Mechanism of the Myocardial Effects of Bretylium

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In their original study concerning the cardiovascular responses to bretylium, Boura and Green noted that this drug produced a small positive inotropic response in the guinea pig ventricle strip. Subsequently, Aviado and Dil found that bretylium, transiently but consistently, increased the force of contraction of the dog heart, an observation recently confirmed by others. Definitive data are not available which demonstrate the mechanism of this increase. Some authors have suggested that the drug has a direct effect on the myocardium, whereas others have suggested that bretylium elicits its effects by the release of catecholamines from myocardial tissue. The present experiments were designed to determine: (a) whether the initial, transient, positive inotropic effect seen with the injection of bretylium is accompanied by a myocardial release of catecholamines; and (b) whether the failure of the heart to respond to sympathetic nerve stimulation following bretylium is due to the myocardial depletion of, or to failure to release, catecholamines.

Methods

The details of the preparation and techniques employed have been discussed previously. However, the essentials of the methods are as follows:

A vagotomized canine preparation under pentobarbital anesthesia (30 mg./Kg.) was employed in which the total vena caval return is diverted into a reservoir and then pumped at a predetermined constant flow into the main pulmonary artery, so as to bypass the right heart. The main pulmonary artery is ligated immediately distal to the pulmonary valve so that the right ventricle receives only the right coronary venous outflow and ejects it via a cannula into a recording rotameter circuit, from which it is returned to the venous reservoir. Except where indicated, the heart was paced by bipolar electrodes attached to the left atrium in order to maintain a constant stroke volume. Left ventricular end-diastolic (LVED) and aortic pressures were recorded continuously.

Stimulation of the cardiac sympathetic efferent nerves was accomplished by bilateral stimulation of the stellate ganglia to which all rami had previously been sectioned. In some experiments, the postganglionic fibers were stimulated to exclude any ganglionic effects of bretylium.* All bretylium injections were given into a femoral vein.

Coronary venous blood samples were obtained from the rotameter circuit, where it emptied into the venous reservoir. Arterial samples were drawn from a femoral artery. The analysis of both arterial and coronary venous plasma catecholamines was carried out by Crout's modification of the trihydroxyindole technique of Lund.* All drugs used were demonstrated in vitro not to interfere with the catecholamine analysis.

Results

The data presented are the results of 12 experiments. A representative example of the cardiodynamic response to the injection of bretylium is shown in figure 1. Immediately following the injection of the drug at a constant heart rate and cardiac output, there occurred a slight transient decrease in arterial pressure and coronary blood flow, and then a rise above the control levels. Although mean aortic pressure showed only a small change, there was a widening of pulse pressure; also left ventricular end-diastolic pressure declined, indicating increased contractility. During these changes, an increased concentration of catecholamines was found in coronary venous blood; in fact, during the first sample period following the bretylium injection, the pulmonary valve so that the right ventricle收到了 only the right coronary venous outflow and injects it via a cannula into a recording rotameter circuit, from which it is returned to the venous reservoir. Except where indicated, the heart was paced by bipolar electrodes attached to the left atrium in order to maintain a constant stroke volume. Left ventricular end-diastolic (LVED) and aortic pressures were recorded continuously.

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injection, the coronary catecholamine output exceeded the input, a response often observed during direct cardiac sympathetic nerve stimulation. An increased coronary output of catecholamines during, or immediately following, the injection of bretylium was observed in each of six experiments. With time, the cardiodynamic responses returned to, or near, the preinjection levels, while the coronary venous catecholamine output declined. Throughout the period following the bretylium injection there was a progressive rise in arterial plasma catecholamine concentration.

Figure 2 shows a comparison between the responses to direct cardiac sympathetic nerve stimulation and the injection of bretylium. In both instances, compared to the control samples obtained between these two interventions, there was an increased coronary venous output of catecholamines. However, while cardiac nerve stimulation produced an immediate rise in pulse pressure, coronary flow, and heart rate, the injection of bretylium was accompanied by an initial transient decrease in blood pressure and coronary flow as in figure 1. Although, in this experiment, there was a modest slowing of heart rate in response to bretylium, the usual response was a tachycardia, as shown in figures 3 and 6. Figure 2 also shows the effect of bretylium on the release of catecholamines during cardiac nerve stimulation. Following the injection of bretylium, nerve stimulation...
produced substantially diminished cardiodynamic responses and little or no indication of myocardial catecholamine release in the four animals studied. This is also shown in the experiment of figure 3. The increased catecholamine concentrations found in the coronary venous samples during sympathetic nerve stimulation in the presence of bretylium in figure 2, and in the second sample of figure 3, can be attributed to the increased arterial level. These experiments further show that bretylium did not prevent the myocardial extraction of catecholamines. Figure 4 shows a plot of the myocardial extraction of catecholamines versus catecholamine input from five experiments before and subsequent to the injection of bretylium. The points obtained after bretylium appear to fall on the same curve but are higher than those obtained before bretylium, indicating that this drug does not directly modify the ability of the myocardium to extract catecholamines.

To ascertain whether or not the myocardial extraction after bretylium represents utilization primarily, experiments were done in which bretylium was injected after the administration of such doses of dichloroisoproterenol (DCI) which have been shown to block the myocardial utilization of catecholamines. One such experiment is shown in figure 5. DCI prevented neither the initial myocardial release of catecholamines following the injection of bretylium nor the later return to the control pattern of extraction (see point 4 in fig. 4). However, as observed with cardiac sympathetic nerve stimulation after the administration of DCI, the myocardial extraction of catecholamines was diminished.

$^{*}$L-(3, 4) (Dichloroisoproterenol) 2-Isopropylamino ethanol HCl—Lilly 20522, supplied by Dr. Irwin Slater of the Eli Lilly Research Laboratories.
cardiac release of catecholamines by bretylium was also not associated with evidence of increased contractility. Of interest was the decrease in heart rate which occurred when bretylium was injected after DCI, a finding of only one of the experiments in which bretylium was given in the absence of DCI. The rise in arterial catecholamine concentration following the injection of DCI can reasonably be attributed to adrenal medullary release secondary to carotid sinus hypotension; the increased coronary venous concentration of the sample taken immediately prior to the bretylium injection probably reflects a high catecholamine content of the myocardial binding sites.

The persistence of myocardial catecholamine extraction in the presence of bretylium even after the injection of DCI indicated that the heart could still store these amines and that the bretylium block was not a result of catecholamine depletion. Further evidence that the failure of the heart to release catecholamines during sympathetic nerve stimulation after bretylium is not a result of catecholamine depletion is shown in figure 6. Following the injection of bretylium, there was the usual myocardial release of catecholamines and an increase in arterial catecholamine levels; the samples taken prior to the Tyramine injection also showed the usual pattern of myocardial catecholamine extraction. Tyramine then produced a release of myocardial catecholamines and evidence of a cardiac sympathomimetic effect. There was also an increased arterial catecholamine concentration.

Discussion

The present experiments demonstrate that the immediate, positive inotropic effect of bretylium is associated with a release of myocardial catecholamines, a response also observed during cardiac sympathetic nerve stimulation. They also show that the failure of cardiac sympathetic nerve stimulation to produce a sympathomimetic response after bretylium cannot be attributed solely to depletion but must also encompass a failure to release catecholamines. As shown by Yelnosky and Mortimer and the present experiments, the cardiac response to bretylium is blocked by dichloroisoproterenol, further suggesting that its positive inotropic and chronotropic actions are mediated via the beta adrenergic receptors.

In a recent study by Gaffney and co-workers, it was concluded that the positive inotropic effect of bretylium was the result of a direct effect of the drug on the myocardium rather than secondary to the myocardial release of catecholamines. This was based on the observation that the chronically, completely denervated heart, which presumably contains no catecholamines, showed a positive inotropic response to bretylium to approximately the same extent as the inner-
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vated heart. However, since this denervated preparation has been shown to be hypersensitive to infused catecholamines, and since the present experiments often showed an increased arterial plasma catecholamine concentration following bretylium, it is suggested that the above results may be secondary to the release of catecholamines from peripheral stores which reach and stimulate the heart.

The initial transient changes in arterial pressure found after the injection of bretylium has been observed by others. As shown by Yelmosky and Mortimer and the present experiments, it is not prevented by DCI. These same authors observed that it was not blocked by Dilozane but reported, on the basis of preliminary experiments, that the response was blocked by atropine.

The observations that bretylium prevented the myocardial release of catecholamines during cardiac sympathetic nerve stimulation but did not prevent myocardial catecholamine extraction even in the presence of DCI, and that Tyramine still caused a release of catecholamines from the myocardium following the injection of bretylium, are of interest with respect to elucidating the mechanism of the myocardial storage of these amines. They suggest that bretylium acts by effecting a block between the nerve impulse (or substances released by the nerve impulse) which impinges on the catecholamine binding sites, and the sites themselves. They also suggest that Tyramine acts distal to this point, perhaps directly on the binding sites. Such a suggestion has been made by Burn and Rand, based on the observations of Hukovic, that Tyramine produces vasoconstriction of the rabbit ear after the constrictor response to nerve stimulation had been blocked by bretylium.

Boyd and co-workers observed that norepinephrine reversed the bretylium block of the vas deferens of the guinea pig and suggested that bretylium accumulated where norepinephrine is believed to be stored. However, at least with respect to the heart, this accumulation, if it occurs, does not appear to be primarily related to the mechanism of action of bretylium, since after the injection of this drug, and in the presence of DCI which blocks catecholamine utilization, a significant myocardial extraction of catecholamines was observed during the period in which sympathetic stimulation was not accompanied by a demonstrable release of myocardial catecholamines. Also, as a result of blocking release but not storage, bretylium could increase the total myocardial content of catecholamines.

Boyd and co-workers have also reviewed the evidence, which supports the suggestion of Burn and Rand, that acetylcholine is the transmitter which is released from post-ganglionic sympathetic nerve fibers and impinges upon the norepinephrine stores causing norepinephrine to be released. Part of this evidence is that of Boura and Green,
who found that bretylium changed the chronotropic response of the heart during stimulation of the cardio-accelerans nerve from tachycardia to bradycardia, and that the bradycardia was blocked by atropine. Also, Hukovic observed that in the presence of atropine, bretylium blocks the accelerating action of acetylcholine on the atria. In the present experiments, the response of heart rate to sympathetic nerve stimulation after bretylium was variable, showing both slowing and little change. However, certain observations are available which are not consonant with the suggestion of Burn and Rand: (a) Sympathetic nerve stimulation after DCI has little or no effect on heart rate and is accompanied by a release of myocardial catecholamines but, as shown in figure 5, when bretylium is injected after DCI, a bradycardia occurs in the presence of an increased concentration of coronary venous catecholamines. (b) The chronically denervated heart which contains no catecholamines, and presumably no nerve endings, shows a negative chronotropic response to bretylium. (c) Bretylium has muscarinic effects on the vas deferens of the guinea pig and produces a transient dilatation of the kidney when injected into the renal artery (Gilmore, unpublished observations). (d) Von Euler and Lishajko observed that, whereas Tyramine and reserpine caused a release of norepinephrine from granules obtained from bovine splenic nerves, acetylcholine did not. The bradycardia observed during stimulation of the cardiac sympathetics following the injection of bretylium may result from the release of bretylium previously taken up by the storage sites which are associated with sympathetic nerve endings.

The data herein indicate that bretylium has parasympathomimetic effects. They also suggest that the responses attributed, by Burn and Rand, to acetylcholine in the presence of bretylium may, at least in part, be due to a parasympathomimetic action of bretylium on the heart which is uncovered when either the beta actions of catecholamines are blocked or when the heart contains no catecholamines which can be released by bretylium. The above observations do not necessarily indicate that acetylcholine does not act as the transmitter between postganglionic sympathetic nerve endings and the catecholamine storage sites. They, however, do point out that some of the effects attributed to acetylcholine in the presence of bretylium may have been due to the muscarinic effects of bretylium itself.

Summary

The increased myocardial contractility resulting from the injection of bretylium has been shown to be accompanied by a myocardial release of catecholamines. Cardiac sympathetic nerve stimulation following bretylium is associated with neither an increased contractility nor a release of myocardial catecholamines. The failure to release catecholamines under these conditions does not result from depletion, but rather from a block of the nerve release mechanism.

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