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Studies of the antagonism of guanethidine by methamphetamine

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GUANETHIDINE, a well-known antihypertensive agent, possesses two distinct properties: (1) blockade of the adrenergic neuron[1] and (2) depletion of catecholamine from heart, spleen, and intestine.[2] The former property has been attributed to its bretylium-like action,[1] *i.e.* the inhibition of release of the neurohumoral agent from the terminals of the adrenergic neuron. The depletion of catecholamine by guanethidine requires the presence of the depleting agent in the tissue.[3] After a single administration of guanethidine, maximal depletion occurs after 4 hr and is maintained for approximately 18 to 24 hr, after which the catecholamine concentration begins to rise. Kuntzman *et al.*[3] reported that, after a single administration, guanethidine is present in tissue for 18 to 24 hr. It was reported recently that the adrenergic neuronal blocking property of guanethidine is antagonized by several sympathomimetic amines[4] and cocaine. This antagonism appears to be competitive in nature.[5] If it is assumed that the antagonism between the sympathomimetic amines and guanethidine is competitive, it may be possible that the depletion of catecholamines by guanthidine and the uptake of guanethidine by tissue are decreased in the presence of sympathomimetic amines. This present investigation demonstrates that in the presence of the sympathomimetic amine, methamphetamine, there is a decrease in the depletion of the catecholamine and in the uptake of guanethidine.

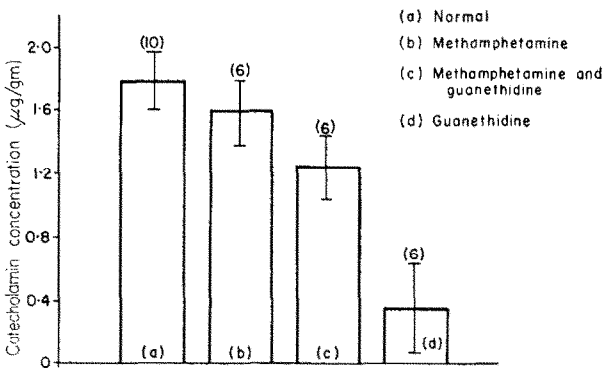

FIG. 1. Levels of catecholamines in hearts of rabbits pretreated for 4 hr with either guanethidine (12·5 mg/kg, i.v.), methamphetamine (20·0 mg/kg, i.p.), or both. Concentration, µg/g tissue ± SD. Numbers in parentheses represent the number of animals used in the experiment.

New Zealand white rabbits, 2·0 to 2·5 kg, of either sex, were used. An aqueous solution of guanethidine was injected (12·5 mg/kg) into the marginal ear vein; methamphetamine was injected intraperitoneally (20·0 mg/kg). When both drugs were used in one animal, guanethidine was injected first and immediately followed by methamphetamine.

The degree of depletion of catecholamine was measured in cardiac tissue from animals pretreated for 4 hr with the various test compounds. The fluorometric analysis of the catecholamine was done

according to the procedure of Shore and Olin[6] with the modifications of Wiegand and Perry[7] and von Euler and Lishajko.[8] The distribution of guanethidine was measured in cardiac tissue from rabbits which were pretreated for 2 hr with the test drugs. The guanethidine was assayed according to a procedure described by Costa.[9] These time periods—*i.e.* pretreatment for 4 hr and 2 hr for catecholamine and guanethidine assay, respectively—were chosen for several reasons. First, it is known that maximal depletion of catecholamine from heart occurs in approximately 4 hr.[3] Second, the knowledge of the distribution of guanethidine is more significant at the time when the depletion is actively occurring than when the depletion is maximal.

The catecholamine concentration (expressed in micrograms/per gram of tissue ± SD) of normal rabbit heart tissue was 1·80 ± 0·38 μg/g (Fig. 1) whereas in hearts from rabbits treated with guanethidine, the catecholamine concentration was 0·35 ± 0·28 μg/g,, or 19% of normal. In rabbits pretreated with methamphetamine, the concentration of catecholamine was 1·59 ± 0·23 μg/g, or 88% of normal. In rabbits receiving both methamphetamine and guanethidine, the concentration of catecholamine was 1·24 ± 0·21 μg/g, or 68% of normal. The concentration of catecholamines in tissue of rabbits pretreated only with guanthidine is significantly different ($P < 0·001$) from all other groups tested.

Tissue levels of guanethidine in heart tissue from rabbits which received only guanthidine was 7·68 ± 1·95 μg/g tissue (Table 1). In tissue from rabbits that were pretreated with guanethidine and

TABLE 1. LEVELS OF GUANETHIDINE IN HEARTS OF RABBITS*

Drug	Concentration (μg/g tissue ± SD)
Guanethidine (12·5 mg/kg, i.v.)	7·68 ± 1·95
Guanethidine (12·5 mg/kg, i.v.) and methamphetamine (20·0 mg/kg, i.p.)	4·66 ± 1·98

* Pretreated for 2 hr with either guanethidine or methamphetamine and guanethidine. Value, μg/g tissue ± SD, is the mean obtained from four animals.

methamphetamine, the level of guanethidine was 4·66 ± 1·98 μg/g. The difference in levels of guanethidine between the two groups is approximately 40% and is significant ($P < 0·05$).

The results given here indicate that methamphetamine effectively antagonizes the depletion of catecholamine by guanethidine and the uptake of guanethidine by tissue. Callingham and Cass[10] recently reported that bretylium and cocaine reduced the depletion of norepinephrine from rat heart and spleen produced by either reserpine or guanethidine. They proposed that bretylium probably acts by preventing the release of the catecholamine. The antagonism of guanethidine by methamphetamine is not likely due to a bretylium-like action since methamphetamine itself causes the release of the catecholamine from its stores. Also, methamphetamine has been reported to antagonize the adrenergic neuronal blocking action of bretylium.[11] A possible explanation is that there is a competition between the adrenergic neuronal blocking agents and the sympathomimetic amines for receptors. The result reported here demonstrated that, because of the competition between guanethidine and methamphetamine, there is decreased uptake of guanethidine by tissue. Concomitantly, this decreased uptake resulted in a lesser degree of catecholamine depletion from cardiac tissue.

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Effect of amethopterin and vincaleukoblastine on urinary 4-amino-5-imidazolecarboxamide*

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THE ribonucleotide of 4-amino-5-imidazolecarboxamide (AIC) has been established as a key intermediate in purine biosynthesis.[1] AIC, a by-product formed by the breakdown of the ribonucleotide, has been shown to appear in the urine of humans and other mammals.[2–4] In normal individuals, AIC is comparable to creatinine in the constancy of its excretion,[3] suggesting that fundamental homeostatic mechanisms govern the rate of purine biosynthesis and the rate of AIC excretion. In acute leukemia, however, the excretion of AIC is about two times normal,[5, 6] and the recovery of an administered load of AIC is much less than normal.[5] These facts imply a heightened rate of purine biosynthesis in acute leukemia, which is consistent with an increased rate of synthesis of leukocytes.

Folic acid derivatives are required for two steps in purine biosynthesis; one is in the conversion of glycinamide ribonucleotide to α-N-formylglycinamide ribonucleotide, and the other is in the conversion of 4-amino-5-imidazolecarboxamide ribonucleotide to 4-formamidoimidazolecarboxamide ribonucleotide.[7] The folic acid derivatives in each case act as carriers of a formyl residue.

Antifolic acid compounds, such as aminopterin and amethopterin are useful in the treatment of certain neoplastic diseases, particularly acute leukemia. If these drugs act by interfering with purine biosynthesis, one might expect a change in AIC excretion after their administration. This paper reports some preliminary studies on AIC excretion in rats given amethopterin, aminopterin, and vincaleukoblastine, a third compound useful in the treatment of leukemia but with a completely unknown mechanism of action.

Wistar rats, weighing approximately 200 to 250 g, were placed in metabolic cages, allowed food and water *ad lib.*, and the urine collected in dilute acid. Creatinine values on the 24-hr urine specimens were determined by Taussky's modification of the Jaffe reaction and AIC determined by the method previously described.[3] The drugs were administered intraperitoneally, and did not interfere with either creatinine or AIC analyses.

Some representative results on individual rats are given in Table 1. After a single dose of amethopterin (1·5 to 5 mg/kg), both the absolute excretion of AIC and the ratio of AIC to creatinine increased two- to seven-fold. Some 20 rats were studied and each showed this effect. At lower doses the effect was transient, lasting only 1 to 2 days, but at 5 mg/kg the value increased until the third day when both animals succumbed. In another experiment, two rats were given 2·5 mg amethopterin/kg for 2 days and 7 mg/kg on the third. Both rats died on the fifth day, but the AIC excretion did not rise after the third day.

Aminopterin, another antifolic acid compound, was administered to four rats at a dose of 0·2 mg/kg. No effect on either AIC excretion or creatinine excretion was observed at this dosage.

A different effect on AIC excretion was observed when vincaleukoblastine (VLB), an antileukemic drug with an unknown mode of action, was administered (Table 1). Two rats were given 0·5 mg VLB/

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