Effects of tumor growth on taste-aversion learning produced by antitumor drugs in the rat

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A number of commonly used antitumor drugs (Cytoxan, Adriamycin, cytosine arabinoside, Vincristine) were evaluated for their ability to act as unconditioned stimuli (USs) in a taste aversion conditioning paradigm. With one exception (cytosine arabinoside), discussed in the text, drugs which induce nausea and vomiting when administered to humans (Cytoxan, Adriamycin) produced significant saccharin aversions in rats; a drug which does not produce these symptoms (Vincristine) did not produce saccharin aversions. In later studies, the effect of a chronic disease state (tumor growth) on the acquisition of learned taste aversions was evaluated. Rats bearing moderate or advanced transplantable tumors were comparable to healthy controls in their formation of conditioned taste aversions. Therefore, the chronic malaise produced by a growing tumor neither attenuated nor exaggerated the taste aversions induced by Cytoxan or Adriamycin injections. Results are discussed in terms of their implications for human taste aversion learning.

Conditioned taste aversions have been detected following the administration of a wide variety of drugs (see Riley & Baril, 1976). One drug frequently employed in taste-aversion experiments, the alkylating agent cyclophosphamide (Cytoxan) (Garcia, Ervin, & Koelling, 1967; Garcia, Hankins, Robinson, & Vogt, 1972; Wilcoxin, Dragoin, & Kral, 1971), is presumably effective because it produces symptoms of GI discomfort (i.e., nausea; emesis). Cytoxan is also one of several chemotherapeutic drugs with similar side effects [gastrointestinal (GI) toxicity] which are widely used clinically in the treatment of neoplastic diseases (Goodman & Gilman, 1970). This suggested that patients receiving treatment for certain types of cancer might form aversions to foods consumed before such treatments.

Bernstein (1978) recently tested this hypothesis and reported that pediatric cancer patients developed significant aversions to a novel ice cream flavor when it was consumed prior to GI toxic drug treatments. These aversions were long-lasting and were evident even though patients had received numerous prior drug exposures. Since drug preexposure is known to attenuate or eliminate taste-aversion conditioning in the rat (Elkins, 1974; Goudie, Taylor, & Atherton, 1975), the observation of these taste aversions in cancer patients was somewhat surprising. It suggested

that humans may be particularly susceptible to forming learned taste aversions. On the other hand, it is possible that susceptibility to forming learned taste aversions is limited to the clinical population studied, and not to all humans. Little is known about the effect of a chronic disease state, such as tumor growth, on the acquisition of learned taste aversions. The chronic malaise produced by a growing tumor could (1) sensitize organisms and amplify taste-aversion learning, (2) mask the aversive effects of the US and attenuate taste-aversion learning, or (3) have no effect. The present studies were aimed at investigating this question. The first studies examined normal rats to determine whether a variety of anti-tumor agents, administered in dosages similar to those in clinical use, would act as USs in taste-aversion conditioning. Later studies compared the taste-aversion learning of healthy rats and rats bearing transplantable tumors.

EXPERIMENT 1

The drugs tested were Cytoxan, doxorubicin (Adriamycin, an antibiotic), cytosine arabinoside (a pyrimidine analog antimetabolite), and Vincristine (a vinca alkaloid.) These drugs, with the exception of Vincristine, produce considerable nausea and vomiting when administered to humans (Goodman & Gilman, 1970).

Method

Experiment 1a. Thirty, naive, syngeneic Wistar-Furth (W/Fu) male rats were housed in individual stainless steel cages with free

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Figure 1. Percent saccharin (saccharin/total fluid $\times 100$) consumed during two-bottle preference test as a function of drug treatment. Mean total fluid consumption in preference test: saline = 16.3 ml; Vincristine = 14.7 ml; ARA-C (cytosine arabinoside) = 16.3 ml; Adriamycin = 15.3 ml; Cytoxin = 13.8 ml.

access to Purina Rat Chow throughout the experiment. This strain of rats readily accepts tumor transplants from other individuals of the same strain, as used in Experiment 2. The animals were divided randomly into five groups. For 12 days, all rats received water for only 30 min a day. Water was presented in graduated drinking tubes at the front of the cage. The position of the drinking tube was alternated each day. On the 13th day, all rats received a .1% aqueous solution of sodium saccharin instead of water, during the usual 30-min drinking session. Immediately after the 30-min drinking session, each rat was injected intraperitoneally (IP) with one of the following solutions: physiological saline (1 ml/kg); Cytoxin (40 mg/kg) (a gift of Keith Wheeler, Mead Johnson); Vincristine (.10 mg/kg); Adriamycin (4.0 mg/kg) (a gift of Luigi Lenaz, Adria Laboratories); or cytosine arabinoside (6.0 mg/kg). Dosages were converted on a mg/kg basis from human therapeutic dosages (Goodman & Gilman, 1970). Dosages given the rats were twice the typical human dose in an effort to compensate for differences in both route of administration (clinically these drugs are administered intravenously) and metabolic rate. On the 14th day, all rats were given a 30-min choice between .1% saccharine solution and tap water, placed randomly in the right and left positions on each animal's cage. A forced sampling procedure was used in which the rats sampled each solution for at least 10 sec at the beginning of the 30-min drinking bout while the other tube was briefly removed. Following this procedure, the animals had free access to both solutions for the remainder of their 30-min bout. Preference scores were calculated for each rat by dividing the volume of intake of saccharine solution by the total volume of intake during the 30-min preference test.

Experiment 1b. Thirty additional rats were exposed to taste aversion conditioning as in the previous study with the addition of two water-drug control groups. Three groups were similar to those in the previous study, with animals exposed to saccharine solution and injected with physiological saline (saccharin-saline group); Cytoxin (20 mg/kg) (saccharin-Cytoxin group), or Adriamycin (saccharin-Adriamycin group). Two additional groups were exposed to plain tap water prior to being injected

with either Cytoxin (20 mg/kg) (water-Cytoxin group) or Adriamycin (4 mg/kg) (water-Adriamycin group). All other procedures were the same as described in Experiment 1a.

Results

Experiment 1a. Results of the preference test are depicted in Figure 1. Control rats which received saline injections during conditioning had an overwhelming preference for the saccharin solution. The overall effect of drug treatments on saccharin preference was significant [ANOVA, $F(4,25) = 19.8$, $p < .005$]. An analysis of the difference between control intakes and each treatment mean (Edwards, 1972, p. 149) indicated a significant depression of saccharin preference in animals that received a single IP injection of Adriamycin ($p < .01$) or Cytoxin ($p < .01$). Saccharin preference after cytosine arabinoside appears somewhat reduced, but this difference is not significant ($p < .10$). Vincristine injections did not affect saccharin consumption.

Experiment 1b. Results of the preference test are depicted in Figure 2. Control groups (saccharin-saline, water-Cytoxin, and water-Adriamycin) had higher preferences for the saccharin solution than did the experimental groups, which had consumed saccharin prior to either Cytoxin or Adriamycin injections. An analysis of variance indicated a significant overall effect of conditioning treatment [$F(4,25) = 25.37$, $p < .01$]. Planned comparisons confirm that the dif-

Figure 2. Percent saccharin consumed during two-bottle preference test as a function of conditioning treatment. Mean total fluid consumption during the preference test was: saccharin-saline = 20.0 ml; saccharin-Cytoxin = 16.3 ml; saccharin-Adriamycin = 15.0 ml; water-Cytoxin = 22.5 ml; water-Adriamycin = 16.8 ml.

ferences in saccharin preference between the three control and two experimental groups is significant ($p < .01$). These results indicate that the reduction in preference seen in the experimental groups is not a nonspecific effect of drug treatment.

Discussion

A number of commonly used antitumor drugs were evaluated for their ability to act as USs in a taste-aversion conditioning paradigm. Dosages selected were based on those in clinical use, although conversions based on species-specific drug metabolism were not attempted. Results were, with one exception, consistent with the idea that drugs which induce nausea and vomiting in humans (i.e., Cytosin, Adriamycin) produced significant aversions in rats. The observation that cytosine arabinoside, unlike the other GI toxic drugs, did not produce significant aversions is probably due to a species difference between rat and human in metabolism of this drug. Lenaz and Philips (1970) and others (Wodinsky & Kessler, 1965) have reported that cytosine arabinoside is less toxic in the rat than in other species due to its incomplete phosphorylation in the rat. All three drugs were associated with aversions in humans (Bernstein, 1978).

Vincristine, which does not produce nausea, did not produce saccharin aversions. This is consistent with studies in humans (Bernstein, 1978; Bernstein, Wallace, Bernstein, Bleyer, Chard, & Hartman, 1979), where control patients receiving Vincristine did not develop taste aversions. A number of drugs which do not produce nausea have been reported to produce conditioned taste aversions (Gamzu, 1977), indicating that GI toxicity may be a sufficient, but not a necessary, condition for the establishment of taste aversions. It is possible that larger doses of Vincristine would produce significant aversions without GI toxicity.

EXPERIMENT 2

In order to determine whether tumor growth interacts with taste aversion conditioning, rats with transplantable tumors were exposed to a conditioning procedure like the one in Experiment 1.

Method

Two polyoma virus-induced sarcomas (PW-739 and PW-7410) were used; these tumors grow progressively and are lethal to animals in 8-10 weeks. These two tumors produce different effects on food intake and body weight. The growth of the PW-739 tumor is associated with considerable anorexia and weight loss, while the PW-7410 tumor does not appear to induce reductions of food intake or weight (Bernstein, Samson, & Sigmundi, Note 1).

Experiment 2a. Fifty-four naive, syngeneic W/Fu female rats were divided randomly into three groups. All rats had a small (2×2 cm) patch of skin exposed on their right flanks. The tumor-free control rats were injected subcutaneously in this patch with .01 ml of physiological saline. Experimental rats received .1 ml

Figure 3. Percent saccharin consumed during two-bottle preference test as a function of tumor and drug treatments. Open bars are Cytoxin treated groups; striped bars are saline controls. Mean total fluid consumption during test: no tumor-Cytoxin = 16.5 ml; no tumor-saline = 14.2 ml; 739 tumor-Cytoxin = 15.3 ml; 739 tumor-saline = 19.2 ml; 7410 tumor-Cytoxin = 16.7 ml; 7410 tumor-saline = 15.3 ml.

subcutaneous injections of either PW-7410 or PW-739 tumor cells in a saline solution at a concentration of 10^6 cells/ml.

Following injection, the animals were given unrestricted access to Purina Rat Chow and water for approximately 6 weeks while the tumors grew. The animals were then housed individually, and the habituation and conditioning procedures described in Experiment 1 were followed. On Day 13, the control (no tumor), PW-739, and PW-7410 groups were each randomly divided in half. Immediately following the 30-min exposure to saccharin solution, one-half of the rats in each group received .1 ml of physiological saline and the remaining half received Cytoxin (20 mg/kg). On Day 14, all groups received a two-bottle preference test as described in Experiment 1.

Experiment 2b. Experiment 2a was essentially repeated with an additional 54 W/Fu female rats, but these animals were studied at an earlier stage of tumor growth and with Adriamycin (4 mg/kg) replacing Cytoxin. Conditioning took place after 4 weeks of tumor growth.

Results

Experiment 2a. A number of tumor-bearing animals died before testing so final group sizes were: 739-control (N = 5), 739-Cytoxin (N = 6), 7410-control (N = 3), 7410-Cytoxin (N = 3), no tumor-control (N = 6), no tumor-Cytoxin (N = 6). Saccharin preference during testing is depicted in Figure 3. Cytoxin-treated groups had a preference for water, while saline-treated animals preferred saccharin in the two-bottle choice test. An analysis of a factorial experiment with unequal ns for various treatments (Edwards, 1972, p. 220) yielded a significant overall treatment effect [$F(5,23) = 5.13$, $p < .005$] and a significant drug effect [$F(1,23) = 24.8$, $p < .005$]. Tumor effects and tumor-drug interactions were not significant ($F < 1.0$). Total fluid consumption of tumor-bearing animals did not differ from controls.

Figure 4. Percent saccharin consumed during two-bottle preference test as a function of tumor and drug treatments. Open bars are Adriamycin-treated groups, striped bars are saline controls. Mean total fluid consumption during test: no tumor-Adriamycin = 17.2 ml; no tumor-saline = 20.2 ml; 739 tumor-Adriamycin = 15.6 ml; 739 tumor-saline = 21.9 ml; 7410 tumor-Adriamycin = 16.2 ml; 7410 tumor-saline = 20.7 ml.

Experiment 2b. Final group sizes for this study were: 739-control (N=8), 739-Adriamycin (N=7), 7410-control (N=8), 7410-Adriamycin (N=9), no tumor-control (N=6), no tumor-Adriamycin (N=6). Results of saccharin preference testing are depicted in Figure 4. As in Experiment 2a, overall treatment effects [$F(5,38) = 7.86, p < .005$] and drug effects [$F(1,38) = 34.4, p < .005$] were highly significant. Tumor effects [$F(2,38) = 1.86, p < .25$] and tumor-drug interactions ($F < 1.0$) were not significant.

These results replicate the findings reported in Experiment 1 that Cytoxan and Adriamycin are effective unconditioned stimuli in the establishment of conditioned aversions to saccharin. They further indicate that tumor growth does not appear to have an effect on the magnitude of learned aversions.

Discussion

These studies examined the effect of a chronic disease state (advanced tumor growth) on the acquisition of conditioned taste aversions. Tumor growth is associated with a variety of physiological (Costa, 1977) and behavioral (Bernstein et al., Note 1; Morrison, 1976) effects, including enlargement of spleen and adrenals, anorexia, and activity depression. Yet rats bearing moderate and advanced tumors were comparable to healthy controls in their formation of conditioned taste aversions. Therefore, the chronic malaise produced by a growing tumor neither attenuated nor exaggerated the taste aversions induced by Cytoxan or Adriamycin injections. It is possible that more sensitive tests would reveal effects of tumor growth on this learning.

These findings are important in interpreting the results of human clinical studies. The toxic nature of the USs employed in taste aversion conditioning studies have precluded controlled experimental investigation of this learning in healthy human subjects. Since cancer patients are frequently exposed to these treatments as therapy they are appropriate subjects for examining the acquisition of learned taste aversions in humans. These patients have been shown to have a strong propensity to form conditioned taste aversions to familiar as well as novel foods (Bernstein, 1978; Bernstein et al. 1979; Bernstein & Webster, Note 2) even when they have received many drug preexposures. Whether results of these studies generalize to healthy humans depends on whether their disease state interacts with taste-aversion learning. Although not conclusive, the studies reported here suggest that tumor growth does not amplify taste-aversion learning. Anecdotal evidence (Garb & Stunkard, 1974) would also tend to support the conclusion that susceptibility to forming conditioned taste aversions is a characteristic of humans, in general, and not just cancer patients.

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