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MECHANISM OF THE POSITIVE INOTROPIC RESPONSES TO BRETYLIUM AND GUANETHIDINE

BY

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Isolated, atropinized, rat atria exhibited positive inotropic responses to bretylium, guanethidine and tyramine. These responses were prevented by treatment of the animal with reserpine, or by addition of dichloroisoprenaline to the organ bath. The positive inotropic effects of these compounds on atria from reserpinized animals were restored by incubation of the tissue with noradrenaline. On the basis of these findings it is concluded that the cardiac stimulation by bretylium, guanethidine and tyramine involves the release of catechol amines. The usually reported increase in sensitivity of the myocardium from reserpinized animals to noradrenaline was not observed. The influence of bretylium and guanethidine on cardiac uptake and release of noradrenaline was also studied with the rat. Guanethidine decreased the concentration of catechol amines and inhibited the uptake of exogenous noradrenaline, while bretylium had no effect on either. The decrease in concentration of cardiac catechol amines produced by guanethidine was prevented by treatment of the animal with bretylium or with 1-phenyl-2-hydrazinopropane (pheniprazine), a monoamine oxidase inhibitor.

Bretylium and guanethidine produce a hypotension in dogs and cats (Boura & Green, 1959 ; Maxwell, Mull & Plummer, 1959 ; Maxwell, Plummer, Povalski & Schneider, 1960) which is preceded by a transient pressor response, particularly when blood pressure is low and the doses are small (Gillis & Nash, 1961). It has been suggested that these actions are mediated by the release of catechol amines. Both compounds interfere with transmission at the adrenergic nerve endings and enhance the actions of injected catechol amines (Boura & Green, 1959 ; Maxwell *et al.*, 1960). Since the cardiac effects of these compounds in intact animals may be modified by reflex responses, an isolated atrial preparation has been used, thus eliminating these indirect actions and allowing a direct examination of the site and mechanism of the cardiac stimulation by these compounds.

The actions of bretylium and guanethidine have been compared with those of tyramine, because tyramine is thought to release noradrenaline from the sympathetic nerve endings by a direct action on the storage granules containing noradrenaline (Schumann, 1960).

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METHODS

Male albino rats of the Holtzman strain, weighing 225 to 250 g, were killed by a blow at the base of the neck, decapitated, and the hearts rapidly removed. Atria were freed of ventricular muscle, connective tissue, fat and blood vessels, then suspended in a modified Tyrode solution (Lee & Shideman, 1959) maintained at 28° C and containing 2.9×10^{-6} M atropine sulphate. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the bathing fluid via a sintered glass plate at the bottom of the bath. The amplitude of isometric contractions (resting tension of approximately 0.5 g) and the rate of spontaneous beat were recorded. Drugs were added to the bath after the amplitude of contraction had become constant, and responses were calculated as percentage changes of the amplitude just prior to the addition of the drug.

The concentrations of catechol amines in the ventricular myocardium were determined by the trihydroxyindole fluorimetric procedure of Shore & Olin (1958), and are expressed as μg of noradrenaline per g of fresh tissue.

The following drugs were used: (−)-noradrenaline bitartrate, tyramine hydrochloride, guanethidine sulphate, bretylium tosylate, and atropine sulphate. The doses of noradrenaline are given in terms of free base. Unless otherwise indicated, doses of other drugs are expressed in terms of the salt used.

RESULTS

Influence of drugs on atria from the normal rat. Tyramine, bretylium, guanethidine and noradrenaline increased the amplitude of contractions of isolated atrial preparations. The full effects were reached within 1 to 2 min after addition of the drug, were fairly well maintained in the presence of the drug, but were readily abolished by washing the preparation with Tyrode solution. The results of these experiments are summarized in Table 1. A significant increase in atrial rate was also observed, but there was considerable variability between the responses of different preparations.

TABLE 1

POSITIVE INOTROPIC EFFECTS OF TYRAMINE, BRETYLIUM, GUANETHIDINE, AND NORADRENALINE ON ISOLATED RAT ATRIA

Rats received reserpine (1.5 mg/kg) intraperitoneally 16 hr prior to each experiment. Responses were determined before and after incubating the atria with noradrenaline (2 μg/ml.) for 30 min. The number of experiments on which each mean is based is indicated in parentheses

		Increase in size of contraction (Mean % change, ± standard error)		
			Atria from reserpinized rats	
Drug	Concentration in bath (μg/ml.)	Atria from normal rats	Before incubation with noradrenaline	After incubation with noradrenaline
Tyramine	0·25	50·9± 7·7 (12)	2·5±1·1 (6)	22·1±3·2 (6)
Bretylium	5·0	33·6± 6·1 (12)	4·1±1·1 (6)	17·8±2·4 (6)
Guanethidine	2·5	40·8± 5·4 (12)	5·3±1·2 (6)	22·2±0·89 (6)
Noradrenaline	0·05	105·0±12·65 (6)	75·2±2·7 (6)	45·8±4·5 (6)
Dichloroisoprenaline	2·0	36·4± 8·2 (6)	—	—

Influence of dichloroisoprenaline (1-(3,4 dichlorophenyl)-2 isopropyl-aminoethanol hydrochloride) on the positive inotropic activities of tyramine, bretylium and guanethidine. Dichloroisoprenaline blocks the cardiac responses to noradrenaline and adrenaline (Moran & Perkins, 1958). In order to determine whether adrenergic receptors are involved in the cardiac stimulation by bretylium and guanethidine, the influence of dichloroisoprenaline on their actions was examined. Addition of

dichloroisoprenaline (2 μg/ml.) to the muscle bath increased both the amplitude of contractions and the rate. After a short time, the amplitude of contractions returned to a level slightly greater than that prior to the addition of the drug and remained there for some time, even though the atria were washed repeatedly. After the inotropic effects of dichloroisoprenaline had been dissipated, the addition of noradrenaline (0.05 μg/ml.) failed to elicit the usual positive inotropic responses. Similarly, tyramine, bretylium, and guanethidine failed to stimulate the heart.

Influence of drugs on atria from rats treated with reserpine. Recent studies have indicated that reserpine releases catechol amines from their storage sites, including those in the myocardium (Paasonen & Krayer, 1958 ; Lee & Shideman, 1959). Rats which had received 1.5 mg/kg of reserpine (Serpasil, Ciba) intraperitoneally 14 hr previously were killed and their ventricles removed for determination of the concentration of catechol amines. Myocardial tissue from ten such animals contained a concentration of catechol amines of 0.11 μg/g (s.e. of mean, ±0.03). The concentration for a group of eighteen untreated animals was 1.04 μg/g (s.e. of mean, ±0.03). On the atria from rats treated with reserpine, none of the test drugs induced a significant inotropic effect. It is interesting that atria from rats treated with reserpine did not show any increased sensitivity to noradrenaline, but were less responsive to tyramine.

Additional evidence for the participation of catechol amines in the cardiac stimulation by bretylium and guanethidine derives from experiments in which atria from reserpinized rats were exposed to noradrenaline (2 μg/ml.) for 30 min. After these atria had been washed, tyramine, bretylium, or guanethidine once more stimulated the heart (Table 1).

Effect of bretylium and guanethidine on release and uptake of catechol amines by the rat heart. Rats were given 25 mg/kg of bretylium or guanethidine intramuscularly. The animals were killed 2 hr later and their hearts analysed for catechol amines. In some rats, noradrenaline (1.5 mg/kg) was administered intraperitoneally, 30 min after the drugs. Bretylium had no effect on the endogenous myocardial catechol amine levels nor on the uptake of injected noradrenaline by the heart, but each was reduced by guanethidine (Table 2).

TABLE 2

EFFECT OF BRETYLIUM AND GUANETHIDINE ON UPTAKE OF NORADRENALINE BY THE RAT HEART

Noradrenaline was injected intraperitoneally (1.5 mg/kg) 30 min after bretylium or guanethidine; the rats were killed 1.5 hr later and the concentration of cardiac catechol amines determined. Other animals were killed 2 hr after drug administration. Catechol amine concentrations are expressed in terms of noradrenaline. Drugs, other than noradrenaline, were injected intramuscularly. An asterisk indicates the mean is significantly different ($P<0.01$) from that of control animals

Treatment	Dose (mg/kg)	No. of animals	Catechol amine concentration (μg/g of fresh tissue, mean± standard error)
None	—	18	1·06±0·03
Bretylium	25	11	1·08±0·05
Guanethidine	25	5	0·75±0·03*
Noradrenaline	1·5	18	1·76±0·08*
Bretylium and noradrenaline	25+1·5	12	1·77±0·08*
Guanethidine and noradrenaline	25+1·5	5	0·99±0·13

Block of the release of catechol amines caused by guanethidine by a monoamine oxidase inhibitor or by bretylium. Certain monoamine oxidase inhibitors and bretylium partially inhibit the release of catechol amines by reserpine in the rat heart (Bhagat & Shideman, unpublished). In order to determine whether such a monoamine oxidase inhibitor and bretylium similarly inhibit the release of catechol amines by guanethidine, the following experiments were performed. Rats were given 10 mg/kg of pheniprazine (1-phenyl-2-hydrazinopropane, Catron) intramuscularly 16 hr before guanethidine (25 mg/kg, intramuscularly). Bretylium (5 mg/kg) was injected into the tail vein 30 min before guanethidine (5 mg/kg, intravenously). The animals were killed 2 hr after the injection of guanethidine, and the concentrations of catechol amines in the hearts were determined. The results of these experiments are summarized in Table 3. Treatment with 1-phenyl-2-hydrazinopropane or with bretylium prevented the depletion of myocardial catechol amines by guanethidine.

TABLE 3

REVERSAL OF GUANETHIDINE-INDUCED RELEASE OF CARDIAC CATECHOL AMINES IN THE RAT BY PHENIPRAZINE AND BRETYLIUM

When pheniprazine or bretylium was given in conjunction with guanethidine, the former was injected 16 hr and bretylium 30 min before guanethidine. Each animal was killed 2 hr after guanethidine, and the concentration of cardiac catechol amines determined. Catechol amine concentrations are expressed in terms of noradrenaline. An asterisk indicates the mean is significantly different ($P<0{\cdot}01$) from that of control animals. i.m., intramuscular; i.v., intravenous

Treatment	Dose (mg/kg)	Route	No. of animals	Catechol amine concentration (μg/g of fresh tissue, mean $\pm$ standard error)
None	—	—	18	$1{\cdot}06 \pm 0{\cdot}03$
Guanethidine	25	i.m.	5	$0{\cdot}75 \pm 0{\cdot}03$*
Guanethidine	5	i.v.	6	$0{\cdot}85 \pm 0{\cdot}02$*
Bretylium	5	i.v.	8	$1{\cdot}07 \pm 0{\cdot}06$
Pheniprazine	10	i.m.	10	$1{\cdot}62 \pm 0{\cdot}06$*
Pheniprazine and guanethidine	10 25	i.m. i.m.	6	$1{\cdot}61 \pm 0{\cdot}1$*
Bretylium and guanethidine	5 5	i.v. i.v.	8	$1{\cdot}09 \pm 0{\cdot}05$

DISCUSSION

Burn & Rand (1958a, 1958b) found that tyramine showed no pressor action in animals whose catechol amine stores had been depleted by reserpine, but that after an infusion of noradrenaline, the pressor action of tyramine was restored. They postulated that tyramine produces its sympathomimetic effects by the release of noradrenaline from storage sites. This hypothesis has received much support and the findings have been confirmed by several workers. Recently Bhagat & Shideman (unpublished) have found that tyramine can reduce the concentration of catechol amines in the heart of the intact rat. Previous reports (Lockett & Eakins, 1960) have suggested such a possibility. Thus, there is direct evidence for the release of catechol amines by tyramine in the intact animal.

The present results suggest that bretylium, guanethidine and tyramine stimulate the heart of the rat by similar mechanisms. The blocking effect of dichloroisoprenaline on their actions on isolated atria suggests the participation of adrenergic

receptors. The failure of bretylium, guanethidine and tyramine to elicit responses in atria from animals pretreated with reserpine suggests the participation of an adrenaline-like substance. It seems reasonable to conclude that these drugs liberate catechol amines from the heart, a conclusion supported by the fact that exposure to noradrenaline of atria from rats treated with reserpine restores responsiveness to these compounds. The latter fact also suggests that, when the catechol amine stores of the heart have been depleted by reserpine, some degree of replenishment can be realized by administration of noradrenaline. That the heart can take up and store catechol amines agrees with the findings of other workers (Raab & Gigee, 1955; Axelrod, Weil-Malherbe & Tomchick, 1959; Muscholl, 1960). Muscholl (1960) has reported that reserpine prevents the uptake of noradrenaline by the rat heart. However, Campos & Shideman (1962) have shown that small amounts of noradrenaline are taken up by the dog heart after treatment of the animal with reserpine. Under these conditions most of the noradrenaline is in the soluble fraction of the cell, apparently in sufficient amounts to account for restoration of the heart's responsiveness to agents such as tyramine.

Chronic sympathetic postganglionic denervation reduces the noradrenaline content of smooth or cardiac muscle (Goodall, 1951; von Euler & Purkhold, 1951; Burn & Rand, 1959), increases its sensitivity to injected noradrenaline, and decreases its reactivity to tyramine (Bülbring & Burn, 1938). Pretreatment with reserpine causes similar changes (Burn & Rand, 1958a). Burn & Rand (1959) therefore postulated that both the increased sensitivity to noradrenaline and the reduced sensitivity to tyramine are a direct consequence of the depletion of noradrenaline stores. Our results are not entirely consistent with these findings, since atria from rats pretreated with reserpine showed no increase in sensitivity of either the inotropic or chronotropic actions of noradrenaline. However, our results agree with those of Fleming & Trendelenburg (1960), who showed that reserpine quickly depletes the tissues of catechol amines but does not increase the sensitivity to noradrenaline until considerable time has elapsed. Our results also substantiate the conclusion of Fleming & Trendelenburg (1960) that the decreased sensitivity to tyramine is related to depletion of catechol amine stores but not to the increase of sensitivity to noradrenaline.

Our results indicated that bretylium and guanethidine differ in their effects upon uptake and release of catechol amines *in vivo*. Guanethidine depleted, while bretylium had no effect on, the levels of endogenous catechol amines in the rat heart, results which agree with those of Cass & Spriggs (1961). Guanethidine also impaired the uptake of noradrenaline while bretylium had no such effect. The results with bretylium differ from those of Herting, Axelrod & Patrick (1962), who found that this drug impairs the uptake of [^{3}H]-noradrenaline. A difference in the route of administration for noradrenaline could account for this discrepancy, but it seemed unlikely.

The depletion of catechol amines from the rat heart by guanethidine was prevented by treatment of animals with pheniprazine, a monoamine oxidase inhibitor, or by bretylium. Although the effects of these compounds are similar, they cannot be attributed to an inhibition of monoamine oxidase, since

bretylium has no such action (Herting *et al.*, 1962). Inhibitors of monoamine oxidase increase the concentration of catechol amines in the rat heart (Crout, Creveling & Udenfriend, 1961 ; Goldberg & Shideman, 1962), an effect not produced by bretylium. These differences could be explained if inhibitors of monoamine oxidase impaired the release and facilitated the uptake of noradrenaline ; actions for which there exists some evidence (Bhagat & Shideman, unpublished). Although bretylium blocked the release of catechol amines, it had no effect on the uptake of noradrenaline by the heart. These actions are consistent with the increase in cardiac catechol amine content following the administration of pheniprazine, and the lack of effect of bretylium.

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