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NaCl and LiCl Efficacy in the Induction of Aversion for Quinine and Saccharin Solutions Immediately Following Injection

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KUTSCHER, C. L., W. A. WRIGHT AND M. LISCH. *NaCl and LiCl efficacy in the induction of aversion for quinine and saccharin solutions immediately following injection.* PHARMAC. BIOCHEM. BEHAV. 6(5) 567–569, 1977. – Rats 24-hr water deprived were injected IP with a fixed amount (10 ml/kg) of solutions of various concentrations of LiCl and NaCl in dosage ranges which in previous experiments either increased or had no effect on water intake. Intake of 0.01% QHCl decreased with increasing concentrations of both NaCl and LiCl. On a molar basis, LiCl was more effective. LiCl also produced an aversion to a palatable solution, 0.1% sodium saccharin; however, NaCl produced no aversion over the dosage range which can be tolerated by the animals.

Gut LiCl NaCl Quinine Saccharin Taste aversion

BURKE, Mook and Blass [4] found that the injection of a strongly hypertonic NaCl solution into water-deprived rats increased water intake, but decreased the intake of a dilute and novel quinine hydrochloride (QHCl) solution in one-bottle tests. These authors suggested that the selective aversion for QHCl (which they called hyperreactivity) was precipitated by cellular dehydration produced by the NaCl injection. The existence of the selective aversion for QHCl was confirmed by Kutscher and Wright [10]; however, they questioned the cell dehydration hypothesis because intraperitoneal injections of LiCl also caused selective reduction in QHCl intake although producing no cellular dehydration. It was suggested that the large dose of NaCl needed to produce aversion may indicate that the crucial internal state may be malaise (perhaps gastrointestinal) rather than cellular dehydration [10,12]. It was suggested that this aversion may be unconditioned aversion which appears when the animal ingests the test solution for the first time [10]. Perhaps this aversion is mediated by illness as is conditioned aversion [6].

The objectives of the following experiment were: (a) to generate dose-response curves in order to provide a direct comparison of the efficacy of LiCl and NaCl in the production of induced aversion; (b) to determine if this aversion can occur for a palatable (saccharin) solution as well as for an unpalatable (QHCl) solution.

METHOD

General

Two hundred and fifty-five naive, female hooded rats were used in this experiment. They were 3–6 months old and weighed 170–329 g and were housed in groups of 5 before the start of testing. Twenty-four hr before testing, each rat was weighed and transferred to an individual steel holding cage (19.2 × 22.8 × 12.0 cm) which contained food, but no water. When 23 3/4-hr water deprived, rats were removed from holding cages, injected (10 m/kg) with LiCl, NaCl, or water and were placed into individual drinking boxes (24.0 × 16.8 × 14.4 cm) composed of steel floor and walls and hardware cloth tops. The floor of each cage was covered with clay bedding (Sol Speedi Dri Absorbent). Drinking fluid was offered 15 min following injection in a 100-ml gas measuring tube graduated in 0.2 ml and fitted with a stainless steel drinking spout. The length of the drinking test session was 1 hr. In order to adapt animals to the drinking boxes, each animal was placed in the drinking box when 24-hr water deprived and offered water once, at least 4 days before the drinking water.

Drinking boxes were located within the animal colony. Lights were on for 12 hr/day. Temperature was maintained at 21 ± 2°C. Air was humidified during the winter months.

Dose-Response Curves for QHCl Intake

Seven rats were injected intraperitoneally (IP) with each of the following LiCl concentrations: 0, 0.03, 0.06, 0.12, 0.18, 0.24, and 0.50 M. Eight rats were injected IP with each of the following NaCl concentrations: 0, 0.15, 0.325, 0.50, 1.00 and 1.50 M. All injectates were mixed in demineralized water. The drinking solution was 0.01% QHCl mixed in demineralized water.

Dose-Response Curves for Saccharin Intake

The LiCl concentrations injected IP were: 0, 0.06, 0.12, 0.24, 0.37, and 0.50 M. Ten rats were injected with the 0 and 0.50 M LiCl. Seven rats were tested with each of the other LiCl amounts. The NaCl injection concentrations used were 0, 0.15, 0.325, 0.50, 1.00 and 1.50 M. Five rats were tested at each concentration level. The drinking solution was 0.1% sodium saccharin mixed in demineralized water.

Gross Activity

Rats were injected IP when 23 3/4 hr water deprived with one of the following: water, 0.5 M NaCl, 1.5 M NaCl and 0.5 M LiCl. The latter two were chosen because they produced an aversion for QHCl. Fifteen min after injection, rats were transferred individually to a Lehigh Valley Activity chamber in which interruption of light beams was measured for 5 min in a circular, darkened compartment. Six rats were studied under each of the 4 injection conditions.

RESULTS AND DISCUSSION

All data were analyzed with analysis of variance. Comparisons between pairs of means were evaluated with Tukey tests [7]. Dose-response curves for NaCl and LiCl injections on intake of QHCl are shown in Fig. 1 and for intake of saccharin in Fig. 2. Intakes of QHCl decreased monotonically as a function of increasing NaCl ($F = 6.19$, $p<0.01$) and LiCl dosage ($F = 6.39$, $p<0.01$), but LiCl was more potent, in molarity units. Significant inhibition of QHCl intakes occurred when NaCl concentrations exceeded 0.5 M. Substantial inhibition was found for LiCl concentrations as low as 0.12 M. The depressions in drinking seen here are selective since it has been shown previously that hypertonic NaCl injections increase drinking of water in water-deprived rats [1] and the NaCl-induced increment is apparent within a 60-min drinking period [8]. LiCl concentrations in the range used here either potentiate water intake (Wright, unpublished study) or produce no significant change in water intake [10].

For those animals offered saccharin to drink, intakes declined as a function of increasing LiCl dosage ($F = 6.54$, $p<0.01$), but NaCl injections up to 1.5 M NaCl produced no significant change in intake ($F = 0.75$, NS). Although NaCl injections slightly higher than 1.5 M have been used [4,10], we found such injections often result in general debilitation of the animal (piloerection, inactivity, diarrhea, unresponsiveness) and thus may have limited experimental usage.

The relative low potency of the NaCl injections to induce aversion for QHCl suggest that whatever the necessary internal condition required to produce aversion, NaCl is relatively ineffective in producing it in spite of the massive water shifts produced. Kutscher and Wright [10] found that a NaCl injection adequate to produce reliable, selective depression of QHCl produced an increase of over 15% in serum osmolality in water-deprived rats and that increase persisted (in the absence of opportunity to drink) with some slight attenuation over 195 min. If cellular dehydration consequent to NaCl injection were the necessary condition for production of the selective aversion, it is unlikely that such a mechanism would have any utility in the life of the animal since its threshold for operation

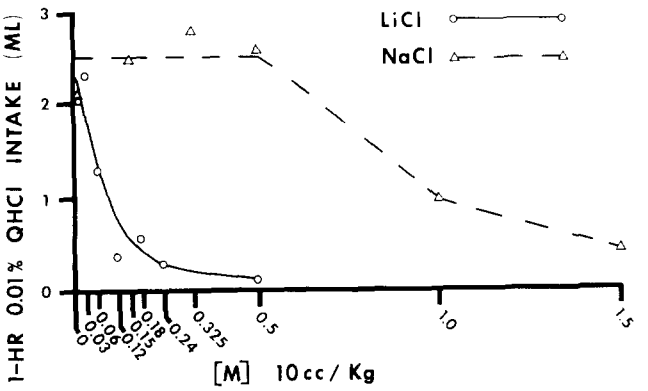

FIG. 1. Mean intakes of QHCl for 24-hr water deprived rats injected IP with various dosages of LiCl or NaCl.

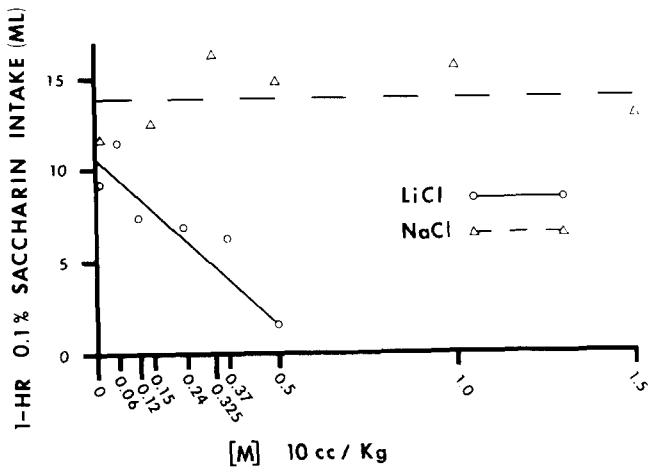

FIG. 2. Mean intakes of saccharin for 24-hr water deprived rats injected IP with various dosages of LiCl or NaCl.

would probably not be reached even under extreme dehydration. Ninety-six hr of water deprivation resulted in only a 4% rise in serum osmolality [9]. Other factors militate against an osmotic basis for the selective aversion. The 0.12 M Li Cl concentration produced aversion even though approximately isotonic to concentration of body fluids. Also, Li^+ does not produce cellular dehydration according to postinjection measures of blood characteristics. NaCl and LiCl have opposite effects on plasma osmolality, plasma proetin, and hematocrit [10]. Unlike the Na^+cation which tends to remain in the extracellular space, Li^+ may accumulate inside cells [11,14]. The high NaCl concentration needed to produce aversion may be due to the lower efficacy of the NaCl in producing the internal disturbance (perhaps illness) required to produce aversion.

A comparison of saccharin intakes (Fig. 2) with QHCl intakes (Fig. 1) shows that the production of NaCl-induced aversion is a joint function of the concentration of NaCl injected (over the range which is tolerable to the rats) and the nature of the solution being ingested. The 1.0 and 1.5 M concentrations which produced aversion for QHCl produced no aversion whatever for saccharin. The failure of the strongly hypertonic NaCl injections used to increase saccharin intake is probably due to the interaction of the internal consequences of the salt injection with the taste of

the saccharin, since NaCl injections can increment water drinking [1,8]; however, a possible ceiling effect imposed by limiting drinking to one hr is a possibility.

Within the range of LiCl concentrations used, definite aversion for saccharin was produced showing that LiCl-induced aversion is possible for a palatable solution just as has been previously shown for an unpalatable (0.01% QHCl) solution [10].

It is important to determine the relationship between the taste aversion induced here by NaCl or LiCl injections to the conditioned aversion which has been demonstrated by these agents under somewhat different test conditions [13]. We have previously [10] called the type of aversion shown here as unconditioned aversion since it is exhibited soon after the injection of the agent (we used a 15-min delay) upon the animal's very first exposure to the test solution. The possibility remains, however, that conditioning may conceivably play a role in the generation of the aversion. The rat may begin to drink the solution at a time when the injection-produced internal disturbance is well established and conditioned aversion for the solution being ingested may be generated during the drinking period. It is difficult to give a definitive answer to this question without knowing what are the minimal amounts of CS exposure (time or volume ingested) required to produce conditioned aversion. It seems likely that at least some of the aversion seen in the present experiment may be indeed unconditioned because exposure to the potential CS was very limited. For example, three of the five rats injected with 0.5 M LiCl drank no measurable amount of QHCl over the 60 min test period. They all tasted the test fluid and retreated from the drinking spout. For the five rats injected with 0.5 M LiCl and drinking saccharin, one drank no measurable amount and two drank only 0.1 ml.

The activity scores provide an index of the extent of bodily disturbance produced by injection concentrations effective in producing aversion. Dosages which significantly reduced QHCl intakes also decreased activity. Mean 5-min activity level of rats receiving 0.5 M LiCl (mean ± SD = 199 ± 36 counts) was significantly ($p<0.05$) lower than that of rats receiving water (482 ± 86); however, rats receiving isomolar (0.5 M) NaCl did not have an activity level (442 ± 92) different from those water injected. The 1.5 M NaCl injected resulted in activity (321 ± 95) lower ($p<0.05$) than water injected animals, but not statistically different from activity produced by 0.5 M LiCl. The superior potency of LiCl over NaCl in decreasing activity and in reducing drinking of novel solutions thus resides in some property other than osmolality.

The results reported above and in a previous paper [10] suggest that some as yet unspecified change in the organism, perhaps gastrointestinal distress, causes the animal to reject either an unpalatable (QHCl) or a palatable (saccharin) solution while intake of water or perhaps very familiar sapid solutions are not affected. The injections need not be made IP in order to produce aversion. Subcutaneous (SC) injections of 0.5 M LiCl were just as effective in diminishing saccharin intake as those made IP. Also, 1.5 M NaCl and 0.5 M LiCl made SC were just as effective in diminishing QHCl intake as those made IP. Although it is not known if gastrointestinal distress is a necessary or even a sufficient cause in the production of aversion, it is clear that the noxious substance need not be topically applied to the gut, as results with an IP injection. It is possible that LiCl or NaCl could influence gut motility systemically after entering circulation at the site of the SC injection.

The adaptive significance of the injection-induced aversion remains to be determined. The reluctance of both wild and domestic rats to ingest avidly a food substance containing a novel taste has been termed neophobia and is seen as adaptive in that restricted intake of a new food substance may limit the possibility of being poisoned [2], particularly in an animal which cannot regurgitate such as the rat. If the animal is under the influence of a toxic agent, intake of an unfamiliar food could be even more restricted than in the healthy animal [3]. Such an illness-enhanced neophobia could serve to restrict the animals intake to very familiar foods which the animal has learned are safe during a time of illness when it may be particularly vulnerable to poisoning.

Since these experiments were completed, similar findings have been reported by Domjan [5] which are compatible with findings in this and a previous paper [10].

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