Beta-Receptor Blockade and Myocardial Effects of Cardiac Glycosides

By Jan Koch-Weser

ABSTRACT

Because of suggestions that norepinephrine release from cardiac sympathetic nerves contributes to the myocardial action of digitals, the effect of beta-receptor blockade on these actions was determined. 10−6 M propranolol did not alter the basal contractility of isolated kitten papillary muscles or of atrial strips from kittens, guinea pigs, rabbits, dogs, and chickens but decreased by 85% their inotropic response to norepinephrine released by tyramine or high-intensity electrical stimulation. The same concentration of propranolol had no effect on cumulative inotropic concentration-effect curves for ouabain, acetylstrophanthidin, and digoxin in these preparations. Presence of propranolol did not modify the actions of cardiac glycosides on the time course of isometric contraction. Beta-receptor blockade was without effect on decreases in active tension and increases in resting tension induced by toxic concentrations of cardioactive steroids. The frequency of digaloxin-induced automaticity in guinea pig atrial strips and kitten papillary muscles was significantly decreased by propranolol, presumably because of its direct electrophysiologic actions. Exposure of cat hearts to digoxin in vivo and to ouabain in vitro had no effect on myocardial norepinephrine concentrations. It is concluded that release of myocardial norepinephrine plays no role in the actions of cardiac glycosides on the heart.

KEY WORDS isolated heart muscle propranolol ouabain inotropic concentration-effect curves acetylstrophanthidin contracture myocardial automaticity myocardial norepinephrine rabbits dogs chickens cats guinea pigs

During the past decade many investigators have suggested that cardiac glycosides release myocardial norepinephrine and that this release contributes to their actions on the mechanical and electrical performance of the heart (1-10). This theory has been based on findings of lowered myocardial norepinephrine concentration after exposure to cardiac glycosides (1, 9, 11-13), of decreased ability of cardiac glycosides to enhance myocardial contractility (3-5, 7, 10, 14, 15) or to induce arrhythmias (2, 16-20) after pretreatment with reserpine, and of interference by beta-receptor antagonists with positive inotropic (4, 6, 21) or arrhythmogenic (6, 10, 22-28) actions of cardiac glycosides. Other investigators have not confirmed the depletion of myocardial norepinephrine by cardiac glycosides (10, 18, 29) or any decrease in their positive inotropic effect after reserpine (2, 17, 18, 20, 27, 28, 30-32) or beta-receptor
blockade (27, 28, 32-35). The inhibitory effect on digitalis arrhythmias of previous treatment with reserpine is generally accepted and that of some beta-receptor-blocking agents is unquestioned, but neither of these observations establishes that digitalis normally plays any role in their genesis.

In view of this continuing controversy, we determined the effect of specific and high-grade beta-receptor blockade with propranolol on mechanical and electrical responses of isolated heart muscle to therapeutic or toxic concentrations of cardiac glycosides. Because of past reports of differences in this regard between species and among various glycosides and aglycones (16, 19, 21, 24, 26, 30), we studied three cardioactive steroids and atrial and ventricular myocardium from five species. The effect of in vivo and in vitro exposure to cardiac glycosides on myocardial norepinephrine concentration was also examined.

Methods

Papillary muscles were obtained from the right ventricles of 58 kittens with a median weight of 610 g. To ensure adequate oxygenization of the central fibers (36), only papillary muscles of less than 0.6 mm² cross-sectional area were included. Cross-sectional area was estimated on the basis of weight at the end of each experiment, assuming the muscle to be a cylinder with a specific gravity of one. Length of the muscles, measured under the resting tension employed throughout the experiment, averaged 8 mm. Atrial strips were taken from the left atria of kittens, guinea pigs, rabbits, puppies, and chickens. They averaged 0.5 mm thick, 10 mm long, and 6 mm wide. Immediately after the animals were killed by a sharp blow on the skull, the hearts were removed and dissected in oxygenated solution at room temperature. Suitable papillary muscles were removed together with a small button of adjacent ventricular wall and a short piece of chorda tendinea. The mural end of each muscle was fixed to a muscle holder by a plastic clamp and the tendinous end was tied with a short silk thread to a wire extending upward to a Statham transducer (model G7B-0.75-350). The long axis of the atrial strips was similarly fixed. Resting tension of all preparations was maintained throughout the experiment at one-half of that resting tension which at the beginning of the experiment was associated with maximum development of tension. It varied between 100 and 300 mg depending on the cross-sectional area of the preparation.

Isometric mechanograms were recorded on a Sanborn model 964 oscillographic recording system equipped with model 350-1100B carrier preamplifiers. Maximum rate of development of tension, time to peak tension, and relaxation time were determined from Polaroid photographs of Tektronix dual-beam oscilloscope records at a sweep speed of 200 mm/sec. All measurements were made during regular rhythm and after tension development had reached a steady stage after mounting of the preparation and after addition of all drugs.

All preparations were initially quiescent unless excited electrically. Electrical stimuli were delivered through two punctate platinum electrodes contacting the surface of the muscle just above the point of clamping. The stimuli were delivered from a constant-current pulse amplifier (Argonaut LRA 048) triggered by a specially designed unit based on Tektronix pulse and waveform generators. Stimulation consisted of rectangular 5-msec pulses. Except when the effect of increases in the intensity of stimulation was studied, their amplitude did not exceed the resting threshold of each preparation (0.13 to 0.30 ma) by more than 10% in order to minimize the release of endogenous norepinephrine from sympathetic nerve endings in the preparation (36, 37).

The muscles were suspended in 50 ml of a modified Krebs solution of the following composition (before equilibration with CO₂): in mEq/liter, Na⁺ 140; K⁺ 5; Ca²⁺ 4.5; Mg²⁺ 2; Cl⁻ 98.5; SO₄²⁻; and in mEq/liter, HCO₃⁻ and H₂CO₃, 29; HPO₄²⁻ and H₂PO₄⁻, 1; fumarate 5; pyruvate 5; L-glutamate 5; glucose 10; and insulin 5 IU/liter. The solution was continuously oxygenated and stirred by passage of finely divided bubbles of a mixture of 95% O₂ and 5% CO₂. After equilibration with this mixture, the pH was 7.4. The perfusing solution was replaced by fresh solution at 1-hour intervals or whenever the concentration of a drug was changed. The solution was always maintained at a temperature of 37.5 ± 0.1°C. Langendorff preparations of kitten hearts were perfused with the same solution and maintained at 37°C. In experiments involving norepinephrine, 10⁻⁵ M dissolved ethylene diamine tetracetae was added to the solution to slow the oxidation of the catecholamine (36).

Leverantrenol, tyramine, atropine, propranolol, ouabain, acetethylcholanthin, and digoxin were added directly to the perfusing solution after dilution of stock solutions. Dilutions were made in such a way that the volume of drug solution added never exceeded 0.1% of the organ bath volume. Propranolol was always left to act for 1 hour before subsequent drugs were added.
Concentration-effect curves for the cardioactive steroids were determined by cumulative increases in drug concentration. The next higher concentration was added as soon as the full effect of the previous concentration had been reached.

Myocardial norepinephrine concentrations were determined by the spectrophotofluorometric method of Crout (38). The results were corrected for the recovery of norepinephrine (89 ± 5%).

Results

Direct and Beta-Receptor-Blocking Actions of Propranolol.—The direct inotropic effects of $10^{-8}$M to $10^{-6}$M propranolol on kitten papillary muscles and on guinea pig atrial strips were determined in order to select the highest possible concentration which had no direct effect on tension development. No positive inotropic effect was seen at any concentration. Propranolol had no negative inotropic effects at concentrations below $2 \times 10^{-6}$M, provided the preparations were excited with stimuli of barely suprathreshold intensity to avoid release of norepinephrine from sympathetic nerve endings. Higher concentrations increasingly depressed myocardial contractility.

The degree of antagonism of $10^{-6}$M propranolol to the positive inotropic effects of exogenous or endogenous norepinephrine was determined in the same types of isolated heart muscle. Figure 1 shows the increase in tension development by three papillary muscles exposed to levarterenol, tyramine and high-intensity electrical stimulation. These inotropic responses were fully and repeatedly reproducible after washout of norepinephrine but were largely blocked 1 hour after addition of $10^{-6}$M propranolol. In three groups of six kitten papillary muscles, $10^{-6}$M propranolol reduced the effects of these concentrations of l-norepinephrine and tyramine and of this rate of electrorelease of norepinephrine to 13%, 16%, and 17% of their original magnitude. The corresponding figures in 14 atropinized (5 × $10^{-5}$M) guinea pig atrial strips were 10%, 13%, and 14%.

These results show that $10^{-6}$M propranolol does not alter the basal contractility of the myocardial preparations used in this study but greatly diminishes the inotropic actions of added or released norepinephrine. Accordingly, the positive inotropic effect of any drug whose action is wholly or in part mediated by release of norepinephrine would be appreciably reduced by $10^{-6}$M propranolol.

Effect of Propranolol on Ouabain Inotropism.—Figure 2 shows the positive inotropic action of five concentrations of ouabain on kitten myocardium in normal solution and in the presence of propranolol. The results are from experiments during which the preparations contracted at frequencies at which the contractility of kitten ventricular myocardium is low and that of kitten atrial muscle is pessimal (39). Thus the absolute and, particularly, the relative positive inotropic effects of

![Figure 1](http://circres.ahajournals.org/)

**FIGURE 1**
Reduction by propranolol of inotropic responses of kitten papillary muscles to added or released norepinephrine. Contraction frequency 60/min. A: Three muscles in normal solution. B: Same muscles 60 min after addition of $10^{-6}$M propranolol. I: l-norepinephrine $3 \times 10^{-7}$M added at arrows; II: tyramine, $10^{-5}$M added at arrows; III: between arrows stimulation intensity raised to five times threshold and an extra stimulus placed into each refractory period of the muscle.
Lack of Effect of Propranolol on Maximum Positive Inotropic Action of Ouabain in Kitten Myocardium

Failure of beta-receptor blockade to alter inotropic concentration-effect curves of ouabain in kitten myocardium. Contraction frequency: atrial strips, 10/min; papillary muscles, 2/min. Means and standard errors of 16 preparations in all four groups.

Cardiac glycosides are very large in both (40). Other contraction frequencies at which basal contractility is much higher and the maximum positive inotropic effect of ouabain is much smaller were examined in six preparations each (Table 1). At no frequency did 10⁻⁶M propranolol have any effect on basal contractility or alter the positive inotropic effect of any concentration of ouabain.

Analysis of high-speed isometric mechanograms of atrial strips and papillary muscles showed the positive inotropic effects of all concentrations of ouabain to result from increases in the rate of development of tension. No definite changes in the time to peak tension or in the relaxation time were detected. Exposure to 10⁻⁶M propranolol did not modify the ouabain-induced changes in the time course of tension development.

Eight kitten papillary muscles contracting 10/min were exposed to 3 × 10⁻⁶M propranolol. This concentration depressed basal contractility by 18% but had no influence on the cumulative inotropic concentration-effect curve of ouabain.

Other Cardioactive Steroids.—Two other cardioactive steroids gave the same results. Effective beta-receptor blockade with 10⁻⁶M propranolol had no apparent effect on the positive inotropic action of acetylstrophanthidin in kitten myocardium (Fig. 3). Cumulative inotropic concentration-effect curves for digoxin (5 × 10⁻⁶M-2 × 10⁻⁵M) were determined in 16 atrial strips and 18 papillary muscles of kittens. The presence of 10⁻⁶M propranolol in the solution perfusing half of the preparations did not significantly alter the mean increases in tension development.

Other Species.—Identical results were obtained with myocardium from other species. Guinea pig atrial strips showed the same positive inotropic response to seven concentrations of ouabain in the absence and in the presence of propranolol (Fig. 4).Cumulative inotropic concentration-effect curves for acetylstrophanthidin and digoxin in 14 guinea pig atrial strips each were similarly unaffected by beta-receptor blockade. Finally, the inotropic effects of ouabain on atrial strips of rabbits (12), dogs (6) and chickens (6) were not appreciably modified by 10⁻⁶M propranolol.

Table 1

<table>
<thead>
<tr>
<th>Contraction Frequency (beats/min)</th>
<th>Normal solution</th>
<th>Propranolol (10⁻⁶M)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atrial Strips</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>108 ± 8</td>
<td>104 ± 10</td>
</tr>
<tr>
<td>1</td>
<td>176 ± 11</td>
<td>172 ± 13</td>
</tr>
<tr>
<td>10</td>
<td>948 ± 44</td>
<td>942 ± 49</td>
</tr>
<tr>
<td><strong>Papillary Muscles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>479 ± 32</td>
<td>472 ± 36</td>
</tr>
<tr>
<td>10</td>
<td>106 ± 8</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>100</td>
<td>32 ± 4</td>
<td>26 ± 6</td>
</tr>
</tbody>
</table>

*As % of original active tension at each frequency.
PROPRANOLOL AND DIGITALIS ACTION

Lack of effect of propranolol on inotropic concentration-effect curves of acetylstrophanthidin in kitten myocardium. Contraction frequency: atrial strips, 10/min; papillary muscles, 2/min. Means and standard errors of 14 preparations in all four groups.

Toxic Mechanical Effects.—Beta-receptor blockade also failed to influence the time course and the extent of the toxic effects of cardioactive steroids on the mechanical behavior of myocardium. In kitten papillary muscles contracting 30 times/min, contracture and complete failure of tension development occur during an exposure of less than 30 minutes to 3 x 10^{-6} M acetylstrophanthidin. The addition of 10^{-6} M propranolol 1 hour previously had no apparent effect on the decrease in active tension or the increase in resting tension (Fig. 5). This conclusion was confirmed with other concentrations of acetylstrophanthidin and ouabain in 10 other kitten papillary muscles and in 12 guinea pig atrial strips.

Ectopic Automaticity.—In contrast to its failure to alter the mechanical effects of cardiac glycosides, propranolol markedly reduced their ability to induce ectopic impulse formation in isolated myocardium. Properly prepared guinea pig and kitten left atrial strips and kitten papillary muscles showed no spontaneous activity in normal perfusing solution. During exposure to increasing concentrations of cardiac glycosides, temporary or permanent automaticity was usually induced in guinea pig atrial strips, occasionally in kitten papillary muscles, and very rarely in kitten atrial strips. The presence of 10^{-6} M propranolol significantly reduced the incidence of spontaneous contractions in guinea pig atrial strips and almost completely prevented them in kitten papillary muscles (Fig. 6).

Effect of Ouabain on Myocardial Norepinephrine Concentration.—Daily intraperitoneal administration of 25 or 50 μg/kg of digitoxin for 1 week to cats had no effect on the norepinephrine concentration of atrial or ventricular myocardium (Table 2). Langendorff preparations of cat hearts were perfused with normal solution and with two concentrations of ouabain, and samples of the left
ventricle were taken periodically for analysis. Myocardial norepinephrine concentration in all three groups decreased significantly during 2 hours of perfusion, but the ouabain concentration of the perfusing solution was without effect (Table 3).

Discussion
The conclusion that cardiac actions of digoxin are partly mediated by release of catecholamines from body stores (1-10) has been suggested by effects of various interventions on the electrical and mechanical behavior of the heart under the influence of cardiac glycosides. Alternate explanations seem more likely for some of these observations, while others could not be confirmed by subsequent investigators.

TABLE 2
Lack of Effect of Digoxin for One Week on Norepinephrine Concentration (ng/g) in Cat Myocardium

<table>
<thead>
<tr>
<th>Daily digoxin dose</th>
<th>Right atrial</th>
<th>Left atrial</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.64 ± .16</td>
<td>1.66 ± .19</td>
<td>2.06 ± .18</td>
<td>1.61 ± .11</td>
</tr>
<tr>
<td>25 µg/kg</td>
<td>2.56 ± .34</td>
<td>1.58 ± .27</td>
<td>1.62 ± .31</td>
<td>1.69 ± .19</td>
</tr>
<tr>
<td>50 µg/kg</td>
<td>2.29 ± .26</td>
<td>1.98 ± .39</td>
<td>2.18 ± .25</td>
<td>1.71 ± .16</td>
</tr>
</tbody>
</table>

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Failure of Ouabain Perfusion of Cat Langendorff Preparation to Affect Left Ventricular Norepinephrine Concentration (ng/g)

<table>
<thead>
<tr>
<th>Min of perfusion (min)</th>
<th>Controls (ng/g) ± SE</th>
<th>Ouabain (10^{-5})M (ng/g) ± SE</th>
<th>Ouabain (2 \times 10^{-5})M (ng/g) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.64 ± .12</td>
<td>1.59 ± .18</td>
<td>1.71 ± .16</td>
</tr>
<tr>
<td>30</td>
<td>1.51 ± .18</td>
<td>1.67 ± .24</td>
<td>1.74 ± .21</td>
</tr>
<tr>
<td>60</td>
<td>1.45 ± .19</td>
<td>1.50 ± .15</td>
<td>1.60 ± .21</td>
</tr>
<tr>
<td>120</td>
<td>1.26 ± .13</td>
<td>1.28 ± .12</td>
<td>1.42 ± .15</td>
</tr>
</tbody>
</table>

Number in parentheses is number of experiments.

Treatment with reserpine before exposure to cardiac glycosides has been found by many (2, 16-20) though not all (5, 14, 28, 31) investigators to reduce the arrhythmogenic potential of these agents. Any ability of previously given reserpine to counteract the arrhythmogenic potential of digitals does not allow the conclusion that in the untreated animal or patient digitals causes arrhythmias in part by releasing catecholamines. First, in the presence of normal myocardial norepinephrine stores, some of the arrhythmogenic actions of cardiac glycosides would be expected to summate with electrophysiologic effects of norepinephrine released by sympathetic nerve activity unrelated to the presence of cardiac glycosides (2, 41). Under the conditions of many such experiments, tonic sympathetic release of norepinephrine must have been quite high. Secondly, reserpine may modify the electrical properties of the myocardium independently of its norepinephrine-depleting action (2, 14, 17, 20).

The unquestioned effectiveness of many beta-receptor antagonists in preventing or abolishing experimental or clinical digitalis-induced arrhythmias is even less evidence for any norepinephrine release by cardiac glycosides. Again, these compounds will antagonize the electrophysiologic effects of norepinephrine released by tonic sympathetic nerve activity which can facilitate some arrhythmogenic actions of digitals. Secondly, those beta-receptor-blocking agents which most effectively antagonize digitals-induced rhythm disturbances have quinidine-like properties which are largely responsible for this action.

More to the point is any influence on the positive inotropic action of cardiac glycosides of procedures which remove myocardial norepinephrine or prevent its cardiac action. The effect of pharmacologic depletion of norepinephrine stores on this action remains controversial. Some investigators have reported that after reserpine pretreatment the positive inotropic effects of cardiac glycosides are decreased (3-5, 7, 10, 15, 42, 43). Others found this to be true only after doses larger than those required to deplete myocardial norepinephrine (30) or not to be reversed by repletion of the stores with norepinephrine (3). Commonly, previous treatment with reserpine had no effect on digitalis inotropism (2, 17, 20, 27, 28, 30-32), and occasionally it enhanced the positive inotropic effect (14, 18). It seems probable that these conflicting results reflect differences in the intensity of reserpine treatment, in the temporal relationship between exposure to reserpine and to digitals, and in the importance of sympathetic tone in the controls not given reserpine.

Any ability of previous treatment with reserpine to decrease the inotropic effects of cardiac glycosides is almost certainly unrelated to its depletion of norepinephrine stores in the heart. Cardiac denervation effectively removes myocardial norepinephrine, but the effects of cardiac glycosides on the mechanical behavior of surgically (31, 43, 44) or immunologically (20) denervated and on that of normal myocardium are indistinguishable. The mechanism by which reserpine can interfere with digitals inotropism remains unclear, but the interference cannot reasonably be cited as evidence that digitalis action is mediated by norepinephrine release.
If digitalis released norepinephrine from sympathetic nerve endings in normal myocardium, blockade of beta receptors should decrease its positive inotropic action. Dichloroisoproterenol, a partial beta-receptor agonist rather than a pure antagonist, has been reported not to influence (21, 33) or to decrease (4, 21) the positive inotropic effect of cardiac glycosides. Pronethalol, a purer beta-receptor antagonist, caused no major changes in the positive inotropic action of cardiac glycosides on rabbit atria (34), on the dog heart-lung preparation (28) and on the dog heart in situ (32). In the latter preparation, the inotropic effect of strospeside was increased after doses of pronethalol which depressed myocardial contractility (45). Loubatières et al. (6) found that doses of propranolol which depress myocardial contractility abolished or prevented the positive inotropic action of ouabain on the dog heart in situ. The positive inotropic effect of ouabain on rabbit atria was slightly reduced by propranolol (34), but the preparations were excited with three times threshold voltage and propranolol decreased tension development in the absence of ouabain. Fratz et al. (27) reported that 10–4 propranolol, which depressed the basal contractility of guinea pig atria, did not alter K-strophanthin inotropism but gave no details. Proscillaridin A produced a greater increase in V_{ma,i} of the dog left ventricle in situ after treatment with propranolol (35), probably because its effect was not diminished by reflex withdrawal of sympathetic tone.

We chose a concentration of propranolol which had no effect on the basal contractility of isolated heart muscle but reduced the positive inotropic action of released norepinephrine by 83 to 86%. This high degree of beta-receptor blockade had no effect on the inotropic concentration-effect curves of all three cardioactive steroids examined. In atrial and ventricular muscle of five species, ouabain caused the same increase in tension development in the absence and presence of propranolol. Beta-receptor blockade also failed to modify the toxic actions of cardiac glycosides on the mechanical behavior of heart muscle. The conclusion seems inevitable that norepinephrine release from sympathetic nerve endings in the myocardium plays no role in the positive inotropic action of digitalis.

This conclusion is further supported by major differences in details of the inotropic actions of digitalis and norepinephrine. While both increase the maximum intensity of the active state, norepinephrine abbreviates the time to maximum intensity far more than cardiac glycosides (46). As a result, cardiac glycosides increase the maximum rate of development of tension to about the same extent as the maximum force of isometric contraction, while norepinephrine increases the former much more than the latter (36, 39). Secondly, the effect of contraction frequency on the magnitude of the inotropic actions of norepinephrine and of cardiac glycosides is very different (30, 47, 48).

Administration of cardiac glycosides to rats (1, 9), guinea pigs (11, 12), or rabbits (13) has been reported to lower their myocardial norepinephrine concentration. However, the concentrations used in most of these experiments were toxic to lethal and the effects may have reflected nonspecific stress. Other investigators found that ouabain caused no decrease of norepinephrine concentration in the hearts of cats (10, 15) or rabbits (29). In our experiments exposure of cat hearts in vivo and in vitro to high nontoxic concentrations of cardiac glycosides had no effect on their norepinephrine concentrations. Although cardiac glycosides could release norepinephrine from the heart without lowering its norepinephrine concentration, there is no convincing evidence for such an action.

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References

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