Effects of Sodium Nitroprusside and Nitroglycerin on Tension Prolongation of Cat Papillary Muscle during Recovery from Hypoxia

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SUMMARY Because of recent studies suggesting that vasodilators affect ventricular compliance, we studied the effect of sodium nitroprusside and nitroglycerin on the mechanical performance of 21 isolated cat papillary muscles. The muscles were stimulated isometrically at 36 beats/min. Sixteen of the muscles were made hypoxic (95% N₂, 5% CO₂) for 50 minutes and then reoxygenated. Sodium nitroprusside (10⁻⁴ M) added to four of these muscles prior to hypoxia substantially diminished the tension prolongation (both the time to peak tension, TTP, and the time for tension to fall to ½ its peak value, RT₅₀) that characterizes recovery from hypoxia. TTP and RT₅₀ measured 2 minutes after reoxygenation were 300 ± 20 msec and 528 ± 26 msec for the control muscles compared to 208 ± 13 msec and 248 ± 22 msec for the muscles pretreated with nitroprusside. Nitroprusside had no effect on the fall and recovery of peak developed force or on the rise and fall of resting force. Furthermore, nitroprusside had no effect on the above parameters in normoxic muscles. We also found that nitroprusside in concentrations of 10⁻⁴ M and nitroglycerin in concentrations of 10⁻⁷ M had little or no effect on tension prolongation. The results of the study indicate that nitroprusside is capable of blocking the tension prolongation that occurs during recovery from hypoxia and may prevent the incomplete myocardial relaxation thought to characterize this phenomenon. Since nitroprusside had no effect on tension prolongation, it is possible that other factors also may be important in the apparent increase in left ventricular compliance associated with administration of vasodilators to patients.

Vasodilators have become widely used in the treatment of left ventricular failure complicating acute myocardial infarction,1, 2 mitral regurgitation,3, 4 and primary myocardial disease.5, 6 There has been a number of studies concerning the effects of vasodilators on systolic performance1, 6 but little is known about their effects on diastolic properties of the left ventricle. Recent studies in man indicate that vasodilators are capable of altering the left ventricular diastolic pressure-volume relationship so that, for a given volume, the pressure is lower during vasodilator administration.4, 6, 10 One possible explanation for this finding is that vasodilators may exert a direct relaxant effect on ventricular muscle similar to their known relaxant effect on vascular smooth muscle. The present study was designed to explore this possibility and examine the effects of sodium nitroprusside and nitroglycerin on parameters of myocardial relaxation in isolated cat papillary muscle preparations. The results indicate that nitroprusside is capable of blocking the tension prolongation that occurs during recovery from hypoxia but that nitroglycerin has no effect on tension prolongation.

Methods

Twenty-one papillary muscles were removed from right ventricles of cats anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). The muscles were removed and promptly placed in baths containing Krebs' bicarbonate buffer which was maintained at a constant temperature of 30°C and gassed with 95% O₂ and 5% CO₂. Each muscle was stimulated isometrically (36 beats/min) using DC electrical pulses of 5-msec duration and a voltage 10% above threshold delivered through platinum electrodes placed parallel to each muscle for massive stimulation. Force and a stimulus artifact were recorded on a multichannel oscillograph (Brush Mark 200). Peak developed force, resting force, the time from the onset of force development to peak isometric force (TTP), and the time for isometric force to fall to ½ its peak value (RT₅₀) were measured directly. Length-active tension curves were obtained after equilibration for 1–2 hours, and for each experiment the muscle was maintained at the length at which developed force was maximal (Lₘₐₓ).

A flow diagram outlining the experimental protocol for the 21 muscles is shown in Figure 1. Sixteen of the muscles were made hypoxic. After control measurements of force development, hypoxia was produced by rapidly replacing the 95% O₂ and 5% CO₂ with 95% N₂ and 5% CO₂. This has been shown in previous studies to reduce the PO₂ of the bath from 600 to 15 mm in 1 minute.11 The changes in the isometric force tracing were recorded during 50 minutes of hypoxia; then the muscles were reoxygenated with 95% O₂ and 5% CO₂ and measurements were recorded for another 40–60 minutes. In eight muscles (paired hypoxic experiments, Fig. 1), this cycle was repeated after the muscles were washed with Krebs' bicarbonate buffer. In four of these experiments sodium nitroprusside (10⁻⁴ M) was added to the bath immediately prior to the first hypoxic period and in the other four experiments nitroprusside was added immediately prior to the second hypoxic period. For two muscles sodium nitroprusside was added to the bath in concentrations of...
Experiments was reduced when the muscles were pretreated with nitroprusside, as shown in Figure 2B and the right panel of Figure 3. With hypoxia peak developed force and the duration of tension fell, and resting force rose to a similar extent as for the control muscles. The marked tension prolongation (TTP and RT\textsubscript{H}) which then returned toward control values after pretreatment with 10\textsuperscript{-6} M nitroprusside, peak developed force and the duration of tension fell with hypoxia, similarly to the control muscles; 2 minutes after reoxygenation the tension prolongation was substantially less than that seen in the control muscles.

10\textsuperscript{-4} M prior to hypoxia, and for six muscles nitroglycerin (10\textsuperscript{-5} M) was added to the bath prior to hypoxia.

In five experiments cumulative dose-response curves were obtained for nonhypoxic muscles by increasing the concentration of sodium nitroprusside (10\textsuperscript{-4} to 10\textsuperscript{-3} M) and observing changes in resting force, developed force, TTP, and RT\textsubscript{H}. For all of the experiments with sodium nitroprusside, the baths were protected from ambient light with aluminum foil.

Control and nitroprusside data from the paired experiments were compared using a paired t-test. Comparison of the control with the nitroprusside and nitroglycerin data from the unpaired experiments was made using an unpaired t-test. Differences were labeled as significant when P values were less than or equal to 0.05.

Results

The response of the isolated cat papillary muscle beating isometrically at 36 beats/min at 30°C to a 30-minute period of hypoxia followed by reoxygenation, is shown in Figure 2A and the left panel of Figure 3. With hypoxia peak developed force and the duration of tension (both TTP and RT\textsubscript{W}) fell while resting force rose. Following reoxygenation there was an immediate marked prolongation of tension development (TTP and RT\textsubscript{W}) which then returned toward control values over 20–40 minutes. Peak developed force and resting force returned toward baseline over 20–60 minutes. When sodium nitroprusside was added to the bath in concentrations of 10\textsuperscript{-6} M the muscles were then made hypoxic, peak developed force and the duration of tension fell, and resting force rose to a similar extent as for the control muscles. However, the marked tension prolongation (TTP and RT\textsubscript{W}) that characterized recovery from hypoxia in the control experiments was reduced when the muscles were pretreated with nitroprusside, as shown in Figure 2B and the right panel of Figure 3.

We performed eight paired experiments similar to the one shown in Figure 3. In half of these, nitroprusside was added to the bath prior to the first period of hypoxia and in half nitroprusside was added prior to the second period of hypoxia. One muscle deteriorated during the second part of the experiment, leaving seven paired experiments, the results of which are summarized in Figure 4. Tension prolongation (TTP and RT\textsubscript{W}) following recovery from hypoxia was less when the muscles were pretreated with 10\textsuperscript{-4} M nitroprusside and this difference was statistically significant for RT\textsubscript{W}.

There were no significant differences in the fall and recovery of peak-developed force. The increase in resting force with hypoxia was greater when the muscles were pretreated with nitroprusside but this was significant at only one point (Fig. 4). Because there was some tendency for the tension prolongation following the second period of hypoxia to be less than that following the first period of hypoxia, control muscles and muscles pretreated with sodium nitroprusside also were compared during the first period of hypoxia and reoxygenation only (unpaired experiments); the results are summarized in Figure 5. During recovery from hypoxia, both RT\textsubscript{W} and TTP were significantly less for the nitroprusside-treated muscles compared to control muscles. There were no significant differences in the fall and recovery of peak developed force or the rise and fall of resting force. In two muscles pretreated with 10\textsuperscript{-4} M sodium-nitroprusside,
Figure 3. Parameters of isometric contraction in one muscle during two periods of hypoxia and reoxygenation with and without nitroprusside. There was a marked prolongation of the time for force to fall to \( \frac{1}{3} \) its peak value (RT\(_3\)) and the time to peak force (TTP) during the control period, but this prolongation was substantially reduced when the muscle was pretreated with sodium nitroprusside. The fall and recovery of peak developed force and the rise and recovery of resting force with hypoxia and reoxygenation were similar in the control period and when the muscle was pretreated with nitroprusside.

Figure 4. Summary of seven paired experiments similar to the one shown in Figure 3. In four of the experiments nitroprusside was added to the bath prior to the first hypoxic period, and in three experiments nitroprusside was added prior to the second hypoxic period. The results during the control period are compared with the results when the muscles were pretreated with sodium nitroprusside. The data are given as mean ± SEM. The fall and recovery of peak developed force during hypoxia and reoxygenation were similar in the control period and when the muscles were pretreated with nitroprusside. The rise of resting force with hypoxia was higher with nitroprusside, but this was significant at only one point (*). The time for force to fall to \( \frac{1}{3} \) its peak value (RT\(_3\)) was substantially prolonged during recovery from hypoxia in the control muscles and this prolongation was reduced with nitroprusside. Time to peak force (TTP) was also prolonged during recovery from hypoxia in the control muscles and this prolongation was less, but not statistically different, with nitroprusside. Differences that were statistically significant (\( P < 0.05 \)) are labeled with asterisks (*).
there was no significant reduction in tension prolongation during recovery from hypoxia.

Six muscles were pretreated with $10^{-4}$ M glyceryl trinitrate (nitroglycerin), and a comparison between them and control muscles is shown in Figure 6. Nitroglycerin had no significant effect on tension prolongation, the fall and recovery of peak developed force, or the rise and recovery of resting force.

The effects of nitroprusside on nonhypoxic muscles were evaluated in five experiments. Nitroprusside in concentrations of $10^{-5}$ to $10^{-4}$ M had no detectable effect on peak developed force, resting force, $RT_H$, or $TTP$.

Discussion

Sodium nitroprusside and nitroglycerin are known to exert a direct relaxant effect on vascular smooth muscle, but there is little information about their effects on cardiac muscle relaxation. Our study indicates that nitroprusside in concentrations of $10^{-8}$ M blocks the tension prolongation that occurs during recovery from hypoxia but that nitroglycerin has no significant effect on tension prolongation. The effects of nitroprusside appear to be quite selective, since nitroprusside had no significant effect on the fall and recovery of peak developed force or the rise and fall of resting force and no effect on nonhypoxic muscles.

The phenomenon of tension prolongation during recovery from hypoxia was first described by Tyberg et al. and Bing et al. in 1970 and 1971, but its mechanism remains unclear. Relaxation requires a reduction in the effective intracellular calcium concentration, and the delay in relaxation may be due either to an excessive amount of calcium released or to a reduced rate of uptake by the sarcoplasmic reticulum. It is thought that the duration of tension is also influenced by the duration of the cardiac action potential plateau and that the maintenance of a depolarized membrane favors continued entry of calcium into the cell and a prolongation of contractile activity. Hypoxia and reoxygenation are associated with shortening and prolongation of the action potential plateau, and this suggests that changes in the duration of tension during hypoxia and recovery could be partially related to events taking place at the cell membrane. However, if this were the only mechanism involved, an augmentation rather than a depression of contraction might be observed during recovery from hypoxia. Alternatively, transient changes in the intracellular environment during recovery from hypoxia, such as changes in pH and energy stores, might influence the uptake of calcium by the sarcoplasmic reticulum and influence tension.
prolongation. There also is some evidence that mitochondrial respiratory activity may play an important role in the tension prolongation that occurs during recovery from hypoxia. Brooks et al.\textsuperscript{17} demonstrated tension prolongation in mitochondria-rich mammalian heart muscle but not in skeletal muscle or turtle or eel ventricular strips, and they found that respiratory inhibitors, such as KCN, rotenone, and actinomycin A, abolished tension prolongation in mammalian heart muscle. This evidence suggests the importance of functioning mitochondria in the prolongation of tension, although it is unclear how this relates to a presumed prolongation of elevated intracellular calcium levels.

The mechanism by which sodium nitroprusside alters tension prolongation is unclear. There is no information about the effects of nitroprusside on the action potential plateau, but there is some information relating to the effects of nitroprusside on the sarcoplasmic reticulum. Studies with isolated rat aorta have shown that nitroprusside has a relaxant effect independent of changes in calcium ion influx and that there is enhanced calcium binding to microsomal fractions in the presence of nitroprusside.\textsuperscript{18} This suggests that the site of action of nitroprusside may be at the sarcoplasmic reticulum in vascular smooth muscle and this could be true for cardiac muscle as well. Alternatively, sodium nitroprusside [Na\textsubscript{3}Fe(CN)\textsubscript{5}NO\cdot2H\textsubscript{2}O] or its metabolite, thiocyanate, may prevent tension prolongation by respiratory inhibition of the mitochondria similar to the respiratory inhibition seen with KCN.

Both sodium nitroprusside and nitroglycerin are rapidly metabolized in man and therapeutic concentrations are unknown.\textsuperscript{19} Concentrations of nitroprusside required to relax rabbit aortic strips contracted by potassium chloride and norepinephrine are 10\textsuperscript{-6} and 10\textsuperscript{-4} M, respectively,\textsuperscript{11} and concentrations required to relax isolated bull veins contracted with norepinephrine are 10\textsuperscript{-8} to 10\textsuperscript{-6} M.\textsuperscript{19} Nitroglycerin relaxes strips from large and small coronary arteries in concentrations of 10\textsuperscript{-8} to 10\textsuperscript{-5} M and 10\textsuperscript{-6} to 10\textsuperscript{-5} M, respectively.\textsuperscript{19} The concentrations of sodium nitroprusside and nitroglycerin used in our experiments (10\textsuperscript{-4} M) are similar to concentrations required to relax vascular smooth muscle in these studies in vitro.

This study may have clinical relevance for patients with ischemic heart disease and congestive heart failure. The tension prolongation that occurs during recovery from hypoxia in isolated muscle preparations may explain certain phenomena observed in the intact ischemic heart. Patients with ischemic heart disease, for example, present evidence of impaired ventricular relaxation\textsuperscript{51} and reduced ventricular compliance\textsuperscript{52} in response to angina induced by pacing, and this might be explained by a prolongation in the duration of contraction associated with transient periods of hypoxia. Relaxation may also be impaired in patients with congestive heart failure.\textsuperscript{53} Although this is not directly related to the tension prolongation seen in isolated hypoxic muscles, the

\textbf{Figure 6} Summary of data from six muscles pretreated with 10\textsuperscript{-4} M nitroglycerin compared with four control muscles during hypoxia and reoxygenation. There were no significant differences between the two groups in peak developed force, resting force, time for force to fall to 1/4 its peak value (RT\textsubscript{50}) or time to peak tension (TTP).
mechanism of impaired relaxation of hypoxia and failing muscle may be the same at the subcellular level, i.e., a defect in calcium uptake by the sarcoplasmic reticulum. If sodium nitroprusside blocks the tension prolongation that occurs during recovery from hypoxia in isolated muscle preparations, it may be capable of influencing the incomplete relaxation seen in patients with ischemic heart disease and congestive heart failure. This might explain the increased left ventricular compliance seen in patients with congestive heart failure during the administration of sodium nitroprusside. Preliminary data, however, has shown that sublingual nitroglycerin also can produce an apparent increase in left ventricular compliance in some patients with coronary artery disease. Since nitroglycerin in the present study had no effect on tension prolongation during reoxygenation, this suggests that other factors may be important in causing the apparent change in compliance induced in patients by vasodilators. Vasodilators may reduce myocardial ischemia by improving the relationship between myocardial oxygen supply and demand and may increase left ventricular compliance on this basis. Vasodilators decrease right heart pressures, and experimental studies have suggested that a reduction in right heart pressures can produce a shift in the left ventricular pressure-volume relationship (reduced left ventricular end-diastolic pressure at the same end-diastolic volume) presumably through subtle shifts in the ventricular septum. Vasodilators also reduce the perfusion pressure in the coronary arteries and their intramural branches and studies on animals have shown that a reduction in coronary perfusion pressure may be associated with increased left ventricular compliance. Therefore, it may be that hemodynamic factors also play an important role in the apparent increase in left ventricular compliance associated with vasodilator administration.

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References

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