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BRIEF COMMUNICATION

Must Antidepressants Be Anticholinergic to Inhibit Muricide?

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STRICKLAND, J. A. AND J. P. DAVANZO. *Must antidepressants be anticholinergic to inhibit muricide?* PHARMACOL BIOCHEM BEHAV **24**(1) 135–137, 1986.—Two classical tricyclic antidepressants possessing anticholinergic side effects, amitriptyline and desipramine, were compared to newer antidepressants lacking such activity, mianserin, trazodone, and bupropion, for their ability to inhibit muricidal behavior. As has been previously shown for the tricyclic antidepressants, the newer antidepressants without anticholinergic activity also depressed mouse-killing behavior. Scopolamine HBr, at a dose which lacked antimuricidal activity, was tested for its ability to potentiate the antimuricidal effect of these antidepressants. Although potentiation was not demonstrated, there was a trend for scopolamine to enhance the antimuricidal effect of all drugs tested, regardless of whether or not they had anticholinergic activity.

Muricide Anticholinergics Antidepressants Scopolamine potentiation

SEVERAL models of animal aggression are used to screen drugs for psychoactive effects. The muricidal rat is commonly used to screen drugs for antidepressant action, as antidepressants selectively inhibit this behavior without causing debilitation [4,10]. Anticholinergic, antihistaminic, and amphetamine-like drugs also depress muricidal behavior. The classical tricyclic antidepressants exhibit varying degrees of anticholinergic side effects, thus limiting the therapeutic utility of these drugs. Hence, it was desirable to develop antidepressants without anticholinergic activity. Mianserin, trazodone, and bupropion are antidepressants exhibiting little or no anticholinergic effects [1, 2, 12]. To our knowledge, neither trazodone nor bupropion have been examined for their efficacy in blocking muricidal behavior. Kopera [7] reported that mianserin has little effect on muricidal aggression. Kamioka and Sakai [6] also found that mianserin was not effective in blocking muricidal aggression induced by olfactory bulbectomy. Onodura and Ogura [10], on the other hand, show that mianserin does suppress muricidal aggression induced by thiamine deficiency. Because the traditional antidepressants possessing anticholinergic side effects are known to inhibit muricidal behavior and some of the "newer" antidepressants are not anticholinergic and have not been tested in this model (or results are contradictory), our first objective was to determine whether these newer antidepressants would inhibit muricide.

Another model of animal aggression, the isolation-induced fighting mouse, is useful for screening antipsychotic and anxiolytic drugs. Antidepressants, antihistamines, certain autonomic drugs, and analgesics also inhibit mouse fighting, but at larger, often behaviorally impairing doses [3]. Scopolamine HBr is among these agents. In extensive studies with this model, DaVanzo [4] showed that scopolamine, at a dose which fails to block aggression, potentiated the ability of all other agents tested to inhibit mouse fighting. He also showed that the ability of scopolamine to potentiate such a variety of drugs in this action was not due to inhibition of metabolism, but the exact mechanism of potentiation was not determined. Therefore, our second objective was to determine whether scopolamine would potentiate the effects of antcholinergic, as well as nonanticholinergic, antidepressants to inhibit another type of aggression, muricide.

METHOD

Male Sprague-Dawley rats (250-275 g) from an inhouse colony were housed separately in self-cleaning wire cages (18×18×24 cm) in an environmentally controlled room with a 12/12 hour light-dark cycle. Rats were allowed free acess to water and standard laboratory rat chow. After 1–2 weeks of social isolation rats were tested for muricidal aggression by placing a white mouse in each of their cages. Thereafter, tests were conducted 1–2 times per week. All muricide tests began in the early afternoon and continued for 4 hours. Any rat killing a mouse within this period was termed "muricidal." To increase the recruitment of muricidal animals food was withheld 18–20 hours before the test. Latency to kill the mouse was recorded to the nearest 0.5 min. To satisfy our criteria for use in the drug study any particular

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TABLE 1

ABILITY OF SELECTED ANTIDEPRESSANTS TO ANTAGONIZE MURICIDAL BEHAVIOR

Drug	ED_{50} ±S.E. (mg/kg IP)	N	Drug + Scopolamine HBr* ED_{50} ±S.E. (mg/kg IP)	N
Amitriptyline	4.57 ± 7.54	18	2.75 ± 0.70	30
Desipramine	10.00 ± 0.27	36	0.80 ± 0.25	18
Mianserin	10.47 ± 2.47	24	2.29 ± 0.86	18
Trazodone	7.24 ± 0.76	24	1.91 ± 2.55	30
Bupropion	15.31 ± 1.62	36	7.41 ± 4.03	18

*Dose of 0.05 mg/kg.

TABLE 2

THE EFFECT OF SELECTED ANTIDEPRESSANTS† ON LATENCY TO KILL AND EATING BEHAVIOR

Treatment	Latency to Kill ±S.E.M. (min)	N	Amount of Carcass eaten ±S.E.M. (g/min)	N
Control	7.2 ± 1.9	30	0.20 ± 0.03	26
Amitriptyline	18.8 ± 5.6*	6	0.24 ± 0.08	3
Desipramine	22.8 ± 4.7*	6	0.00 ± 0.00	2
Mianserin	21.3 ± 5.5*	6	0.13 ± 0.01	2
Trazodone	17.5 ± 5.3*	6	0.11 ± 0.04	4
Bupropion	24.8 ± 3.9*	6	0.07 ± 0.07	2

†Doses comparable to antimuricidal ED_{50}.
Significantly different from control: *$p<0.05$.

rat must kill a mouse during 3 consecutive tests, the last kill occurring within 10 min. Rats satisfying these criteria were divided into groups of 6.

All drugs were solubilized in distilled water and doses were calculated according to the weight of the free base. Food was withheld 18 hours before drug administration to allow for more consistent drug absorption. Drugs were injected intraperitoneally. Animals that were administered scopolamine in addition to an antidepressant received 2 separate injections. Muricide tests began 30 min after drug injection and continued for 30 min. The number of rats killing mice was recorded and the latency to kill to the nearest 0.5 min was noted. Rats that did not kill a mouse within the test period were assigned a latency of 30 min. The amount of the mouse carcass consumed was also recorded. Each rat received several drugs with at least a one week washout period between doses. The drug schedule was arranged so that no rat received the same dose of a particular drug twice. The drugs used were scopolamine HBr, amitriptyline HCl, mianserin HCl, and desipramine HCl, all from commercial suppliers. We are grateful for the donations of bupropion HCl by Wellcome Research Laboratories and trazodone by Bristol-Myers Pharmaceutical Research and Development Division.

Before drugs were tested for their antimuricidal effects, their ability to cause behavioral impairment was assessed by several tests performed on nonmuricidal rats of the same strain and weight range as the muricidal rats. Our tests were patterned after those of Swinyard *et al.* [13]. Rats were tested 30 min after the injection of antidepressant and their performance was compared to that of untreated animals. The combination of scores for each of these tests was used to assess the degree of behavioral impairment produced by several doses of each drug. We used this information to bracket a range of doses for the muricide test that would not be debilitating. The dose of antidepressant causing only the slightest behavioral impairment was used as the highest dose for the muricide test. The ED_{50} for each drug was calculated by the method of Miller and Tainter [9] using the percent inhibition of muricide at 3–6 doses of each drug. The test for parallelism between the dose-response of each drug with and without scopolamine was done according to the method of Litchfield and Wilcoxon [8]. In addition, regression lines for each dose-response curve (log dose vs. probit) were calculated and parallelism was determined by comparing the slopes of the regression lines of each antidepressant alone with that of its combination with scopolamine. To determine whether the antidepressants were different from each other in their abilities to affect other aspects of this behavior, the latency to kill and the amount of the mouse carcass eaten, these variables were compared at the antimuricidal ED_{50} of each drug by Analysis of Variance. Pair-wise comparisons were performed using the Duncan new multiple range test. A test statistic with $p<0.05$ was accepted as statistically significant. The amount of mouse carcass eaten was expressed as the amount eaten per unit of time left in the 30 min test to allow for the fact that some animals had a longer period of time to eat than others, depending upon how fast they killed the mouse.

RESULTS AND DISCUSSION

Two months of social isolation and regular muricide testing yielded an average of 44% muricidal animals for our particular Sprague-Dawley colony. Four groups of animals obtained at different times yielded various rates of muricide: 28%, 35%, 75%, and 38%. Walsh [14] and others have shown that various strains of rats exhibit different rates of mouse-killing and Walsh also verifies that even different groups of rats from the same colony vary in their frequency of isolation-induced muricide.

The ED_{50}'s determined for the antimuricidal effects of the selected antidepressants are shown in Table 1. The ED_{50}'s for amitriptyline and desipramine are comparable to those determined by Horovitz [5] which were 5.1±1.2 mg/kg and 9.8±2.5 mg/kg, respectively. Sofia [11] also reported a similar value for desipramine, 11.9 mg/kg, 95% confidence limits: 6.3–22.4 mg/kg. Our results indicate that drugs without anticholinergic activity, specifically trazodone, bupropion, and mianserin, also inhibit muricidal behavior. Therefore, the anticholinergic property possessed by many antidepressants is not required for inhibition of mouse-killing. Our results do not agree with others who have reported that mianserin does not inhibit muricidal behavior. We cannot explain this discrepancy in the case of Kopera [7] as no details of the muricide model were given, but Kamioka and Sakai [6] used a model substantially different from ours. They found that mianserin would not inhibit muricide induced by olfactory bulbectomy while we showed that it does inhibit muricide induced by social isolation. Onodura and Ogura [10] used yet

another model of muricidal aggression, that induced by thiamine deficiency, and also found that mianserin inhibited muricide. Our ED_{50} value (10.47±2.47 mg/kg) was similar to theirs (7.0 mg/kg, 95% confidence limits: 3.8–13.0 mg/kg).

Analysis of Variance to compare the latency to kill of the control group with that of the antidepressants at doses comparable to the antimuricidal ED_{50} revealed a significant difference, $F(5,54)=4.77$, $p<0.001$. The Duncan new multiple range test indicated a significant difference between the latency of the control group and the latency of each treatment group ($p<0.05$), but there was no significant difference between the antidepressant groups (Table 2). Analysis of Variance to compare the eating behavior (grams carcass eaten/min) of all groups with antidepressant doses at the antimuricidal ED_{50} showed that there was no significant difference between the groups, $F(5,33)=1.20$, $p<0.33$.

Very small doses of scopolamine had an antimuricidal effect. As little as 0.1 mg/kg reduced the incidence of muricide to 33%. Yoshimura and Ueki [15] reported that much higher doses of scopolamine were required to inhibit muricidal behavior. They showed that a dose of 2 mg/kg only depressed muricidal behavior by 25% while 8 mg/kg was required to effect a 75% reduction in the incidence of mouse-killing. The dose of scopolamine determined to be without antimuricidal effect in our study was 0.05 mg/kg. Its lack of effect was demonstrated on two separate occasions. This dose is one-tenth that determined to be noneffective at blocking fighting behavior in mice [4].

As Table 1 shows, the noneffective dose of scopolamine reduced the ED_{50} of each drug tested. Although the reduction was questionable in the case of amitriptyline, it was dramatic for desipramine. There was no apparent pattern for the ability of scopolamine to enhance the antimuricidal effect of these drugs. The drug possessing the most anticholinergic activity, amitriptyline, was enhanced no more than its less anticholinergic congener, desipramine, and together their actions were augmented no more than the drugs without anticholinergic effects, mianserin, trazodone, and bupropion. The Litchfield-Wilcoxon test for parallelism of the dose-response data of each drug with and without scopolamine was not conclusive. Linear regression analysis showed that trazodone was the only antidepressant for which the regression lines of the dose-responses were significant. The comparison of slopes revealed that the dose-response with scopolamine was not parallel to that of trazodone alone. Because the regression lines for the other drugs were not significant, the slopes of their dose-responses with and without scopolamine could not be compared. In effect, both methods of analysis yielded the same result. The inability to establish parallelism indicates that this must be a nonspecific effect. We were unable therefore to conclude that scopolamine truly potentiates the action of these drugs in the muricide model as DaVanzo [4] showed in the fighting mouse model. But even so, it is clear that there is a trend for scopolamine to enhance the effect of each of these drugs.

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