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Role of Serotonin in the Blockade of Muricidal Behavior by Tricyclic Antidepressants[1]

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EISENSTEIN, N., L. C. IORIO AND D. E. CLODY. *Role of serotonin in the blockade of muricidal behavior by tricyclic antidepressants*. PHARMAC. BIOCHEM. BEHAV. 17(4) 847–849, 1982.—Dose-response curves for the ability of three tricyclic antidepressants, imipramine, desmethylimipramine and amitriptyline to inhibit muricidal behavior were measured after treatment with a tryptophan-free diet and after administration of p-chloroamphetamine. Both treatments, which have been reported to specifically reduce central levels of serotonin, decreased the ability of the drugs to inhibit muricide. The results suggest that all three antidepressants block muricide in part through their effects on serotonin.

Serotonin Muricide Antidepressants Mechanism of action

THE role of serotonin (5-HT) in muricidal behavior in rats has been well established. Manipulations that reduce brain serotonergic function such as administration of parachlorophenylalanine (PCPA) which depletes brain 5-HT [5, 14, 17], lesions of the serotonergic neurons of the raphe nucleus [10,20], and administration of 5,6- and 5,7-dihydroxytryptophan which destroy serotonergic nerve terminals [4] have all been shown to induce muricidal behavior in rats. As a corollary, pharmacologic manipulations that enhance brain serotonergic function inhibit established muricide. For example, agents that specifically increase 5-HT levels like the 5-HT precursor 5-hydroxytryptophan [3], quipazine (a 5-HT agonist), fluoxetine (a 5-HT uptake inhibitor) and fenfluramine (an agent that releases 5-HT) [8] all inhibit muricidal behavior. Monoamine oxidase inhibitors [5] which inhibit the degradation of 5-HT (as well as dopamine (DA) and norepinephrine (NE)), tricyclic antidepressants [6,11] which block uptake of 5-HT, DA and NE and stimulants such as amphetamine [12,18] which release 5-HT, DA and NE and antihistamines such as tripelennamine and chlorphreniramine [1] also block muricide.

This study was undertaken to evaluate the extent to which inhibition of muricide produced by tricyclic antidepressants is a result of an increase in brain serotonergic function. To do this, dose-response curves were obtained for three standard antidepressants in the presence of a tryptophan free diet reported to reduce central levels of 5-HT [2] and after treatment with p-chloroamphetamine (PCA), an agent that depletes brain 5-HT with more specificity than PCPA [16] and that does not induce muricide in non-killer rats [15].

METHOD

Male Long Evans rats (Blue Spruce Farms, Altamont, NY) weighing 225 to 275 g were used. At the start of the experiment, the animals were housed individually and allowed to adapt to laboratory conditions for 5 days. Food (Lab Blox, Wayne) and water were available ad lib and a 12 hour light/dark cycle was maintained. All rats were deprived of food for 58 hours and then tested for mouse killing. A male CF1 mouse, 20–24 g, was placed in the rat's cage and each rat was given 30 minutes to kill the mouse. Any rat who failed to kill within 30 minutes was discarded. Following this first trial, food was again made available ad lib throughout the entire study. All rats who killed were tested again on each of four consecutive days and any rat who failed to kill on any day was discarded.

In the diet experiments, rats were deprived of all food for 24 hours prior to testing and a feeder containing either a tryptophan-free (TF) diet or a tryptophan-free diet containing 0.3% tryptophan (TFC) (ICN Pharmaceuticals) was placed in each rat's cage. Biggio [2] reported that rats placed for 24 hours on a tryptophan-free diet produced a 58% decrease of brain 5-HT that persisted for an additional 24 hours. At testing time, the diet was removed and the amount of food consumed by each rat recorded. Then, each rat was injected intraperitoneally with the test drug. Five minutes later a male CF1 mouse was placed in each rats' cage and each rat was allowed 30 min to kill the mouse. Each treatment group contained 15 rats. To determine whether the TF diet alone either induced muricide in non-killer rats or blocked muricide in established killers, a control group of ten

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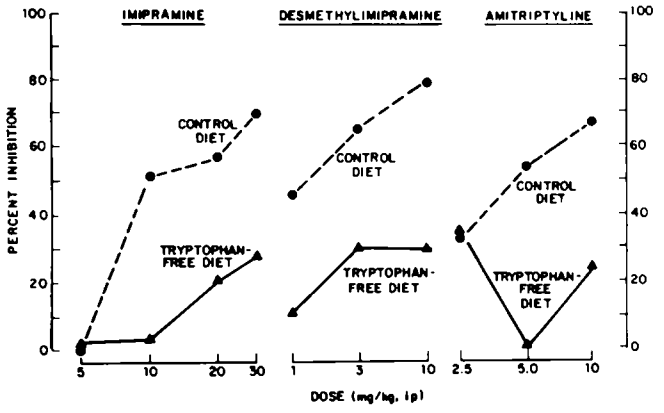

FIG. 1. Effects of tryptophan-free diet on dose-response curves for imipramine, desmethylimipramine and amytriptyline. Each point represents the percent of rats who failed to kill mice. Fifteen rats were tested for each data point.

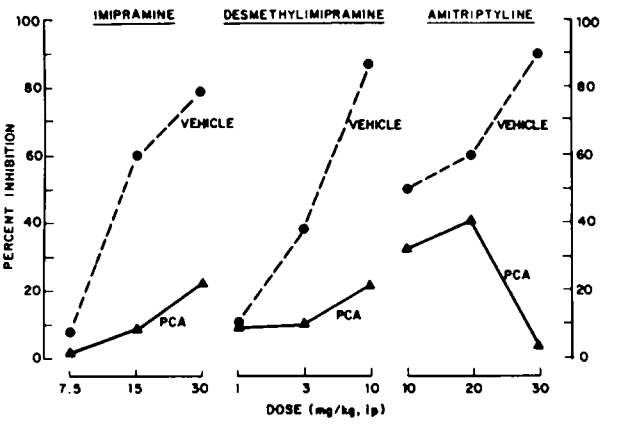

FIG. 2. Effects of 24 hr pretreatment with parachloroamphetamine on dose response curves for imipramine, desmethylimipramine and amytriptyline. Each point represents the percentage of the 15 rats who failed to kill mice.

non-killer rats and ten killer rats received both the same treatment and testing as the rats receiving the tryptophan free diet.

In the PCA experiments, different groups of 15 rats each were dosed with either vehicle or PCA at 5 mg/kg IP. This dose has been shown [16] to produce a 50% reduction in brain 5-HT 24 hours after administration. Twenty-four hours later, all rats were injected with a test dose of the antidepressant and tested 30 minutes later for muricide. To determine whether PCA induced muricide in non-killer rats, ten non-killer rats were injected intraperitoneally with 5 mg/kg of PCA and tested 24 hours later for muricide.

In the diet and PCA experiments, the antidepressants (as hydrochloride salts) and dose ranges (expressed as weight of the base) were imipramine at 5 to 30 mg/kg IP, desmethylimipramine at 1 to 10 mg/kg IP and amitriptyline at 2.5 to 30 mg/kg IP. All drugs were suspended in aqueous 0.4% methylcellulose solution.

RESULTS

Figure 1 reveals that the tryptophan-free diet was approximately equiactive in shifting to the right the dose response curves of each of the tested antidepressants. Analysis of variance demonstrated that this effect for all 3 drugs was statistically significant, $F=37.46$, $p<0.001$, ANOVA. Individual comparisons using the Mantel-Haenszel calculation [7] revealed that the individual differences between diet-control were statistically significant for all three antidepressants ($p<0.001$).

Among the 20 control rats who received the TF diet, none of the 10 established muricidal rats failed to kill mice and none of the 10 non-killers killed mice.

As can be seen in Fig. 2, pretreatment with PCA, 5 mg/kg ip, reduced the inhibition of muricide produced by all three antidepressants. Analysis of variance revealed this treatment to be statistically significant, $F=30.63$, $p<0.001$, ANOVA, for all three antidepressants. The Mantel-Haenszel analysis failed to reveal significant differences between drugs. As in the first experiment, individual comparisons between treatment and control for each drug revealed significant differences ($p<0.01$). This treatment with PCA at 5 mg/kg ip did not induce muricide behavior in ten non-killer rats.

DISCUSSION

Administration of a tryptophan-free diet and PCA at 5 mg/kg to rats, blocked the inhibition of muricide induced by antidepressants. Since these manipulations result in specific depletion of brain 5-HT and neither manipulation induces muricide in rats, these results indicate that the ability of the tricyclic antidepressants to block muricide in the rat is mediated in part by increasing serotonergic function. This is not surprising in light of previous reports implicating 5-HT in the induction of muricide. However it is also clear from the data reported here that neither the tryptophan-free diet nor pretreatment with PCA completely blocked the inhibition of muricide. It may be that neither treatment reduced central 5-HT levels enough to completely prevent the anti-muricidal effects of the tricyclic antidepressants or that the anti-muricidal effects of these drugs are also mediated in part by a second mechanism e.g., blockade of the uptake of NE or DA.

It is surprising that both treatments were approximately equipotent in blocking all three antidepressants because of the different potencies of these drugs in blocking 5-HT uptake: AMI > IMI > DMI. It might have been expected that DMI would have been affected least by the reduction in serotonin and that less of an effect on the inhibition of muricide would have been seen. Since they were blocked equally well, there may be one part of the pathway, by which muricide may be inhibited, common to all three drugs that is mediated by serotonin.

The results showing that PCA at 5 mg/kg did not induce muricide behavior in non-killer rats agrees with the results of Miczek *et al.* [15]. We also did not induce muricide behavior in non-killer rats with a 24-hour TF diet, although muricide behavior has been induced in non-killer rats remaining on a tryptophan-free diet for 6 days [9] or 2–4 weeks [19]. It is not known why our manipulations did not evoke this behavior in

rats whereas manipulations such as PCPA 5,6-dihydroxytryptophan, or raphe lesions do induce muricide. It may be that pretreatment with PCA at 5 mg/kg IP or the 24 hour TF diet did not cause 5-HT depletion sufficient enough to evoke muricide in these studies. Treatments such as PCPA, 5,6-dihydroxytryptophan, or raphe lesions may cause a greater degree of depletion. In addition, these latter manipulations are not as specific, sometimes interfering with other systems, e.g., adrenergic function, which may be required for induction of muricide.

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