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Selective Blockade of Muricidal Activity in the Rat by Anorectic Agents

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Abstract □ The ability of several anorectics to block selectively mouse killing in the rat was examined. A significant ratio between the muricide ED_{50} and the ED_{50} of a drug to produce debilitation in rotarod performance is a measure of selectivity. The mouse-killing behavior of a rat can be blocked with certain drugs at doses that do not render the animal debilitated. This selectivity, determined by the ratio of ED_{50}'s, was found in the four anorectics examined as follows: diethylpropion > dextroamphetamine > aminorex > fenfluramine.

Keyphrases □ Anorectic agents—ability to block mouse killing in rats, determination of selectivity, correlated with decreased rotarod performance □ Muricidal activity—selective blockade by anorectic agents, rat □ Mouse-killing behavior—killer rats, effect of four anorectic agents, determination of selective blockade

Horovitz *et al.* (1) demonstrated that several stimulants, antidepressants, and antihistamines block mouse-killing (muricide) behavior in rats. Barnes *et al.* (2) and Gogerty *et al.* (3) found that stimulants which possess anorectic activity abolish mouse-killing behavior. The stimulant-anorectic dextroamphetamine was shown by Horovitz's group and later by Sofia (4) to block selectively muricide behavior in the rat. Selectivity was observed when the dose of a drug causing muricidal blockade was significantly smaller than that resulting in debilitation of the animal. Selectivity was measured by the ratio between the ED_{50} (MKD_{50}) dose for mouse-killing blockade and the ED_{50} (NTD_{50}) for neurotoxicity (4), as found by the inability of the rat to perform on the rotarod *via* the method of Dunham and Miya (5).

Table I—Effects of Anorexigenic Compounds on Rat Rotarod Performance and Muricide Behavior

Drug	Muricide Blockade, MKD_{50}, mg./kg.[a]	Rotarod Debilitation, NTD_{50}, mg./kg.[a]	NTD_{50}/ MKD_{50}
Aminorex	1.67 (0.97–2.86)	12.1 (6.74–21.8)	7.25
Dextro-amphetamine	0.46 (0.30–0.72)	4.64 (1.82–11.8)	10.09
Diethylpropion	3.83 (2.13–6.89)	68.1 (no limits)	17.78
Fenfluramine	3.16 (1.69–5.92)	14.7 (8.5–25.3)	4.65

[a] MKD_{50} and NTD_{50} are given with fiducial limits in parentheses.

In the present experiment, the mouse-killing selectivity of other anorectic compounds was investigated. Rotarod performance was again used as a measure of neurotoxicity.

EXPERIMENTAL

The anorexigenics, aminorex, dextroamphetamine, diethylpropion, and fenfluramine, were tested in rats for their muricide-blocking ability and debilitation of performance on the rotarod as described in the following procedures.

Mouse Killing—Male Wistar rats[1], weighing 150–200 g. at the start of the experiment, were used. Food and water were freely available to each individually caged rat. To determine mouse-killing ability, a mouse was placed in each rat's cage for 10 min. This 10-min. period for the kill was gradually reduced to 5 sec. Eventually, only those rats that killed consistently and instantly upon confrontation with the mouse were saved for future drug studies. These animals, approximately 15% of the original group, were termed "killer rats."

Saline was administered intraperitoneally to condition the rats to the injection procedure and to eliminate any rats that did not kill after placebo administration. Mice were placed in the rats' cages 30, 60, and 120 min. after the saline injection. The same procedure was followed during the ensuing drug studies.

For a drug session, a single dose of one of the four drugs dissolved in saline was injected. A mouse was introduced to the rat at the three time intervals for a maximum of 30 sec. to test for muricide behavior. At the end of the 30 sec., all mice were removed from the cages. If a mouse was not killed at any of the three time intervals, muricidal behavior was considered blocked.

Killer rats were used for several drug injections. Therefore, at least 2 weeks elapsed between drug administrations. Between drugs, saline was given several times following the same procedure. Four rats were used for each dose of a drug, and the data were analyzed for an ED_{50} for mouse-killing behavior (MKD_{50}) by Horn's (6) method.

Rotarod Test—Wistar rats were also used for rotarod testing. Housing and food conditions were the same as in the mouse-killing experiments. Each rat was placed on a rotarod moving at a rate of approximately 4 r.p.m. and trained to maintain himself for 120 sec. Approximately 10% of the rats had difficulty in this conditioning and were discarded from the experiment. The rats were then intraperitoneally administered one dose of the four drugs to be examined. Thirty, 60, and 120 min. later, each animal was tested for its ability to remain on the rotarod for a 30-sec. period. If a rat fell off the rotating rod during any of the three testing sessions, he

[1] Harlan Industries, Cumberland, Ind.

was said to be debilitated. Four rats were used for each dose of drug, and the data were analyzed for NTD_{50} in rotarod performance by Horn's (6) method.

RESULTS

The selectivity of mouse-killing behavior can be calculated by dividing the rotarod NTD_{50} dose by the muricide MKD_{50} dose. A ratio close to or below 1.00 indicates a nonspecific blockade of mouse killing, the muricidal block being indistinguishable from debilitation or depression of the animal. A ratio significantly greater than 1.00 indicates selectivity of a drug, mouse-killing blockade at a nonneurotoxic dose.

The effects of the four anorectic compounds examined for muricide blockade and rotarod performance are given in Table I. With dextroamphetamine, the smallest dose (0.46 mg./kg.) of all the anorectics tested was needed to produce mouse-killing blockade in 50% of the rats and also the smallest dose was needed for rotarod debilitation. However, the separation between those two doses was great enough to produce a ratio of NTD_{50}/MKD_{50} of 10.09. Only the ratio for diethylpropion was larger (17.78) than that of amphetamine. Aminorex (7.25) and fenfluramine (4.65) also demonstrated ratios well above 1.00.

DISCUSSION

Of the four anorexigenic agents tested, only the dextroamphetamine data can be compared with results of other investigators. Even though different methods of calculation of the MKD_{50} and the NTD_{50} were used, Sofia (4) and Horovitz *et al.* (1) demonstrated a ratio significantly separating the antimuricide and neurotoxic doses for dextroamphetamine. Our data confirm this separation.

All of the anorexigenic drugs examined in this study illustrated selectivity determined by the NTD_{50}/MKD_{50} ratio in mouse killing in the rat. Muricide behavior was blocked at doses that did not render the rat physically debilitated. The relative selectivity of the anorectics studied is as follows: diethylpropion > dextroamphetamine > aminorex > fenfluramine.

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Synthesis and *In Vitro* Antiplaque Activity of Methylene Homologs of Chlorhexidine

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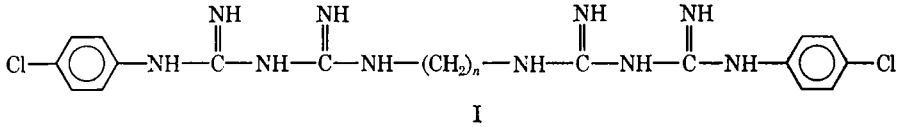
Abstract □ Five straight-chain homologs of chlorhexidine, containing from two to 12 methylene groups, were synthesized and evaluated along with chlorhexidine for *in vitro* antiplaque activity. Maximum antiplaque activity was exhibited by the compounds containing six (chlorhexidine) and 12 methylene groups.

Keyphrases □ Chlorhexidine methylene homologs—synthesized and tested *in vitro* for antiplaque activity □ Antiplaque activity—synthesis and *in vitro* testing of chlorhexidine methylene homologs □ Plaque inhibitors—synthesis and *in vitro* testing of chlorhexidine methylene homologs

Dental plaque is a soft, tenacious, bacterial deposit which forms on the surface of teeth. A close correlation exists between dental plaque and the principal diseases of the mouth: caries and periodontal disease (1). The high incidence of these diseases among the general population (2) is ample evidence that the current approaches to plaque control based on the use of mechanical aids are not effective. In this report, some efforts to develop long-acting chemical inhibitors of plaque formation are described. Recent *in vitro* and clinical studies (2–5) have shown that chlorhexidine [1,6-bis-(N^5-*p*-chlorophenyl-N^1-biguanido)hexane, I, $n = 6$], an antibacterial bisbiguanide, is an effective inhibitor of dental plaque. Three factors are apparently responsible for the antiplaque activity of chlorhexidine: (*a*) the antibacterial activity of chlorhexidine (6), (*b*) the ability of chlorhexidine to bind to the tooth surface (7), and (*c*) the largely bacterial nature of plaque (8).



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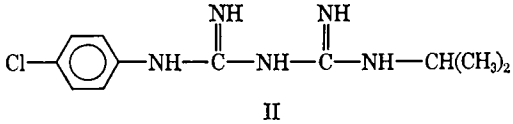

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