Learned aversion and rearing movement in rats given LiCl, $PbCl_2$ or NaCl

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Summary. The effects of an i.p. injection of 0.15 M LiCl resembled closely the effects of another toxic substance, 0.005 M $PbCl_2$, on conditioned aversion to saccharin and rearing movements in male rats. The findings suggest that nonspecific aversive after-effects of a LiCl injection can account fully for prompt effects of LiCl on rearing behavior.

The use of lithium salts to treat mental disorders has stimulated interest in the effects of lithium on behavior in laboratory animals. Many studies on behavior in rats use lithium treatments which can cause strong learned aversions due to prompt unpleasant after-effects of the treatments[2-4]. Consequently, some changes in behavior observed soon after an injection of lithium may be caused mainly by nonspecific toxic effects of the lithium treatment rather than specific actions of lithium on behavioral mechanisms. The present experiments were carried out to determine whether LiCl has a specific action on vertical locomotor activity (rearing movements) or whether effects of LiCl on rearing movements can be accounted for fully by nonspecific toxic effects[5].

Experiment 1. Materials and methods. 48 male albino Wistar rats weighing 250–320 g were used. They were housed individually in clear plastic cages (42 × 26 × 16 cm) in a thermostatically controlled room (23 °C) on a 12-h light-dark cycle (lights on from 08.00 h to 20.00 h) with free access to rat chow pellets. The rats received tap water to drink for 15 min each day for 8 days. On day 9, they received 0.1% saccharin to drink in place of water for 15 min followed by an i.p. injection (2.5, 5, 10 or 20 ml/kg) of 0.15 M NaCl, 0.15 LiCl or 0.005 M $PbCl_2$ at room temperature. Each dose of each solution was given to 4 rats. On days 10 and 11, the rats received water to drink for 15 min a day. On day 12, the rats received 0.1% saccharin to drink for 15 min. Water and saccharin intake were measured on days 11 and 12, respectively. The rat's behavior and general appearance was observed after injections. Anova was used to determine whether the main effects (doses and salt treatments) and the interaction (doses × salt treatments) were statistically significant. When the Anova was significant, comparisons between pairs of means were carried out[6].

Results. Rats given 5 ml/kg or more of 0.15 M LiCl or 0.005 M $PbCl_2$ or 20 ml/kg of 0.15 M NaCl looked sick soon after the i.p. injection. They were inactive, curled up or prostrate, and unalert for from 15 min to 2 h after injection. Rats given the other doses of 0.15 M LiCl, 0.005 M $PbCl_2$ and 0.15 M NaCl were not noticeably affected by the injections.

Figure 1 shows the amounts of water and saccharin drunk on days 11 and 12, respectively, by the rats injected with NaCl, LiCl and $PbCl_2$ on day 9. Water intake was not affected significantly by the dose or the salt treatments. However, a statistically significant interaction occurred ($p < 0.05$), mainly due to a signficant reduction in water intake in rats given the highest dose of $PbCl_2$ on day 9 ($p < 0.05$). Saccharin intake was affected significantly by the dose ($p < 0.05$) and the salt treatment ($p < 0.01$). A significant interaction also occurred ($p < 0.01$). Saccharin intakes by the rats given LiCl or $PbCl_2$ were significantly reduced compared to rats given NaCl ($p < 0.01$ and 0.05, respectively), while saccharin intakes by rats given LiCl or $PbCl_2$ did not differ significantly from each other. The extent of the reduction in saccharin intake depended on the dose of salt administered; greater reductions occurred after the 2 highest doses than after the 2 lowest ones ($p < 0.01$).

Experiment 2. Materials and methods. 30 male albino Wistar rats weighing 240–270 g were used. They were housed and fed as described in experiment 1. The rats received tap water to drink for 15 min each day for 8 days and received 0.1% saccharin to drink in place of water for 15 min on day 9, as in experiment 1. An i.p. injection (10 mg/kg) of 0.15 M NaCl, 0.15 M LiCl or 0.005 M $PbCl_2$ at room temperature was given to groups of 5 rats either 5 min or 11 h after drinking saccharin on day 9. On days 10, 11 and 12, the rats received water to drink for 15 min. On day 13, the rats received 0.1% saccharin to drink for 15 min and the amount drunk was recorded. The statistical analysis was as described in experiment 1.

Results. Table 1 shows the effects of the time of injection of NaCl, LiCl and $PbCl_2$ after drinking saccharin on day 9 on the intake of saccharin on day 13. The time of injection and the salt treatments affected saccharin intake significantly ($p < 0.01$). The interaction was also significant ($p < 0.05$). Saccharin intake was significantly less in groups given an injection of LiCl or $PbCl_2$ 5 min after drinking saccharin compared to the group given NaCl 5 min after drinking saccharin ($p < 0.001$ and 0.01, respectively). In addition, saccharin intake on day 13 was significantly less in the group given LiCl than in the group given $PbCl_2$ 5 min after drinking saccharin on day 9 ($p < 0.05$). No significant differences in saccharin intake occurred on day 13 between the groups given NaCl, LiCl or $PbCl_2$ 11 h after drinking saccharin on day 9. Saccharin intake on day 13 was significantly less in groups given $PbCl_2$ and LiCl 5 min after drinking saccharin on day 9 compared to the groups given $PbCl_2$ and LiCl 11 h after drinking saccharin ($p < 0.05$ and 0.01, respectively). No significant differences occurred between the day 13 saccharin intakes in rats given NaCl 5 min or 11 h after drinking saccharin on day 9.

Experiment 3. Materials and methods. 36 male albino Wistar rats weighing 300–330 g were used. They were housed as described in experiment 1 with free access to rat chow pellets and tap water. The test employed by Johnson[7] to determine the effect of LiCl on rearing movements in rats was used. It was carried out on a blind basis. A rear was recorded by a trained observer each time the rat raised its head at least 9 cm above the floor of the vertical transparent tube, 46 cm tall and 23 cm internal diameter. Each rat was tested for 5 min. The rats received an i.p. injection (2.5, 10 or 20 ml/kg) of 0.15 M NaCl, 0.15 M LiCl or 0.005 M $PbCl_2$ at room temperature 15 min before tests. Each dose of each solution was given to 4 rats. The statistical analysis was as described in experiment 1.

Results. Figure 2 shows the effects of NaCl, LiCl and $PbCl_2$ on rearing movements in rats. Rearing was affected significantly by the dose ($p < 0.05$) and by the salt treatments ($p < 0.025$). The interaction was not statistically significant. Rearing movements decreased significantly as the dose of the solution was increased ($p < 0.05$). Rearing movements were significantly decreased in rats given LiCl or $PbCl_2$ compared to those given NaCl ($p < 0.05$), while there was no significant difference in rearing between rats given LiCl or $PbCl_2$.

Discussion. The results of experiments 1 and 2 show that 0.15 M LiCl and 0.005 M $PbCl_2$ produced similar, but not identical, dose- and time-dependent aversions to saccharin

Amount of 0.1% saccharin drunk (ml/rat) during a 15-min test on day 13 in rats given an i.p. injection of 0.15 M NaCl, 0.15 M LiCl or 0.005 M $PbCl_2$ either 5 min or 11 h after drinking saccharin for 15 min on day 9

Treatment	Time of injection	
	5 min	11 h
NaCl	14.2 ± 1.1	14.6 ± 2.9
LiCl	2.2 ± 0.8[a,b,c]	12.6 ± 2.9
$PbCl_2$	6.4 ± 1.6[a,c]	11.8 ± 1.1

Values are means ± SEM for 5 rats per group. [a], Significantly less than the corresponding NaCl value; [b], Significantly less than the corresponding $PbCl_2$ value; [c], Significantly less than the corresponding 11-h value. See text for levels of significance.

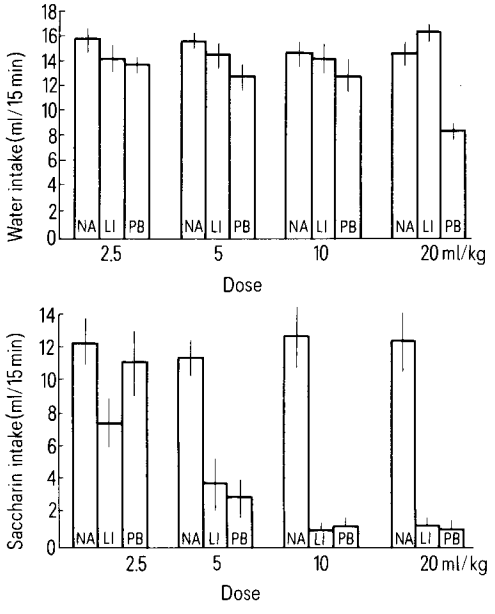

Fig. 1. Mean water (upper) and saccharin (lower) intake (±SEM) in the test for learned aversion in rats given an i.p. injection of 0.15 M NaCl (NA), 0.15 M LiCl (LI) or 0.005 M $PbCl_2$ (PB) immediately after drinking saccharin 2 and 3 days respectively before the 15-min drinking period.

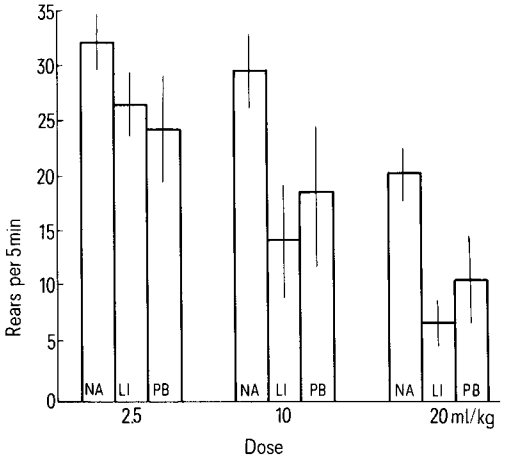

Fig. 2. Mean number of rears (± SEM) in rats given an i.p. injection of 0.15 M NaCl (NA), 0.15 M LiCl (LI) or 0.005 M $PbCl_2$ (PB) 15 min before the 5-min test.

in rats; an injection of LiCl or $PbCl_2$ soon after the rats drank saccharin led to a similar dose-dependent decrease in saccharin intake during the test carried out 3 days later, and led to suppression of saccharin intake during a test carried out 4 days later, while neither LiCl nor $PbCl_2$ injected 11 h after drinking saccharin affected subsequent saccharin intake reliably. Thus, both salt solutions (0.15 M LiCl and 0.005 M $PbCl_2$) produced conditioned aversion to saccharin. It is to be noted, however, that while the conditioned aversions produced by LiCl and $PbCl_2$ were similar in many respects, the conditioned aversion produced by LiCl appeared to be stronger than the one produced by $PbCl_2$ since in experiment 2 saccharin intake was suppressed more by LiCl than by $PbCl_2$.

The concentration of $PbCl_2$ used was arrived at by trial-and-error. The similarity of the prompt effects of an i.p. injections of 0.15 M LiCl and 0.005 M $PbCl_2$ on the appearance of the rats, and the similarity of the dose- and time-dependence of conditioned aversions produced by an injection of equal volumes of these salt solutions, suggests that the treatments had comparable unpleasant after-effects in the rats.

The results of experiment 3 showed the effects of 0.005 M $PbCl_2$ on rearing movements in rats to be very similar to the effects of 0.15 M LiCl, since a similar dose-dependent decrease in rearing occurred after an i.p. injection of an equal volume of either solution. Of course, the close similarity of the effects of $PbCl_2$ and LiCl on learned aversions and rearing movement does not prove that the effect of LiCl on rearing was caused by nonspecific toxic actions of the lithium treatment[8]. Nevertheless, the present findings show that the prompt effects of LiCl on rearing movements in rats can be accounted for fully by nonspecific toxic after-effects of the lithium treatment. The outcome of the present study prompts the question 'What is an appropriate control group for use in studies on behavior in laboratory animals given LiCl?' Studies on behavior in rats given lithium salt solution typically use an equal dose of a corresponding sodium salt solution as the control treatment. However, the sodium salt treatment fails to provide a control for nonspecific unpleasant actions of the lithium salt, because sodium salts are much less toxic than lithium salts[9]. The present findings suggest that inclusion of $PbCl_2$ and other compounds that can cause prompt unpleasant after-effects similar to those produced by lithium salts, may be useful as 'toxicity-control' treatments in studies on behavior in rats given lithium salts and may provide a means to distinguish between nonspecific toxic actions of lithium and specific actions of lithium on behavioral mechanisms.

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2 M. Nachman, J. comp. physiol. Psychol. *73*, 22 (1970).
3 M. Nachman and J.H. Ashe, Physiol. Behav. *10*, 73 (1973).
4 D.F. Smith, in: Annual Research Reviews, Lithium and animal behavior, vol. 1. Ed. D.F. Horrobin. Eden Press, Montreal 1977.
5 D.F. Smith, Experientia *32*, 1320 (1976).
6 E.F. Lindquist, in: Design and Analysis of Experiments in Psychology and Education. Houghton Mifflin Company, Boston 1956.
7 F.N. Johnson, Experientia *28*, 533 (1971).
8 D.J. Ingle, Perspect. Biol. Med. *15*, 254 (1972).
9 N.N. Samoilov, Russian Pharmac. Toxic. *5*, 266 (1970).