Anion Manipulation: A New Antiarrhythmic Approach

Action of Substitution of Chloride With Nitrate on Ischemia- and Reperfusion-Induced Ventricular Fibrillation and Contractile Function

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The role of anions in the initiation of ischemia- and reperfusion-induced arrhythmias is unknown. We examined the antiarrhythmic effects of isotonic substitution of extracellular Cl\(^-\) with NO\(_3^-\) by using the rat Langendorff preparation (n=12 per group). During 30 minutes of regional ischemia, the incidence of ventricular fibrillation (VF) was reduced from 50% in hearts perfused with control solution (containing a Cl\(^-\):NO\(_3^-\) ratio of 100:0) to 25%, 0% (p<0.05), 0% (p<0.05), and 0% (p<0.05) by perfusion with solution containing Cl\(^-\):NO\(_3^-\) ratios of 75:25, 50:50, 25:75, and 0:100, respectively. The incidence of reperfusion-induced VF was also reduced from 58% to 25%, 8% (p<0.05), 8% (p<0.05), and 0% (p<0.05), respectively. Similar effects were produced in hearts reperfused after briefer durations of ischemia (10 or 15 minutes). Substitution of NO\(_3^-\) for Cl\(^-\) also facilitated spontaneous termination of VF. Heart rate and occluded zone size were not affected by anion substitution. Coronary flow was affected by NO\(_3^-\), but changes did not correlate with arrhythmias. During ischemia, electrocardiographic changes indicative of class III activity (widening of the ventricular complex) were produced by anion substitution. These changes occurred selectively in the ischemic tissue with no significant influence before ischemia onset. However, the relation between this effect and arrhythmia reduction was not linear and a cause–effect relation is therefore unlikely. In separate groups of hearts (n=12 per group), switching from 100:0 to 0:100 Cl\(^-\):NO\(_3^-\) solution or vice versa 10 seconds after coronary occlusion or just before reperfusion demonstrated that 1) protection against ischemia-induced VF resulted partly from an action in the ischemic zone and partly from an action in the nonischemic zone, and 2) protection against reperfusion-induced VF occurred principally from an action occurring during reperfusion and within the reperfused tissue. To assess whether benefit was offset by deleterious effects on contractile function in nonischemic tissue, we constructed Starling curves in isolated rat hearts. The 0:100 Cl\(^-\):NO\(_3^-\) solution had no effect on compliance or contractility at physiological end-diastolic pressures but reduced the slope of the peak systolic pressure–volume relation by -20% as end-diastolic pressure was increased above 10 mm Hg. In conclusion, anions appear to play a hitherto unrecognized role in arrhythmogenesis in ischemia and reperfusion. Manipulation of anion homeostasis may represent a novel target for antiarrhythmic drug development. (Circulation Research 1992;70:617–632)

**Key Words** • anions • antiarrhythmic • arrhythmogenesis • Cl\(^-\) • compliance • ischemia • contractility • NO\(_3^-\) • reperfusion • ventricular fibrillation

Ischemia-induced ventricular fibrillation (VF) is a major cause of sudden cardiac death. Ischemia-induced arrhythmias have been shown to respond to numerous drugs in animal models. For example, class I, II, III, and IV antiarrhythmics as well as \(\alpha\)-adrenoceptor antagonists, 5-hydroxytryptamine receptor antagonists, and numerous other classes of drugs have all been reported to have antiarrhythmic activity. However, owing to various side effects, none has proven useful as a prophylactic agent for routine use in patients with coronary artery disease at risk of myocardial ischemia; ironically, the most effective antiarrhythmic agents, the class III drugs, appear to have the greatest potential for causing side effects. For this reason it is apparent that there is an acute need for a new approach to prevent ischemia-induced VF.

Reperfusion-induced arrhythmias in animal models are characteristically unresponsive to drugs that are

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known to be of benefit against ischemia-induced arrhythmias. Although the role of reperfusion-induced VF in sudden death is not established, it is recognized that spontaneous reperfusion (occurring, for example, in vasospasm) is capable of eliciting VF in humans. For these reasons, it would be advantageous if novel means for prevention of reperfusion-induced VF could be developed.

Modification of cation homeostasis has proven a logical target for intervention in the setting of ischemia and reperfusion. Ischemia-induced arrhythmias are susceptible to inhibition by drugs that inhibit sodium, calcium, and potassium currents, 2 and reperfusion-induced arrhythmias can be attenuated by lowering extracellular calcium concentration 3 or raising extracellular potassium concentration. However, little information is available concerning the antiarrhythmic potential of manipulation of anion homeostasis during ischemia or reperfusion. To our knowledge, the only relevant study concerns the activity of 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS), a blocker of Cl⁻-HCO₃⁻ exchange, which was found to inhibit ischemia-induced and reperfusion-induced arrhythmias. 4

In the present study we have attempted to examine whether manipulation of the anion content of the extracellular milieu is capable of inhibiting ischemia- and reperfusion-induced VF. This was achieved by replacing Cl⁻ with NO₃⁻ in perfusion media. Possible indirect mechanisms responsible for any antiarrhythmic effects were evaluated by contiguous assessment of relevant antecedent hemodynamic and electrocardiographic variables. An attempt was made to determine the site of antiarrhythmic action against ischemia-induced arrhythmias (ischemic versus nonischemic tissue) and to discern whether any reduction in reperfusion-induced arrhythmias resulted from an action during reperfusion (direct action) or from an action during ischemia (indirect action) by crossover perfusion. Finally, to test whether any benefit was offset by potentially deleterious effects on contractility or compliance in nonischemic tissue, we measured systolic and diastolic function by constructing Starling curves in additional groups of hearts.

Materials and Methods

**Animals and Experimental Methods**

Male Wistar rats (220–260 g, Banting and Kingman) were anesthetized with diethyl ether, and 200 IU sodium heparin was administered intravenously. After 30 seconds each heart was quickly excised and immersed in ice-cold perfusion solution (constituents described below) to induce rapid arrest. The aorta was cannulated for Langendorff perfusion. A Ag-AgCl wire electrode was inserted into the left ventricular muscle mass (in the center of the region to become ischemic) to record a unipolar electrogram (ECG) that was recorded against a lead attached to the aortic cannula. This electrode arrangement gave a clear P wave and ventricular complex. The ECG was continuously displayed on a Gould pen recorder at 5 mm/sec chart speed and a Gould digital storage type 1421 oscilloscope at 100 mm/sec sweep speed. Permanent chart recordings at fast speed (100 mm/sec) were taken 1) periodically (for estimating heart rate and ECG analysis), 2) during the onset of arrhythmias, and 3) continuously for the period 10 seconds before the start of reperfusion until 3 minutes after the start of reperfusion. For studies of regional ischemia a traction-type coro nary occluder 11 consisting of a silk suture threaded through a polyethylene guide cannula was used for coronary occlusion. The suture was positioned loosely around the left main coronary artery according to Heimburger 12 as modified for in vitro experimentation. Each heart was allowed to stabilize for 10 minutes before coronary occlusion. After the designated period of ischemia, the occluder was released and the heart was reperfused for 3 minutes. In some experiments global ischemia was used. This was achieved by occluding the aortic inflow line. To prevent sinus bradycardia during global ischemia, heart rate was maintained by atrial superfusion. 14

**Verification of Coronary Occlusion and Reperfusion**

Two independent methods were used to verify occlusion during regional ischemia and to delineate the ischemic (occluded zone) tissue from the nonischemic tissue, as described previously. First, coronary flow was recorded by timed collection of the coronary effluent and occlusion was verified by comparing flow 1 minute before occlusion with flow 1 minute after occlusion. Second, at the end of each experiment (3 minutes after the start of reperfusion) the perfusion solution was replaced by a solution of sulfan blue dye B.P.C. (Disulphine blue, ICI). Each heart received 5 ml diluted dye (500 mg in 110.9% saline), and the coronary occluder was retightened. Perfusion solution was then reintroduced for 3 minutes, leaving dye trapped in the occluded zone. After excision of the atria and excess mediastinal tissue, the occluded zone was dissected from the nontouched nonischemic zone. The occluded zone was weighed and quantified as a percentage of the total ventricular weight. Reperfusion was also verified by two independent methods, as described previously. First, uniform staining of the occluded zone by dye was taken to indicate effective reperfusion. Second, coronary flow during reperfusion was measured and compared with preocclusion flow. Rat atra receive their blood supply almost exclusively from extracoronary vessels, whereas the ventricles are supplied by the left and right coronary arteries; coronary flow in the rat Langendorff preparation represents, almost exclusively, ventricular flow. For this reason occluded zone size determined by the dye method correlates with occluded zone size determined by the flow reduction method. For the sake of simplicity we have expressed occluded zone size on the basis of the dye method only.

**Composition of Perfusion Solutions**

Standard Cl⁻-containing solution (constituents in millimolars: NaCl 118.5, NaHCO₃ 25.0, KCl 4.0, MgSO₄ 1.2, CaCl₂ 1.4, and glucose 11.1) was modified to contain Cl⁻:NO₃⁻ ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 by isotonic substitution of Cl⁻ salts with NO₃⁻ salts (KNO₃, Ca(NO₃)₂, and NaNO₃). The composition of the other constituents was fixed. Calculated osmolality was identical in each group. Temperature of the solutions was maintained at 37°C. The solutions were bubbled with 5% CO₂–95% O₂. Measured pH was 7.4 in
all solutions. Throughout the text, “$\text{NO}_3^-$ substitution for $\text{Cl}^-$” has been abbreviated to “NCS.”

**Experimental Protocol**

*Concentration–response and time–response study.* The severity of reperfusion-induced arrhythmias is highly dependent on the duration of preceding ischemia.\textsuperscript{16,17} Some interventions, for example, manipulation of extracellular calcium,\textsuperscript{18} are known to confer benefit only when reperfusion takes place at specific times. We have therefore examined four durations of regional ischemia (5, 10, 15, and 30 minutes) in conjunction with each of the five solutions described above; thus, a total of 20 groups of hearts were used. Each heart was used only once, and 12 hearts were used per group as in previous studies; thus a total of 240 hearts were used. We used hierarchical, nested, randomized block design, analogous to a Latin square within a Latin square\textsuperscript{19} in the design of the study, as described previously.\textsuperscript{8} Five randomization tables were constructed. Each table contained four “experimental units” corresponding to the four ischemic durations arranged into 12 “blocks.” The choice of solution was determined by reference to a sixth randomization table from which two solutions were chosen for each experimental run (typically eight rats). The experimental operator was blinded to the nature of the solution. Records were identified by a number code only, and therefore analysis was carried out without knowledge of the solution used. Codes were broken on completion of analysis. The group of hearts exposed to 30 minutes of regional ischemia was used for examining the effect of anion manipulation on susceptibility to ischemia-induced arrhythmias.

*Crossover study with regional ischemia.* With respect to ischemia-induced arrhythmias, if any protection were conferred by NCS in the foregoing protocol, it would not be possible to differentiate between an action on the ischemic versus the nonischemic zones. A second protocol was therefore used to resolve this question. Because collateral flow in the rat heart is less than 5%,\textsuperscript{20} we set out to trap either $\text{Cl}^-$ or $\text{NO}_3^-$ solution in the ischemic zone with delivery of either solution to the adjacent nonischemic zone.

The outlets of two glass perfusion columns were connected inferiorly with a malleable plastic Y-connector, which delivered perfusate via a single aortic cannula to a heart that was perfused in the Langendorff mode. One column contained standard $\text{Cl}^-$-containing solution and the other column contained solution with complete $\text{Cl}^-$ substitution by $\text{NO}_3^-$. With this arrangement it was possible to rapidly switch the solution delivered to the heart by clamping one or other limb of the Y-connector and simultaneously declamping the other limb. The measured dead space was 0.2 ml. Coronary flow in perfused rat hearts before, during, and after regional ischemia ranges from approximately 4 to 18 ml/min.\textsuperscript{8} Thus, the lag time for complete exchange of coronary perfusion ranged between 0.67 and 3 seconds.

Four groups of 12 rats were studied. In each case a 10-minute period of control perfusion was followed by 30 minutes of regional ischemia. In the first group, after an initial 10-minute period of control perfusion with $\text{NO}_3^-$-containing solution ($\text{Cl}^-$:$\text{NO}_3^-$ ratio, 0:100), the left coronary artery was occluded trapping $\text{NO}_3^-$-containing solution in the ischemic zone. Ten seconds after coronary occlusion the solution was replaced by $\text{Cl}^-$-containing solution ($\text{Cl}^-$:$\text{NO}_3^-$ ratio, 100:0). The hearts continued to receive $\text{Cl}^-$-containing solution for the remainder of the 30-minute period of ischemia with delivery of $\text{Cl}^-$-containing solution to the nonischemic region. In the second group, $\text{Cl}^-$-containing solution was delivered during the 10-minute period of control perfusion and trapped in the ischemic zone after occlusion, in a manner equivalent to $\text{NO}_3^-$ trapping in the first group. This was followed, 10 seconds after occlusion, by replacement of the $\text{Cl}^-$-containing solution with $\text{NO}_3^-$-containing solution that was delivered throughout the remainder of the experiment. Thus, $\text{Cl}^-$-containing solution was trapped in the ischemic region while $\text{NO}_3^-$-containing solution was delivered to the nonischemic region. In the third group, $\text{NO}_3^-$-containing solution was delivered during the 10-minute period of control perfusion and during the 30-minute period of regional ischemia. Thus, $\text{NO}_3^-$-containing solution was trapped in the ischemic region and was delivered to the nonischemic region. In the fourth group, $\text{Cl}^-$-containing solution was delivered during the 10-minute period of control perfusion and during the period of regional ischemia. Thus, $\text{Cl}^-$-containing solution was trapped in the ischemic region and was delivered to the nonischemic region. Hearts were randomized to treatment, and analysis of records was carried out blindly.

*Crossover study with global ischemia.* The arterial supply of the rat ventricle has been shown to possess few functional collaterals.\textsuperscript{20,21} However, even a small amount of collateral flow would lead to the possibility of contamination of solution trapped in the ischemic zone by the solution delivered to the nonischemic zone. To overcome this potential problem when examining reperfusion arrhythmias, we used a model of global ischemia.\textsuperscript{14} In this model, the variability of outcome of reperfusion encountered in some global ischemia models\textsuperscript{22} is overcome by the use of atrial superfusion; this effectively maintains ventricular temperature, sinus rate, and, most importantly, ventricular beating rate, at levels close to those encountered in regionally ischemic hearts.\textsuperscript{14} The result of this is an increase in the incidence of reperfusion-induced VF in control hearts to a level that resembles that observed in hearts reperfused after regional ischemia.\textsuperscript{14} Two perfusion chambers filled with either 100:0 or 0:100 $\text{Cl}^-$:$\text{NO}_3^-$ solutions were arranged with an inferiorly placed malleable plastic Y-connector so the solutions could be easily crossed over. Hearts were randomized to one of four groups. In the first group, $\text{Cl}^-$-containing solution was delivered during a 10-minute control period, which was followed by 10 minutes of global ischemia with continuous atrial superfusion. Each heart was then reperfused with $\text{Cl}^-$-containing solution. Thus, $\text{Cl}^-$-containing solution was trapped in the ischemic tissue and $\text{Cl}^-$-containing solution was delivered during reperfusion. In the second group, $\text{NO}_3^-$-containing solution ($\text{Cl}^-$:$\text{NO}_3^-$ ratio, 0:100) was delivered during control perfusion (and trapped in the ischemic tissue) and also during reperfusion. In the third group, $\text{Cl}^-$-containing solution was used for the initial 10-minute control perfusion and was trapped in the heart during global ischemia, whereas $\text{NO}_3^-$-containing solution was delivered during reperfusion. In the fourth group, $\text{NO}_3^-$-containing solution was
delivered during the initial 10-minute control perfusion and trapped in the heart during global ischemia, whereas Cl−-containing solution was delivered during reperfusion. Hearts were randomized to treatment, and analysis of records was carried out blindly.

It should be noted that we were unable to use the global ischemia model for crossover studies concerning ischemia-induced arrhythmias since an adjacent nonischemic zone is necessary for arrhythmogenesis in this setting.23

**Diagnosis and Quantification of Arrhythmias**

Diagnosis and quantification of arrhythmias conformed to the guidelines of the Lambeth Conventions.24 The incidence of ventricular premature beats (VPBs), bigeminy, salvos, ventricular tachycardia (VT), and VF was recorded. VT was defined as a run of four or more VPBs. Individual deflections in a run of VT were not included as VPBs. VF was defined as ventricular rhythm with no recognizable QRS complex, in which signal morphology changed from cycle to cycle, and for which it was impossible to estimate heart rate. Signal morphology was evaluated from fast chart speed pen recordings in conjunction with an oscilloscope. Sustained VF was defined as an episode lasting for longer than 2 minutes, as described previously14; 2 minutes of continuous VF is uniformly fatal in rats in vivo, even if defibrillation is subsequently successful.25

**ECG Analysis**

Two measurements were made to analyze the ECG: 1) the duration between onset of the P wave and onset of the ventricular complex (PR interval), and 2) the width of the ventricular complex. No separate T wave is seen in the rat ECG, thus conventional measurement of the QT interval is impracticable. This is because repolarization begins in the apex of the ventricles before depolarization is complete in other parts of the ventricles, secondary to the brief duration of the ventricular action potential in the rat.27 Our measurement of the width of the ventricular complex was made at 90% repolarization and is defined as QRST90. Figure 1 shows a representative QRST complex and indicates how QRST90 is measured.

**Exclusion Criteria**

A total of 404 hearts, of which 336 were retained, were used for these studies. Sixty-eight preparations (17%) had to be excluded for reasons listed below. Stability criteria. Unstable preparations were excluded. A stable preparation was defined as having (5 minutes before occlusion) a sinus rate of at least 290 beats per minute, a coronary flow of at least 9 ml/min, and an absence of arrhythmias. Stability criteria were not fulfilled by five hearts. Ventricular rate (in the global ischemia group). Any heart with a ventricular rate less than 150 beats per minute 9 minutes after the beginning of global ischemia and 1 minute before reperfusion was excluded in accordance with previous observations in this model in which it was found that in control hearts this criterion was absolutely essential for occurrence of reperfusion-induced VF.14 This led to exclusion and replacement of 40 hearts. Censoring. Twenty-three hearts not in sinus rhythm at the moment of reperfusion were excluded from the reperfusion study and replaced, as described previously,14 since it would have been impossible to determine whether ischemia or reperfusion was responsible for arrhythmias occurring during reperfusion. The resultant censoring and selection generated three subsets of hearts: 1) a subset suitable for the study of ischemia-induced arrhythmias (the hearts used in the first attempt to fill each "cell" of the randomization table, some of which were not in sinus rhythm at the time of reperfusion); 2) a subset suitable for the study of reperfusion-induced arrhythmias (the hearts that were in sinus rhythm at the moment of reperfusion); and 3) all hearts entered into the study.

**Measurement of Contractile Function**

Many antiarrhythmic agents have negative inotropic actions that detract from their therapeutic efficacy.26 Evidence from studies in isolated myocardial tissue preparations suggests that substitution of Cl− for other anions may actually be positively inotropic.29–31 To determine whether any antiarrhythmic effects of NCS
were offset by impairment of contractility in the non-ischemic (uninvolved) region, we evaluated isochoric left ventricular systolic and diastolic function in separate groups of rat hearts perfused in the Langendorff mode.

A small compliant, but nonelastic, balloon was made from plastic wrapping film (Clingfilm) and connected via a length of polypropylene tube to a pressure transducer (Spectramed P23XL, Statham, UK). The balloon was made slightly more capacious than the left ventricle (typically 0.4 ml) to ensure that, as it was filled, any increase in the measured pressure was caused by an increase in ventricular wall tension and not balloon wall tension. An opening was cut in the left atrium and used for insertion of the balloon catheter into the left ventricle. The catheter was inflated with distilled water. With the balloon inflated, isochoric pressure recordings of left ventricular systolic and diastolic pressures were made before and after intervention.

A total of four solutions were used: standard Cl−-containing solution or solution modified by complete NCS or by addition of 10 μM epinephrine or 1 μM verapamil. The latter two interventions were used as standards for testing the sensitivity of the preparation as a model for detecting negative and positive inotropic activity and for gauging the extent of any effects of NO3−.

A randomized design was used, and nine hearts were used for each intervention. After 15 minutes of control perfusion with standard Cl−-containing solution (during which time the intraventricular balloon was inserted), isochoric ventricular pressure was measured. A Starling curve was constructed with 10-μl volume increments to the balloon. The volume required to achieve a diastolic pressure of 10 mm Hg under these baseline conditions (vol0) was noted, and the ventricle was then unloaded by deflation of the balloon. Ten minutes later, the solution was switched either to an identical Cl−-containing solution (time-matched control group) or to one of the three interventions. After 15 minutes the balloon was reinflected to vol0, and then the Starling curve was reevaluated.

For this study, a stable preparation was defined as having (at the end of the initial perfusion with control solution) a sinus rate of at least 290 beats per minute, a coronary flow of at least 9 ml/min, and a developed pressure (systolic minus diastolic) of greater than 100 mm Hg at vol0. Stability criteria resulted in the exclusion of five hearts of an original 41 entered into the study.

There is evidence that the slope of the peak isochoric pressure–volume relation (EFS) is a useful measure of left ventricular systolic function. We determined EFS as a best-fit linear regression line. A second measure of systolic function (contractility) was calculated as the systolic minus diastolic (“developed”) pressure. This variable, determined at a “high physiological” end-diastolic pressure (10 mm Hg), was presented to put into perspective any changes occurring in EFS since it was expected that determination of EFS would require incrementation of end-diastolic pressure beyond the range normally encountered in the heart in vivo.

Isochoric diastolic pressure is determined in part by left ventricular diastolic compliance (e.g., Reference 34). To gain a measure of any change in compliance we recorded the diastolic pressure at vol0 (i.e., using the same intraventricular volume that produced a diastolic pressure of 10 mm Hg during the control perfusion).

All values were expressed as mean±SEM. Groups were compared by modified parametric analysis (see below), which was paired when values were compared before and after switching solutions.

**Statistics**

Statistical analyses were based on previously published guidelines. Gaussian-distributed variables (which included heart rate, coronary flow, and indexes of contractile function) were expressed as mean±SEM and were subjected to analysis of variance. If treatment constituted a significant source of variance, each group of hearts with differing Cl−:NO3− ratios was compared with the control standard Cl−-only group by using Dunnett’s test. Data transformation was necessary to permit analysis of means for variables not Gaussian distributed. The duration of VT, the number of VPBs, and the time of onset of first reperfusion-induced arrhythmia were log10 Gaussian distributed and were therefore log10-transformed, as described previously. Only those hearts that exhibited particular rhythm disturbances were used in the calculation of mean±SEM values for variables such as log10 onset time. Group percent incidences of arrhythmias were compared using Mainland’s contingency tables. We took p<0.05 to indicate a statistically significant effect.

**Results**

**Ischemia-Induced Arrhythmias**

Statistically significant concentration-dependent reductions in the incidences of ischemia-induced arrhythmias were produced by NCS. The incidence of VF was reduced by 50% by perfusion with solution containing a Cl−:NO3− ratio of 75:25; thus, only a modest NCS produced a large antiarrhythmic effect. Higher proportions of NO3− abolished VF (Figure 2a). The incidences of other ventricular arrhythmias were also reduced in a concentration-dependent manner. If arrhythmias are ranked in a subjective order of severity (VF>VT>S>BG>VPB, where S is salvos and BG is bigeminy) it can be seen that NO3− substitution was proportionately more effective against the more severe arrhythmias, with increasingly higher NO3− concentrations required for inhibition of less-severe arrhythmias (Figure 2a). The mean latency to the onset of the first ischemia-induced arrhythmia (usually VPB) was not found to be altered by NCS (Figure 2b).

**Reperfusion-Induced Arrhythmias**

Owing to the bell-shaped relation between susceptibility to reperfusion-induced arrhythmias and the duration of preceding ischemia, hearts were subjected to one of four durations of regional ischemia (5, 10, 15, or 30 minutes). NCS led to statistically significant concentration-dependent reductions in the incidences of reperfusion-induced VF (Figure 3) and VT (Figure 4) in hearts subjected to 10, 15, and 30 minutes of ischemia. The incidence of sustained VF was also reduced by NCS in a concentration-dependent manner (Figure 5). To reveal the statistical significance of the latter effect it was necessary to “bin” data from hearts subjected to
different durations of ischemia, owing to the low incidence of VF with high concentrations of NO$_3^-$ (Figure 5). The less severe reperfusion-induced arrhythmias tended to be exacerbated by NCS (VPBs are shown in Figure 6), although this can be explained by "unmasking" as a consequence of the reduction in the incidence of VT and VF.

Reperfusion-induced arrhythmias in hearts subjected to 5 minutes of ischemia were affected differently. In controls, the incidences of arrhythmias were lower than in hearts reperfused after longer durations of ischemia and NCS had a concentration-dependent proarrhythmic effect. This trend was seen for all classes of arrhythmias; it was negligible in the case of VF (Figure 3) and VT (Figure 4) but was more evident for the less severe arrhythmias and reached statistical significance for VPB incidence (Figure 6). In contrast to the situation with hearts reperfused after 10 or 15 minutes of ischemia (see above), the proarrhythmic effect on VPBs in hearts reperfused after 5 minutes of ischemia cannot be attributed to unmasking by inhibition of VT and VF, making the proarrhythmic effect unequivocal.

The latency to onset of the first reperfusion-induced arrhythmia was increased in a concentration-dependent manner by NCS in hearts reperfused after 10, 15, or 30 minutes of ischemia (Figure 7). However, in hearts reperfused after 5 minutes of ischemia, the opposite effect was seen, with increasing proportions of NO$_3^-$ shortening the latency to onset of the first arrhythmia (Figure 7).

**Heart Rate, Coronary Flow, and Occluded Zone Size**

To determine whether the antiarrhythmic effects of NCS were some consequence of altered hemodynamics, we measured heart rate and coronary flow throughout the experiment and occluded zone size at the end. Heart rate fell slightly during the course of the experiment in all groups (Table 1). NCS had little effect on heart rate, and the Cl$^-$/NO$_3^-$ ratio did not correlate with heart rate at any stage in the protocol (Table 1). Not surprisingly, therefore, heart rate did not correlate with arrhythmia incidence.

NCS had a time-dependent effect on coronary flow, causing a progressive fall in the nonischemic region. This effect was concentration dependent (Figure 8). Although the fall in flow occasionally reached statistical significance, there was no correlation between flow and arrhythmias ($p=NS$).

Calculation of recovery of flow in the reperfused zone revealed a hyperemic response in control hearts reperfused after 5 or 10 minutes of ischemia (compare values in Figure 9 with equivalent values in the nonischemic region shown in Figure 8), with a progressively smaller hyperemic response in control hearts reperfused after 15 or 30 minutes. NCS caused a concentration-dependent inhibition of the hyperemic response, such that recovery of coronary flow was significantly reduced by 100% NCS in hearts reperfused after 5 minutes of ischemia (Figure 9). A decline in hyperemic response occurred in controls during reperfusion after longer periods of ischemia (15 or 30 minutes). As a consequence, an antihyperemic effect of NCS was not demonstrable in hearts reperfused after 15 or 30 minutes of ischemia (Figure 9). Despite their often being statistically significant, the effects of NCS on recovery of flow can be regarded as pathophysiologically unimportant with respect to the mechanism by which NCS inhibits reperfusion-induced VF, since there was no correlation between mean flow recovery and VF incidence ($p=NS$). Furthermore, there was no correlation between recovery of flow and latency to onset of the first reperfusion-induced arrhythmia; although recovery of flow (Figure 9) and arrhythmia onset (Figure 6) were both delayed by NCS in hearts reperfused after 10 minutes of ischemia, the opposite was true in hearts reperfused after 5 minutes of ischemia (with arrhythmia onset occurring sooner despite a reduction in recovery of flow).

NCS had no effect on occluded zone size (Figure 10). This was no surprise since collateral flow is negligible in rat hearts.  

**ECG Changes**

NCS had a triphasic effect on QRST$_{90}$ (Figure 11) that was dependent on when the values were recorded. Before the onset of ischemia, no changes were re-
corded. During the first minute of ischemia, width remained constant in the 100:0 Cl⁻:NO₃⁻ group but was shortened in a concentration-dependent manner by NCS. The effect was statistically significant when the 100:0 Cl⁻:NO₃⁻ group was compared with the 0:100 Cl⁻:NO₃⁻ group (p<0.05). Thereafter, there was an increase in the width of the interval, which was markedly potentiated by NCS in a concentration-dependent manner. Widening was statistically significant throughout the remainder of the experiment when the 100:0 Cl⁻:NO₃⁻ group was compared with the 0:100 Cl⁻:NO₃⁻ group (p<0.05). The groups perfused with intermediate proportions of NO₃⁻ exhibited intermediate intervals (which were significantly prolonged versus the 0:100 Cl⁻:NO₃⁻ group from 20 minutes after the start of ischemia; p<0.05), but it was not possible to demonstrate a graded concentration dependence. Consequently, the incidences of ischemia-induced arrhythmias did not correlate with interval width measured during peak arrhythmia susceptibility (10 or 15 minutes after occlusion).

A small widening of the PR interval (Figure 11) produced by NCS (significant only in the 0:100 Cl⁻:NO₃⁻ group at 30 minutes after occlusion) failed to correlate with ischemia-induced arrhythmia incidence.

Assessment of the relation between ECG shape and reperfusion arrhythmias was complicated by the fact that arrhythmias appeared within a few seconds of the start of reperfusion and were sustained for variable periods; such censoring gave rise to data selection and unequal group sizes for ECG data. By 1 minute after the start of reperfusion there was considerable variation between groups in the numbers of hearts in which sinus rhythm had resumed. However, in the case of QRST₉₀ width, clear differences between groups were evident, and values during reperfusion have been included in Figure 11. Although reperfusion caused a rapid reversal of QRST₉₀ widening, values remained significantly increased by NCS compared with preischemic values (particularly in the case of the 0:100 Cl⁻:NO₃⁻ group). Furthermore, QRST₉₀ width correlated inversely with the incidence of reperfusion-induced VF (p<0.05). In the case of the PR interval, values during reperfusion have been omitted from Figure 11 owing to the large standard errors associated with diminished group size (especially in the all-Cl⁻ controls), as explained above.

ECG intervals can be expected to be influenced by heart rate. We made no correction for heart rate in the present study, but none was required since NCS had no effect on heart rate (Table 1).

**Crossover Study With Regional Ischemia**

To assess whether ischemia-induced arrhythmias had been inhibited by an action in the ischemic tissue or the surrounding nonischemic tissue, crossover studies were performed. In hearts with Cl⁻ trapped in the occluded zone and NO₃⁻ delivered to the nonischemic zone the incidences of VT and VF were reduced to 17% (p<0.05) and 0% (p<0.05) from control values (Cl⁻ in the ischemic and nonischemic zones) of 100% and 67%, respectively. Hearts receiving NO₃⁻ throughout the ex-
experiment had no VT or VF. This implied that the antiarrhythmic effect was mediated via an action in the nonischemic zone. However, in hearts with NO$_3^-$ trapped in the ischemic zone and Cl$^-$ delivered to the nonischemic zone, VF incidence was also reduced to 17% (p<0.05), although the incidence of VT was unchanged (100%).

Crossover Study With Global Ischemia

To assess whether effects of NCS on reperfusion-induced arrhythmias occurred as a result of actions during reperfusion or actions during the preceding periods of ischemia, crossover studies were performed in hearts subjected to 10 minutes of global ischemia. Atrial superfusion was maintained throughout the experiment. This kept the ventricular beating rate above 150 beats per minute throughout ischemia in all hearts. There were no significant differences between the four groups in terms of the incidence of reperfusion-induced VT. In hearts perfused before ischemia with 100:0 Cl$^-$:NO$_3^-$ solution and reperfused with this solution, the incidence was 100%, and in hearts perfused and reperfused with 0:100 Cl$^-$:NO$_3^-$ solution, the value was 83%. Similarly, values in hearts perfused with Cl$^-$ and reperfused with NO$_3^-$ or vice versa were 75% and 92%, respectively. However, the incidence of VF was reduced by NCS. Furthermore, this required NO$_3^-$ to be present during reperfusion. The VF incidence in the Cl$^-$ perfusion, Cl$^-$ reperfusion group was 92% and was significantly reduced to 8% (p<0.05) in the NO$_3^-$ perfusion, NO$_3^-$ reperfusion group. A reduction also occurred in the Cl$^-$ perfusion, NO$_3^-$ reperfusion group, to 50% (p<0.05), but in the group perfused with NO$_3^-$ and reperfused with Cl$^-$, there was no significant reduction in VF incidence (75%). This indicates that NCS inhibits reperfusion-induced VF largely

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**FIGURE 4.** Reperfusion-induced ventricular tachycardia (VT). Group incidences in hearts reperfused after 5, 10, 15, or 30 minutes of regional ischemia are shown.

**FIGURE 5.** Reperfusion-induced sustained ventricular fibrillation (VF). "Binned" group incidences (data for hearts subjected to 5, 10, 15, and 30 minutes of ischemia having been combined) are shown.
via an action mediated during reperfusion and within the reperfused region.

**Contractile Function**

End-diastolic pressure at vol$_{10}$, developed pressure at vol$_{10}$ and E$_{ES}$ are shown in Table 2. In all hearts the linear correlation coefficient for E$_{ES}$ was greater than 0.95, indicating that this measure of contractility was derived from the linear portion of the Starling curve.$^{34}$ NO$_3^-$ (100% NCS) was without effect on end-diastolic pressure or developed pressure at vol$_{10}$ (Table 2). However, when end-diastolic pressure was increased above 10 mm Hg, contractility was slightly impaired by NO$_3^-$ since E$_{ES}$ was reduced by 20% (Table 2). In contrast to NO$_3^-$, verapamil caused a large and significant increase in end-diastolic pressure (+400%) and large and significant reductions in developed pressure (−72%) and E$_{ES}$ (−55%) (all p<0.05). Epinephrine had opposite effects to verapamil (Table 2). The three measures of contractile function remained constant when solution was switched from Cl$^-$ to an identical Cl$^-$ solution (Table 2), thereby establishing that the act of switching solutions was not a significant source of variance.

Values of heart rate and coronary flow (not shown) in the unloaded state were similar in the NO$_3^-$ and Cl$^-$ groups to values observed in the arrhythmia experiments. In the verapamil group there was a significant fall in heart rate (of approximately 85 beats per minute) and in the epinephrine group a significant elevation (of approximately 42 beats per minute) with changes persisting during exposure to interventions. Verapamil caused a slight increase in coronary flow that was statistically significant when compared with that of the Cl$^-$ group (in which flow declined slightly during the course of the experiment, as it did in the arrhythmia studies).

**Discussion**

This is the first report of a potentially new antiarrhythmic approach involving manipulation of anion homeostasis. Although NCS has no obvious therapeutic potential of its own, the present data suggest that selective manipulation of anion homeostasis in the myocardium may represent a novel target for antiarrhythmic drug development; because available drugs for prevention of VF are inadequate for prevention of sudden cardiac death,$^{38}$ this has potentially far-reaching therapeutic consequences.

**Action of NCS on Ischemia-Induced Arrhythmias**

NCS had concentration-dependent antiarrhythmic activity during ischemia, particularly against the most severe arrhythmias (VT and VF). The ability of VF to sustain once it occurred was also inhibited by NCS.

Several potential mechanisms of action of NCS against ischemia-induced arrhythmias can be ruled out. Because we found no relation between arrhythmias and coronary flow, heart rate, or occluded zone size (variables that play an important role in determining arrhythmogenesis in our model)$^{8,14,27,39}$ then antiarrhythmic activity cannot be attributed to bradycardia, coronary vasodilatation, or reduction in the size of the ischemic emulsion.
ischemic region. Indeed, vasodilatation of coronary collaterals can be ruled out as an antiarrhythmic mechanism a priori since the rat heart is deficient in such vessels. Also, latency to onset of arrhythmias, a variable that is increased by anti-ischemic interventions that slow the rate of development of electrophysiological changes, was not affected by NCS. Therefore, the antiarrhythmic effects of NCS cannot be attributed to anti-ischemic actions. By examining the site of antiarrhythmic action of NCS (ischemic versus nonischemic tissue) by performing perfusion crossover studies, it was revealed that actions of NCS in both the nonischemic and the ischemic tissue alone appeared to be sufficient to account for inhibition of ischemia-induced VF, whereas inhibition of VT appeared to result exclusively from an action in the nonischemic zone. These data indicate that NCS inhibits ischemia-induced arrhythmias by directly affecting the electrophysiological con-
sequences of ischemia (via actions in the ischemic zone and the adjacent uninvolved zone) rather than by influencing the underlying causes of this dysfunction, the time course of its development, or ancillary variables (heart rate, coronary flow, and occluded zone size), which can determine or modify its extent and severity.

**Actions of NCS on Reperfusion-Induced Arrhythmias**

The effects of NCS on reperfusion arrhythmias were dependent on the duration of preceding ischemia. In hearts subjected to 10, 15, or 30 minutes of ischemia, NCS reduced the incidences of reperfusion-induced VT and VF. As with ischemia-induced arrhythmias, there was no correlation between antiarrhythmic effects and antecedent hemodynamic variables (changes in heart rate, occluded zone size, or recovery of coronary flow during reperfusion). NCS reduced recovery of coronary flow in hearts reperfused after 5 or 10 minutes of ischemia; however, it has been shown previously that reductions over the range encountered in the present study do not affect reperfusion-induced arrhythmia incidence. The delayed onset of reperfusion-induced arrhythmias produced by NCS in hearts reperfused after 10, 15, or 30 minutes of ischemia was not related to recovery of flow.

In hearts reperfused after 5 minutes of ischemia (in which VT and VF incidence was low in control and NCS hearts), we observed a true proarrhythmic effect of NCS on VPB incidence. Thus, the so-called bell-shaped susceptibility curve (the relation between incidence of

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**TABLE I. Heart Rates Measured 1 Minute Before Reperfusion**

<table>
<thead>
<tr>
<th>Duration of ischemia (minutes)</th>
<th>Ratio of Cl$^-$ to NO$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100:0</td>
</tr>
<tr>
<td>5</td>
<td>262±10</td>
</tr>
<tr>
<td>10</td>
<td>263±9</td>
</tr>
<tr>
<td>15</td>
<td>249±5</td>
</tr>
<tr>
<td>30</td>
<td>238±14</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between anion ratio groups.
reperfusion-induced arrhythmias and duration of preceding ischemia) was shifted to the left by NCS. This was a paradox in view of the consistent antiarrhythmic effects against all types of arrhythmia observed during ischemia and against VT and VF during reperfusion after 10–30 minutes of ischemia. Changes in the shape or location of the bell-shaped curve give an indication of the mechanism of action of an antiarrhythmic intervention. For example, bradycardia shifts the curve to the right, indicative of an anti-ischemic action.39 Drugs that shift the curve downward (i.e., inhibit arrhythmias independently of the duration of preceding ischemia) can be regarded as having a specific action on the arrhythmogenic stimulus of reperfusion (most likely an electrophysiological action); the new antiarrhythmic RS6865 appears to fall into this class.40 In the present study, the shift in the curve substantially downward (VT and VF) and to the left (VPB) by NCS indicates either that NCS speeds up the time course of development of susceptibility to reperfusion-induced arrhythmias or that it has a selective effect on different arrhythmogenic triggers, inhibiting best those operative 10 or more minutes after the onset of ischemia but having no effect (or even facilitating) those operating with shorter (5-minute) durations of ischemia. Further work is required to elucidate which hypothesis is correct.

Crossover perfusion studies with a model of global ischemia in which reperfusion-induced arrhythmias arise from within the reperfused zone14 showed that inhibition of reperfusion-induced arrhythmias by NCS resulted from a specific action on the arrhythmogenic triggers operating during reperfusion within the reperfused zone, rather than by indirect actions via some action during ischemia (consistent with the apparent lack of anti-ischemic activity) or within the uninvolved zone.

Together, these data indicate that NCS inhibits reperfusion-induced arrhythmias via direct modification of the electrophysiological consequences of reperfusion mediated by an action in the reperfused tissue in a manner dependent on the underlying arrhythmogenic trigger mechanism (which varies according to the duration of preceding ischemia).

**Insight Into Mechanism of Action From Changes in the ECG**

During ischemia, NCS widened the QRSTwt interval. It has previously been shown that NCS can prolong action potential duration at 90% (and 50%) repolarization in papillary muscle.41 If widening of QRSTwt by NCS reflects prolongation of action potential duration by NCS, then antiarrhythmic activity may have resulted from an associated prolongation of refractory period. However, this is unlikely to be the case because 1) drugs that produce significant widening of the QT interval, such as amiodarone, are not necessarily effective in preventing VF during ischemia or reperfusion in the rat heart42 and 2) tedisamil, an Ito blocker,43 has no effect on the incidence of VF in ischemia or reperfusion,44 despite its ability to widen the QRST interval in the rat heart to an even greater extent than did NCS in the
present study. Therefore, widening of QRST and prevention of VF by NCS in the rat heart appear to be casually rather than causally related. On the other hand, QRST widening may have played a role in the ability of NCS to reduce the incidence of sustained VF since amiodarone and tedisamil both possess similar activity in the rat heart. Indeed, the "chemical defibrillatory" effect of tedisamil has been attributed entirely to QRST widening. However, the cellular mechanism by which NCS mimics the ECG effects of amiodarone and tedisamil remains to be determined and may be complicated in the setting of acute ischemia by possible dissociation between action potential duration and refractory period, as demonstrated in the His bundle. It is of interest to note, however, that these ECG effects occurred during ischemia only, and no significant change in configuration was present before ischemia onset. This is strong evidence that, in the case of VF, NCS has "site selectivity" of action mediated in the involved region, in accordance with evidence from the crossover studies discussed above.

Changes in the PR interval were slight and unrelated to the antiarrhythmic effects. Because PR interval is prolonged by drugs that inhibit the fast and slow inward current, this would appear to rule out inhibition of these currents as the mechanism of antiarrhythmic action of NCS.

**Cellular Mechanism of Action: Hypotheses**

The present study does not permit a full assessment of the cellular mechanism of action of NCS. One possibility concerns intracellular pH. During ischemia, a fall in intracellular pH coincides with the appearance of arrhythmias. The former may contribute to the latter by virtue of its ability to shorten action potential duration. Because NCS prolongs action potential duration and QRST (the present study) and because substitution of extracellular Cl by other anions can increase intracellular pH via alteration of Cl−-HCO3− exchange, it is possible that inhibition of Cl−-HCO3− exchange may have contributed to the antiarrhythmic effects of NCS via amelioration of ischemia-induced falls in intracellular pH and action potential duration shortening. This cannot be established directly because the Cl−-HCO3− exchange current cannot be measured in a beating heart. However, in indirect support, recent studies have shown that specific blockers of Cl−-HCO3− exchange such as SITS inhibit ischemia-induced and reperfusion-induced arrhythmias in the isolated rat heart.

**Contractile Function**

We measured systolic and diastolic function in separate groups of hearts to examine whether the antiarrhythmic effects of NCS might be offset by a detrimental influence on contractile function. Over the normal range of end-diastolic pressure, NCS was without effect on contractility or compliance. However, at high "nonphysiological" end-diastolic pressures, EES was reduced by approximately 20% by total NCS. This effect, although statistically significant, was much less than that with verapamil (55% reduction).
Furthermore, NCS had no effect on developed pressure at control end-diastolic volume, whereas verapamil produced a 72% reduction. The effect of NCS on E\textsubscript{cgs} possibly represents a “garden hose” mechanism, since in arrhythmia studies NCS caused a slight fall in coronary flow of similar magnitude to the change in E\textsubscript{cgs}, although we did not undertake to explore this possibility further. From these data we conclude that if anion manipulation represents a new approach to attenuation of arrhythmias in acute ischemia/reperfusion, it is not substantially compromised by any inherent negative influence on contractile function in nonischemic tissue. Nevertheless, from our data, the possibility remains that in the setting of more chronic cardiac dysfunction in which end-diastolic pressures are abnormally elevated (e.g., in heart failure), complete NCS may elicit a negative inotropic response and, possibly, hemodynamic compromise; in view of the potential benefits of anion manipulation against arrhythmias it would be worthwhile to perform in vivo studies to evaluate this possibility and to determine whether arrhythmic effects in the acute phase of ischemic heart disease can be achieved by anion manipulation without significant impairment of hemodynamic function.

Coronary Flow

The lack of NCS-induced vasodilation was, perhaps, surprising, in view of the established effects of organic nitrate compounds on coronary flow. It is known that tolerance develops to the vasodilator effects of glyceryl trinitrate in the microgram per kilogram range in rats in vivo within 60 minutes of intravenous administration,\(^4^9\) so with NO\textsubscript{3}\(^-\) concentrations in the 30–100 mM range, one might speculate that tolerance may have developed very rapidly in the present studies. We examined the possibility of rapid tolerance to coronary vasodilator effects of NCS in some additional experiments. We observed a non–statistically significant increase in coronary flow with 100% NCS 3 minutes after the start of perfusion of isolated rat hearts (19.8±1.2 versus 17.5±1 ml/min/g); this increase disappeared by 10 minutes (13.8±3 versus 13.9±1 ml/min/g, respectively). This tends to support the rapid tolerance hypothesis. However, it should be noted that our experiments are the first to examine coronary flow when NO\textsubscript{3}\(^-\) is substituted for Cl\(^-\) in the extracellular space, and this situation is not necessarily directly comparable to administration of organic NO\textsubscript{3}\(^-\) compounds in the presence of fixed Cl\(^-\) concentration, so substantial and sustained coronary vasodilation in the present study was not necessarily expected.

Was possible tolerance to NO\textsubscript{3}\(^-\) vasodilation relevant to the effects of NCS on arrhythmias? In terms of coronary flow changes, this would seem unlikely, since flows were within the normal range for the preparation, and coronary steal effects are not relevant with constant pressure perfusion.\(^1^4\) However, at the cellular level, NO\textsubscript{3}\(^-\) tolerance may involve depletion of sulfhydryl moieties.\(^5^0\) Might this have some relevance to arrhythmogenesis? We doubt so, since N-acetylcysteine, an agent that has the opposite effect to NO\textsubscript{3}\(^-\) and repletes cellular sulfhydryl levels via elevation of cysteine levels\(^5^1\) inhibits (rather than facilitates) arrhythmogenesis during ischemia and reperfusion in the rat.\(^5^2\)

Species Considerations

Use of rat models for the study of arrhythmias and other responses to myocardial ischemia has recently been reviewed.\(^2^7\) It is clearly inappropriate to extrapolate findings from a single animal species to humans. As discussed in detail previously,\(^8\) rats have a faster basal heart rate and unusual cardiac electrophysiological characteristics (such as a short QT interval) compared with other species. Furthermore, phase 1 arrhythmias occur a few minutes later after the onset of ischemia in rat than in dog or pig and do not show well-defined 1A and 1B subpeaks.\(^2^3,2^5,2^7\) Regardless of these factors, in the rat, ischemia and reperfusion elicit arrhythmias that share characteristics and responsiveness to interventions that are broadly similar to arrhythmias elicited in other species; thus there is no a priori reason why rat arrhythmia data should be regarded as anomalous.\(^1^7,2^7\)

As noted previously,\(^2^7\) clinical relevance of any findings from rat (or indeed from any other animal species) can be proven only when the relevant clinical data become available. At present, direct data concerning the relation between anions and arrhythmogenesis in humans are lacking. The only available data are indirect\(^5^3\) and indicate that there is a strong association between a low incidence of sudden cardiac death (in certain regions of the United Kingdom) and high levels of NO\textsubscript{3}\(^-\) in drinking water.\(^5^4\) It has not been determined whether this association is casual or causal. We plan to explore this further by modifying anion content of rat
diet and inducing regional ischemia and reperfusion in vivo.

Data from a second species may serve to support present findings. The choice of the second species requires consideration of numerous factors. We have performed some additional studies with rabbit, a species with negligible coronary collateral flow, thus potentially suitable for bioassay of antiarrhythmic interventions. Hearts (n=6 per group) were subjected to 30 minutes of regional ischemia and 5 minutes of reperfusion. Occluded zone sizes were similar to those in rat (42±5% of total ventricular weight in controls and 41±6% with complete NCS).

FIGURE 11. ECG changes in hearts subjected to 30 minutes of regional ischemia. Values are mean±SEM. Statistical significance has been omitted for clarity. In the case of the interval of the width of the ventricular complex at 90% repolarization (QRST<sub>90</sub>) (top panel), values before occlusion were not significantly different from one another. After 5 minutes of ischemia, QRST<sub>90</sub> intervals were significantly prolonged in the all-NO<sub>3</sub><sup>-</sup> group (when compared with all-Cl<sup>-</sup> controls), and they remained so for the remainder of the experiment. Prolongation also occurred in the other NO<sub>3</sub><sup>-</sup>-perfused groups, significant (compared with the all-Cl<sup>-</sup> controls) 20, 25, and 30 minutes after the start of ischemia. During reperfusion, QRST<sub>90</sub> intervals remained prolonged only in the groups receiving the two highest ratios of NO<sub>3</sub><sup>-</sup> to Cl<sup>-</sup>. In the case of the PR interval (bottom panel), significant widening (compared with the all-Cl<sup>-</sup> controls) occurred only with 100% NO<sub>3</sub><sup>-</sup> substitution for Cl<sup>-</sup> and only at 30 minutes after the onset of ischemia.

TABLE 2. Contractile Function in Nonischemic Hearts

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Values before intervention</th>
<th>Values after intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DP (mm Hg)</td>
<td>SP-DP (mm Hg)</td>
</tr>
<tr>
<td>Cl&lt;sup&gt;-&lt;/sup&gt;</td>
<td>10</td>
<td>122±5</td>
</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>10</td>
<td>121±6</td>
</tr>
<tr>
<td>Verapamil</td>
<td>10</td>
<td>121±4</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>10</td>
<td>117±4</td>
</tr>
</tbody>
</table>

Values of diastolic pressure (DP), developed systolic minus diastolic pressure (SP-DP), and the linear regression slope between ventricular volume and systolic pressure (E<sub>E5</sub>) recorded before and after intervention. Perfusion solution before intervention was standard Cl<sup>-</sup> solution. Interventions, delivered from a separate reservoir, were identical Cl<sup>-</sup> solution, solution modified by complete substitution of Cl<sup>-</sup> with NO<sub>3</sub><sup>-</sup>, solution modified by addition of 1 µM verapamil, or solution modified by addition of 10 µM epinephrine.

*p<0.05 vs. value before intervention.
Ischemia-induced VPB incidence fell from 100% to 50% with NCS, and reperfusion-induced VT incidence fell from 66% to 17%, findings similar to those from rat studies. However, VF did not occur in either group, limiting the scope of findings.

Conclusion

VF elicited by ischemia could be almost fully abolished by NCS. The effect was not secondary to changes in heart rate, coronary flow, or occluded zone size, indicating an action mediated at the cellular level. Reperfusion-induced VF could also be almost fully abolished by NCS by an action mediated at the cellular level. The antiarrhythmic effect depended on an action during reperfusion, was mediated in the reperfused region, and was unrelated to heart rate or recovery of coronary flow. In hearts reperfused after a very brief period of ischemia (5 minutes), the NCS increased the VPB incidence. NCS had no detrimental effects on systolic or diastolic function in nonischemic tissue. However, at abnormally high end-diastolic pressures, NCS had slight negative inotropic activity. NCS may represent the basis of a new antiarrhythmic approach.

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