Dose : response effects on norepinephrine-depletion of reserpine and of guanethidine in bretylium-pretreated rats[1]

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INFSI *et al.*[2] noted that the depletion of heart norepinephrine induced by intraperitoneal reserpine (100 μg/kg) was reduced after pretreatment of the animals with subcutaneous bretylium (10 mg/kg) at 18 hr and 2 hr before reserpine injection. Kuntzman *et al.*[3] developed their discussion of the mode of action of guanethidine and reserpine on the basis of data which showed that i.p. bretylium (200 mg/kg) prevented the depletion of heart norepinephrine induced by i.p. guanethidine (5 mg/kg twice daily for 5 days) and that pretreatment with the bretylium-like compound, BW 392C60 (N-*o*-chlorobenzyl-N′,N″-dimethylguanidine, 15 mg/kg), prevented the depletion of norepinephrine by i.p. guanethidine (100 mg/kg) but not by i.p. reserpine (1 mg/kg).

In the present work we show that these apparent discrepancies are due primarily to the difference in doses of the drugs employed.

EXPERIMENTAL

Medication procedure

Adult female Sprague Dawley strain rats (Charles River Breeding Farms) were pretreated i.p. twice daily with 20 mg bretylium/kg for 2 days. After the fourth administration of bretylium, groups of seven or eight rats each were given intraperitoneal doses, per kilogram, of 0·0625, 0·25, or 1·0 mg reserpine; or 2, 8, or 32 mg guanethidine.

The control animals, not pretreated with bretylium, were given, i.p., per kg, 0·156, 0·625, or 2·5 mg reserpine; or 0·156, 0·625, 2, or 8 mg guanethidine.

Analytical method

For the estimation of the norepinephrine content of the rat hearts, pooled tissue from groups of seven or eight Sprague-Dawley strain female rats was homogenized with 0·1 N HCl, extracted with NaCl-saturated butanol, and the solvent phase, in turn, extracted with 0·01 N HCl as in the Shore and Olin procedure.[4] To 0·5 ml of the extract, 0·5 ml of 0·2 M phosphate buffer was added and the solutions mixed. Then 0·1 ml of 0·25% potassium ferricyanide solution was added to oxidize the norepinephrine to noradrenochrome, and the solutions mixed agian. After a minute or two, 1 ml of a freshly prepared alkaline ascorbate reagent containing 2% ethylendiamine[5] was added and the fluorescence measured in an Aminco-Bowman spectrophotofluorometer at activation and fluorescence wavelengths of 400 and 520 mμ respectively.

RESULTS AND DISCUSSION

It may be seen from Fig. 1 that each drug manifests a dose:response relationship when given over

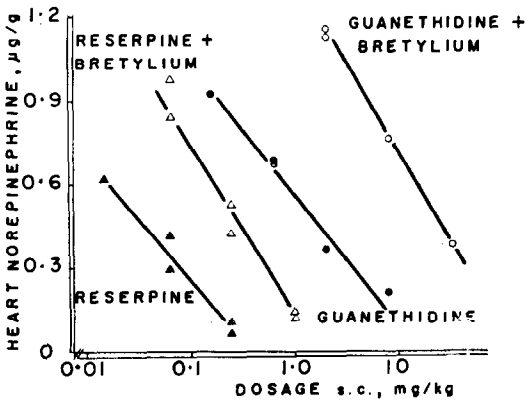

FIG. 1. Norepinephrine-depleting effects of reserpine or guanethidine in the heart tissue of bretylium-pretreated rats.

a suitable range. Reserpine exerted a maximal or near-maximal effect in the absence of bretylium pretreatment when given at about 0·25 mg/kg. After bretylium pretreatment, about four times as much reserpine was required to achieve a comparable effect. In the absence of bretylium pretreatment about 8 mg guanethidine/kg exerted a near-maximal effect. The bretylium-pretreated animals required about 32, or more, mg guanethidine per kg to achieve a similar response.

Kuntzman *et al.*[3] state that the "guanethidine and reserpine appear to release norepinephrine by different mechanisms since bretylium, a strongly basic compound, counteracts guanethidine but not reserpine in releasing norepinephrine." It is clear from Fig. 1 that there are not qualitative differences between the bretylium–reserpine, bretylium–guanethidine interactions. A fourfold increase in the dose of either guanethidine or reserpine is required to overcome the effects of 20 mg bretylium/kg given i.p. daily for 2 days.

In our view the similarity of the dose:response curves of guanethidine and reserpine does not furnish insight into the mode of action of these bases. While it may be true that the mechanisms whereby reserpine and guanethidine effect catecholamine depletion are different, the evidence adduced by Kopin and Gordon,[6, 7] based on tissue norepinephrine release studies, offers much stronger support for this hypothesis than the interaction of these drugs with bretylium.

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Brain amines: Response to physiological stress*

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LEVELS of serotonin and norepinephrine in brain respond reliably and selectively to certain pharmacological agents, but a reliable differential response of both amines to nonpharmacological procedures has not been noted. Consequently, several different stressors were investigated for their effects on both monoamines in the brains of 200-g, male, Sprague-Dawley rats.[1]

Norepinephrine and serotonin were assayed in the same brain by fluorescence spectrometry with the method of Mead and Finger[2] and double blind control procedures were used. Changes found by this method were confirmed by use of other standard methods[3, 4] for each of the amines including bioassay for serotonin.[5] In each stress experiment a minimum of four control and four experimental animals was assayed until more than 50 rats had been studied. There were no differences in brain

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