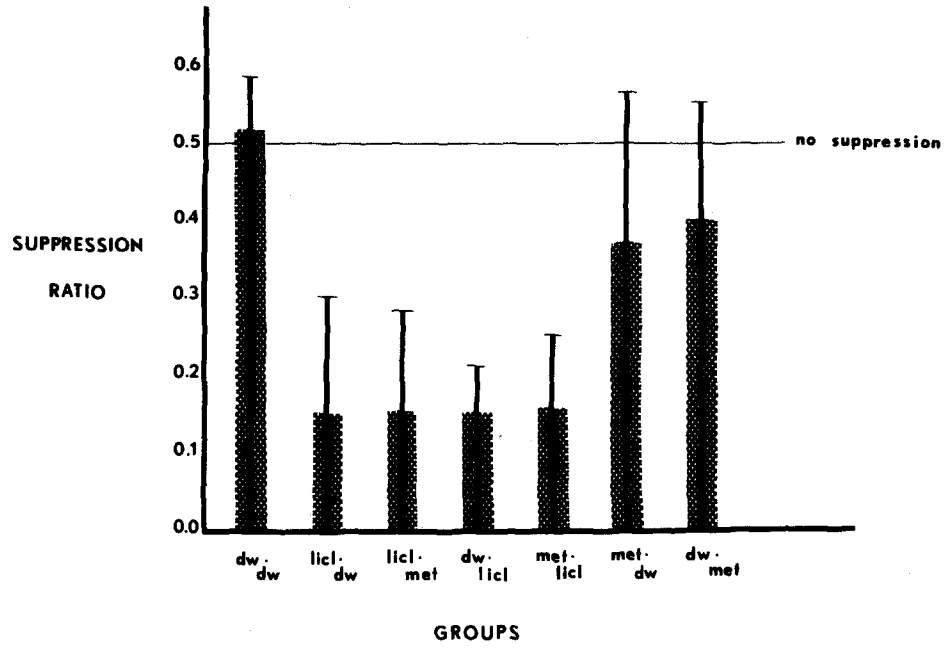

FIG. 1. Mean suppression ratios and standard deviations of group saccharin intakes in Experiment 1.

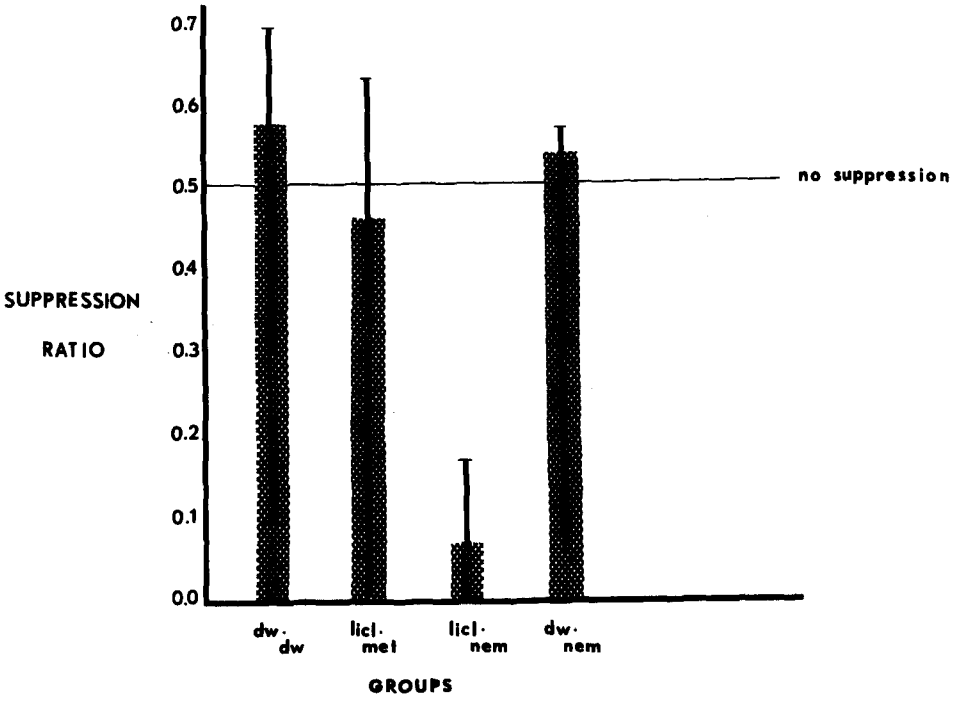

FIG. 2. Mean suppression ratios and standard deviation of group saccharin intakes in Experiment 2.

Results and Discussion

Seizures were observed in 83% of the animals receiving Metrazol. A total of 7 animals had to be replaced for either failing to meet the water test criterion, or dying following tonic convulsions. Nembutal produced anesthesia (loss of the righting reflex) within approximately 3 minutes of injection.

The data were analyzed as previously, and are presented in Fig. 2. Two groups from the previous experiment (Met-LiCl and LiCl-Met) have been added to Fig. 2 to illustrate the time-dependent effect of Metrazol.

An overall analysis of variance indicated significant differences between the 6 groups, $F(5,30) = 15.03$, $p<0.01$. Following the computation of Z scores, it became apparent

that animals receiving Metrazol did not suppress saccharin intakes on Day 8 (LiCl-Met-0 min, Z = 0.26; LiCl-Met-3 min; Z = 0.99; Met-LiCl-2 min; Z = 0.24; p's>0.10 in all cases). These data suggest that Metrazol can interfere with LiCl induced conditioned aversion. Significant suppression was observed in the LiCl-Nem-0 min (Z = 3.63; $p < 0.01$) while no effect, or a slight faciliatation were observed in the DW-DW and DW-Nem-0 min groups. These latter findings suggest that Nem does not impair LiCl induced conditioned aversion, nor does it induce conditioned aversion.

GENERAL DISCUSSION

The effect of Metrazol (40 mg/kg) on a LiCl induced conditioned aversion was investigated. The results indicate a time-dependent Metrazol effect. When Metrazol is administered 2 min before, immediately following, or 3 min after LiCl injection, conditioned aversion to a novel taste is impaired. Metrazol administered 10 min before or after the LiCl injection, on the other hand, does not interfere with the development of a conditioned aversion.

At least two interpretations of these data are possible. First, Metrazol may interfere with consolidation of memory in a time-dependent manner. One implication of this suggestion is that in some respects, the nature of the neurobiological mechanism underlying acquisition of a conditioned aversion may be similar to other forms of learning. Data reported with ECS [8,10] are in agreement with this possibility.

Second, the close temporal proximity of the pharmacological effects of Metrazol to the LiCl toxicosis may modify or attenuate the LiCl effect, and presumably affect acquisition. The findings of Ahlers and Best [1] indirectly and some aspects of the present data directly make this a less likely interpretation. Injection of Metrazol 120 min after CS-UCS pairing has been reported [1] to impair conditioned aversion induced by apomorphine. In our study, injection of Met 3 min after LiCl administration also impaired the development of a conditioned aversion. In this group, LiCl produced ataxia was already apparent when the Met injection occurred, and seizures were typically observed 2 min later, well after the apparent toxicosis. In addition, simultaneous onset of drug effects may not necessarily imply attenuation of toxicosis and impairment of acquisition. Although Nem is an inadequate control for the pharmacologic effect of Met, its behavioral effects coincided with and lasted for the duration of the LiCl effect, but interference was not observed.

These findings are also relevant to an issue recently investigated by Kral and Beggerly [8]. Is the impairment of conditioned aversion following an interfering agent the result of interference with the CS trace, a proactive effect on the UCS, or interference with some association mechanism? The fact that Metrazol given 10 min after the novel saccharin taste and 10 min prior to LiCl failed to interfere with the CS-UCS association argues against interference with a hypothetical association mechanism.

Furthermore, the fact that Metrazol was given 10 min after the CS in both Met-LiCl and LiCl-Met-0 groups, but only produced interference in the latter group suggests that interference with the CS trace also probably did not occur.

The present data show that Metrazol impairs conditioned aversion in a time-dependent manner. The essential variable appears to be a close temporal proximity of the drug administration to the UCS, whether it is given before or after the CS-UCS association. Both prograde and retrograde impairments have been reported following the use of this drug in other learning paradigms [13,14]. It appears both of these phenomena are occurring in the present study.

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