Glycoside Inotropy in the Absence of an Increase in Potassium Efflux in the Rabbit Heart

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ABSTRACT

The inotropic effect of 1.25 x 10^{-6} M acetylstrophanthidin (ACS) and the influx and efflux of labeled potassium (^{42}K^+) were studied in the arterially perfused rabbit interventricular septum under control conditions and during respiratory acidosis. An increase in the CO_2 content of the gas mixture with which the modified Ringer’s solution was equilibrated from 5 to 30% reduced the perfusate pH from 7.37 to 6.66. The increment in developed tension in the presence of ACS was 3.0 ± 0.2 g (N = 10) under control conditions, but it was greater, 7.1 ± 0.9 g (N = 9) during acidosis (P < < 0.001). The net K^+ loss due to an increase in K^+ efflux was 1.8 ± 0.2 mmoles/kg wet weight in control experiments but only 0.1 ± 0.1 mmoles/kg wet weight under acidic conditions (P < < 0.001); in seven of nine experiments in respiratory acidosis, no increase in K^+ efflux occurred despite a marked positive inotropy. In three septums, K^+ influx was reduced by ACS during respiratory acidosis. These results demonstrate that during acidosis ACS inhibits sodium-potassium adenosinetriphosphatase (Na^+-K^+ ATPase) and causes an inotropic effect but does not increase K^+ efflux. K^+ efflux cannot be linked to calcium (Ca^{2+}) influx or regarded as the controlling factor of glycoside-induced inotropy. The results give further support to the proposal that digitalis-induced inotropy is secondary to an enhancement of a Na^+-Ca^{2+} exchange system.

Although the mechanism by which glycosides exert an inotropic effect on the myocardium still remains a matter of dispute, several facts have been well substantiated. Glycosides specifically inhibit the sodium-potassium adenosinetriphosphatase (Na^+-K^+ ATPase) within the cell membrane (1, 2), and the degree of inhibition is closely related to the extent of the inotropic effect (3). Concurrent with these drug actions, an augmentation of calcium (Ca^{2+}) influx occurs, and Ca^{2+} is the presumed mediator of the increase in myocardial contractility (4, 5). However, it remains unclear and controversial how precisely the inhibition of Na^+-K^+ ATPase is linked to an increase in Ca^{2+} influx. Two different hypotheses have been proposed. Langer and Serena (4) and Langer (6) hold that the augmented Ca^{2+} influx arises from stimulation of a Na^+-Ca^{2+} carrier. The stimulation is secondary to a raised intracellular Na^+ concentration resulting from the inhibition of Na^+-K^+ ATPase. The increase in K^+ efflux observed, coincident with glycoside inotropy, is an increase in the passive K^+ “leak” associated with the net gain of intracellular Na^+ and is not primary to the greater Ca^{2+} influx and the inotropic effect. Morad and Greenspan (7) have put forward an alternative hypothesis in which the outward movement of K^+ is linked directly to the inward movement of Ca^{2+}, and they discount the coupling of Na^+ and Ca^{2+}. An inotropic effect should always, therefore, be accompanied by an increase in K^+ efflux. The fundamental difference between the two proposals is that Langer and Serena (4) link the increase in Ca^{2+} influx to the intracellular Na^+ concentration whereas Morad and Greenspan (7) link it to an increase in K^+ efflux. The experiments reported in the present paper demonstrate that under certain conditions, namely an acidosis caused by an increase in CO_2 (respiratory acidosis), the inotropic effect of glycosides is apparent in the absence of a change in K^+ efflux. The theory proposed by Morad and Greenspan (7) is untenable without modification under these circumstances.

Methods

Perfused Septal Preparation. — The preparation used in these experiments was the arterially perfused rabbit interventricular septum which has been previously described (8, 9). Adult male New Zealand white rabbits were heparinized and anesthetized with sodium pentobarbital. The thorax was rapidly opened, and the heart was excised and placed in warm, oxygenated perfusate. The septal artery was cannulated within 2-4 minutes.
The triangular piece of tissue was mounted between opposing Harmon forceps, and the apex was connected to a strain-gauge transducer (Statham Instruments). A reservoir of perfusate raised 80-200 cm above the preparation provided sufficient hydrostatic pressure to perfuse the septum with a modified Ringer's solution (10). The flow was maintained constant throughout each experiment by small changes in the height of the reservoir. Flow was measured by timing effluent drops of a constant size, and the mean flow varied between 1.1 and 1.4 ml/min g⁻¹ wet weight. The temperature of the perfusate in the reservoir equaled that of the laboratory and varied between 23 and 25°C. The perfusate could be warmed by a heating coil around a steel needle immediately adjacent to the cannula. The temperature of the tissue was measured using a needle thermistor (Yellow Springs Instrument Company), and it was kept constant in each experiment between 25 and 26°C. Septums weighed 0.9-1.4 g and were stimulated at 42-60 beats/min.

Solutions and Chemicals.—The modified Ringer's solution used as the perfusate had the following millimolar composition: Na⁺ 142, K⁺ 5.0, Ca²⁺ 1.5, Mg²⁺ 1.0, Cl⁻ 117, H₂PO₄⁻ 0.4, HCO₃⁻ 28, and dextrose 5.6. The solution was continuously bubbled in the reservoir with a 5% CO₂-95% O₂ or a 30% CO₂-70% O₂ gas mixture. "K⁺" (New England Nuclear Corporation) was made up in an amount of K⁺-free perfusate calculated to maintain the overall concentration at 5.0 mM. Acetylstrophanthinidin (ACS) was obtained from Lilly Laboratories.

Measurements.—The pH of the perfusate was measured on a sample drawn anaerobically into a glass syringe from a stopcock close to the cannula but before the heating coil so that the sample was obtained at room temperature. The pH was measured on a BMSI and PHM 72 (Radiometer). Buffer standards (Instrumentation Associates) were used to calibrate the electrode. The pH thus measured was adjusted to the exact temperature of the tissue using known values for the solubility of CO₂ at different temperatures (11) and a blood-gas calculator (Radiometer, type BCGI). This procedure was validated in experiments in which pH and carbon dioxide tension measurements were made at the temperature of the tissue. No significant difference could be detected between values obtained with perfusate at tissue temperature and those adjusted by the preceding procedure to the tissue temperature. The "K⁺" activity of the effluent collected in planchets during isotopic washout experiments was counted using a G-M probe and counter (Nuclear Chicago, Ultrascaler II). Tissue "K⁺" was continuously monitored by a lead-collimated G-M probe (Atomic Accessories, model 222A Geiger tube), placed at a distance of 1-2 cm from the muscle.

Experimental Procedure.—A period of 120 minutes was allowed for equilibration of the septums before any experimental intervention. The muscles were labeled with "K⁺" for 30-55 minutes. The washout was started with nonisotopic solution, the effluent drops were collected in individual planchets, and the activity was counted, corrected for decay, plotted semilogarithmically, and analyzed. The tissue activity was measured simultaneously with the G-M probe. During the washout, the perfusate was switched for 8 minutes to a solution containing ACS. The advantage of simultaneous plots obtained from the effluent drops and the tissue probe is that a detailed analysis of K⁺ movements is possible. If the plot from the tissue probe is linear and parallel to the plot of the effluent, then the isotope must be distributed homogeneously in a single kinetic compartment. If, following an intervention, the slope of the plot for the tissue probe is unchanged but the plot of the effluent deviates from a straight line, a transient unsteady state exists involving a net loss or gain of the ionic species that is labeled with the isotope (4).

In separate experiments, the uptake of "K⁺" was followed continuously with the same tissue probe, and the effect of ACS was studied. Samples of the control and experimental solutions were obtained, weighed, and counted to confirm that no difference existed in their specific activities.

The net loss of K⁺ from the septum, attributable to an increased efflux in washout experiments, was calculated using previously described methods (4). Results are expressed as means ± SE. Differences between groups of results were analyzed using an unpaired Student’s t-test.

Results

Previous experiments (10) have shown that 2 hours is sufficient time for this preparation to reach a steady state. Over the next 5 hours, K⁺ content, water content, and muscle function remain constant. In experiments in which septums were exposed to 30% CO₂ repeatedly or for a continuous period up to 2 hours, there was no evidence of irreversibility of the reduced tension development. These observations indicate that the experimental preparation did not deteriorate.

The effect on developed tension of an increase in the CO₂ content of the gas mixture with which the perfusate was equilibrated from 5 to 30% is illustrated in Figure 1. A rapid loss of tension occurred, and after a small undershoot a new steady state was established. The effect was completely reversible.

During the experiment (Fig. 1), a respiratory acidosis was introduced while the uptake of "K⁺" by the tissue was followed with the G-M probe. The pH of the perfusate fell from 7.39 to 6.67. After the intervention, the slope of the uptake curve appeared to increase slightly; this effect probably reflects a net uptake of K⁺ which occurs secondary to respiratory acidosis and has been previously reported (10). After 68 minutes, ACS (1.25 × 10⁻⁶M) was introduced in the presence of the G-M probe. The pH of the perfusate fell from 7.39 to 6.67. After the intervention, the slope of the uptake curve appeared to increase slightly; this effect probably reflects a net uptake of K⁺ which occurs secondary to respiratory acidosis and has been previously reported (10). After 68 minutes, ACS (1.25 × 10⁻⁶M) was introduced in the presence of the G-M probe.

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three different septums. The experiments demonstrate that ACS causes an inotropy in the presence of respiratory acidosis and that concurrently $^{42}\text{K}^+$ influx is markedly reduced. These effects are the same as those observed in the presence of ACS in the normal pH range (4).

The washout of $^{42}\text{K}^+$ from a septum under normal conditions is shown in Figure 2. The perfusate was equilibrated with 5% CO$_2$, and the pH was 7.36. Between 22 and 30 minutes, the muscle was exposed to ACS ($1.25 \times 10^{-6}$M). After 6 minutes of the washout, the logarithmic plot for the tissue probe (counts/min) remained straight and parallel to the effluent plot (counts/min min$^{-1}$) before and after ACS. The parallelism indicates that $^{42}\text{K}^+$ in the septum is distributed in a single compartment that is kinetically homogeneous. After exposure of the septum to ACS (Fig. 2), the plot of the effluent deviated above the straight line by an average of 11 SD for each point; the deviation indicates an increase in $^{42}\text{K}^+$ efflux. At the same time, the plot for the tissue probe remained linear. This combination of results means that in relation to the total tissue counts the increment in efflux was small and that no detectable alteration of the steady-state exchange rate of $^{42}\text{K}^+$ occurred. Therefore, the increased efflux, apparent in the effluent plot, can only be attributed to a net loss of K$^+$ from the tissue.

An identical experiment is shown in Figure 3 except that during the last 10 minutes of the labeling period and the entire washout the perfusate was equilibrated with 30% CO$_2$. The pH was reduced to 6.67, and ACS ($1.25 \times 10^{-6}$M) was introduced between 22 and 30 minutes. It is clear that the respiratory acidosis abolished the net loss of K$^+$.
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attributable to an increase in efflux that occurred in the presence of ACS at normal pH (compare Fig. 2). Nevertheless, the inotropic effect of ACS was still present and was greater in the acidic condition.

The experiments were repeated in 12 septums, and 19 washouts were performed. The pH of the perfusate when it was equilibrated with 5 or 30% CO₂ ranged from 7.35 to 7.38 in 10 washouts and from 6.64 to 6.70 in 9 washouts, respectively. In 7 septums after the initial washout, the muscle was relabeled with K⁺, and a second washout was performed under the opposite acid-base condition. The initial washout was in the presence of 5% CO₂ in 2 septums and in the presence of 30% CO₂ in 5 septums. The experimental order did not affect the results in these 7 paired washouts. In those experiments performed during respiratory acidosis, the septums were labeled under control conditions except for the final 10 minutes of the labeling period which was in the presence of 30% CO₂. The dose of ACS was 1.25 × 10⁻⁶M in 14 experiments, 1.0 × 10⁻⁶M in 4 experiments, and 0.8 × 10⁻⁶M in 1 experiment. The results are summarized in Figure 4. The increment of developed tension expressed in absolute units (ΔP) was greater in the presence of 30% CO₂ than it was in the presence of 5% CO₂ (P < < 0.001). The developed tension before the introduction of ACS was less in respiratory acidosis than it was under normal conditions because of the depressant effect of CO₂ (Fig. 1). The percent increase in developed tension was, therefore, very much greater during acidosis: 87 ± 10% vs. 14 ± 1% (P < < 0.001). At the same time that the inotropic effect of ACS was occurring, there was, under control conditions, a net loss of K⁺ which was quantified by measuring the area under the effluent curve (Fig. 2). This calculated net loss represents only that due to the increased K⁺ efflux caused by the ACS. It does not include the contribution of a reduced K⁺ influx (Fig. 1) to the total loss of K⁺ from the tissue (see Discussion). The calculated net loss was 1.8 ± 0.2 mmoles/kg wet weight in the presence of 5% CO₂ but only 0.1 ± 0.1 mmoles/kg wet weight in the presence of 30% CO₂ (P < < 0.001). In 7 of the 9 experiments in which ACS was introduced during respiratory acidosis, it was not possible to detect any increase in K⁺ efflux (Fig. 3).

Discussion

The present experiments demonstrate that at a control pH of 7.4 ACS, a cardioactive steroid, has an inotropic effect on the myocardium and causes a net loss of K⁺ attributable to an increase in K⁺ efflux as well as an inhibition of K⁺ influx (Figs. 1 and 2). The net loss in ten experiments due to an increase in efflux was 1.8 ± 0.2 mmoles/kg wet weight, which is a value similar to that previously reported by Langer and Serena (4) in the same preparation. In the presence of respiratory acidosis, however, there was a marked reduction in efflux, and in seven of nine experiments a complete absence of the increase in K⁺ efflux was seen (P < < 0.001) even though the increment in developed tension, expressed either in absolute units or as a percent of the tension before glycoside administration, was greater than it was under control acid-base conditions (P < < 0.001). At the same time, K⁺ influx was reduced in acidosis, demonstrating that the ability of ACS to inhibit Na⁺-K⁺ ATPase was unaffected (Fig. 1); the reduction was similar to that previously reported under control acid-base conditions (4). During respiratory acidosis, therefore, ACS inhibits Na⁺-K⁺ ATPase and exerts an inotropic effect, but it does not cause an increase in K⁺ efflux.

These facts are particularly pertinent to the controversy concerning the precise mechanism by which glycosides cause an inotropic effect in cardiac muscle. Langer and Serena (4) have shown that exposure of the arterially perfused rabbit septum to ACS results in a net gain of Na⁺ and Ca²⁺ but a net loss of K⁺. These changes are

Effect of a pH change (respiratory acidosis) on the positive inotropy and the net loss of K⁺ that occur in the presence of ACS. In acidosis, the increase in tension is greater (P < < 0.001) expressed in absolute units (ΔP) or as a percent of the tension (%ΔP) prior to the introduction of ACS. The net loss of K⁺ which is caused by ACS under control conditions is almost undetectable in respiratory acidosis (P < < 0.001). Circulation Research, Vol. 37, September 1975.
apparent whenever an inotropic effect is observed, and they occur in the absence of toxic effects including the development of contracture. The magnitude of the ionic movements is related to the size of the inotropic effect. They have postulated that the increase in Ca$^{2+}$ influx is due to the raised intracellular Na$^+$ concentration resulting from the inhibition of Na$^+$/K$^+$ ATPase. A direct causal sequence would then be present between the inhibition of a single enzyme, Na$^+$/K$^+$ ATPase, and the occurrence of inotropy.

An alternative hypothesis has been proposed by Morad and Greenspan (7). From experiments in frog cardiac muscle using the voltage clamp technique, they have postulated that glycosides have two primary effects, an inhibition of the Na$^+$/K$^+$ ATPase and a direct action to increase the K$^+$ permeability of the cell membrane. They have suggested that the increase in K$^+$ efflux observed in the presence of glycosides is due to both mechanisms and linked directly to an increase in Ca$^{2+}$ influx which is the final mediator of the inotropic effect. The proposal requires that there be a mechanism closely linking K$^+$ efflux and Ca$^{2+}$ influx. Any role for the Na$^+$ ion is thought to be excluded by the finding that glycoside-induced inotropy is still present after frog heart muscle has been treated with tetrodotoxin. This argument is open to criticism, because, although tetrodotoxin specifically blocks the fast Na$^+$ channel associated with the upstroke of the action potential (12), it is not effective in blocking Na$^+$ exchange through a slow channel. Therefore, even in the presence of tetrodotoxin, a considerable Na$^+$ exchange could occur during the period of the action potential (13).

The essential feature of the mechanism of glycoside-induced inotropy proposed by Morad and Greenspan (7) is that the control of Ca$^{2+}$ influx is linked to K$^+$ efflux and not to the intracellular Na$^+$ concentration. If the hypothesis is correct, an increase in K$^+$ efflux should be apparent whenever glycosides produce an inotropic effect. The present experiments clearly show that this sequence of events does not occur when ACS is administered to cardiac muscle during respiratory acidosis: a larger than control inotropic effect was observed in the absence of an increase in K$^+$ influx (Fig. 4). The hypothesis, at least under these conditions, is untenable, and by exclusion the present results afford support for the earlier hypothesis of Langer and Serena (4). Extending their theory, the effect of the acidosis is to eliminate the passive K$^+$ leak and leave unaltered the inhibition of the Na$^+$/K$^+$ pump. This effect is demonstrated by the almost total prevention of the active process of K$^+$ uptake (Fig. 1). Since K$^+$ uptake is linked to active Na$^+$ efflux, the action of ACS is associated with a net gain in intracellular Na$^+$, which provokes an increase in Ca$^{2+}$ influx.

In a previous paper (10) we have shown that the predominant effect of respiratory acidosis on K$^+$ homeostasis is an inhibition of passive efflux. It is this effect which we suggest causes the abolition of the glycoside-induced increase in K$^+$ efflux in respiratory acidosis (Figs. 2 and 3). Langer and Serena (4) have shown that the total tissue loss of K$^+$ following glycoside administration is due to a 42% inhibition of K$^+$ influx and a 65% increase in K$^+$ efflux. The increased passive K$^+$ efflux has been attributed to an increase in intracellular Na$^+$ concentration. The net loss of K$^+$ from the septum, calculated by measuring the area under the effluent curve in Figure 2, is, therefore, an underestimate of the true total tissue net loss of K$^+$, since it represents only that caused by an increase of efflux and not that due to the reduction of K$^+$ influx, which has been shown still to occur after exposure to ACS in respiratory acidosis (Fig. 1). Because of this reduction in influx, ACS in acidosis should cause a total tissue net loss of K$^+$ smaller in magnitude than that under control conditions. The hydrogen ion only prevents that part of the total tissue net loss of K$^+$ which is attributable to an increase in K$^+$ efflux. Na$^+$ exchange in respiratory acidosis has also been studied; it is unaltered when the extracellular pH declines from 7.4 to 6.8 (10). If the inotropic effect of glycosides is due to a net gain of intracellular Na$^+$, then it should not be reduced by respiratory acidosis. In fact, the inotropic effect of the same dose of ACS was increased during acidicotic conditions compared with that during control conditions (Fig. 4).

It may be argued that the absence of an increase in efflux of the isotope $^{42}$K$^+$ in the presence of glycoside during acidosis does not entirely discount the possibility of Ca$^{2+}$/K$^+$ exchange as proposed by Morad and Greenspan (7), because acidosis might expose a small additional compartment in the cell which is only slowly exchangeable with the extracellular space and contains unlabeled K$^+$. Glycoside might then, at least in theory, cause an increase in the efflux of cold K$^+$ from this compartment in exchange for Ca$^{2+}$; such an increase in K$^+$ efflux would be undetected. There is no experimental basis for such an assertion, however.

In these experiments (Figs. 2 and 3) and in those in which K$^+$ washouts have been continued for longer periods (4), no additional slowly exchanging

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K⁺ compartment was detected either under control conditions or during acidosis. The septums were exposed to acidic conditions for the last 10 minutes of the labeling period so that such a compartment would at least be partly labeled. An increase in efflux should be discernible since the minimum detectable efflux which can be observed with this method is 0.2 mmol/liter tissue water (4). Furthermore, if a small, very slowly exchanging compartment accounts for an undetected efflux of unlabeled K⁺ under acidic conditions, then it cannot be argued that the increased efflux of labeled ⁴²K⁺ under control conditions is necessarily linked to the increased influx of Ca²⁺ responsible for the inotropic effect of the glycoside (7). There can be no doubt (Figs. 2 and 3) that the increased efflux of ⁴²K⁺ under control conditions from whatever cellular compartment is absent in acidosis.

These conclusions are relevant to several clinical situations. Glycoside-induced dysrhythmias have been attributed to the associated net loss of K⁺ which causes partial depolarization of the myocardial cell (6). Since respiratory acidosis reduces the increase in K⁺ efflux and hence part of the net K⁺ loss (Fig. 3), it might be expected to protect the myocardium against the onset of such dysrhythmias, which, if the converse argument were valid, would be more common in respiratory alkalosis. The toxicity of glycosides is indeed known to be increased in respiratory alkalosis, irrespective of the extracellular K⁺ concentration (14, 15).

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