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BRIEF COMMUNICATION

Conditioned Saccharin Taste Aversion Induced by Mycotoxins in Rats: Lack of Effect of Ochratoxin A[1]

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CLARK, D. E. AND P. J. WELLMAN. *Conditioned saccharin taste aversion induced by mycotoxins in rats: Lack of effect of ochratoxin A*. PHARMACOL BIOCHEM BEHAV **32**(3) 819–821, 1989.—The present study examined the putative aversive action of ochratoxin A (OA) using a conditioned saccharin aversion paradigm. Adult male rats consumed a 0.1% saccharin solution then were treated (IP) with either a 5% $NaHCO_3$ vehicle (negative control), 32 mg/kg LiCl (positive control) or 0.75, 1.5 or 3.0 mg/kg OA. Twenty-four hours later, the rats were given a choice between tap water and the 0.1% saccharin solution. Rats treated with the vehicle or any of the doses of OA exhibited a marked preference for the saccharin solution, whereas the rats treated with LiCl exhibited a marked rejection of the saccharin solution. The implications of these data for an understanding of mycotoxicosis are discussed.

Rats Conditioned saccharin aversion Feed refusal Anorexia Mycotoxins Ochratoxin A Behavioral toxicity Taste aversion

MYCOTOXINS are biologically active secondary metabolites produced by several fungal species on sorghums and other grains commonly used as components in feed for livestock and poultry as well as in the human diet. Ochratoxin A (OA) is a mycotoxin produced by *Aspergillus* and *Penicillium* species and has been found worldwide as a contaminant in animal feed and human food (6). Reported manifestations of ochratoxicosis include impaired renal and hepatic function, enteric and gastric necrosis, and loss of body weight in species such as rats, mice, dogs, pigs, ducks, rainbow trout, and hens (1, 6, 8, 10, 12).

Weight loss or reduced weight gain is consistently observed in rats treated chronically with OA-contaminated feed at dose levels of 0.75 to 2.0 mg/kg (1). However, the reduced weight gains noted in OA-treated rats is accompanied by feed refusal or rejection of the adulterated diet. Although changes in ingestive behavior may be indicative of mycotoxin exposure [cf. (2)], recent studies have suggested that toxicity of mycotoxins can also be gauged using the conditioned taste aversion paradigm in which a novel saccharin flavor is paired with mycotoxin exposure resulting in avoidance of the saccharin flavor. Clark *et al.* (3), for example, demonstrated that vomitoxin (deoxynivalenol) readily induced a strong conditioned saccharin aversion in rats at low dose levels. To further assess the toxicity of OA, the aversiveness of acutely administered OA (0.75, 1.5 and 3.0 mg/kg) was compared with that of a positive control (LiCl, 32 mg/kg) using a conditioned taste aversion paradigm (3,7) in the present study.

METHOD

Animals

Animal care, handling and euthanasia were in accordance with procedures established and approved by the Animal Use Committee, Texas A&M University. The animals were 40 adult male Sprauge-Dawley albino rats (Timco, Inc., Houston, TX) weighing between 164 and 196 g at the beginning of the experiment. The rats were individually housed in standard polycarbonate rodent cages (Lab Products, Bryan, TX) in a colony room maintained at 23 ± 1 degrees C under a continuous lighting schedule. The rats were allowed ad lib access to chow pellets (Purina Rat and Mouse

[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

Diet No. 5001; Ralston Purina Co., St. Louis, MO) and to tap water except as specified in the procedures below.

Injection Materials

A vehicle solution was prepared using 5% $NaHCO_3$ dissolved in sterile distilled water. The OA solutions were prepared by dissolving crystalline OA (Sigma Chemical Co., St. Louis, MO) into the vehicle to produce final concentrations of 0.75, 1.5 and 3.0 mg/ml. The LiCl solution consisted of 32 mg/ml dissolved in the $NaHCO_3$ vehicle. All injections were given IP in a volume of 1.0 ml/kg.

Procedure

The rats were maintained in the colony for a 7-day acclimation period prior to the start of the study. Food and water were provided ad lib during the acclimation period. The rats were handled and weighed daily during this period.

The rats were, then, trained to consume their daily portion of water during a 30-minute period on 7 consecutive baseline days. Water was provided from a calibrated drinking tube (Wahmann) in a separate drinking test cage. Each test cage was identical to the home cage and was located in the colony room on a separate rack. In each test cage, one or more metal sipper tubes could extend through the wire mesh that formed the top of the cage. Water intakes during the 30-minute test interval were recorded to the nearest ml for each rat. Body weights were recorded to the nearest g daily. The rats were allowed access to food, but not water, in the home cage during the 23.5-hr intertest intervals. At the end of the baseline phase, five groups of rats (n = 8 per group) of comparable water intake were formed using the average water intake recorded for each rat during baseline days 5–7. The groups exhibited comparable mean group water intakes within each group. These matched groups were then randomly assigned to the treatment conditions of this experiment.

On Day 8 (the conditioning day), the rats were offered a 0.1% saccharin solution in place of water during the 30-minute test period. Immediately after consumption of the saccharin solution, each rat was injected with the $NaHCO_3$ vehicle or with either 0.75, 1.5 or 3.0 mg/kg OA. To provide a comparison with a substance known to induce conditioned saccharin aversion (7), a positive control group received an injection of LiCl (32 mg/kg). On Day 9, the rats were offered a choice between tap water and the 0.1% saccharin solution from calibrated drinking tubes during a 30-minute test period to measure the strength of the conditioned aversion to saccharin. The two-bottle choice data were analyzed as saccharin consumption ratios (i.e., volume of 0.1% saccharin consumed divided by total fluid intake). Using this ratio, consumption of only saccharin would yield a value of 1.0, whereas complete rejection of the saccharin would result in a ratio of 0.0. Because OA failed to induce a conditioned saccharin aversion (see the Results section below), extinction trials in which the rats are allowed to select either water or 0.1% saccharin (after eating an unadulterated diet) to determine the magnitude of an aversive effect via resistance to extinction were not conducted.

RESULTS

Table 1 depicts baseline water intake, saccharin consumption of Day 8 and saccharin consumption ratios, as well as total fluid intake on Day 9 for the treatment groups of this experiment. Separate one-way analyses of variance revealed no significant differences among the groups with regard to either baseline water consumption (collapsed for each rat across Days 5–7) or consumption of 0.1% saccharin on Day 8. Analyses of variance computed using the saccharin consumption ratio data from Day 9 did, however, reveal a significant treatment effect, $F(4,35) = 10.4$, $p<0.001$. The saccharin consumption ratio for the vehicle (negative control) group was 0.64 but was 0.06 for the LiCl (positive control) group. Subsequent a posteriori Tukey's pair-wise comparisons among the treatment means (5) revealed no significant differences between the vehicle group and any of the groups treated with OA, $Q(35) \leq 3.6$, $p>0.05$. In contrast, rats treated with LiCl at 32 mg/kg exhibited a significant rejection of the saccharin solution relative to the vehicle control group, $Q(35) = 7.1$, $p<0.01$. Finally, there were no significant differences among the groups with regard to total fluid consumption on Day 9. Thus, all groups drank similar amounts of fluid on the test day. The differences among the treatment groups were related to pattern of consumption: the lithium chloride group drank primarily water, whereas the remaining groups drank primarily saccharin.

TABLE 1

MEAN GROUP BASELINE WATER INTAKE (AVERAGED ACROSS DAYS 5–7), SACCHARIN INTAKE (DAY 8) AND SACCHARIN INTAKE RATIOS (DAY 9) FOR RATS TREATED WITH EITHER VEHICLE,[1] LITHIUM CHLORIDE,[2] OR 0.75, 1.5 OR 3.0 mg/kg OA

		Average Fluid Intake (ml)			
Group	N	Water (Days 5–7)	Saccharin (Day 8)	Saccharin Ratio (Day 9)	Total Intake (Day 9)
Vehicle	8	13.0 ± 1.0[3]	12.8 ± 0.9	0.64 ± 0.09	15.5 ± 0.6
LiCl	8	12.7 ± 0.9	12.6 ± 0.8	0.06 ± 0.04*	14.8 ± 1.7
0.75 OA	8	13.1 ± 0.7	13.9 ± 1.5	0.72 ± 0.06	15.1 ± 0.6
1.5 OA	8	13.0 ± 0.8	11.3 ± 1.5	0.57 ± 0.11	15.0 ± 0.5
3.0 OA	8	13.1 ± 0.9	11.2 ± 1.0	0.59 ± 0.09	14.5 ± 1.2

[1]Vehicle = 5% $NaHCO_3$ in sterile distilled water. [2]Lithium chloride (32 mg/kg). [3]Mean ± SD. *$p<0.01$: comparison with vehicle control group (2-tailed Tukey *t*-test).

DISCUSSION

Mycotoxins are frequently implicated in feed refusal and weight loss in a variety of animal species. Chronic exposure to dietary OA results in reduced feed consumption. Munro *et al.* (8), for example, determined that OA-contaminated diets resulted in reduced food intake and body weight gains in rats at OA levels between 9.6 ppm (1.3 mg/kg) and 24 ppm (2.4 mg/kg). These results suggest that OA contamination results in altered feeding behavior (decreased food consumption) with consequential decrease in body weight gain.

In the present study, the influence of various treatment levels of OA on conditioned saccharin aversion in adult rats was determined to establish whether acute exposure to OA would result in significant aversion to a novel flavor paired with OA exposure. The results failed to demonstrate that IP injection of OA at either 0.75, 1.5 or 3.0 mg/kg would induce a conditioned saccharin aversion in adult male rats. A significant conditioned taste aversion was, however, established using a moderate dose of LiCl (32 mg/kg). This positive control using LiCl strengthens the assertion that acute exposure to OA at these dose levels did not result in an aversive state sufficient to produce conditioned saccharin aversion.

The lack of effect of OA on taste aversion was surprising given the report of reduced feed consumption and body weight following repeated exposure of rats to OA-contaminated feed (8). The present failure to obtain a conditioned taste aversion to ochratoxin is not the result of our use of a submaximal dose. Berndt and

Hayes (1) observed marked reductions in body weight in rats treated with OA at 0.75 and 2.0 mg/kg. Similar changes in body weight were noted by Munro *et al.* (8) at ochratoxin-A levels ranging from 1.3 to 2.4 mg/kg. Moreover, these dose levels were found to reduce feeding by 18–47%. Thus, our use of a dose of ochratoxin at 3.0 mg/kg greatly exceeds the level required to alter feeding behavior and body weight. The present data suggest that the feed refusal syndrome noted in ochratoxicosis is unlikely to be due to general malaise, but rather may result from a change in diet palatability by ochratoxin-A.

Other mycotoxins also cause reduced feeding activity, reduced body weight and conditioned taste aversion. Rappold *et al.* (11), reported that aflatoxin b_1 produced a strong aversive effect in rats and Clark *et al.* (3) demonstrated that rats fed vomitoxin (deoxynivalenol)-contaminated diets exhibit rapid acquisition of conditioned saccharin aversion. The report of a malaise-inducing action of vomitoxin has been replicated and extended by the work of Ossenkop *et al.* (9), who demonstrated that taste aversion induced by vomitoxin is attenuated after experimentally-induced destruction of the area postrema. These differential effects of OA and vomitoxin in conditioned saccharin aversion paradigms suggest dissociable mechanisms of action (e.g., perhaps taste versus postingestion malaise).

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