Intracellular Na\(^+\) Activity and Positive Inotropic Effect of Sulmazole in Guinea Pig Ventricular Myocardium

Comparison With a Cardioactive Steroid

Robert Schmied, Ge-Xin Wang, and Michael Korth

Recent studies suggest that inhibition of Na\(^+\),K\(^+\)-ATPase may contribute to the positive inotropic action of the imidazopyridine sulmazole. Therefore, we investigated the effect of sulmazole and its stereoisomers and for comparison the effect of the cardioactive steroid dihydroouabain (DHO) on intracellular Na\(^+\) activity by means of Na\(^+\)-sensitive microelectrodes. In the resting papillary muscle of the guinea pig, (±)-sulmazole increased intracellular Na\(^+\) activity (a\(^{\text{INa}}\)) within 15–20 minutes by 0.5±0.1 (n=3), 1.3±0.1 (n=7), 2.7±0.2 (n=6), and 4.9±0.5 (n=6) mM at 60, 100, 300, and 1,000 \(\mu\)M, respectively. (+)-Sulmazole was more effective than the racemate; a\(^{\text{INa}}\) was increased by 1.2±0.3, 2.1±0.3, and 4.0±0.2 mM at 60, 100, and 300 \(\mu\)M, respectively (n=2 for each concentration). In the contracting papillary muscle (0.2 Hz), (+)- and (±)-sulmazole (600 and 1,000 \(\mu\)M) produced a maximum positive inotropic effect that exceeded that of DHO by 11% and 8%, respectively. As an inotropic agent, (+)-sulmazole was almost twice as potent as the racemate. The maximum direct inotropic effect of (−)-sulmazole (1,000 \(\mu\)M) amounted to only 14% of the DHO maximum and was, in contrast to the racemate and (+)-sulmazole, antagonized by 3 \(\mu\)M carbachol. (−)-Sulmazole did not affect a\(^{\text{INa}}\). DHO increased a\(^{\text{INa}}\) by 0.9±0.1 (n=5), 1.5±0.1 (n=7), 2.4±0.1 (n=5), 2.8±0.2 (n=4), and 3.8±0.2 (n=4) mM at 30, 50, 80, 100, and 120 \(\mu\)M, respectively. The increase in a\(^{\text{INa}}\) versus the positive inotropic effect of various concentrations of (±)-sulmazole, (+)-sulmazole, and DHO could be fitted by linear regression (r=0.970). The results demonstrate that the rise in a\(^{\text{INa}}\) presumably caused by Na\(^+\) pump inhibition, exclusively determined the positive inotropic effect of (±)-sulmazole and its (+)-isomer. A cAMP-dependent mechanism was probably responsible for the small inotropic effect of (−)-sulmazole. (Circulation Research 1991;68:597–604)

The search for a more effective pharmacotherapy of congestive heart failure has led during the past decade to a number of new positive inotropic–acting compounds. Among these, the imidazopyridine sulmazole has attracted considerable attention because its inotropic effect appeared to rely on a new mechanistic principle. Sulmazole and, later, adibendan and pinmobendan have been shown to exhibit positive inotropic effects even in myocardial cells devoid of cell membrane.\(^1\) It was suggested that the cardiotoxic action of sulmazole may involve a direct action on the myofibrils because the Ca\(^{2+}\) sensitivity of the myofibrils is increased by the drug.\(^2\) Solaro and Rüegg\(^3\) found that sulmazole increased the affinity of myofibrillar troponin C for Ca\(^{2+}\). A new aspect, however, was added to the inotropic mechanism when Endoh et al\(^4\) reported that inhibition of phosphodiesterase may contribute to the positive inotropic effect of sulmazole.

In a recent study, van Meel et al\(^5\) could demonstrate that the Ca\(^{2+}\)-sensitizing effect of sulmazole was confined to the (+)-stereoisomer, whereas both isomers were equally effective in inhibiting phosphodiesterase. Therefore, it was concluded that the higher inotropic efficacy of the (+)-isomer relative to that of the (−)-isomer was due to an increase of the Ca\(^{2+}\) affinity of troponin C. In the same study, it was also demonstrated that sulmazole was a weak inhibitor of Na\(^+\),K\(^+\)-ATPase; the effect was also confined to the (+)-isomer. Although the authors discussed the possibility that inhibition of the Na\(^+\) pump by

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high concentrations of (±)-sulmazole and its (+)-isomer may contribute to the positive inotropic effect, its role was considered rather marginal. The same conclusion was derived by Allan et al., who reported little or no effects on sarcolemmal Na⁺,K⁺-ATPase and Na⁺-Ca²⁺ exchange by sulmazole. Honerjäger et al.⁷ however, found that sulmazole, besides phosphodiesterase inhibition, produced a significant inhibition of Na⁺,K⁺-ATPase at all inotropically effective sulmazole concentrations. They concluded that both effects, increase in cellular cAMP and increase in intracellular Na⁺ activity (a'Na), may be important for the positive inotropic effect.

Because of its toxic effects, sulmazole has been withdrawn from clinical trials. However, as a reference compound for newly developed imidazopyridines and for drugs that sensitize contractile proteins to Ca²⁺, sulmazole is still widely used in basic research. In the present study, we directly investigated the contribution of Na⁺,K⁺-ATPase inhibition to the positive inotropic effect of (±)-sulmazole and its stereoisomers by measuring a'Na in guinea pig papillary muscles by means of Na⁺-sensitive microelectrodes. To bring the findings with sulmazole into perspective, the concentration-dependent effects of the cardioactive steroid dihydroouabain on a'Na and force of contraction were also investigated. Evidence is presented that the positive inotropic effect of (±)-sulmazole and its (+)-stereoisomer is due to a rise in a'Na whereas that of (−)-sulmazole is very likely due to a cAMP-dependent mechanism.

Materials and Methods

Materials

Stock solutions of sulmazole were prepared with distilled water. (±)-Sulmazole, (+)-sulmazole, and (−)-sulmazole were from Thomae, Biberach, FRG; carbamylcholine chloride (carbachol) was from Sigma, Munich, FRG; sodium iodophore I (ETH 227) and N,N-dimethyltrimethylsilylamine were from Fluka, Buchs, Switzerland; dihydroouabain was a gift from J. Heusser, Hommel AG, Adliswil, Switzerland.

Preparations

Guinea pigs of either sex weighing 250–350 g were killed by cervical dislocation. If not stated otherwise, the animals were pretreated with reserpine (5 mg·kg⁻¹ body wt i.p. 24 hours before the experiment) to avoid the release of endogeneous catecholamines. Right ventricular papillary muscles (diameter, 0.5–0.8 mm) were rapidly excised from the isolated heart and mounted in a two-chambered organ bath with internal circulation of the bath solution (volume, 50 ml) as described by Reiter.⁸ The bath solution was constantly gassed and kept in circulation by 95% O₂-5% CO₂; the temperature was maintained at 35°C, pH 7.5. The composition of the bath solution was (mM) NaCl 115, KCl 4.7, MgSO₄ 1.2, CaCl₂ 3.2, NaHCO₃ 25, KH₂PO₄ 1.2, and glucose 10.

Isometric Contraction Recording

The muscles were stimulated at their base through two punctate platinum electrodes with square wave pulses of 1 msec and an intensity slightly above threshold. Force of contraction was recorded isometrically by means of an inductive force transducer (Q-11, 10 p, Hottinger Baldwin Meßtechnik, Darmstadt, FRG) connected to an oscilloscope and a pen recorder. The resting force was kept constant at 4 mN throughout the experiment. An equilibration period of at least 1 hour at a stimulation frequency of 1 Hz preceded each experiment. Subsequently, the frequency of stimulation was lowered to 0.2 Hz, and the drug intervention was started as soon as force of contraction had reached a steady state. The following parameters of the isometric contraction were evaluated: peak force of contraction; positive inotropic effect, that is, increase in force over its basal value; and relaxation time determined at 10% of peak force of contraction. Contractions were considered rested-state contractions when the period of rest was long enough that strength and time course of the contraction were not influenced by previous activity of the muscle (usually after 15 minutes).

Electrophysiological Measurements

To measure a'Na, papillary muscles were mounted horizontally in a perfusion chamber (volume, 0.4 ml) perfused at a constant rate of 5 ml·min⁻¹. Exchange of bath solution was complete within 1 minute. The voltage recording electrodes had tip resistances of 20 MΩ, and small tip potentials, when filled with 3 M KCl, acidified to a pH of 2 with HCl. The construction and calibration of the Na⁺-sensitive microelectrodes with the neutral ion exchange resin ETH 227⁰ have been described in detail elsewhere.¹⁰ The muscles were impaled with a conventional electrode and a Na⁺-sensitive microelectrode so that the impalements were as close as possible. From the potentials measured with the two microelectrodes, the a'Na of the cell was calculated by means of the following equation:

\[ E'_{Na} - E_m = E_o + S \log(a'_{Na} + k_{Na,K}a'_{K}) \]

where E'Na is the transmembrane potential measured with the Na⁺-sensitive microelectrode with respect to the reference electrode in the bath, E_m is the transmembrane potential measured with the conventional microelectrode, E_o is a constant potential of the Na⁺-sensitive microelectrode, and S is the slope of the Na⁺-sensitive microelectrode (ranging from 50 to 61 mV per decade) as determined in NaCl solutions containing 0.1 mM EGTA. k_{Na,K} is the selectivity coefficient of the Na⁺-sensitive microelectrode, which ranged from 0.01 to 0.02 for K⁺:Na⁺ in a mixture of 10 mM NaCl/140 mM KCl. a'K is the intracellular K⁺ activity, which was 110 mM, as experimentally determined with K⁺-sensitive electrodes in three papillary muscles. Before and after each experiment, the electrodes were calibrated.
at 35°C with pure solutions of NaCl and with mixtures of NaCl and KCl, with the sum (Na\(^+\)+K\(^+\)) kept constant at 150 mM. Any change in calibration meant that the experiments were discarded. Conventional and Na\(^+\)-sensitive microelectrodes were connected to a dual-channel high-impedance electrometer (model 773, World Precision Instruments, New Haven, Conn.). The signals were displayed separately and electronically subtracted on a pen recorder and on digital panel meters. The panel meter readings were used for calculating \(a'_{\text{Na}}\).

**Statistics**

The data are presented as arithmetic mean±SEM. Significance tests were performed using Student's *t* test for paired or unpaired observations. Differences between means were regarded statistically significant at *p*<0.05.

**Results**

**Inotropic Effects of Sulmazole**

Figure 1 shows the concentration-dependent (10–1,000 \(\mu\)M) positive inotropic effects of the racemate and of the two stereoisomers of sulmazole. The inotropic effect is expressed as the fraction of the maximum positive inotropic effect of dihydrouobain determined in the same muscle. The positive inotropic effect of (+)-sulmazole (111±7% at 600 \(\mu\)M, *n=5*) was significantly higher than the maximum inotropic effect obtained with dihydrouobain. (+)-Sulmazole (1,000 \(\mu\)M) was almost equieffective with dihydrouobain (108±15%, *n=5*), whereas the increase in force of contraction induced by (−)-sulmazole (1,000 \(\mu\)M) amounted to only 14±5% of the dihydrouobain maximum (*n=6*). Increasing the bath concentration of (+)-sulmazole to 1,000 \(\mu\)M and that of (−)-sulmazole to 3,000 \(\mu\)M resulted in a small further increase in force of contraction, which was accompanied by a marked prolongation of relaxation time (up to 180% of control, not shown). However, since the muscles usually became spontaneously active under this condition, a new steady state could not be determined. Linear extrapolation from the experimentally determined points on the (+)-sulmazole curve to the curve of the (+)-stereoisomer in Figure 1 revealed that concentrations of 100, 300, 600, and 1,000 \(\mu\)M (±)-sulmazole were equieffective with concentrations of 60, 220, 350, and 530 \(\mu\)M (+)-sulmazole. This increase in inotropic potency by a factor of nearly 2 could be expected since (+)-sulmazole was mainly responsible for the positive inotropic effect of the racemate (see Figure 1). As can be seen from the superimposed isometric contraction curves in Figure 2a, (+)-sulmazole prolonged relaxation time in a concentration-dependent fashion. At 600 \(\mu\)M (+)-sulmazole, a prolongation of relaxation time by 34±9% (*n=3*) occurred as compared with 25±6% determined with the maximally effective dihydrouobain concentration on the same muscles (*n=3*). Figure 2b shows that the (−)-isomer of sulmazole barely affected the shape of contraction. In three preparations, (−)-sulmazole (1,000 \(\mu\)M) slightly decreased relaxation time by 11±4%. As further demonstrated in Figure 2b, carbachol (3 \(\mu\)M) decreased the positive inotropic effect of 1,000 \(\mu\)M (−)-sulmazole by 50%. Similar results were obtained in two other preparations exposed to 3 \(\mu\)M carbachol in the presence of 1,000 \(\mu\)M (−)-sulmazole. In contrast to (−)-sulmazole, the positive inotropic ef-
effect of various concentrations of (±)- and (+)-sulmazole was not affected by carbachol (not shown).

Effect of Sulmazole on a'Na

The concentration-dependent effect of (±)-sulmazole on a'Na and on resting membrane potential is shown in Figure 3. Application of 100 μM (±)-sulmazole to the superfusing solution caused a'Na to increase from 6.8 to 7.9 mM within 15 minutes. When the concentration of (±)-sulmazole was raised to 300 and 1,000 μM, increases in a'Na from 6.0 to 8.5 mM and from 6.5 to 11.4 mM were obtained. As further shown in Figure 3, membrane resting potential depolarized during superfusion with 100, 300, and 1,000 μM (±)-sulmazole by 0.5, 1.5, and 2.5 mV, respectively. After the effect of each (±)-sulmazole concentration on a'Na was stable, the muscle was again superfused with drug-free solution, and a'Na was returned to its predrug control level (Figure 3). Reversal of a'Na during washout was usually complete within 20–30 minutes.

Figure 4 summarizes the effects of (±)-sulmazole on the increase in a'Na over the predrug control level and shows that 60 μM (±)-sulmazole was the lowest concentration at which a significant increase in a'Na was observed. In three experiments, a'Na was found to be 6.1±0.2 mM before and 6.6±0.2 mM 15 minutes after the addition of 60 μM (±)-sulmazole (p<0.05). In 19 additional papillary muscles a'Na rose from 6.5±0.1 to 7.7±0.2 mM in the presence of 100 μM (n=7), from 6.5±0.2 to 9.2±0.3 mM in the presence of 300 μM (n=6), and from 6.2±0.2 to 11.1±0.5 mM in the presence of 1,000 μM (n=6) (±)-sulmazole.

Figure 5 shows a typical experiment in which the effect of (+)-sulmazole on a'Na was compared with that of (−)-sulmazole on the same preparation. (+)-Sulmazole (300 μM) caused a'Na to increase from 6.2 to 10.1 mM and membrane resting potential to decline from −82 to −81 mV. Switching to drug-free solution, both a'Na and resting potential returned toward control levels. Subsequent superfusion of the preparation with (−)-sulmazole (300 μM) was without effect on a'Na and membrane resting potential.

Effects of Dihydroouabain on Na+ Activity

Figure 6 shows the concentration-dependent effect of dihydroouabain on a'Na and membrane resting potential in quiescent papillary muscles. Threshold
concentration for a rise in \( a'_{Na} \) was 30 \( \mu \)M dihydroouabain; \( a'_{Na} \) rose within 15 minutes from 6.3 to 7.2 mM. Exposure to 50, 80, and 120 \( \mu \)M dihydroouabain resulted in an increase in \( a'_{Na} \) from 6.3 to 7.6 mM, from 6.4 to 8.6 mM, and from 6.4 to 10.5 mM, respectively. A summary of increases in \( a'_{Na} \) over the predrug control level is shown in Figure 4. In a total of 25 preparations, dihydroouabain increased \( a'_{Na} \), from 5.9±0.2 to 6.8±0.3 mM at 30 \( \mu \)M \((n=5)\), from 6.2±0.2 to 7.7±0.3 mM at 50 \( \mu \)M \((n=7)\), from 6.3±0.1 to 8.7±0.2 mM at 80 \( \mu \)M \((n=5)\), from 6.1±0.1 to 8.9±0.2 mM at 100 \( \mu \)M \((n=4)\), and from 6.7±0.2 to 10.5±0.1 mM at 120 \( \mu \)M \((n=4)\). On return to drug-free solution, \( a'_{Na} \) declined within 15–20 minutes to the predrug control value (Figure 6). As further demonstrated in Figure 6, membrane resting potential was not affected by 30 \( \mu \)M dihydroouabain (−82 mV throughout the experiment), whereas at 50, 80, and 120 \( \mu \)M dihydroouabain, depolarizations of 1, 1.5, and 2.5 mV occurred, respectively.

Relation Between Positive Inotropic Effect and \( Na^+ \) Activity

From Figure 4, it can be inferred that 100 \( \mu \)M \((±)-sulmazole\) produces the same increase in \( a'_{Na} \) as does 50 \( \mu \)M dihydroouabain. Since the experiments shown in Figure 4 were carried out on different muscles, the effects of \((±)-sulmazole\) and of dihydroouabain on \( a'_{Na} \) were investigated in the same preparation. Figure 7 shows such an experiment in which \( a'_{Na} \) rose within 10 minutes from 5.9 to 7.6 mM in the presence of 100 \( \mu \)M \((±)-sulmazole\). After \( a'_{Na} \) had returned to the control value during washout, \( a'_{Na} \) rose again within 8 minutes from 6.0 to 7.7 mM in the presence of 50 \( \mu \)M dihydroouabain. As can be seen from Table 1, concentrations of \((±)-sulmazole\) and of dihydroouabain that produced identical increases in \( a'_{Na} \) when given sequentially to the same papillary muscle produced the same increase in force of contraction in isometrically contracting papillary muscles paced at a frequency of 0.2 Hz. Besides steady-state

![Figure 5](image-url) **Figure 5.** Recordings showing effectiveness of \((±)-sulmazole\) (300 \( \mu \)M) and failure of \((-)-sulmazole\) (300 \( \mu \)M) to increase intracellular \( Na^+ \) activity \((a'_{Na})\) and to decrease membrane resting potential \((E_m)\). The same quiescent papillary muscle from a reserpine-pretreated guinea pig was used for both runs.

![Figure 6](image-url) **Figure 6.** Recordings showing concentration-dependent increase in intracellular \( Na^+ \) activity \((a'_{Na})\) and decrease in resting membrane potential \((E_m)\) induced by the cardioactive steroid dihydroouabain. Note that all effects could be reversed by washing the muscles with drug-free solution. Different resting papillary muscles from reserpine-pretreated guinea pigs were used for each dihydroouabain concentration.

![Figure 7](image-url) **Figure 7.** Recording showing identical increases in intracellular \( Na^+ \) activity \((a'_{Na})\) induced inotropically equieffective concentrations of \((±)-sulmazole\) (100 \( \mu \)M) and dihydroouabain (50 \( \mu \)M). The same resting papillary muscle from a reserpine-pretreated guinea pig was used for both runs.
Table 1. Identical Increases in Intracellular Na⁺ Activity and in Force of Rested- and Steady-State Contractions by (+)-Sulmazole and Dihydropyridine

<table>
<thead>
<tr>
<th>Condition</th>
<th>Δ(a_{Na}^{i}) (mM)</th>
<th>Rested-state contraction (%)*</th>
<th>Steady-state contraction (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Sulmazole (100 μM)</td>
<td>1.3±0.1 (4)</td>
<td>...</td>
<td>23.4±6.3 (4)</td>
</tr>
<tr>
<td>Dihydropyridine (50 μM)</td>
<td>1.4±0.1</td>
<td>...</td>
<td>24.1±7.2</td>
</tr>
<tr>
<td>(+)-Sulmazole (300 μM)</td>
<td>2.7±0.2 (4)</td>
<td>14.6±1.9</td>
<td>98.4±2.5</td>
</tr>
<tr>
<td>Dihydropyridine (100 μM)</td>
<td>2.7±0.2</td>
<td>15.0±4.5</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Δ\(a_{Na}^{i}\) increase in intracellular Na⁺ activity over the predrug control level. Numbers in parentheses denote number of experiments. Force and Δ\(a_{Na}^{i}\) values are from different rested- and steady-state contractions from identical papillary muscles.

*Positive inotropic effect given as percent of the maximum steady-state effect of 100 μM dihydropyridine at 0.2 Hz.

inotropic effects, Table 1 shows that contractions elicited after a rest period of 45 minutes, during which either 300 μM (+)-sulmazole or 100 μM dihydropyridine was present for the last 30 minutes, were also nearly identically augmented by both drugs (rested-state contractions). Figure 8 summarizes the results of Figures 1 and 4 by showing the correlation between increase in \(a_{Na}^{i}\) over the predrug control level and the positive inotropic effect of various concentrations of (+)-sulmazole, (+)-sulmazole, and dihydropyridine. All data points could be fitted by linear regression with a correlation coefficient of 0.970. This relation indicates that the increase in \(a_{Na}^{i}\) was very likely the determinant mechanism of the positive inotropic effect of the cardiac steroid dihydropyridine and of the imidazopyridines (+)- and (+)-sulmazole.

Discussion

The present study demonstrates that (+)-sulmazole and its stereoisomer (+)-sulmazole increased \(a_{Na}^{i}\) in guinea pig papillary muscles, whereas the (-)-isomer of sulmazole had no measurable effect on \(a_{Na}^{i}\). The rise in \(a_{Na}^{i}\) induced by the racemate and the (+)-isomer occurred in a concentration-dependent fashion and correlated with the positive inotropic effects. Contrary to (-)-sulmazole, (+)-sulmazole and (+)-sulmazole have been demonstrated to inhibit Na⁺,K⁺-ATPase in sarcolemmal particles from guinea pig and cat hearts.5,7,11 Thus, Na⁺ pump inhibition probably accounts for the increase in \(a_{Na}^{i}\).

Besides Na⁺,K⁺-ATPase inhibition, (+)-sulmazole has been shown to inhibit myocardial cAMP and cyclic GMP phosphodiesterase.4,11,12 This enzyme exists as at least three isoenzymes, of which the most important, with regard to the positive inotropic effect, seems to be that described as phosphodiesterase III.13-15 Like other recently developed cardiotonic agents, (+)-sulmazole showed some selectivity for the phosphodiesterase III isoenzyme.6,7,13,16 In a recent study, the stereoisomers of (+)-sulmazole were shown to be roughly equipotent with respect to cAMP and cGMP phosphodiesterase inhibition in crude heart preparations of the guinea pig, but (+)-sulmazole was more potent as a positive inotropic–acting agent, indicating that an additional mechanism is effective.5 The present study confirms this observation, the increase in force of contraction induced by 300 μM (+)-sulmazole amounted to nearly 93%, whereas that of 300 μM (-)-sulmazole amounted to only 5% of the dihydropyridine maximal.

Figure 8. Graph showing relation between the positive inotropic effect and the increase in intracellular Na⁺ activity (Δ\(a_{Na}^{i}\)) induced by (+)-sulmazole, (+)-sulmazole, and dihydropyridine. Symbols represent arithmetic means, with SEM shown as horizontal and vertical bars. Positive inotropic effects were determined separately for (+)-sulmazole, (+)-sulmazole, and dihydropyridine in five, five, and 10 papillary muscles, respectively, driven at a frequency of 0.2 Hz. Determinations of \(a_{Na}^{i}\) were carried out for each drug concentration on a different resting preparation. Concentrations were as follows: 100 μM (●) (n=7), 300 μM (●) (n=6), and 1,000 μM (●) (n=6) (+)-sulmazole; 100 μM (△) (n=2) and 300 μM (▽) (n=2) (+)-sulmazole; and 30 μM (○) (n=5), 50 μM (○) (n=7), 80 μM (△) (n=5), 100 μM (▽) (n=4), and 120 μM (○) (n=4) dihydropyridine, where n is the number of determinations. Ordinate scale represents positive inotropic effect, expressed as percent of the value observed with the maximally effective concentration of dihydropyridine. Values of contraction force were obtained from the experiments shown in Figure 1. Abscissa scale represents Δ\(a_{Na}^{i}\) (range, 5.9–7.1 mM). Reserpine-pretreated guinea pigs were used for all experiments. Calculated linear regression plot Δ\(a_{Na}^{i}\) versus positive inotropic effect: y = -12.5 + 27.6x; r = 0.970.
mum. When carbachol was used to probe the functional importance of phosphodiesterase inhibition for the positive inotropic effects of (±)-sulmazole and its stereoisomers, only the effect of (−)-sulmazole could be antagonized by the muscarinic receptor agonist. Thus, it appears that inhibition of phosphodiesterase by (±) - and (+)-sulmazole is inadequate to explain even in part their inotropic actions. In contrast to the present study, attenuation of maximally 18% of the positive inotropic effect of (±)-sulmazole by carbachol has been reported in guinea pig papillary muscle. It is possible that the disparity of findings is due to the fact that catecholamine-depleted preparations had been used in the present study. The observation that the concentration–effect curves of isoprenaline and histamine were shifted to the left by (±)-sulmazole has been taken as evidence that (±)-sulmazole acts as an inhibitor of phosphodiesterase. This effect, however, does not prove that sulmazole’s own positive inotropic effect is also due to phosphodiesterase inhibition, since inhibitors like methylxanthines and papaverine are able to enhance the positive inotropic effect of isoproterenol already at concentrations that are not yet associated with an increase in force of contraction. In canine ventricular muscle, (±)-sulmazole has been reported to produce a positive inotropic effect in the presence of β-adrenoceptor blockers that was partially antagonized by carbachol. This finding indicates that a cAMP-dependent mechanism as a contributing factor to the positive inotropic effect of sulmazole may be more important in the dog than in the guinea pig. 

There is also some evidence to suggest that imidazopyridines increase the sensitivity of the contractile proteins of myocardial cells to Ca2+. (±)-Sulmazole was the first compound with a positive inotropic action that was found to increase myocardial force and shortening velocity at a given constant intracellular Ca2+ concentration. This observation was extended by Solaro and Rüegg, who observed an increase in Ca2+ binding by cardiac myofibrillar proteins and an activation of myofibrillar ATPase activity in the presence of (±)-sulmazole. Recent observations by van Meel et al. demonstrate that the increase in Ca2+ sensitivity of skinned myocardial fibers was confined to the (+)-isomer of sulmazole. In aequorin-injected dog ventricular trabeculae, however, Blinks and Endoh showed that (±)-sulmazole increased the amplitude of the intracellular Ca2+ transient almost over the entire range of concentrations associated with positive inotropic effects. A decrease of the Ca2+ transient, despite a further small increase in force of contraction, was obtained only at very high concentrations (≥1 mM). In addition, (±)-sulmazole prolonged the aequorin signal at high concentrations, an effect that is opposite what would be expected from a simple increase in the affinity of the myofibrils to Ca2+.

Neither inhibition of phosphodiesterase nor a Ca2+-sensitizing action on the contractile proteins is considered a mechanism for the positive inotropic action of cardioactive steroids. This group of compounds is thought to act solely by inhibition of Na+,K+-ATPase, leading to an elevation of the intracellular Na+ concentration that acts on membrane Na+-Ca2+ exchange to increase the intracellular Ca2+ concentration. As a consequence, the Ca2+ content of the sarcoplasmic reticulum increases, and more Ca2+ is released during depolarization, resulting in an enhanced contraction. The latter has been demonstrated for the cardioactive steroid acetylstrophanthidin in aequorin-injected cardiac cells. In addition, all manipulations that inhibit the Na+ pump or enhance Na+ influx have been shown to enhance force of contraction. Under these conditions, a close relation between aNa and force of contraction has been obtained. In the present study, positive inotropic effect and increase in aNa were compared at various concentrations of (±)-sulmazole, its (+)-stereoisomer, and the cardioactive steroid dihydroouabain. The results clearly demonstrate that the relation between changes in aNa and positive inotropic effect could be fitted for all three compounds by linear regression with a correlation coefficient of r=0.970. Although the increase in aNa determined in quiescent muscles does not reflect the true increase in aNa in contracting preparations, the fact that all data points could be fitted by a single linear regression supports the assumption that a common mechanism of action is responsible for the inotropic effects of the imidazopyridines and dihydroouabain. Any additional positive inotropic effect of (±)-sulmazole and its (+)-isomer unrelated to Na+ pump inhibition, such as inhibition of phosphodiesterase or sensitization of myofibrils to Ca2+, should have resulted in a regression line that is steeper than that obtained for dihydroouabain. It is well known that myocardial activity, by increasing aNa, enhances binding of cardioactive steroids to Na+,K+-ATPase and thereby raises their inotropic efficacy. It is unlikely that (±)-or (+)-sulmazole differ from dihydroouabain in this respect, since rested-state contractions, which are independent of previous myocardial activity, were augmented to the same extent by those concentrations of dihydroouabain and (±)-sulmazole that were equipotent in their steady-state inotropic effects and on aNa during rest (see Table 1). Furthermore, (±)- and (+)-sulmazole as well as dihydroouabain depolarized resting membrane potential in a concentration-dependent fashion, an observation that is good experimental support for Na+ pump inhibition as the common mechanism for the rise in aNa. Racemate and (+)-isomer of sulmazole up to 1 mM and 600 μM, respectively, induced a moderate prolongation of relaxation time (this study and Reference 7). A similar effect was also described for dihydroouabain in the guinea pig papillary muscle. At higher concentrations, however, a marked prolongation of relaxation time and a prolongation of the intracellular Ca2+ signal occurred. This latter effect may be related to a finding of Trube and Trautwein, who reported that sulmazole at concen-
trations greater than 1 mM induced release of Ca\textsuperscript{2+} from sarcoplasmic reticulum and inhibition of Ca\textsuperscript{2+} reuptake. This effect should, at least in the steady state, represent a mechanism that opposes the positive inotropic effect of sulmazole due to Na\textsuperscript{+} pump inhibition.

In conclusion, the present results demonstrate that the positive inotropic effect produced in guinea pig papillary muscle by (±)-sulmazole and its (+)-stereoisomer is due to an increase in a\textsubscript{Na}, which presumably is the consequence of Na\textsuperscript{+} pump inhibition. The small positive inotropic effect of (−)-sulmazole can be explained by a cAMP-dependent mechanism. The results indicate that Na\textsuperscript{+} pump inhibition as mechanism for the positive inotropic action of sulmazole has apparently been underestimated in the literature.

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