Effect of Adrenergic Blocking Agents on Tyramine-Induced Release of Catecholamines from the Cat Heart.* (28053)

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According to present pharmacological concepts, if the pressor action of a sympathomimetic amine is antagonized by an adrenergic blocking agent of the Dibenamine type, *i.e.*, an *alpha*-adrenergic blocking agent(1), the amine is assumed to be acting by combination with peripheral effector sites, *e.g.*, on receptors of vascular smooth muscle. An apparent exception to this concept is the pressor action of mephentermine which is inhibited by Dibenamine(2) and phenoxybenzamine although the constrictor effect of mephentermine injected into the femoral artery of the dog is not influenced by phenoxybenzamine (unpublished observation). The possibility exists that phenoxybenzamine may inhibit the pressor effect of mephentermine and similar sympathomimetic amines by an influence on cardiac effects. Mephentermine has marked cardiac effects(3,4) which are in part or wholly mediated by catecholamine release(5, 6,7). It is desirable, therefore, to determine the effect of *alpha*-adrenergic blocking agents on the release of catecholamines induced by sympathomimetic amines. As a first approach to this problem, the effect of phenoxybenzamine and other agents on catecholamine release was studied in the isolated perfused cat heart. Tyramine was used as the catecholamine releasing agent because it releases fluorometrically determinable amounts of catecholamines in this preparation.

Method. Hearts were removed from cats after anesthesia with pentobarbital sodium and were set up as Langendorff preparations. The perfusion fluid was Krebs-Henseleit Bicarbonate Ringer containing glucose in a concentration of 2 g/l and equilibrated with 95% O_2-5% CO_2. Temperature was maintained at 37°C. Coronary flow was measured by timing the flow into a graduated cylinder with a stopwatch. Force of contraction was measured by a Grass force-displacement transducer and was recorded on a Grass Polygraph.

Samples of 50 ml were collected in graduated cylinders containing 1 ml of 1 N HCl and 0.75 mg of EDTA; the final pH was 5.0. After adsorption onto and elution from alumina columns, the concentration of catecholamines in these samples was determined by the trihydroxyindole method of von Euler and Floding(8). Fluorescence was measured by means of a Turner Fluorometer. In the concentrations used, none of the drugs interfered with the determination of catecholamines.

The general procedure for studying catecholamine release was as follows: The hearts were perfused for one-half hour with Krebs-Henseleit solution (control experiments) or with Krebs-Henseleit solution containing one of the test substances. The perfusion fluid was then changed to one containing tyramine in a concentration of 50 μg/ml plus the test substance. Collection of perfusate for analysis was begun immediately after cardiac effects were seen.

In 5 of the experiments, after perfusing the hearts for one-half hour with Krebs-Henseleit solution containing phenoxybenzamine, they were perfused for an additional one-half hour with phenoxybenzamine-free Krebs-Henseleit solution before addition of tyramine to the latter solution. These experiments will be termed "washed out" experiments.

Results. In the absence of drugs, no catecholamines could be detected in the perfusate; therefore, the basal release produced a concentration of less than 0.1 mμg/ml which was the lower limit of sensitivity of the method. Incorporation of tyramine in a concentration of 50 μg/ml into the fluid perfusing the heart resulted in a mean concentration of catecholamines in the perfusate of 4.4 mμg/ml or a rate of release of 85.5 mμg/min.

The effect of various test substances on re-

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TABLE I. Effect of Various Substances on Release of Catecholamines and Force of Contraction in the Perfused Cat Heart* Produced by Tyramine, 50 μg/ml.

Test substance	Conc of test substance (mg/l)	Conc of catecholamines in perfusate (mμg/ml)	Rate of release of catecholamines into perfusate (mμg min)	% increase in force of contraction
None		4.40 ± 1.04†	85.5	117 ± 46†
Phenoxybenzamine	5	.13 ± .10‡	4.7‡	12 ± 7§
" ‖	5	.48 ± .30‡	14.0‡	10 ± 11§
GD-131	5	.28 ± .21‡	7.6‡	12 ± 5§
Phentolamine	15	6.03 ± 1.36	105.7	172 ± 33
Dihydroergotamine	5	7.70 ± 3.00	89.7	106 ± 50

* Each group represents mean of 5 experiments.
† Mean ± standard error of mean.
‡ Differs from control at $P < .01$ level.
§ Differs from control at $P < .05$ level.
‖ These hearts were perfused for an additional half-hour with phenoxybenzamine-free Kreb's solution before addition of tyramine.

lease of catecholamines produced by 50 μg/ml of tyramine is shown in Table I. Phenoxybenzamine and GD-131 (N-[2-chloroethyl]-N-ethyl-cyclohexanemethylamine) in concentrations of 5 mg/l reduced the release to 0.13 and 0.28 mμg/ml, respectively. These values differ significantly from control values obtained with tyramine alone ($P<0.01$). In the presence of phenoxybenzamine or GD-131, the increase in force of contraction produced by tyramine was only about 10% of the increase obtained with tyramine alone and negative inotropic effects were seen in some experiments. In contrast to this, phentolamine, at a concentration of 15 mg/l and dihydroergotamine (DHE), at 5 mg/l, did not reduce either the concentration of catecholamines released into the perfusate or the rate of release. The increase in force of contraction produced by tyramine in the presence of phentolamine or DHE was not significantly different from that produced by tyramine alone.

In those experiments with phenoxybenzamine in which the hearts were perfused with phenoxybenzamine-free Krebs-Henseleit solution for one-half hour after phenoxybenzamine (washed out), both the catecholamine release and the per cent increase in force of contraction were significantly reduced below control values, and they did not differ significantly from those values obtained in the presence of phenoxybenzamine.

Discussion. In view of the fact that the β-haloalkylamines are highly reactive substances, it might be argued that phenoxybenzamine and GD-131 combine with and inactivate tyramine in the perfusion reservoir. The following considerations render this unlikely: First, assuming a mole-for-mole combination, the phenoxybenzamine (14.7 μM) could combine with only 5.1% of the tyramine (288 μM); clearly not enough to produce the inhibitions seen in these experiments. Second, when the phenoxybenzamine was washed out of the preparation for one-half hour before adding tyramine to the perfusion fluid, neither the inhibition of catecholamine release nor the inhibition of positive inotropic effect was significantly different from those experiments in which tyramine was incorporated into the same solution as the phenoxybenzamine. Thus, the blockade of release of catecholamines must be related to an interaction of phenoxybenzamine with cellular constituents.

Since the tyramine-induced release of catecholamines was markedly inhibited by phenoxybenzamine but not by phentolamine or DHE, experiments were done with GD-131 which is a β-haloalkylamine which lacks peripheral adrenergic blocking activity(9). The ability of this agent to inhibit the release of catecholamines suggests that the blockade is related to the β-haloalkylamine structure rather than to a more specialized structure responsible for *alpha*-adrenergic blockade.

The experiments reported here show that phenoxybenzamine is able to inhibit the release of catecholamines by tyramine in the

isolated cat heart, and that this blockade of release is sufficient to account for the marked reduction in the positive inotropic effect produced by tyramine after phenoxybenzamine. Therefore, phenoxybenzamine does not block cardiac effects of this type of sympathomimetic amine by *beta*-receptor blockade but by blocking the release of catecholamines.

Before one may utilize these results to explain the blockade of the pressor effect of mephentermine, one must show that phenoxybenzamine blocks the cardiac release of catecholamines in the intact animal and that this release is the principal cause of the pressor effect.

Summary. The release of catecholamines produced by tyramine in the isolated cat heart is inhibited by phenoxybenzamine and GD-131 but not by phentolamine or dihydroergotamine. Inhibition of the release of catecholamines is associated with a marked reduction in the positive inotropic effect of tyramine. The possibility is suggested that β-haloalkylamines may interfere with the release of catecholamines *in vivo* as well as inhibiting the effects of catecholamines on effector cells.

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Histo-Physiological Studies on Chick Pancreas as Influenced by Feeding Raw Soybean Meal.* (28054)

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Chernick *et al.*(1) reported that feeding raw soybean meal to chicks caused an enlargement of the pancreas and an increase in its proteolytic content. Distinct morphological changes in the hypertrophic pancreases of rats fed raw soybean meal were reported by Booth *et al.*(2). These authors suggested that the growth depression caused by ingestion of raw soybeans may be due to the excessive loss of critical amino acids contained in the pancreatic enzymes excreted in the feces. However, amino acid supplementation or increasing dietary protein levels failed to correct growth depression and pancreatic hypertrophy in chicks(3,4) or in rats(5). This investigation was conducted to explore the cause of pancreatic hypertrophy, using pilocarpine as an agent to enhance the flow of pancreatic enzymes.

Experimental. Male New Hampshire × Delaware chicks were maintained in a temperature controlled and ventilated laboratory. Chicks used in this study were reared in electrically heated, thermostatically controlled battery brooders with raised wire screen floors. Raw or heat treated soybean meal served as the source of protein in a semi-purified diet(6). Heat treated soybean meal was prepared by passing live steam over the meal, spread in shallow pans, in an autoclave for

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