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BRIEF COMMUNICATION

Deoxynivalenol (Vomitoxin)-Induced Conditioned Taste Aversions in Rats Are Mediated by the Chemosensitive Area Postrema

KLAUS-PETER OSSENKOPP,*†[1] MAURICE HIRST*† AND WILLIAM A. RAPLEY†

**Neuroscience Program and †Department of Pharmacology and Toxicology, University of Western Ontario, London, Ontario, Canada N6A 5C2*

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OSSENKOPP, K.-P., M. HIRST AND W. A. RAPLEY. *Deoxynivalenol (vomitoxin)-induced conditioned taste aversions in rats are mediated by the chemosensitive area postrema.* PHARMACOL BIOCHEM BEHAV 47(2) 363-367, 1994.—The present experiments used a conditioned aversion to a novel saccharin taste to assess the aversive effects of deoxynivalenol (vomitoxin) administration, and to examine the putative mediating role of the chemosensitive area postrema (AP). In experiment 1 adult male rats drank a novel 0.15% saccharin solution followed by injection of deoxynivalenol (n = 7; 0.125 mg/kg, IP) or vehicle (n = 7; propylene glycol, 0.5 ml/kg). In subsequent two-bottle preference tests the rats conditioned with deoxynivalenol displayed significantly ($p < 0.01$) lower absolute and relative saccharin intake levels in comparison to control rats which exhibited a strong preference for saccharin solution. In experiment 2 adult male rats received area postrema ablations (n = 6) or sham lesions (n = 6). On two conditioning days all rats drank a novel 0.15% saccharin solution followed by injections of deoxynivalenol (0.125 mg/kg, IP). In subsequent two-bottle preference tests the sham-lesioned rats displayed a significant ($p < 0.01$) aversion to the saccharin stimulus, relative to the area postrema-ablated rats which exhibited a preference for the saccharin solution. Thus, systemic administration of deoxynivalenol, following a novel taste, induced conditioned taste aversions which were mediated by the area postrema.

Conditioned taste aversion Trichothecene mycotoxin Deoxynivalenol Vomitoxin Area postrema Behavioral toxicology Rats

DEOXYNIVALENOL (vomitoxin; 3,7,15-trihydroxy-12,13-epoxytricothec-9-ene-8-one) is a naturally occurring mycotoxin. It belongs to the trichothecene family of toxins produced by *Fusarium* species, which are well known to be pathogenic to plants producing cereal grain and to cause alimentary toxicosis in farm animals and humans (9,28,30-32). Deoxynivalenol has been found in corn and wheat grown in many parts of the world and is a recognized naturally occurring contaminant of foods and feeds (28,32). A wide range of concentrations of deoxynivalenol has been reported in naturally infected corn and grain samples. Bamburg (2) summarized some of these data and reported levels as high as 50 ppm in corn. Levels as high as 8.5 ppm of deoxynivalenol in wheat (26) were associated with reports of feed refusal, vomiting, and reproductive problems in livestock. This toxin induces emesis in pigs and dogs (7,27) and, when present as a contaminant in feed, causes feed refusal by pigs and rats (7-9, 20,31,32). Orally administered deoxynivalenol leads to decreased food consumption, body weight gain, feed efficiency, and fertility (1,9,15). Consumption of rice infected by the fungus *Fusarium graminearum,* a source of vomitoxin, causes nausea, vomiting, and drowsiness in humans (29).

Strong avoidance conditioning of a novel taste can be produced in animals by pairing ingestion of the novel tasting substance with exposure to toxic agents, such as lithium [e.g., (10,16)]. The suggestion has been made that it is the illness-inducing (i.e., nausea or gastrointestinal disturbance) property of the toxins which make them such effective agents in producing a conditioned taste aversion [CTA; e.g., (10,11)]. Clark et al. (6) examined the effects of deoxynivalenol on feeding and the formation of a CTA in rats. They found that a CTA to saccharin could be established by adulteration of

[1] Requests for reprints should be addressed to Dr. Klaus-Peter Ossenkopp, Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2.

the food with 4–8 ppm of deoxynivalenol and concurrent presentation of a novel saccharin drinking solution. Adulteration of the diet with 8 ppm of vomitoxin also resulted in a transient reduction in food intake.

The area postrema (AP), a circumventricular organ located in the fourth ventricle, has been functionally implicated in a range of physiological and behavioral processes, and especially as a sensor for blood-borne toxins (4). This structure has a reduced blood–brain barrier and is accessible to drugs which do not penetrate to many other regions of the brain. Despite the fact that rats do not vomit (12), the rat area postrema has been shown to mediate the formation of a variety of CTAs [e.g., (3,13,17,18,21,23)], although not all CTAs are abolished following ablation of this structure [e.g., (3,19)].

Within this context it was of interest to determine whether deoxynivalenol could condition a taste aversion when administered systemically and to examine the role of the area postrema in such a conditioning process. On the basis of a previous study (6) we predicted that deoxynivalenol would support a conditioned taste aversion in rats when administered IP. In addition, since a related trichothecene mycotoxin, T-2 toxin, may induce vomiting in cats and dogs (5,14) by stimulation of the area postrema, we predicted that the deoxynivalenol-induced CTA would require an intact area postrema.

GENERAL METHOD

Subjects

Twenty-six sexually mature (330–370 g) Long-Evans male hooded rats (Charles River, Quebec) were individually housed in stainless steel cages. The animals were kept in a colony room maintained at approximately 21°C and a 12-h light/dark cycle with lights on from 0700 to 1900. The animals were maintained on standard laboratory chow (Purina) and tap water, which were available ad lib unless otherwise noted.

Drug

Highly purified (approximately 98% by high-performance liquid chromatography [HPLC]) vomitoxin, in the form of 4-deoxynivalenol, was provided by the laboratory of Dr. H. L. Trenholm, Agriculture Canada, Ottawa. This deoxynivalenol was dissolved in propylene glycol in a concentration of 0.25 mg/ml.

Conditioning Procedure

All animals were adjusted to a 23 h/day water deprivation schedule over a seven-day period. On the conditioning day(s) all rats were given 60 min access to a 0.15% (w/v) sodium saccharin solution. Rapidly following this drinking period some of the rats (group DON, experiment 1; all rats, experiment 2) were given injections of deoxynivalenol (0.125 mg/kg IP). This dose was selected from feeding studies in rats (unpublished data). It is approximately equivalent to the amount available to a 350-g rat in a meal consisting of food pellets (approximately 10 g) containing 5 ppm of deoxynivalenol. The other rats received injections of the vehicle (propylene glycol, 0.5 ml/kg, IP). The two-bottle preference tests consisted of a 60-min choice between a saccharin solution and tap water.

Data Analysis

Data were analyzed with analysis of variance (ANOVA) procedures (SOLO 2.0, BMDP Statistical Software, Inc., Los Angeles) followed by post hoc Newman-Keuls tests. The significance level used for hypothesis testing was $\alpha = 0.05$.

EXPERIMENT 1

The first experiment examined the conditioning of an aversion to saccharin with IP injection of deoxynivalenol. Previous studies had demonstrated conditioned saccharin aversions in rats when deoxynivalenol- (6) or T2-toxin-adulterated (33) food was paired with presentation of a novel saccharin drinking solution. Under these experimental conditions it was difficult to separate the effects of the mycotoxins on illness-enhanced neophobia, sensitization, and CTA. In the present experiment the novel saccharin taste was followed by IP injection of deoxynivalenol, the more typical method of CTA induction (16).

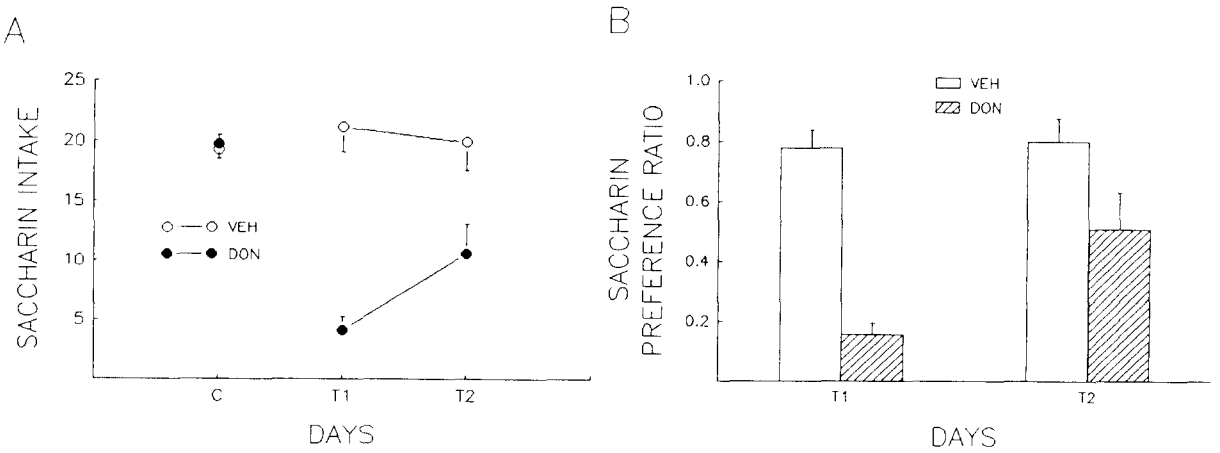

FIG. 1. (A) Group mean saccharin solution intake on the conditioning day (C) and the two-bottle preference tests (T1, T2). Note that on the conditioning day only saccharin solution was available, whereas on the preference test days both saccharin solution and water were available. (B) Group mean saccharin preference ratios obtained during the two-bottle preference tests (T1, T2). Error bars are SE.

METHOD

Conditioning Procedure

On day 8 all rats received 60 min access to the saccharin solution followed rapidly by injection of deoxynivalenol (group DON, $n = 7$) or propylene glycol (Group VEH, $n = 7$). On days 9 and 10 the rats were given tap water for 60 min but otherwise were not disturbed. On days 11 and 12 all rats were given two-bottle preference tests.

RESULTS AND DISCUSSION

Comparison of mean group water intake on the last two days of the water deprivation adjustment period (days 6 and 7) revealed no significant group differences ($p > 0.30$). Mean group saccharin solution intake levels on the conditioning day and the two test days are presented in Fig. 1A. Group VEH maintained a high level of saccharin intake on all days. In contrast, group DON exhibited high levels of saccharin intake on the conditioning day, but much reduced levels on the two test days, indicative of a conditioned aversion to saccharin. This was confirmed by the ANOVA procedure, which revealed a significant Groups × Days interaction, $F(2, 24) = 17.44, p < 0.001$. The group mean difference was not significant on the conditioning day ($p > 0.50$), but the groups did differ significantly on the two test days, $F(1, 12) = 34.57$, $p < 0.001$.

Figure 1B depicts the group data for the two-bottle test procedure (saccharin preference ratios). These data confirmed the existence of a conditioned aversion to saccharin in group DON. The ANOVA indicated a significant groups main effect, $F(1, 12) = 30.14, p < 0.001$, and a significant test day main effect, $F(1, 12) = 5.87, p = 0.032$, as well as a significant Groups × Days interaction, $F(1, 12) = 4.88, p = 0.047$. The significant interaction reflected a fairly rapid reduction in the strength of the CTA by the second test day in group DON. These results are in agreement with a previous demonstration of a deoxynivalenol-induced CTA in rats (6) and pigs (22).

EXPERIMENT 2

Since the results of experiment 1 indicated that IP injections of deoxynivalenol could condition a taste aversion to a novel saccharin taste, it was of interest to examine the possibility that such a CTA was mediated by the area postrema. Previous research (5) had suggested a role for the area postrema in T-2 mycotoxin-induced emesis in cats and dogs. Furthermore, several previous experiments have provided evidence for mediation of toxin-induced CTA by the area postrema in rats (3, 13,21,24,25). Other research in our laboratory has also supported a role for the area postrema in deoxynivalenol-adulterated food rejection in rats (20).

METHOD

Surgical Procedure

Six rats (group APX) received area postrema lesions under sodium pentobarbital anesthesia (Somnotol, 55 mg/kg, IP). The animals were anesthetized and then placed in a headholder which kept the head in a ventroflexed position. The dorsal surface of the brainstem was exposed by retracting the overlying muscles and the atlanto-occipital membrane joining the cranium and spinal column, and enlarging the foramen magnum. The floor of the fourth ventricle was viewed through an operating microscope (Zeiss, OPMI 99), and lesions of the area postrema were made by touching this structure with the tip of a small cautery. The neck muscles and scalp were sutured and the animals were allowed to recover from the operation for 10 days. The other rats (group SHA, $n = 6$) were treated in an identical manner except that the area postrema was not lesioned (sham-lesion procedure).

Conditioning Procedure

On days 8 and 9 all rats were given 60 min access to the saccharin solution. Immediately following these drinking periods all rats received IP injections of deoxynivalenol. Two conditioning days were used in this experiment, since the results of experiment 1 had indicated that deoxynivalenol-induced CTAs showed fairly rapid extinction with a one-trial conditioning procedure. On day 10 the rats were given tap water for 60 min but not otherwise disturbed. On days 11 and 12 all rats were given two-bottle preference tests.

Histological Procedure

At the conclusion of the experiment all lesioned and sham-lesioned rats were deeply anesthetized and then perfused intra-

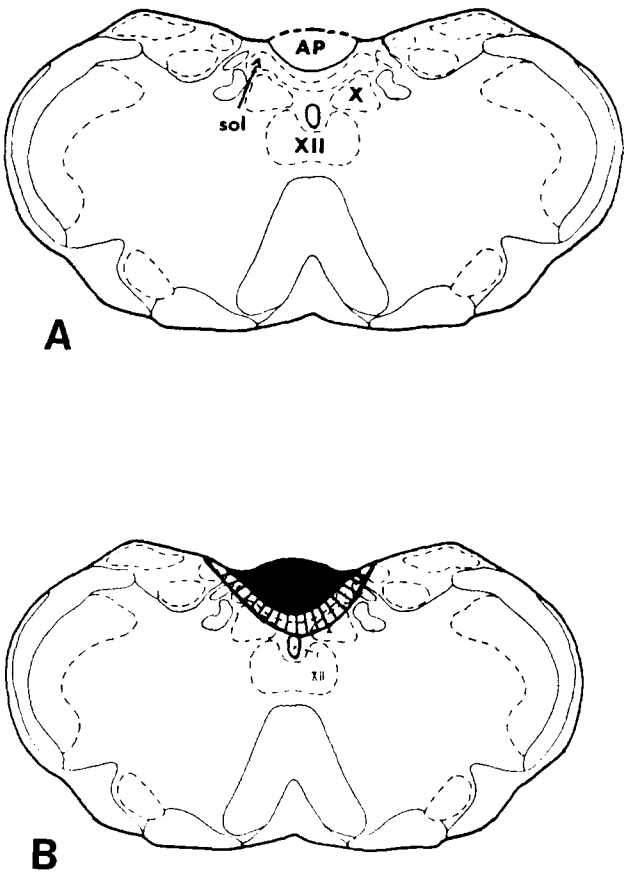

FIG. 2. Representative diagrams of coronal brainstem sections showing the degree of damage following the lesion procedure in group APX. (A) Sham-lesioned (SHA) group. (B) Area postrema-lesioned (APX) group showing the least (darkened area) and greatest (hatched area) amount of tissue damage. Diagrams are from Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.

cardially with isotonic saline followed by a 10% solution of formalin. The brains were removed and stored in formalin for at least two days and then coronal sections 50 μm thick were cut on a freezing microtome at the level of the brainstem containing the area postrema. These sections were mounted on slides and stained with cresyl violet.

RESULTS AND DISCUSSION

Histology

Histological analysis revealed that all of the lesions were successful in producing damage to the AP. However, incomplete damage to this structure was observed in one rat, and the data for this animal were eliminated from any further data analyses. The rest of the lesioned rats had complete lesions of the AP as well as partial damage to the subjacent caudomedial solitary nucleus. The histological sections from the lesioned rats were very similar to those previously published from this laboratory [e.g., (17,18,21)]. Sham-lesioned rats did not exhibit any signs of damage to the area postrema or surrounding areas. Figure 2 depicts the extent of damage for the smallest and largest area postrema lesions.

Conditioned Taste Aversions

Comparison of mean group water intake on the last two days of the water deprivation adjustment period (days 6 and 7) revealed no significant group differences ($p > 0.30$). Mean group saccharin solution intake levels on the two conditioning days and the two taste preference test days are presented in Fig. 3A. An overall ANOVA revealed a significant Groups $\times$ Test Phase interaction, $F(2, 18) = 7.77, p = 0.037$. Additional analyses indicated no significant group differences on the two conditioning days ($F < 1$), but significantly reduced saccharin intake on the two taste preference test days by group APS relative to conditioning phase levels, $F(1, 12) = 66.82$, $p < 0.001$, and relative to group APX, $F(1, 9) = 13.18$, $p < 0.01$. These reduced levels of saccharin intake are indicative of a CTA for group APS, whereas group APX displayed no evidence of a CTA ($F < 1$; taste preference phase intake relative to conditioning phase intake).

Figure 3B depicts the group data for the more sensitive two-bottle test procedure (saccharin preference ratios). These data confirm the significant group difference: groups main effect, $F(1, 9) = 22.40, p = 0.001$. The days main effect and the interaction were not significant. Thus, both the absolute and relative levels of saccharin intake were much higher in the AP-lesioned group indicating that, unlike the sham-lesioned rats, these animals failed to acquire a conditioned aversion to the novel saccharin taste paired with deoxynivalenol injections.

GENERAL DISCUSSION

The major findings of the present experiments are the conditioning of a saccharin taste aversion with IP injections of deoxynivalenol (experiment 1) and mediation of this aversion by the area postrema (experiment 2). The demonstration that deoxynivalenol can condition an aversion to a novel taste in rats is consistent with previous experiments using rats (6) and pigs (22). These earlier studies also obtained vomitoxin-induced CTAs. However, the previous study by Clark et al. (6) used vomitoxin-contaminated food as the unconditioned stimulus to induce a CTA to a saccharin solution concurrently available. The present data showed that it is the postingestional effects of the deoxynivalenol which are responsible for the observed aversions, since these treatments did not occur until after the novel saccharin solution had been consumed.

Refusal of feed contaminated with vomitoxin may occur because the contaminant renders the food unpalatable or because the vomitoxin may produce aversive postingestional effects. The present results provide evidence for the aversive postingestional properties of deoxynivalenol. Additional experiments need to examine the effects of vomitoxin on food palatability independent of the postingestional effects of this toxin.

The observations, that area postrema lesions attenuated/abolished the deoxynivalenol-induced CTAs, are consistent with a number of earlier studies showing a chemosensitive role

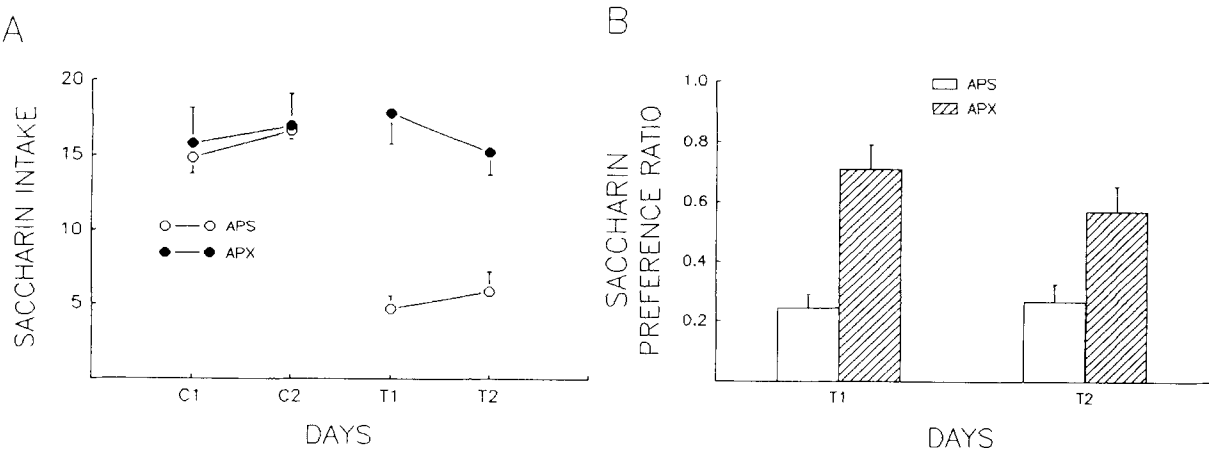

FIG. 3. (A) Group mean saccharin solution intake on the two conditioning days (C1, C2) and the two-bottle preference tests (T1, T2). Note that on the conditioning days only saccharin solution was available, whereas on the preference test days both saccharin solution and water were available. (B) Group mean saccharin preference ratios obtained during the two-bottle preference tests (T1, T2). Error bars are SE.

for this neural structure in detection of blood-borne toxins (4). The absence of a taste aversion following area postrema ablation is unlikely to be a result of deficits in taste, since lesioned rats have been shown to exhibit normal or enhanced CTAs when conditioned with amphetamine (3) and nicotine (19). The present findings are also consistent with previous studies showing that the area postrema may mediate acute emetic effects of the T-2 mycotoxin (5). If the area postrema is responsible for detection of the blood-borne vomitoxin and the stimulation of this structure results in aversive postingestional effects, then pharmacological intervention at the level of this structure may prove to be useful in attempts to control some of the adverse effects of vomitoxin, such as feed refusal and weight loss.

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