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LiCl-Induced Selective Depression of Saccharin Drinking in the Mouse

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HAMBURGER, J. N. AND C. L. KUTSCHER. *LiCl-induced selective depression of saccharin drinking in the mouse.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 651–655, 1979.—Water-deprived mice were injected with various concentrations of LiCl or NaCl 15 min before they were allowed to drink either water or 0.1% saccharin. The NaCl injections produced a dose-dependent increase in intake of both fluids: however, the higher dosages of LiCl produced a selective depression of fluid intakes. Saccharin intakes were depressed for less than one hr but water intakes were not affected. LiCl injections also depressed general activity and produced an apparent shift of water from blood into cells. The LiCl-induced depression of saccharin was not significantly influenced by extensive previous experience with the drinking fluid.

Cell dehydration Drinking LiCl Saccharin Selective depression Taste aversion

HYPERTONIC NaCl injections reliably increase water intakes of water-deprived rats; however, when deprived rats were offered a 0.01% quinine hydrochloride (QHCl) solution instead of water, intakes were depressed compared to control-injected animals [1]. It was suggested that the volume of fluid ingested is a resultant of the taste characteristics of the fluid and certain internal states of the animal, in this case apparently cellular dehydration. The original observation was replicated by us; however, it was found that LiCl is much more effective than NaCl (in terms of molarity) in the production of a transient quinine aversion in rats [9,10]. We have called this phenomenon selective depression since LiCl produced either no change or an increase in water intake. The phenomenon is not specific to QHCl drinking since selective depression has been produced for intake of saccharin [3], NaCl [5] and vinegar [3]

In order to study the generality of the taste aversion observed in the rat, we studied selective depression in another species, the mouse, attempting to identify some of the variables which influence it. Specifically, we were interested in: (1) a comparison of the dose-response relationships for both LiCl and NaCl in the production of selective depression for a palatable saccharin solution; (2) the duration of the taste aversion produced; (3) the effect of previous exposure to the drinking fluid in the establishment of selective depression. This last point is particularly important in the comparison of selective depression to conditioned taste aversion since the latter is usually precluded if the animal has had as little as one trial of experience with the taste fluid intended to be the conditioned stimulus, perhaps because the animal has already learned that the fluid is "safe" [7].

EXPERIMENT 1

In this experiment, the efficacy of NaCl and LiCl injections was compared over the range tolerated by the animal.

METHOD

Apparatus.

Mice were maintained during the experiment in individual cages (15.4 x 12.0 x 14.4 cm). Cages were constructed of steel with wire mesh tops and clay bedding on the floors. Mice were maintained on demineralized water and Purina Chow pellets which were continuously available except during the selective depression test. Lights were on for 14 hr per day. Air temperature was maintained at 20 ± 1°C. and air was humidified during the winter months. Water intakes were measured in 100 ml gas-measuring tubes graduated in 0.2 ml units.

All injections were made IP with 1 ml plastic syringes and no. 25 hypodermic needles. During the drinking tests, the mice were offered either demineralized water or a 0.1% sodium saccharin solution mixed in demineralized water.

Animals.

One hundred-thirty, naive, female white Swiss mice, 60 days of age were used in this experiment. They were bred in the laboratory from CD-1 stock obtained from Charles River.

Procedure

One week before the selective depression tests, mice were removed from plastic group living cages and were placed in the individual steel cages. During this period, on two occasions mice were deprived of water for 24 hr and were given a subsequent 15-min access to water (no food present) in order to facilitate approaching the drinking tube and drinking. For the selective depression tests, mice were water-deprived for 24 hr, weighed and injected with either LiCl or NaCl, 1% deprivation body weight. LiCl concentrations injected were 0, 0.12, 0.25, 0.50, 0.75 M and NaCl concentrations were 0, 0.12, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0

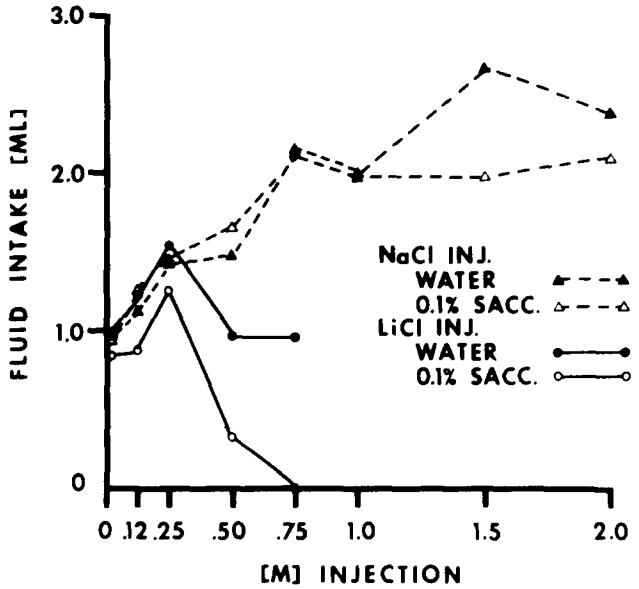

FIG. 1. Water and saccharin intakes as a function of concentration of NaCl and LiCl injection.

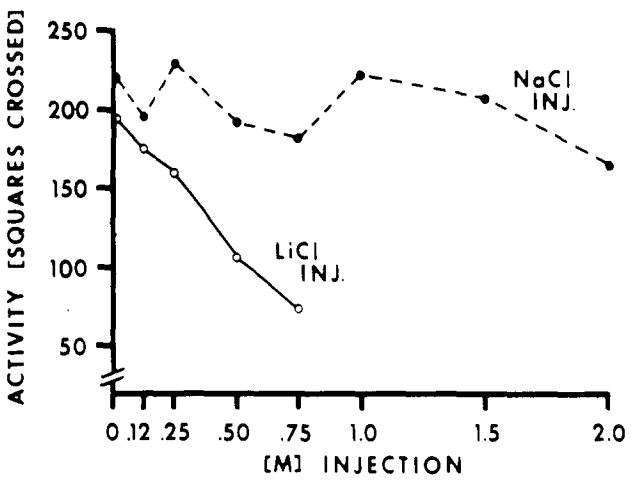

FIG. 2. Three-min activity scores in the open field as a function of concentration of NaCl and LiCl injection.

M. Ten mice were injected with each concentration. Five were subsequently permitted to drink water and 5 were given 0.1% saccharin.

Ten minutes after the injection, mice were removed from the cage and were placed in an open field 91.4 cm square ruled into squares 11.5 cm on a side and painted gray. The open field was surrounded by a fence 30.5 cm high. Illumination was provided by a 75 W white light bulb placed 50 cm above the center of the apparatus floor. Number of lines crossed with the front feet in 3 min was counted for each animal. Mice were subsequently returned to their cages where, 15 min following injection, the drinking tube was inserted and a 15-min drinking period allowed (no food present).

Following this test, food and water were returned for 2 days. Mice were then deprived of water for 24 hr and were offered for 15 min the solution they were given during the selective depression test in order to check for the presence of conditioned taste aversion. After 3 more days of ad lib food and water intakes, mice were water-deprived for 24 hr and were injected with the injection dosage they had received during the selective depression test. Fifteen min following the injection, mice were sacrificed with ether. The pericardial cavity was opened and 0.5 ml of blood was withdrawn from the heart into a heparinized plastic syringe. Blood samples were centrifuged in straight-walled glass serum tubes at 11,500 rev/min for 15 min. Plasma was analyzed for protein concentration on a Bausch and Lomb protein meter and for osmolality on an Osmette osmometer. Hematocrits were determined by measuring height of red blood cells and total column of blood.

RESULTS

The fluid intakes of NaCl-injected and LiCl-injected mice were analyzed separately with a two-way analysis of variance (water and saccharin intakes x injection dosage). Comparisons between individual means were evaluated with a Tukey (a) test (8). For LiCl-injected mice, fluid intakes were influenced by dosage levels of injection, $F(4,40)=8.35$; $p<0.01$, and type of solution ingested, $F(1,40)=18.72$; $p<0.01$. Interaction between these two variables was not significant. Post hoc tests showed that the 0.50 M LiCl dosage depressed saccharin intake below the level of the 0 M LiCl group and the 0.75 M injection virtually abolished saccharin drinking (Fig. 1). Water intakes were not significantly depressed by an LiCl dosage.

In the NaCl-injection condition, water and saccharin intakes increased with increasing concentrations of NaCl $F(7,64)=13.06$; $p<0.01$. Saccharin intakes were not different from water intakes and there was no significant interaction between the two variables.

No evidence of conditioned aversion was seen in both LiCl-injected and NaCl-injected mice, i.e. a significant decrease in test fluid intake as a function of injection concentration.

The activity scores are shown in Fig. 2 with scores from both saccharin and water drinking animals combined at each dosage level. The data from NaCl-injected and LiCl-injected mice were analyzed in separate one-way analyses of variance. Activity decreased significantly as a function of LiCl dosage, $F(4,45)=9,91$; $p<0.01$, but not as a function of NaCl dosage. There was a significant positive correlation between the activity prior to the selective depression test and the amount of 0.1% saccharin solution consumed during the subsequent 15-min drinking test ($r=+0.52$; $p<0.01$) by the LiCl-injected animals. No significant correlation was found between activity and water intakes for the LiCl-injected animals or between saccharin intakes or water intakes and activity for the NaCl-injected animals.

The effects of LiCl and NaCl on blood characteristics are shown in Fig. 3. LiCl injections significantly increased hematocrit $F(4,39)=12.06$; $p<0.01$, and plasma protein concentration, $F(4,38)=8.16$; $p<0.01$, as a function of LiCl dosage, but plasma osmolality was not changed significantly. The pattern of change produced by the NaCl injections was quite different. Plasma osmolality increased as a function of NaCl dosage, $F(7,65)=71.00$; $p<0.01$. No significant change

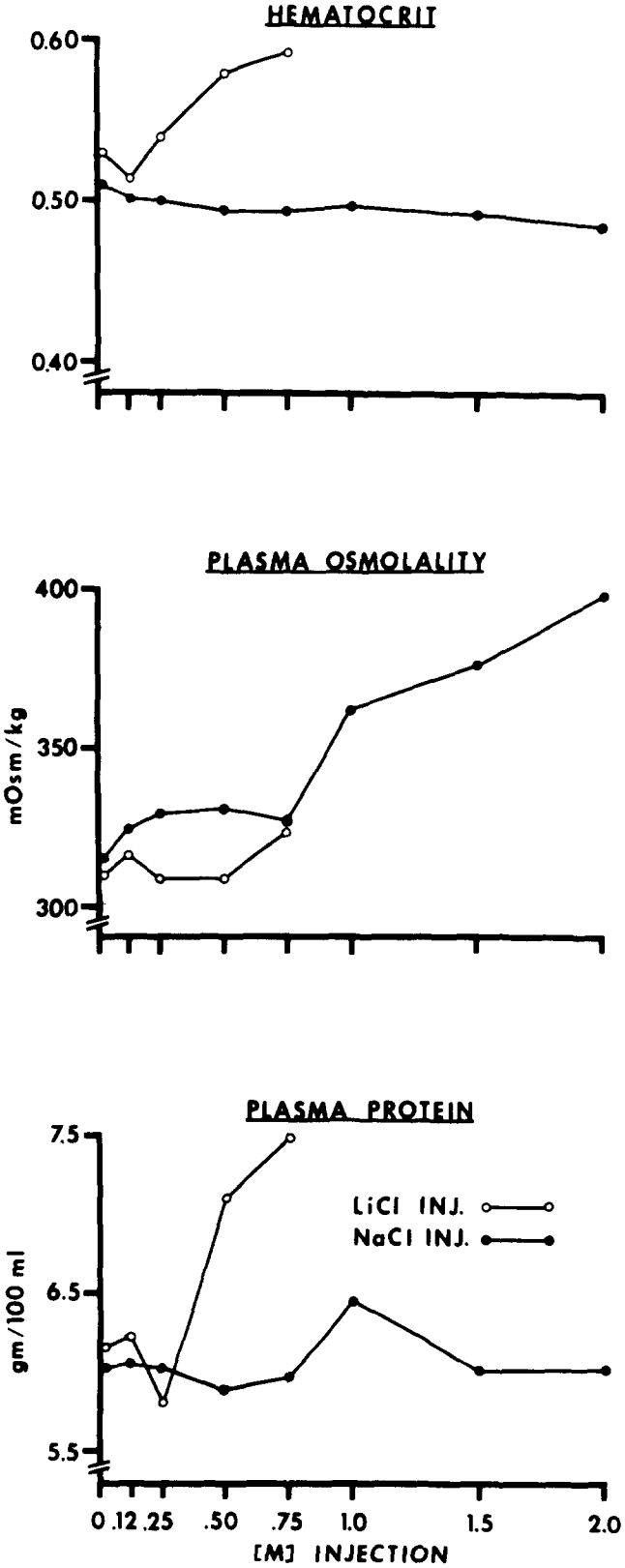

FIG. 3. Blood characteristics 15 min following injection as a function of concentration of NaCl or LiCl injection. This delay is the same as that between injection and the beginning of the selective depression test.

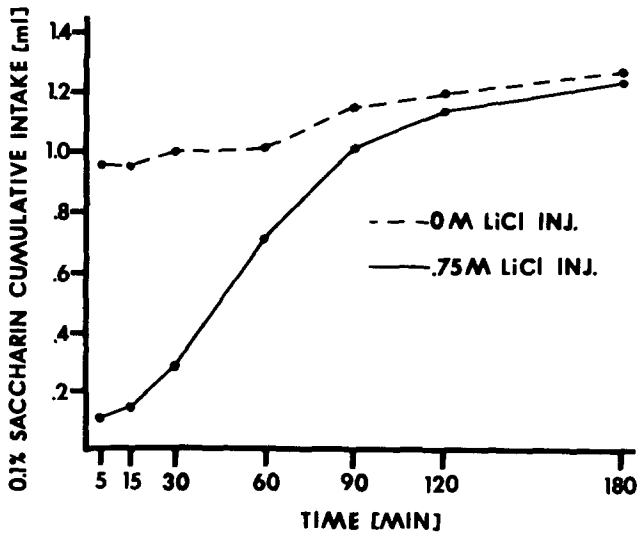

FIG. 4. Cumulative saccharin intakes following LiCl or control injections.

appeared in hematocrit. Plasma protein differed as a function of injection dosage, $F(7,66)=3.20$: $p<0.05$, apparently because the protein level of the 1.0 M-injected group was significantly higher ($p<0.01$) than that of the 0 M group.

EXPERIMENT 2

Clearly, the 0.5 and 0.75 M LiCl dosages produced selective depression as they decreased saccharin intake, but not water intake. In this experiment, we measured saccharin intake for a 3-hr period post-injection to determine when the inhibition of drinking dissipates.

METHOD

Thirty naive, female CD-1 mice were used in this experiment. Preinjection procedures were the same as described above. Fifteen mice were injected with 0.75 M LiCl and fifteen were injected with 0 M LiCl. Fifteen minutes following injection, they were presented with 0.1% saccharin to drink and cumulative intakes were recorded at 5, 15, 30, 60, 90, 120 and 180 min.

RESULTS

For the 0 M LiCl injected group, 76% of the 3-hr saccharin intake occurred in the first 5 minutes with small drafts occurring over the remaining 175 min (Fig. 4). For the LiCl-injected animals, saccharin drinking was depressed compared to the 0 M LiCl group only within the first hr. Comparisons of mean cumulative intakes were shown by t tests to be different at 5, 15, and 30 min ($p<0.001$) only.

EXPERIMENT 3

In this experiment we examined the importance of previous exposure to the drinking fluid on the LiCl-induced aversion.

METHOD

First, the effect of 0–5 saccharin presentations on 15-min drinking periods subsequent to a 24-hr water-deprivation

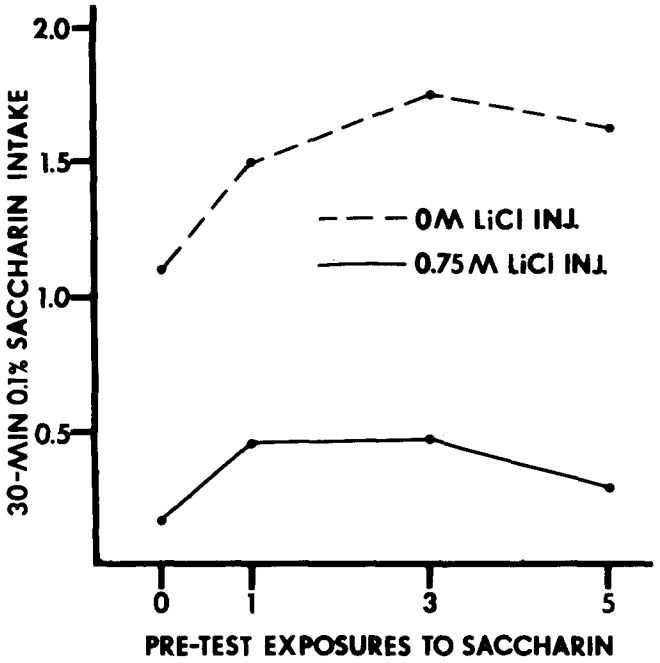

FIG. 5. Saccharin intakes as a function of previous experience with saccharin.

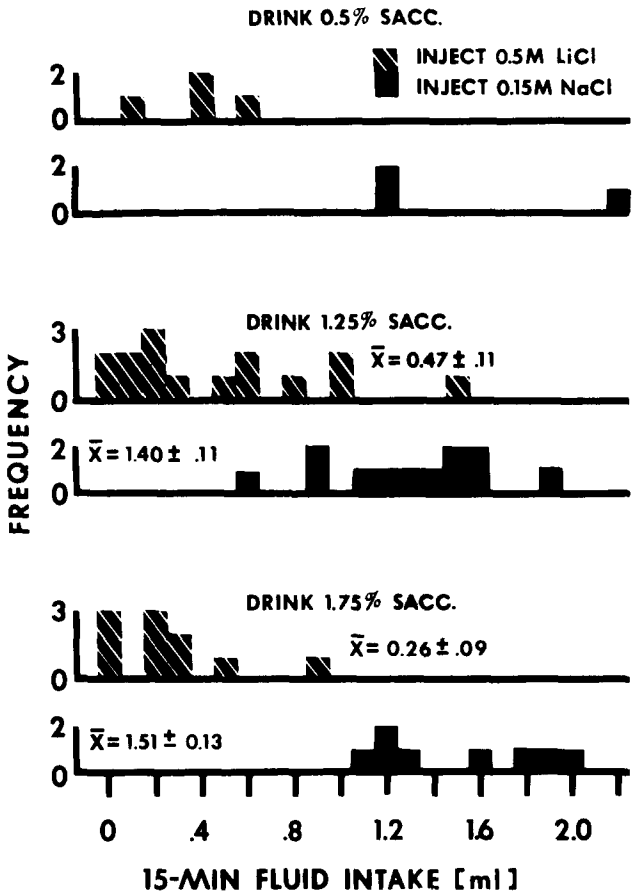

FIG. 6. LiCl-induced depression of drinking in mice with two weeks of exposure to the drinking fluid.

period was studied. Eighty mice were given 7 days to adapt to the individual cages. They were then water-deprived for 24 hr on 5 occasions with 2 days of ad lib water allowed for recovery between deprivations. Food was available continuosly except during the drinking test. Each deprivation period terminated with a 30-min drinking period in which the mice were offered either water or 0.1% saccharin to drink. Twenty mice were given each of the following number of drinking fluid presentations: (a) 5 water; (b) 4 water, then 1 saccharin; (c) 2 water, then 3 saccharin; (d) 5 saccharin. Following the last presentation, mice were given water ad lib and after 2 days, were again 24-hr deprived, injected, delayed 15 min, and then allowed to drink 0.1% saccharin for 15 min. Within each group half the mice were injected with 0.75 M and half were injected with 0 M LiCl.

Since these discrete saccharin exposures did not preclude LiCl-induced depression of saccharin, pre-exposure to the saccharin solution was extended to a longer time interval and to higher concentrations of saccharin in other animals. Wright [11] found that the efficacy of many LiCl dosages to produce selective depression in rats could be enhanced by increasing the concentration of saccharin solution offered.

Fifty-two female mice 100–120 days of age were allowed to drink both water and one saccharin solution (0.5, 1.25, or 1.75%) ad lib for two one-week periods separated by 2 days when only water was given. Weekly intakes of each fluid were determined by weighing the 200 ml bottles which were placed on the cages. Thirty-two of the 52 animals had received two additional weeks of exposure to either another saccharin solution or an NaCl solution in a two-bottle test with water. These 32 mice were evenly distributed among the various groups.

Eleven days after the last weekly 2-bottle test, mice were deprived, injected with either 0 M or 0.5 M LiCl, delayed 15 min and offered the saccharin solution to which they had been exposed for two weeks.

RESULTS

For the mice receiving 0–5 saccharin pre-exposures in discrete 15-min test periods, the intakes of 0.1% saccharin were analyzed in a two-way analysis of variance. As seen in Fig. 5, pre-exposures tended to increase saccharin intake on the test day, $F(3,72)=4.9$; $p<0.01$. LiCl injection significantly reduced saccharin intake, $F(1,72)=177.07$; $p<0.01$, and the interaction between the two variables was not significant. LiCl produced depression of saccharin intake even when 5 pre-exposures to saccharin had been given.

Even when the concentration of saccharin was increased and the pre-exposure duration extended to 2 weeks of continuous access, LiCl still produced depression of saccharin intake (Fig. 6). Relatively few animals were tested with 0.5% saccharin, but the results suggest depression of drinking with LiCl. Depression was seen in the mice drinking 1.25% saccharin, $t(26)=5.74$; $p<0.001$; however, the LiCl depression is most clearly seen in the mice drinking the 1.75% saccharin solution, $t(17)=8.42$; $p<0.001$.

The rather concentrated saccharin solutions used here were preferred to water by the mice over the two weeks of observations. For the 0.5% saccharin solution, mice showed a preference ratio of 87 ± 0.7 (means $\pm$ SE) for the first week

and 91 ± 0.4 for the second. For mice drinking the 1.25% solution, the preference ratios were 77 ± 4.0 and 87 ± 3.3. For those drinking the 17.5% solution, preference ratios were 67 ± 6.1 and 75 ± 4.5.

DISCUSSION

Selective depression of fluid intake observed previously in rats [3, 4, 9, 10] has also been seen in the mouse. LiCl injections which decrease or virtually terminate intake of a dilute saccharin solution have no effect on water intakes. The threshold dosage required to produce a statistically significant depression for saccharin apparently lies between 0.25 and 0.50 LiCl (1% body weight volume). Not every novel, physiologically significant, stimulus will produce selective depression, however. Injections of NaCl which raised mean serum osmolality as much as 30% produced an increase, rather than a decrease, in the intake of saccharin. The suggestion that selective depression is produced by cellular dehydration, an interpretation made from observing intake of aversive QHCl solution [1] may not be applicable in a situation in which a solution preferred to water is used as the test drinking fluid. For the LiCl injection the critical stimulus (or stimuli) producing selective depression is probably not cellular dehydration, but perhaps some aspect of the malaise produced by the toxic action of LiCl [4]. In support of that notion, it should be noted that LiCl produced a dose-dependent decrease in activity and, at the 0.75 M concentration sometimes produced piloerection and diarrhea. In fact, LiCl probably produced no cellular dehydration, but instead apparent cellular hydration with hypovolemia as indicated by the increased hematocrit and increased plasma protein concentration in mice in this study and also in rats [9]. It has been shown in dogs that sodium ions infused intravenously expand plasma, but lithium ions produce hypovolemia since a large fraction of the administered lithium is deposited intracellular carrying plasma water with it [2].

Although the LiCl injections produced physiological and behavioral results in mice similar to those observed in rats [9,10], the findings on NaCl injections are somewhat different. In the rat, we were unable to increase saccharin intake with hypertonic NaCl injections, but intakes of the mouse were double control levels in mice receiving the highest NaCl dosages.

A critical question is the degree to which selective depression represents a learned taste aversion since the disruptive effects of LiCl injection (a potential US) most certainly overlap in time the ingestion of the drinking fluid (a potential CS). Some of the findings in the present study argue against a learning interpretation. First, the 0.75 M LiCl dosage produced only a transient aversion to saccharin. Within two hr, cumulative intakes of the LiCl-injected group had overtaken the intakes of the control-injected group. Second, no evidence of conditioned aversion was found in tests 3 days following the selective depression tests. Third, LiCl-induced depression of saccharin intake was produced in mice which had daily access over a two-week period to a highly concentrated (salient) saccharin solution strongly preferred to water. In conditioned taste aversion experiments, such frequent contact with the taste of a sapid fluid should preclude the formation of the conditioned taste aversion [6].

The failure of preexposure to the taste fluid to preclude selective depression is similar to the findings in rats where 10 [11] or 25 [3] preexposures to the saccharin test solution did not prevent selective depression under the single-bottle test condition. We have recently found, however, that in a two-bottle test situation where the rat has both water and saccharin available, 4 30-min pre-exposures to the saccharin solution precluded LiCl-induced selective depression (Kutscher, Yamamoto, Hamburger; unpublished data). These findings suggest that methodological differences may be critical in studying the variables which influence selective depression.

The data of the present experiment show that selective depression in a one-bottle test can be readily elicited in the mouse by LiCl in a dose-dependent fashion and seems invulnerable to considerable prior experience with the drinking fluid. The critical internal change produced by the LiCl injection has not yet been identified, but it is not cellular dehydration.

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