ABSTRACT

On the basis of studies on cardiac tissue removed from animals treated with antiadrenergic drugs, a number of investigators have suggested that the positive inotropic response to digitalis requires norepinephrine in the cardiac muscle. In the present study the action of strophanthidin was studied in isolated papillary muscles obtained from normal cat hearts, from chronic, totally cardiac-denervated, norepinephrine-depleted hearts, and from reserpine-treated, norepinephrine-depleted cats. Complete force-velocity and length-tension curves were recorded. Following the addition of strophanthidin (1.0 μg/ml) to the bath, maximum isometric tension rose by averages of 2.17 ± 0.32 g/mm² in the normal muscles and 2.65 ± 0.50 g/mm² in the muscles from the denervated cats, but increased significantly less (P < 0.05) in the muscles from the reserpine-treated animals (1.09 ± 0.36 g/mm²). In addition to these changes in isometric tension, strophanthidin increased the maximum velocity of contraction (V_max) to a comparable extent in normal and denervated muscles, with a smaller elevation of V_max in reserpine-treated muscles. Strophanthidin reduced the absolute refractory period to an equal extent in all three groups of muscles. From a comparison of the inotropic responses of the muscles from normal and cardiac-denervated cats it is concluded that cardiac norepinephrine stores and neural integrity are not essential for the positive inotropic effect of strophanthidin or for its effects on the duration of the absolute refractory period. However, it appears that prior reserpine treatment may interfere with the inotropic response to digitalis by a mechanism other than norepinephrine depletion.

ADDITIONAL KEY WORDS

reserpine cardiac denervation
strophanthidin force-velocity curve
absolute refractory period myocardial contractility
anesthetized cats
inotropic action of glycosides

It has been suggested that the digitalis glycosides release norepinephrine (NE) from cardiac sympathetic nerves and that intact stores of the neurotransmitter are therefore required in order for these drugs to exert their full positive inotropic effect.1,2 This suggestion has been based primarily on the demonstration of a reduced response to digitalis in cardiac muscle which had been depleted of its NE stores by reserpine or guanethidine, or in which the response to released NE was blocked with dichloroisoproterenol.1-6 These studies do not, however, exclude the possibility that actions of these drugs, other than NE depletion or adrenergic blockade are responsible for the diminished effectiveness of digitalis. Chronic total denervation of the heart results in profound depletion of cardiac NE stores6 and provides an opportunity to study the effects of digitalis

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on NE-depleted cardiac muscle in the absence of other pharmacologic agents. In the present investigation the effects of digitalis glycosides were determined in papillary muscles obtained from normal cats, in muscles removed from cats depleted of cardiac NE stores by denervation, and in muscles from cats in which NE depletion had been produced by reserpine administration. Thus, the effects of cardiac NE depletion on glycoside action were determined in the absence of other drug actions and the results obtained were compared with those observed when reserpine had been given.

Methods

The papillary muscles were obtained from 10 normal cats, 8 cats which had undergone total cardiac denervation 10 to 20 days previously and 8 cats which had been given reserpine (3 mg/kg) intraperitoneally 48 and 24 hr prior to sacrifice. Total extrinsic cardiac denervation was accomplished by mediastinal neural ablation. At the time of study, the cats were anesthetized with sodium pentobarbital (25 mg/kg) intraperitoneally and papillary muscles from the right ventricle were rapidly removed and transferred to a myograph containing oxygenated Krebs solution while the remaining right ventricular tissue was assayed for NE. The NE, determined spectrophotofluorometrically by the trihydroxyindoleacetic method, was essentially eliminated from the hearts of these cardiac-denervated and reserpine-treated animals and averaged 0.006 ± 0.003 μg/g (SEM) in the right ventricles of each of these two groups; NE averaged 2.13 ± 0.30 μg/g in the normal animals. The depletion of NE in the papillary muscles from the denervated and reserpine-treated animals was further substantiated by the finding that these muscles exhibited only a trivial inotropic response to tyramine.

Methods used for study of the papillary muscles have been described in detail previously, as has the myograph in which they were studied. With this system the force of contraction at any desired muscle length could be determined and simultaneously the extent and velocity of shortening of the muscle at any load could be measured. Initial length of the muscle was established by a small preload which was then kept constant as progressive afterloads were added and the initial velocity of shortening was determined for each afterload. Maximum velocity of shortening (V_{max}) occurred as the muscle lifted the preload alone and when the increasing afterload was such that the muscle could not shorten, maximum isometric tension for that muscle length (P_{o}) was described. The plot of initial velocity of shortening relative to load (or force) comprises the force-velocity relation. The lever could also be made stationary and the force of isometric contraction recorded as muscle length was increased in a stepwise manner, thus describing the length-tension curve.

The muscles were stimulated with square wave DC impulses of 9 msec duration and 1.5 times the threshold voltage delivered through field electrodes placed parallel to the long axis of the muscle. All experiments were carried out at 30°C and the frequency of contraction was set at 12 per min. To assure steady-state performance, a period of 1 hr was allowed between the time the muscles were placed in the myograph and the initial recordings. The function of the papillary muscles remained stable for periods of at least 3 to 4 hr. The experiments on the muscles from the three groups of animals were carried out concurrently rather than sequentially and in an identical manner.

Control force-velocity relations, maximum isometric active tensions measured at the apex (L_{max}) of the length-tension curves and the absolute refractory periods were determined. The active tension was calculated as the difference between the total and resting tensions, and was corrected for the cross-sectional area of each muscle. Following these measurements either 1.0 μg/ml of strophanthidin or 0.25 μg/ml of ouabain was added to the bath. The maximum effects were observed 20 min later with strophanthidin and 1 hr later with ouabain, and the determinations of maximum isometric tension, force-velocity relations, and the absolute refractory period were repeated at these times. In muscles from 5 additional normal cats, reserpine was added to the muscle bath in a concentration of 10 μg/cc 20 min prior to the addition of strophanthidin, and the effects of this drug on the muscles' response to the glycoside were determined. Three other normal cats received 10 mg/kg of reserpine intravenously and 20 min later their papillary muscles were removed and the responses to strophanthidin determined.

Results

ISOMETRIC TENSION

Every muscle studied exhibited some augmentation of isometric tension when exposed to strophanthidin or ouabain. The mean values for the absolute levels of isometric ten-
sion at Lmax observed before and after strophanthidin was added to muscles obtained from the normal, cardiac-denervated, and chronically reserpine-treated cats are reported in table 1. The increments were similar in the normal muscles and the NE-depleted muscles obtained from the cardiac-denervated cats averaging $2.17 \pm 0.32$ and $2.65 \pm 0.50$ g/mm² respectively. However, the responses of the NE-depleted muscles obtained from the cats which had received reserpine on the 2 days prior to study were significantly less than those observed in the other two groups, and averaged $1.09 \pm 0.36$ g/mm². It is of interest that the muscles obtained from the cardiac-denervated cats had control values of tension which were not depressed, but actually somewhat higher than the normal. Furthermore, the control values in the muscles from the cardiac-denervated and chronically reserpine-treated animals were essentially identical, although the increments in tension produced by strophanthidin were significantly lower in the latter group.

The muscles from 2 denervated cats also responded normally to ouabain, the second glycoside studied, with changes in isometric tension of 3.0 and 1.8 g/mm² compared to increments of 2.3, 1.6 and 2.8 g/mm² following addition of this dose of ouabain to muscles from 3 normal cats. The muscles obtained from normal cats given a large dose of reserpine just prior to sacrifice exhibited normal responses when exposed to strophanthidin, with increments in tension with strophanthidin which averaged 3.1 g/mm². The muscles obtained from normal cats which were exposed to reserpine in vitro exhibited increments in tension with strophanthidin which were slightly, but not significantly, lower than normal averaging $1.7 \pm 0.5$ g/mm².

**FORCE VELOCITY RELATIONS**

In addition to augmenting isometric tension, the glycosides also shifted the entire force-velocity curves to a similar extent, increasing the maximum velocity of shortening ($V_{max}$), as well as the isometric tension developed ($P_o$), in the muscles obtained from each of 5 normal and 5 cardiac-denervated cats (fig. 1). However, smaller shifts in the force-velocity curves were observed in the muscles from the 8 chronically reserpine-treated cats. The increments in $V_{max}$ averaged 68% in the normal muscles, 71% in the cardiac-denervated muscles, and only 29% in the muscles from the reserpinized animals.

**ABSOLUTE REFRACTORY PERIOD**

The absolute refractory period of the muscles, i.e., the shortest attainable time interval between a driven and propagated premature stimulus, was always determined at a frequency of 12 contractions per min. Strophanthidin abbreviated the refractory periods in all three groups of muscles (fig. 2), and there were no significant differences among the three groups for the values prior to strophanthidin, following the glycoside, or for the decreases in absolute refractory periods induced by the drug.

**Discussion**

The role played by intact cardiac NE stores in mediating the positive inotropic effect of digitalis has not been settled. Considerable

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Before strophanthidin</th>
<th>After strophanthidin</th>
<th>Percentage Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>6.19 ± 0.50</td>
<td>8.36 ± 0.54</td>
<td>35</td>
</tr>
<tr>
<td>Denervated</td>
<td>8</td>
<td>8.34 ± 0.54</td>
<td>10.99 ± 1.10</td>
<td>32</td>
</tr>
<tr>
<td>Reserpine</td>
<td>8</td>
<td>8.75 ± 0.85</td>
<td>9.84 ± 0.94</td>
<td>12</td>
</tr>
</tbody>
</table>

± = SEM.
data have been presented to support the position that intact cardiac stores of NE are essential for the full positive inotropic effect of digitalis and that the glycosides may exert a part of their positive inotropic effect through release of tissue catecholamines.

Tanz\textsuperscript{1,2} reported that the augmentation of isometric tension produced by ouabain in isolated papillary muscles obtained from cats in which cardiac NE-depletion had been produced by reserpine and guanethidine was substantially less than the augmentation observed in muscles obtained from normal cats. Similar results were obtained when dichloroisoproterenol, a beta-adrenergic blocking agent, was added to normal isolated muscles prior to exposure to the glycoside. He suggested that the release of the positive inotropic substance, NE, is necessary for the full response to digitalis glycosides, and that muscles from which the NE stores have been removed, or in which the adrenergic receptors are blocked, exhibit a decreased response to ouabain. Levy and Richards\textsuperscript{3} have also reported a diminished inotropic response to ouabain in isometrically contracting left atria from reserpinized rabbits. Förster and Stolzenburg\textsuperscript{4} found that pretreatment of guinea pigs with 0.25 mg/kg per day of reserpine for 5 days, decreased the positive inotropic response of isolated atria to digitoxigenin but not to digoxigenin. However, pretreatment of guinea pigs with a lower dose of reserpine (0.1 mg/kg per day, for 12 days) did not alter the positive inotropic response of the atria to either digitalis preparation. Denis and co-workers\textsuperscript{5} showed that ouabain reversed the deterioration of isometric force which they observed in isolated left atria obtained from normal rabbits, but this effect of the drug was not present in atria obtained from reserpine or guanethidine pretreated rabbits, or in atria from normal rabbits when reserpine or guanethidine was added directly to the perfusing fluid.

Conversely, other workers have shown normal inotropic responses to glycosides after beta-adrenergic blockade and in hearts depleted of NE. Moran and Perkins\textsuperscript{10} studied the positive inotropic effect of digoxin on the right ventricular contractile force of open-chest, vagotomized dogs, with and without complete adrenergic blockade, produced by dichloroisoproterenol and found that the positive inotropic effects of the glycoside were
not reduced by adrenergic blockade. Similarly, Levy and Richards\textsuperscript{11} showed that ouabain exerted normal positive inotropic effects on isolated atria of rabbits in the presence of the potent beta-adrenergic receptor blocking agents, pronethalol and propanolol. Also, Roberts and co-workers\textsuperscript{12} showed no difference between the ouabain-produced increase in isometric tension in papillary muscles obtained from normal cats and animals that had been treated with reserpine.

The object of the present investigation was to attempt to resolve the disagreement resulting from the experiments reviewed above. The possibility was considered that the responses to glycosides might have been interfered with, not by the antiadrenergic effects of the drugs employed, but through some other pharmacologic action. In an earlier investigation from this laboratory, it was noted that ouabain resulted in a normal increase of the relative amplitude of right ventricular contractile force in the denervated but otherwise intact dog heart.\textsuperscript{18} However, in this study the changes observed may have reflected, in part, the alterations which the glycoside induced in heart rate, cardiac output and arterial pressure. Moreover, in the intact cardiac-denervated dog the possibility that catecholamines were released from the adrenal medulla and other noncardiac stores could not be excluded. Finally, the position that cardiac stores of NE are essential for the inotropic effect of digitalis is based upon studies performed on isolated heart muscles which have been pharmacologically depleted of NE.\textsuperscript{1-5} Accordingly, it was felt that a definitive answer to this problem could be obtained only by the study of the effects of glycosides on isolated tissue, obtained from NE-depleted denervated hearts which had not been exposed to other drugs. It was observed that the NE-depleted papillary muscles obtained from the denervated animals exhibited normal inotropic responses both to strophanthidin and ouabain. The contractile state of the denervated muscle was not depressed prior to the addition of the glycosides; in fact their isometric tensions tended to exceed the normal values (table 1). Furthermore, the denervated muscles exhibited normal responses to glycosides, both with respect to increase in the force of isometric tension and the velocity of shortening. From these observations it is concluded that the normal positive inotropic response of cardiac muscle to digitalis glycosides does not depend on an intact cardiac NE store. Therefore, it is not necessary to incriminate release of cardiac NE by glycosides as an essential feature of the inotropic action of digitalis.

As in many of the earlier studies, the present investigation revealed both an absolute and relative depression of the inotropic response to glycosides following chronic reserpineization. However, it is clear that these findings cannot be explained by the NE depletion produced by reserpine, since the NE-depleted papillary muscles from cardiac-denervated cats responded in an entirely normal manner. The mechanism by which reserpine appears to interfere with the response to glycosides is not clear. Certainly, muscles from reserpine-treated cats have the capability of responding to other inotropic interventions, as evidenced by their normal responses to sustained paired electrical stimulation, exogeneous NE and increased frequency of contraction.\textsuperscript{9} Neither the direct addition of reserpine to the bathing medium nor the intravenous administration of a large dose to the cat just prior to sacrifice abolished the positive inotropic response to strophanthidin. The isometric tensions in the muscles obtained from the reserpinized animals prior to the addition of strophanthidin were somewhat higher than the normal muscles and the possibility can not be completely excluded that this higher baseline level was responsible for their diminished inotropic responses to the glycoside. This possibility is unlikely since the baseline tensions in the muscles obtained from denervated hearts also exceeded normal and in these muscles the augmentation following strophanthidin was not depressed.

Govier observed that both beta-adrenergic blockade and prior reserpineization blocked the shortening of the refractory period of iso-
lated rabbit atria induced by ouabain and suggested that ouabain induced a decrease in atrial refractory period by release of NE. However, in the present study, strophanthidin decreased the absolute refractory period equally in NE-depleted and normal papillary muscles. Thus, an intact cardiac NE store is not essential for this action of digitalis on ventricular myocardium. Interestingly, while previous reserpinization appeared to interfere with the positive inotropic effect of the glycoside, it did not alter the effects of strophanthidin on this electrophysiologic property.

References
Studies on Digitalis XIV: Influence of Cardiac Norepinephrine Stores on the Response of Isolated Heart Muscle to Digitalis

JAMES F. SPANN, Jr., EDMUND H. SONNENBLICK, THEODORE COOPER, CHARLES A. CHIDSEY, VALLEE L. WILLMAN and EUGENE BRAUNWALD

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