Pharmacology Biochemistry & Behavior, Vol. 13, pp. 133–135. Printed in the U.S.A.

Conditioned Taste Aversion as an Index of Lead Toxicity

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Received 18 April 1980

DANTZER, R. *Conditioned taste aversion as an index of lead toxicity*. PHARMAC. BIOCHEM. BEHAV. **13**(1) 133–135, 1980.—Rats treated with lead acetate following the consumption of a solution with a distinct taste exhibited an aversion to the initially consumed solution. Conditioned taste aversion was reliably induced with 10–20 mg/kg lead acetate. Repeated treatment with lead did not enhance this effect when measured either in forced or in free choice conditions. The utility of the taste aversion procedure for evaluation of toxic agents is discussed.

Lead acetate Conditioned taste aversion Rat

WHEN an animal is given the opportunity to ingest a solution with a specific taste followed by the administration of a toxic agent (e.g. ionizing radiations or lithium chloride), he will subsequently show an aversion to the initially consumed solution [2,3]. This effect is known as a learned taste aversion and has been conceptualized as a form of classical conditioning in which some type of malaise or gastrointestinal distress serves as an unconditioned stimulus.

The compelling nature of this phenomenon has generated considerable interest both from the point of view of behavioral analysis and pharmacology. Many agents have now been reported to be effective in eliciting the aversion, including psychotropic drugs [10], methylmercury [7,12] and rodenticides [9].

The present experiments were initiated to determine the usefulness of the taste aversion procedure for evaluation of acute (Experiment 1) and repeated (Experiment 2) lead intoxication in rats.

EXPERIMENT 1

METHOD

The subjects were 35 male rats of a Wistar strain, weighing 200–250 g. They were housed individually in translucent plastic cages in a thermostatically controlled room (22°) on a 12-hour light-dark cycle (lights on from 8.00 to 20.00). In order to train the subjects to drink on cue in their home cage, a sucrose solution (20% by weight) was made available in 15-min sessions for 7 days. Food deprivation was employed as needed during only the first 5 days to ensure that subjects would ingest the solution. Thereafter either the sucrose solution or sweetened milk (sweetened evaporated milk diluted 3.5 to 1) was presented alternatively on daily 15-min sessions until five milk presentations were made. The fifth milk presentation was followed after 30 min by an IP injection of 127 mg/kg lithium chloride (7.5 ml of a 0.40 M solution per kg of body weight), 1.25, 2.5, 5.0, 10 or 20 mg/kg lead acetate (2 ml/kg) or saline (2 ml/kg). Each treatment was given to 5 rats. The subjects were allowed two days to recover during which 2 sucrose presentations were made spaced 24 hr apart. Seventy two hours after the toxicosis conditioning session, they were presented with the milk solution for a 15-min duration test session.

Sucrose and milk intakes were measured every day throughout the experiment, by weighing the bottle before and at the end of each session.

RESULTS

On the day of the 5th milk presentation, the rats drank an average of 10 ± 0.37 g milk during the 15-min experimental session. Figure 1 shows that, with the exception of the saline-treated animals, all groups showed taste aversion as evidenced by the drastic decrease in milk intake following lithium or lead administration. Milk intake during the conditioning session and the test session were analyzed by a 2-way ANOVA for the treatment factor and the day factor which was treated as a within subject variable. The main effect of treatment administration was significant, $F(6,56)=2.85$, $p<0.05$, as was the main effect of days, $F(1,56)=37.0$, $p<0.001$. The interaction was not significant. Post-hoc intergroup comparisons were performed according to the Newman-Keuls method ($\alpha=0.05$). The lithium group and the 10 and 20 mg/kg lead groups were suppressed relative to saline-treated animals on the test day. Sucrose consumption was significantly decreased 24 hours after the toxicosis conditioning session in the same groups, $F(6,84)=3.62$, $p<0.01$, but did not differ from controls 48 hours after.

EXPERIMENT 2

This experiment investigated whether repeated lead treatment could enhance conditioned taste aversion or induce

[1]This work has received financial support from Institut National de la Recherche Agronomique (Recherches Vétérinaires) and from Ministère de L'Environnement (Grant n° 76/90). The author gratefully acknowledges the skillful technical assistance of Ms. R. M. Bluthé.

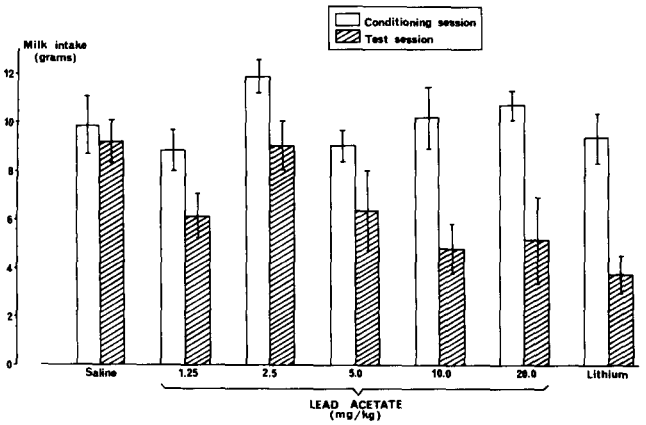

FIG. 1. Average milk intake on the conditioning session and on the test session in rats injected with saline, lithium or different doses of lead acetate after the conditioning session (mean ± SEM). Each experimental group included 5 animals.

tolerance. A forced choice rather than a free choice procedure was used for that purpose in order to ensure maximum fluid consumption throughout the experiment. Rats were maintained on a chronic water restriction regimen and saccharin was prefered to milk as a distinctive taste cue in order to keep the caloric value of the consumed fluid as low as possible.

METHOD

Twenty-eight male Wistar rats were used. Their average weight at the start of the experiment was 275 g. Food was freely available throughout the experiment. After one week of adjustment to the individual caging a procedure of water restriction was initiated : water was presented for 30 min per day, during 5 days. On Day 6, the first toxicosis conditioning session was initiated : a saccharin solution (0.1% by weight) was presented during 30 min instead of water and was followed 30 min later by an IP injection of lead acetate (2 mg/kg, 4 mg/kg, 8 mg/kg, for a volume of 2 ml/kg) or saline (2 ml/kg). Each treatment was given to 7 rats. The remaining four toxicosis conditioning sessions were spaced 3 days apart, water being presented during 30 min, 24 hr and 48 hr after the conditioning session. Seventy-two hours after the last conditioning session, a test session was given during which the saccharin solution was presented for 30 min but was not followed by any injection. Forty-eight hours after this test session, the animals were given a choice of the usual saccharin solution or water during a 30-min exposure.

RESULTS

Figure 2 presents the mean saccharin solution intake in each experimental group during the five successive toxicosis conditioning sessions and the test session. The first conditioning session was not followed by any appreciable fluid rejection (as measured by the amount of saccharin solution consumed on the second conditioning session), even with 8 mg/kg lead acetate, contrasting with the abrupt decrease of fluid intake induced by 10 mg/kg lead acetate in the first experiment. This difference was expected, due to the forced choice procedure used here. Performance was analysed by a 2-way ANOVA for the factors of experimental treatments

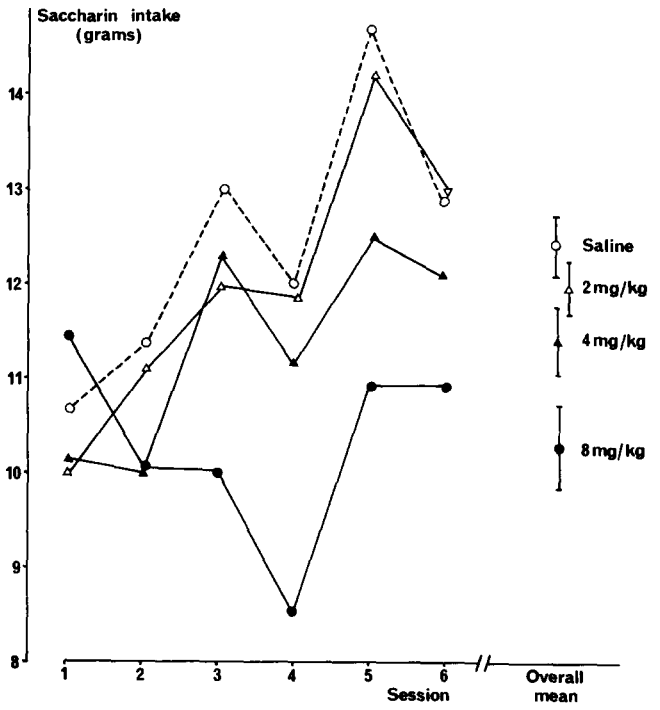

FIG. 2. Average saccharin solution intake in rats treated with saline or various doses of lead acetate after the conditioning sessions. Five conditioning sessions (1–5) were spaced three days apart, followed 72 hours later by a test session (session 6). At the right are the mean values of fluid consumed over the 6 sessions (mean ± SEM). Each experimental group included 7 animals.

and sessions. The main effect of experimental treatment was significant, $F(3,144)=7.32$, $p<0.001$, as was the main effect of sessions, $F(5,144)=5.92$, $p<0.001$. The interaction was not significant. In general, these results reflect the fact that all experimental groups displayed an increase of saccharin intake throughout the experiment, the 8 mg/kg lead-treated group having the lowest consumption. During the same period, water consumption did not differ among experimental groups, $F(3,264)=1.35$.

Performance during the choice session (Fig. 3) was analyzed by a 2-way ANOVA for the factors of experimental treatment and type of fluid (water versus saccharin). The main effect of experimental treatment was not significant, $F(3,48)=0.14$. The main effect of fluid type and the interaction were significant, $F(1,48)=7.68$, $p<0.01$ and $F(3,48)=6.50$, $p<0.001$: saccharin drinking was suppressed in 8 mg/kg lead-treated rats with respect to controls.

Growth of rats over the course of the experiment was not affected by lead treatment, $F(3,120)=1.43$.

DISCUSSION

These experiments demonstrate that lead administration induces some sort of illness which is conditioned to the distinct flavor preceding it. A sufficient dose of lead must be administered in order to reach such an effect (8–10 mg/kg lead acetate IP), but this dosage is without any other adverse effect.

Lead toxicity is affected by many different factors among which the age is the most important [5,6] : adult animals are

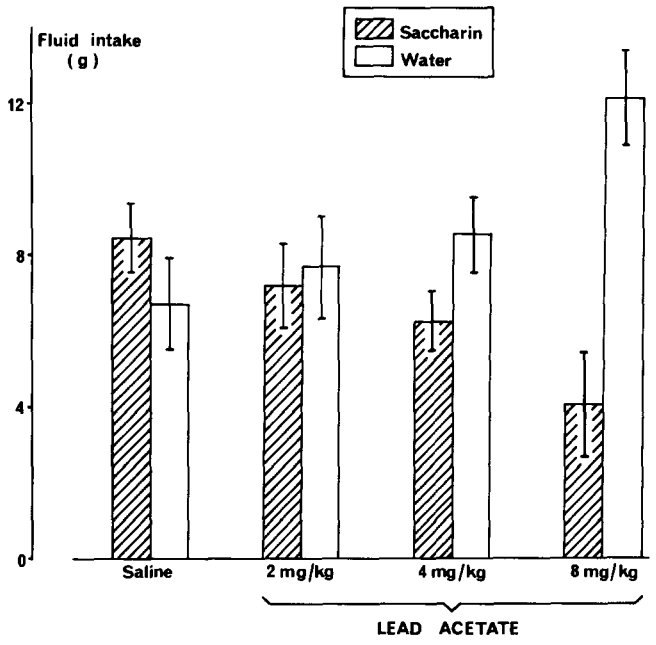

FIG. 3. Average intake of water or saccharin solution on a free choice test run 5 days after the last conditioning session in the repeated lead poisoning experiment (mean ± SEM). Each experimental group included 7 animals.

considerably more resistant to lead poisoning than young animals. In adult animals, inorganic lead does not usually induce any appreciable change in weight gain and food consumption unless administered at very high doses or on a long term basis. For instance, doses of 12 mg/kg lead acetate constitute a fatal dosage for rats only when given daily over a 21-day period [11].

Gastroenteritic symptoms are by far the most frequent characteristics of lead poisoning in adult man and animals [5,6]. They range from slight abdominal discomfort to colics. Development of conditioned aversion to lead acetate is consistent with the idea that gastrointestinal dysfunction is the unconditioned response involved. Such an effect would appear to be more sensitive than other behavioral tests to detect low level lead intoxication, since weaned rats receiving 100 mg/kg lead acetate per day [1] or sheep administered 2–4 mg/kg lead during 27 weeks [13] did not differ from controls in a learning task.

Repeated lead administration was not superior to acute lead intoxication to induce conditioned taste aversion. In the experimental procedure used, saccharin was the only fluid available during the conditioning sessions. Under these forced drinking conditions, rats still developed a conditioned aversion to the saccharin solution as demonstrated by the lower intake of 8 mg/kg lead-treated rats and more specifically by their clear preference for water when given the choice. Lower doses were without adverse consequences in either forced or free choice. There was no apparent tolerance to the effects of lead with repetition of treatment, in contrast with what is observed with most psychotropic drugs [2]. There was also no apparent cumulative effect of lead since suppression of fluid intake was not obtained with lower doses than in the acute lead poisoning experiment. It has been shown that 3 days after an IP injection of lead, 51% of the initial dose is still present in the whole body of adult rats while after 6 days the percentage of Pb retained is 39% [8]. On the third toxicosis conditioning session for instance, this would mean that rats having received a lead dose of 4 mg/kg have a total burden of nearly 8 mg/kg. If lead effects were cumulative, they should exhibit conditioned taste aversion which clearly is not the case. This would mean that the effective dose is the amount of lead administered on the conditioning session and not the cumulative dosage received.

Little is known about the ability of other heavy metals to induce conditioned taste aversion. With methylmercury chloride, the concentrations needed to obtain such an effect are high and accompanied by profound signs of organic mercury toxicosis [7,12]. No study is available concerning the consequences of organic lead intoxication. The potential utility of taste aversion as a method to estimate toxicity clearly requires further work with different toxic agents and it would certainly be worth determining to what extent the specific aspects of lead intoxication contribute to the taste aversion. Attention should also be paid to illness-producing effects of lead in behavioral toxicology studies with this pollutant.

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