Effect of Guanethidine and Bretylium on the Dog Heart-Lung Preparation

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GUANETHIDINE and bretylium are reported to produce hypotension by interference with transmission at the adrenergic neuro-effector junction and to have sympathomimetic effects after intravenous injection in dogs and cats.\(^1\)\(^2\) In the case of guanethidine, Maxwell et al.\(^1\) have suggested that these effects are due to initial catecholamine release. Recently, Cass et al.\(^3\) have shown that guanethidine depletes rabbit and cat hearts of catecholamines. Bretylium is said to have no effects on the catecholamine content of the adrenals or sympathetic ganglia.\(^4\)

Bretylium is reported to have a negative inotropic effect in the Langendorff preparation of the rabbit heart and a small positive inotropic effect upon strips of guinea pig ventricles.\(^2\) It is only in intact dogs that the cardiovascular effects of guanethidine have otherwise been studied.\(^1\)\(^8\) The direct cardiac effects of a drug, such as changes in contractile force, heart rate, or conduction, are difficult to determine in intact animals in which these effects may be modified by reflex responses such as those occurring with changes in blood pressure; these blood pressure changes themselves may affect myocardial contractile force.\(^6\) Measurements of changes in cardiac output in the whole animal are sometimes loosely used as an indication of the inotropic effects of a drug, but changes in output may or may not reflect these effects. The strain-gauge arch applied to the heart may be used in intact or open-chest animals to record changes in the force of contraction. When this is done, changes in diastolic pressure may produce effects upon the contractile force of the heart, but these are usually small.\(^6\)

The heart-lung preparation is suitable for these determinations because it excludes many of the variables discussed above, such as reflexes, changes in blood pressure (peripheral resistance can be held constant), and changes in venous filling pressure (which may be continuously adjusted in the heart-lung preparation).

This paper describes the effect of guanethidine\(^5\) and bretylium\(^\dagger\) on contractile force and heart rate in: (1) the normal heart-lung preparation; and (2) heart-lung preparations made from dogs pretreated with reserpine to deplete their hearts of catecholamines.\(^7\)

Methods

Dogs were anesthetized with sodium pentobarbital given intravenously (30 mg./Kg.). Twenty-two heart-lung preparations were prepared from mongrel dogs of either sex, weighing from 12 to 25 Kg. The Starling resistance was set at 80 mm. Hg; the blood entering the right atrium was kept at 37 to 38.5 C. The volume of blood in the preparation at the outset of each experiment was approximately 800 ml. and the apparent loss from the venous reservoir did not exceed 25 ml. in the course of any one experiment, which always lasted less than one hour. The height of the blood in the venous reservoir was maintained at 18 cm. above the level of the right atrium. At the outset, the venous return was adjusted to produce a systemic output (cardiac output minus coronary flow) of 600 ml. per minute. Direct measurements of systemic output were made in duplicate with a Stolnikov stromuhr at five-minute intervals. A strain-gauge arch (120 ohms) was sutured to the surface of the right ventricle. The placement and tension adjustments were as recommended by Cotten,\(^6\) and the tension was recorded by a Grass polygraph. In some experiments, heart rates were recorded by an electrocardiograph with leads attached directly to the ventricle; in others, rates were determined from the strain-gauge record.

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All drugs were injected through the venous inflow cannula of the heart-lung preparation in a volume of less than 5 ml for each injection and, except where specified, with no more than one drug used in each heart-lung preparation. Doses were injected five minutes apart. All doses are expressed as milligram per heart-lung preparation (approximately 1 Kg. body weight).

Dogs pretreated with reserpine were given 0.1 mg/Kg. intraperitoneally on each of two successive days before the experiments, as recommended by Waud et al. The second injection was given 24 hours before the experiment. In one heart-lung preparation the only dose of reserpine (2 mg.) was given 30 minutes before injection of guanethidine. Dogs pretreated with guanethidine were given 10 mg/Kg. intravenously on each of two successive days prior to the experiment.

In two experiments, heart rate was kept constant by driving the heart through the atria using a Grass rectangular-wave stimulator, while a dose-response curve was obtained with guanethidine. This was done to eliminate the effects of changes in rate upon changes in contractile force.

Results
General Description of Effects

During control observations in each experiment the peak systolic tension recorded by the strain-gauge arch did not vary by more than 1 mm. (out of 10 to 15 mm. response). After the injection of the drug, increases or decreases of 1 mm. or more beyond this limit were interpreted as positive or negative inotropic effects, respectively.

Guanethidine and bretylium each had positive inotropic and chronotropic effects in the normal heart-lung preparation. These effects started within one minute after either drug and were maximal in less than one minute from their initial appearance. The duration of the effects due to minimally effective doses was only about 30 seconds, but effects from maximal doses lasted longer than 30 minutes, after which the experiments were terminated. The rates of onset of inotropic and chronotropic effects of guanethidine and bretylium were similar. This is in contrast to the slower onset of similar effects from reserpine given acutely to the normal heart-lung preparation. Reserpine had a positive chronotropic effect with a slow rate of onset beginning within two to five minutes after injection and reaching a peak in about 30 minutes, as previously reported by Innes et al. A positive inotropic effect of reserpine developed with about the same time course as the rate change.

Spontaneous failure rarely develops in our normal heart-lung preparations during the first hour of operation; those in which drug effects were studied were never used for longer than one hour. All preparations made from animals pretreated with reserpine were in good condition at the beginning of the experiment. In two which were followed for one hour without treatment, no fall in systemic output or myocardial contractile force was seen.

In heart-lung preparations made from dogs pretreated with reserpine, guanethidine had a negative inotropic effect which appeared gradually over three to five minutes at low doses but within one minute at high (5 mg.) doses. In one experiment, a single injection of guanethidine (5 mg.), given after a one-hour control period (stable output and contractile force) caused a rapid, marked decrease in systemic output from 600 to 475 ml. per minute. This negative inotropic effect was still developing 15 minutes after injection of guanethidine. Guanethidine had no effect on heart rate after pretreatment with reserpine. Bretylium in this circumstance still had a small positive inotropic effect but the positive chronotropic effect due to bretylium was replaced by a negative chronotropic effect.

Guanethidine

Figure 1A shows the relation between the dose of guanethidine and the degree of inotropic effect in six untreated and five chronically reserpinized heart-lung preparations. The upper half of the graph (open circles) shows the positive inotropic effect of guanethidine in the untreated heart. The minimally effective dose for an increase in contractile force was 300 µg. in all six experiments, and the maximally effective dose was about 3 mg. In two of these experiments (closed triangles in fig. 1A), the heart rate was kept constant by driving the atria at 240 beats per minute; the effect of guanethidine upon contractile force was similar to that in experiments in.
which the heart rate was allowed to change. The lower half of the graph (closed circles) shows that the positive inotropic effect of guanethidine seen in the normal heart was reversed by pretreating the dog with reserpine. This decrease in contractile force was associated in each experiment with a progressive fall in systemic output. In two such experiments, acetylstrophanthidin (80 µg.) promptly reversed this failure; in three others, epinephrine had its usual positive inotropic and chronotropic effects and returned the output to control values. In one heart-lung preparation, 2 mg. of reserpine given only 30 minutes before guanethidine did not prevent the usual positive inotropic effect of guanethidine.

Figure 1C shows the relation between the dose of guanethidine and heart rate in the heart-lung preparation of normal and chronically reserpinized dogs. Guanethidine had a marked positive chronotropic effect in the normal heart. The heart rate seems less sensitive to guanethidine than the positive inotropic effect. The rise in heart rate was eliminated by pretreatment with reserpine for two days, but was still present in the experiment in which guanethidine was given 30 minutes after 2 mg. of reserpine.

The effects of guanethidine were examined in the heart-lung preparation from dogs pretreated for two days with guanethidine (10 mg./Kg. per day). Figure 2 indicates the results of three such experiments. After pretreatment with guanethidine, guanethidine caused only a negative inotropic effect as measured by both strain-gauge arch and systemic output. This effect appeared after 1 mg. in each experiment but the fall in output did not reach the low levels produced by guanethidine after pretreatment with reserpine (fig. 1A).

Bretylium

Bretylium has a positive inotropic effect in the normal heart-lung preparation at doses above about 300 µg. (fig. 1B, open circles). In two out of three experiments, the effect was greater than that seen with guanethidine. In four experiments, after pretreatment with reserpine, a smaller positive inotropic effect of bretylium persisted (fig. 1B, closed circles). The minimal dose of bretylium for the positive inotropic effect did not appear to have been affected by reserpine pretreatment, but the largest increment in contractile force from bretylium after reserpine pretreatment was reduced to the order of one-fourth of that in the normal heart.

Bretylium has a positive chronotropic effect in the normal heart but the maximal increase in rate is less than that caused by guanethidine (fig. 1D). As with guanethidine, the chronotropic effect is less sensitive to bretylium than is the inotropic effect. In the chronically reserpinized heart, bretylium has a negative chronotropic effect at high doses, in spite of its positive inotropic effect (fig. 1B and D).

Discussion

The usefulness and reliability of the strain-gauge arch for recording a positive or negative inotropic effect have been extensively studied. During drug-induced failure in our experiments, marked increase in the size of the heart occurred. Changes in heart size have been noted to affect the reliability of the strain-gauge arch for measurement of contractile force, primarily when low initial tension is placed upon the muscle fibers between the gauge attachments. This limitation was avoided by using a higher initial tension. Furthermore, in the present experiments direct measurements of systemic output during failure correlated well with the decreased contractile force recorded by the strain-gauge arch. It is known that in the nonfailing heart, positive inotropic effects evident from the strain-gauge arch record are not associated in all cases with an increase in cardiac output.

Bretylium has previously been reported to cause a negative inotropic effect in the rabbit Langendorff preparation and a positive inotropic effect in isolated guinea pig ventricular strips. In blood-perfused hearts, the inotropic effects of guanethidine and bretylium apparently have not been previously determined when venous pressure and arterial pressure have been held constant. These controls are important when studying drugs known to af-
Dose-response relations in heart-lung preparations. (A) Guanethidine on heart contractile force. (B) Bretylium on heart contractile force. (C) Guanethidine on heart rate. (D) Bretylium on heart rate. Open circles: preparations from normal dogs; closed triangles: preparations from normal dogs, heart rate held constant; closed circles: preparations from chronically reserpinized dogs; closed squares: preparations from chronically reserpinized dogs, three experiments with single doses.

Figure 1

feet vasoconstriction and hence possibly venous return, both of which may affect isometric systolic tension. Our experiments in the heart-lung preparation with constant venous pressure and peripheral resistance demonstrate a positive inotropic effect in the normal heart from either guanethidine or bretylium.

Reserpine is known to deplete myocardial catecholamines and, if endogenous catecholamines are important to myocardial function, this effect of reserpine might be expected to alter the stability of the heart-lung preparation. Our experiments demonstrating that heart-lung preparations made from chronically reserpinized dogs were in good condition mechanically, however, do not support this idea. Reserpine acutely administered to the heart-lung preparation has previously been noted to produce a positive chronotropic effect, to this we have added the demonstration that it produces a positive inotropic effect, which develops with about the same time course as the positive chronotropic effect. Innes et al. have attributed the positive chronotropic effect of reserpine to catecholamine release; the same explanation seems reasonable as a working hypothesis for the positive inotropic effect.

Except with respect to the rate of onset of the effects, guanethidine and bretylium have cardiac effects similar to those of reserpine.
i.e., positive inotropic and chronotropic effects. Cass et al. have shown that 12.5 mg./Kg. of guanethidine given to rabbits depletes their hearts of catecholamines after 18 hours. We have shown that pretreatment with guanethidine, which may be expected to do the same for the dog's heart, like similar pre-treatment with reserpine, leaves the heart-lung preparation of the dog in good mechanical condition. We propose that the positive inotropic and chronotropic effects of guanethidine and bretylium are due, at least in part, to catecholamine release, as they probably are for reserpine.

This hypothesis is strongly supported by the finding that pretreatment with reserpine in a manner known to deplete the heart of catecholamines eliminates the positive inotropic and chronotropic effects of guanethidine and much reduces the positive inotropic effect of bretylium, while abolishing its positive chronotropic effect. The alternative hypothesis, that the presence of reserpine, rather than its previous depletion of catecholamines, might have produced these changes, was excluded by the demonstration that reserpine administered only 30 minutes before guanethidine, a time insufficient in the intact dog to produce complete amine release, did not prevent the usual positive inotropic effect of guanethidine. Further support of our proposal comes from the fact that guanethidine, chronically administered in such a way as to deplete the heart of catecholamines, is like reserpine in antagonizing the positive inotropic and chronotropic effects of subsequently administered guanethidine. Although bretylium has been reported to have no effect upon the catecholamine content of the adrenals or the sympathetic ganglion, our results suggest the prediction that in the heart bretylium will be found to resemble reserpine and guanethidine in releasing catecholamines.

The degree of decrease of inotropic and chronotropic effect produced by pretreatment with reserpine is similar for guanethidine and bretylium. These relationships suggest that pretreatment with reserpine, by removing catecholamines, removed a constant factor in the response of contractile force and heart rate to guanethidine and bretylium. A further implication is that when this factor is removed each of these two drugs has characteristic effects upon these functions. In the case of guanethidine, the positive inotropic effect seen in the normal heart-lung preparation (fig. 1A, open circles) would be the difference between an indirect positive inotropic effect caused by amine release and a direct negative inotropic effect. The larger positive inotropic effect of bretylium would represent the sum of the indirect positive inotropic effect from amine release and a further positive inotropic effect of bretylium. The dose of reserpine used in pretreatment is known to cause amine depletion to the extent of about 80 per cent. The residual positive inotropic effect of bretylium might, as alternative explanations, be due to residual amine release or to a direct effect of bretylium on the myocardium.

If we consider the effect of pretreatment with reserpine on the heart rate in a similar manner, we would conclude that guanethidine has no intrinsic effect on heart rate apart from that due to catecholamine release, whereas bretylium has an intrinsic negative chronotropic effect.

These observations in dog heart-lung preparations may prove to be relevant to the treatment of hypertensive patients with guanethidine and reserpine, bretylium and reserpine, or guanethidine alone. However, species variations in drug effects as well as potency are common. Possible clinical implications are apparent in situations where limited cardiac...
reserve is present. In this connection, it may be important that acetylstrophanthidin corrected the failure seen with guanethidine.

There seem to be similarities between guanethidine, bretylium, and reserpine with respect to amine release. As previously noted, guanethidine and bretylium are known to interfere with transmission across the adrenergic neuro-effector junction.1,2 Reserpine too has been shown to interfere with transmission at this junction.11 A similar mechanism for this interference by guanethidine, bretylium, and reserpine may exist. Perhaps the block of transmission common to all three is due not to a prevention of norepinephrine release, as previously suggested,1 but to a depletion of transmitter substance at the adrenergic neuro-effector site.

Summary

Inotropic and chronotropic effects of guanethidine and bretylium have been observed in heart-lung preparations made from normal and chronically reserpinized dogs. Guanethidine (0.3 to 30 mg.) in the untreated preparation had marked positive inotropic and chronotropic effects, whereas after pretreatment with reserpine it had a striking negative inotropic effect and no effect on heart rate. Guanethidine given to preparations made from animals pretreated with guanethidine had a negative inotropic effect smaller than that seen after reserpine pretreatment. Bretylium (0.3 to 30 mg.) in the untreated preparation had both positive inotropic and positive chronotropic effects. In the chronically reserpinized animal, the positive inotropic effect of bretylium persisted though it was reduced to about one-quarter of the original size. The positive chronotropic effect of bretylium in this circumstance was reversed to a negative chronotropic effect. These effects are interpreted as indirect but strong evidence that: (1) guanethidine and bretylium exert at least part of their initial positive inotropic and chronotropic effects by a release of catecholamines; and (2) in the amine-depleted heart, guanethidine has an intrinsic negative inotropic effect, whereas bretylium has an intrinsic positive inotropic effect.

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Addendum

In a recent publication, Aviado and Dil have shown that bretylium increases the force of contraction of the heart in the open-chest anesthetized dog (J. Pharmacol. & Exper. Therap. 129: 328, 1960).

References


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