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Bupropion, d-Amphetamine, and Amitriptyline-Induced Conditioned Taste Aversion in Rats: Dose Effects

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MILLER, D. B. AND L. L. MILLER. *Bupropion, d-amphetamine, and amitriptyline-induced conditioned taste-aversion in rats: Dose effects.* PHARMACOL BIOCHEM BEHAV 18(5) 737–740, 1983.—Nine groups of rats (n=6 per group) were adapted to a daily one-half hour period of water availability. When intake had stabilized, they were allowed access to a 0.1% (w/v) solution of saccharin, and immediately afterward were given IP injections of isotonic saline; bupropion HCl (10.0, 20.0, or 40.0 mg/kg); d-amphetamine-sulfate (0.5, 1.0, 2.0 mg/kg); or amitriptyline HCl (5.0, 10.0, or 20.0 mg/kg) in a volume of 1 ml. The lowest dose of each compound was chosen to be equipotent in screening tests used to identify potential antidepressants. Following 2 days of access to water alone, all groups were given a choice between water and saccharin for 3 consecutive days. All compounds induced taste aversions in a dose-related manner, but amitriptyline induced greater and longer-lasting aversions than either bupropion or d-amphetamine which were equipotent over the dose range studied. As such, this is the first demonstration that bupropion and amitriptyline, two clinically effective antidepressants, can induce taste aversions and replicates as well the common finding that d-amphetamine has substantial taste aversion-inducing properties. The ability of these compounds to induce taste aversions could be mediated through their effects on central catecholaminergic processes although amitriptyline has significant peripheral anticholinergic effects.

Conditioned taste-aversion Antidepressant Bupropion d-Amphetamine Amitriptyline Two-bottle test Extinction Rats

BUPROPION, an aminoketone (see Fig. 1), has shown to have clinically significant antidepressant activity [10,11]. It is effective in animal screening tests for antidepressant activity including the prevention of tetrabenazine-induced sedation, blepharospasm, and fall in rectal temperature [22,23]. Significant increases in locomotor activity also occur but the antidepressant effects are observable at much lower doses than those necessary to produce changes in activity or operant behavior [7,16]. Despite the antidepressant qualities exhibited by bupropion, its biochemical properties are distinct from most commonly used antidepressants, the tricyclics and monoamine oxidase inhibitors. For example, bupropion is only a weak inhibitor of serotonin and norepinephrine reuptake into synaptosomes, and does not inhibit Type A or B forms of monoamine oxidase *in vivo* or *in vitro*. Nor does it have cholinergic effects [12, 22, 23]. The structure of bupropion resembles amphetamine but unlike amphetamine, it has no sympathomimetic or other automomic effects in animals [21]; it does not cause release of catecholamines [12]. Many actions of bupropion appear to be related to its selective inhibition of dopamine re-uptake [7].

Thus, bupropion is unique in its structural, biochemical and functional characteristics and consequently it would be difficult to predict its action in a behavioral paradigm where it is directly compared to both stimulant and antidepressant drugs. For example, Jones *et al.* [17] in a study of the stimulus properties of bupropion hypothesized that because of its antidepressant properties, this compound could be used as a drug cue to evaluate antidepressant activity in a drug discrimination paradigm. However, dose-related gen-

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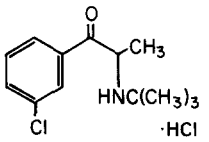

BUPROPION

FIG. 1. The structure of bupropion HCl.

eralization to the psychostimulants, d-amphetamine and methylphenidate and to the non-tricyclic antidepressants, viloxazine and nomifensine, rather than the tricyclic antidepressants, amitriptyline and imipramine, indicated bupropion produced a complex drug cue having both CNS stimulant and antidepressant characteristics. Other studies have not directly compared bupropion to stimulants and antidepressants in the same behavioral paradigm.

Since bupropion shows promise as the prototype for a new class of clinically effective antidepressants, it is of interest to determine whether it exhibits stimulant or antidepressant-like properties in other behavioral paradigms. One possible paradigm which could be used to evaluate these dual properties is the conditioned taste aversion paradigm [6]. When a novel flavor, such as saccharin, is paired with a drug or toxic agent, intake of that novel flavor decreases on subsequent presentations. A wide range of centrally-active compounds, including psychomotor stimulants, opiate analgesics, and antianxiety agents, have been shown to produce conditioned taste aversions [2, 5, 6, 14]. In contrast, the tricyclic antidepressants have not been extensively evaluated for their taste aversion-inducing properties. Berger [2] in a comparison of several drugs, reported that imipramine did not produce an aversion. Bupropion has not been evaluated for its ability to produce a taste aversion and because of its stimulant and antidepressant properties it is difficult to predict its actions. In the present study, bupropion, d-amphetamine, and amitriptyline were compared for their ability to produce a taste aversion. Several doses of each compound were included to allow dose-response comparisons and the persistence of the learned aversion was determined by repeated choice tests.

METHOD

Animals

Sixty male and female adult Sprague-Dawley derived rats (350–450 g), obtained from the Charles River Company (Charles River Breeding Laboratories, Portage, IA) were used in this study. Rats of the same sex were housed 3 to a cage (45×45×20 cm) in which food was available ad lib throughout the study. The animals were housed in a temperature-controlled (21±0.6°C) colony room in which lighting was provided from 0600 to 1800 hr daily.

Apparatus

Taste aversion trials were conducted in ceiling suspended metal cages that measured 24.5×18×18 cm with a 1.3 cm wire mesh floor and front (Wahmann Mfg. Co., Timonium, MD). Fluids were provided in one or two 100 ml graduate glass cylinders (Wahmann Mfg. Co.) with rubber stoppers and straight metal drinking spouts. Each bottle was attached so that a drinking spout could be contacted via one of the two small recesses attached to the front of the cage.

Procedure

At the start of the study rats were placed on a 23.5 hour water-deprivation schedule. Each rat was weighed daily and placed in a test cage in which tap water was available for a 30 minute period. The amount of water consumed was determined by weighing the water bottle with drink tube to the nearest 0.1 g and subtracting this weight from the pre-test bottle weight. Water bottle position was counterbalanced across animals for any one test day and across days for any one animal. Testing was conducted daily Monday–Friday between 1200–1400 hr.

The rats were adapted to the daily test conditions for 7 days with no significant group differences in fluid intake occurring on the final day of adaptation. Then the rats were provided with 0.1% (w/v) sodium saccharin solution for one (exposure) session. Within 5 to 10 minutes following this initial exposure to saccharin the rats were injected IP with d-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO); bupropion HCl (Burroughs Wellcome Co., Research Triangle Park, NC); amitriptyline HCl (Merck, Sharp, and Dohme, West Point, PA), or isotonic saline vehicle. The lowest dose of each compound was equipotent in several standard antidepressant screening tests. The other doses of each compound were two and four times the low dose. Thus, separate groups (n=6 per group) received 0.5, 1.0, or 2.0 mg/kg of d-amphetamine sulfate; 10.0, 20.0, or 40.0 mg/kg of bupropion-HCl; or 5.0, 10.0, or 20.0 mg/kg of amitriptyline-HCl; or saline vehicle. All drugs were administered as the base in a volume of 1 ml. Rats housed together received the same treatment.

During the next 2 daily sessions tap water was available as before (the groups did not differ significantly in daily intake); on the third session following saccharin exposure animals were given a choice between tap water and saccharin solution. In an effort to evaluate the persistence of the aversion, choice tests were repeated for an additional 2 successive days. Data are expressed in terms of absolute volume of fluid intake and saccharin preference (expressed as a proportion of total fluid intake × 100).

RESULTS

Analyses

Separate analyses of variance (ANOVA) comparing each of the 9 treatment groups plus the vehicle control group were performed for each of the 3 choice tests. The dependent variables in the analyses were proportion of saccharin to total fluid intake for each dose group and total fluid intake. To test for differences between dose groups, Duncan's multiple range comparison (alpha=0.05) were performed on a post-hoc basis at each trial.

Overall Effects

In general, d-amphetamine, bupropion, and amitriptyline produced dose-related taste aversions in the first choice test (Fig. 2). Extinction of this taste aversion was demonstrated in the second and third choice tests with the attenuation being more pronounced for all doses of d-amphetamine and bupropion in comparison to amitriptyline. Extinction follow-

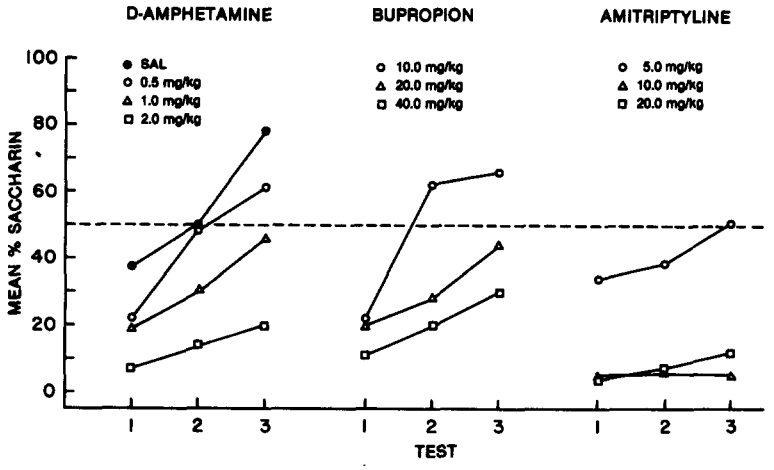

FIG. 2. Flavor aversions induced by bupropion, d-amphetamine, and amitriptyline as a function of dose. The rats (N=6 per group) were given a choice between saccharin and water on three consecutive sessions and the data are expressed as a percent of the total fluid intake. There were no group differences in total fluid intake on any of the choice days (data not shown).

ing amitriptyline occurred primarily for the low dose group. There were no group differences in total fluid intake on any of the three choice tests; the average for the groups total intake was between 20.0 and 25.0 ml per test. Also the groups did not differ in the amount of saccharin consumed on the conditioning trial; the intakes ranged from between 14.0 to 19.0 ml.

Taste Aversion

Choice Test 1. The ANOVA for the Choice Test data indicated a highly significant group effect, $F(9,50)=4.04$, $p<0.006$. Comparisons between the groups indicated the low dose of amitriptyline and the low and medium doses of amphetamine and bupropion were no different than saline. However, the high dose of all three compounds as well as the medium dose of amitriptyline produced substantial decreases in saccharin intake when compared to vehicle control. It should be noted that the vehicle control showed an aversion to saccharin as the percent saccharin consumed by this group was less than 50%, an indication the injection procedure itself may have produced some aversion. An ANOVA for total fluid intake indicated no group difference.

Choice Test 2. The differences between the groups were still apparent on Choice Test 2 as revealed by an overall group effect, $F(9,50)=3.66$, $p<0.0014$. No aversion was apparent in the vehicle control group (saccharin intake was 50% of total intake) and the low doses of the three compounds did not differ from saline. Potency and dose-response relationships were clearly demonstrated in that the saline and low dose groups for all compounds drank significantly more saccharin than the other dose groups. In addition, the medium dose of bupropion and the medium and high dose amphetamine groups consumed more saccharin than the high dose bupropion and amitriptyline groups as well as the high dose amitriptyline group. The groups did not differ in overall fluid intake.

Choice Test 3. Significant taste aversions were still present on Choice Test 3, $F(9,50)=3.92$, $p<0.0008$ although Fig. 2 clearly indicates that some groups showed less aversion on Choice Test 3 than either Test 1 or 2. Significant dose-response and potency relationships were still apparent. All low dose groups and the vehicle control group consumed more saccharin than either the medium or high dose bupropion and d-amphetamine groups. As found in Choice Tests 1 and 2 the medium and high dose amitriptyline groups consumed the smallest amount of saccharin and thus still showed an almost total aversion to saccharin.

DISCUSSION

The three centrally active compounds studied, bupropion, amphetamine, and amitriptyline, all induced dose-related taste aversions. This study is the first to demonstrate that taste aversions can be produced by the two clinically effective antidepressants, bupropion and amitriptyline. The ability of d-amphetamine to induce robust taste aversions is well documented [4, 5, 8] and is reproduced here. In addition, the use of a repeated testing paradigm provides evidence that amitriptyline produced a much more substantial taste aversion than equipotent doses of either of the other compounds. This is shown by the failure of the medium and high dose groups to show any extinction in contrast to the d-amphetamine and bupropion groups. Although the low dose amitriptyline group showed an attenuation of the aversion with repeated testing, the degree of extinction was much less than that shown by either the low dose bupropion or d-amphetamine groups. By the end of extinction testing, the saline, low dose bupropion and d-amphetamine groups were showing a preference for saccharin; this preference was not displayed by the amitriptyline low dose group.

All the compounds tested in this study have central nervous system actions and these actions are generally attributed to their respective abilities to modify or interfere with central catecholamines. The effects of d-amphetamine in the conditioned taste-aversion paradigm have generally been attributed to its central nervous system effects [18] and various studies have attempted to determine the mechanism of action of its aversion producing properties. Several studies have shown that selective depletion of the central catecholamines, dopamine and norepinephrine, will attenuate taste-aversion inducing properties of amphetamine [18] but selec-

tive depletion of either norepinephrine or dopamine does not affect conditioning. Also depletion of these catecholamines does not affect the aversions induced by lithium chloride which are generally considered to be due to emetic properties and mediated through the area postrema, a brainstem emetic region [3]. Conversely, destruction of the area postrema does not prevent amphetamine-induced aversions [19,20]. Cooper *et al.* [7] have shown that most of the behavioral effects of bupropion are due to its selective inhibition of dopamine re-uptake. The destruction of dopaminergic neurons prevents the action of bupropion in locomotor testing and the Porsolt depression model of "experimental helplessness" [21]. It would be of interest to determine if selective destruction of dopaminergic but not noradrenergic neurons would attenuate the taste-aversion inducing properties of bupropion.

Contrary to what would be expected, based on Berger's research [2] with another tricyclic antidepressant—imipramine, amitriptyline was found to induce a robust taste aversion. The difference in methods between this and the Berger study as well as the low dose of imipramine used by Berger could be possible sources of the discrepancy. The present study utilized a two-bottle choice test to determine saccharin preference. The two-bottle paradigm is generally considered more sensitive [9] than the one-bottle method used by Berger and has been used to produce an aversion in less sensitive testing procedures [1,13]. The use of a range of doses or a higher dose might have resulted in imipramine-induced aversions even in the less sensitive one-bottle test.

Amitriptyline has substantial central nervous system effects through its ability to inhibit monoamine oxidase and thereby interferes with the catecholamines, norepinephrine and dopamine, as well as serotonin. Assessment of the taste-aversion inducing abilities of amitriptyline following modification of these catecholaminergic systems would help to determine if its taste aversion-inducing properties are mediated through this mechanism. However, since amitriptyline has substantial cholinergic properties, the mediation of the aversion through a peripheral effect or a combination of peripheral and central effects cannot be ruled out. Since the aversions induced by amitriptyline were substantial and did not attenuate with repeated testing, a combination of central and peripheral actions in the mediation of its taste aversion properties is more likely. Berger [2] has shown with other cholinergically active agents, such as scopolamine, that the peripherally active quarternary derivatives produce greater taste aversions when compared to equipotent doses of their centrally active counterparts.

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