Increased coronary perfusion augments cardiac contractility in the rat through stretch-activated ion channels

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Lamberts, R. R., M. H. P. van Rijen, P. Sipkema, P. Fransen, S. U. Sys, and N. Westerhof. Increased coronary perfusion augments cardiac contractility in the rat through stretch-activated ion channels. Am J Physiol Heart Circ Physiol 282: H1334–H1340, 2002. First published December 13, 2001; 10.1152/ajpheart.00327.2001.—The role of stretch-activated ion channels (SACs) in coronary perfusion-induced increase in cardiac contractility was investigated in isolated isometrically contracting perfused papillary muscles from Wistar rats. A brief increase in perfusion pressure (3–4 s, perfusion pulse, \( n = 7 \)), 10 repetitive perfusion pulses (\( n = 4 \)), or a sustained increase in perfusion pressure (150–200 s, perfusion step, \( n = 7 \)) increase developed force by 2.7 ± 1.1, 7.7 ± 2.2, and 8.3 ± 2.5 mN/mm² (means ± SE, \( P < 0.05 \)), respectively. The increase in developed force after a perfusion pulse is transient, whereas developed force during a perfusion step remains increased by 5.1 ± 2.5 mN/mm² (\( P < 0.05 \)) in the steady state. Inhibition of SACs by addition of gadolinium (10 \( \mu \)mol/l) or streptomycin (40 and 100 \( \mu \)mol/l) blunts the perfusion-induced increase in developed force. Incubation with 100 \( \mu \)mol/l \( \text{N}^\text{O} \)-nitro-l-arginine [nitric oxide (NO) synthase inhibition], 10 \( \mu \)mol/l sodium nitroprusside (NO donation) and 0.1 \( \mu \)mol/l verapamil (L-type \( \text{Ca}^{2+} \) channel blocker) are without effect on the perfusion-induced increase of developed force. We conclude that brief, repetitive, or sustained increases in coronary perfusion augment cardiac contractility through activation of stretch-activated ion channels, whereas endothelial NO release and L-type \( \text{Ca}^{2+} \) channels are not involved.

Mechanotransduction; gadolinium; nitric oxide; papillary muscles; streptomycin

The existence of an interaction between coronary perfusion and cardiac contractility is well established (22). An increase in coronary perfusion with a concomitant increase in filling of the coronary vessels leads to an increase in cardiac contractility and cardiac oxygen consumption known as the Gregg effect (1, 3, 14, 18, 22, 36). Filling of the coronary vessels will change hoop (circumferential) stress in the vessel wall, thereby mechanically deforming the membranes of myocardial cells. Mechanical deformation of cardiomyocytes activates stretch-activated ion channels (SACs) (24, 27, 28, 37, 39, 40), thereby conducting \( \text{Ca}^{2+} \), \( \text{Na}^{+} \), or \( \text{K}^{+} \) cations (7, 8, 35, 37, 39, 40), which may affect the contractile state of the myocardium. Besides hoop stress, a change in perfusion will change also shear stress and may thereby induce endothelium-dependent nitric oxide (NO) release. Because NO has been shown to have positive inotropic effects at low concentration and negative inotropic effects at high concentration (26, 30), there could be an effect of perfusion-induced NO release on the contractility of cardiomyocytes.

Therefore, we investigated the role of SACs and NO on the coronary perfusion-induced increase in cardiac contractility. In isolated perfused papillary muscles of the rat, coronary perfusion pressure was increased via a brief “perfusion pulse,” repetitive perfusion pulses, or a sustained perfusion increase “perfusion step.” The effects of the perfusion increase on isometric force development were tested before and after addition of the SAC blockers gadolinium (III) chloride hexahydrate (Gd³⁺) and streptomycin, the NO synthase (NOS) inhibitor \( \text{N}^\text{O} \)-nitro-l-arginine (l-NNA), the NO donor sodium nitroprusside (SNP), and the L-type \( \text{Ca}^{2+} \) channel blocker verapamil. Our results indicate that a brief, repetitive, or sustained increase in coronary perfusion augments cardiac contractility through activation of stretch-activated ion channels, whereas endothelial NO release and L-type \( \text{Ca}^{2+} \) channels are not involved.

METHODS

Preparation and setup. All animals were treated according to guidelines of the Animal Experimental Committee of the Vrije Universiteit of Amsterdam, The Netherlands. Under ether anesthesia, the hearts of 50 male Wistar rats (300–400 g body wt) were quickly removed and perfused via the aorta with a crystalloid solution (for composition, see below). To prevent contraction of the heart, external \( \text{Ca}^{2+} \) concentration ([\( \text{Ca}^{2+} \)]) was kept at 0.5 and 25 mmol/l, respectively, and 2,3-butanedione monoxime was added. A papillary muscle

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with part of the septum and septal artery was removed from the right ventricle and transferred to the experimental superfusion bath. The septum was clamped on a Perspex plate and the muscle tendon was attached to a force transducer (model KG4, Scientific Instruments; Heidelberg, Germany) with a piece of silk thread. The septal artery was cannulated using a glass cannula and connected to a pressurized reservoir through a pressure difference meter (model SCX01DN, Sensym) for flow measurements. Muscle diameter was determined with the use of a video analyzing system (36).

Bath (superfusion) and pressurized reservoir (perfusion) were filled with identical crystalloid solution containing (in mmol/l) 120 NaCl, 4.9 KCl, 1.2 MgSO4, 1.8 NaH2PO4, 1 CaCl2, 10 glucose, 5 HEPES, 20 NaHCO3, 15 choline chloride, and 0.01 adenosine, and then gassed with 95% O2-5% CO2. Solution was kept at 27°C and pH was set at 7.45.

Muscles were stimulated via a pair of platinum electrodes at 0.2Hz to obtain muscle-isometric contractions. Passive force (F\text{\text{\textit{pass}}}) the force imposed on the resting muscle) and developed force (F\text{\text{\textit{dev}}}; the difference between the total force production during contraction and F\text{\text{\textit{pass}}}) were measured at 95% L\text{\text{\textit{max}}} with L\text{\text{\textit{max}}} being the muscle length at which maximal isometric force was developed.

**Experimental protocols**. After an equilibration period of 60 min in control conditions, different perfusion protocols were tested to investigate adaptation processes and to exclude interference of preload changes. In the first protocol, a brief increase (3–4 s) from low (10 cmH2O) to high (80 cmH2O) coronary perfusion pressure (P-pulse or perfusion pulse) was applied between two isometric contractions. In the second protocol, coronary perfusion pressure was increased from low-to-high perfusion and sustained until force development reached a steady state (perfusion step). In four muscles of the Gd3+ group (see **Experimental protocols**), we performed an additional perfusion protocol to investigate a different application of the stretch (perfusion pressure). Four muscles were exposed to 10 consecutive perfusion pulses, and each pulse was given between two isometric contractions (repetitive perfusion pulses).

The minimal perfusion pressure of 10 cmH2O was used because at lower perfusion pressures the vasculature would collapse completely, resulting in endothelial damage, and thus affecting contractile state. The maximal perfusion pressure of 80 cmH2O (~65 mmHg) was used because this reflects normal perfusion pressure in the septal artery and it is the perfusion pressure at which the increase in F\text{\text{\textit{dev}}} is maximal.

The perfusion protocols were performed in control conditions and were repeated after 30 min of incubation with one of the pharmacological agents. A first group of papillary muscles (n = 7) was incubated with Gd3+ (10 μmol/l), a nonspecific stretch-activated ion channel blocker. A second group of papillary muscles (n = 10) was incubated with the NOS inhibitor L-NNA (100 μmol/l), and a third group (n = 6) was incubated with the NO donor SNP (10 μmol/l). To investigate the role of SACs, a fourth group of muscles (n = 8) was incubated with streptomycin (40 and 100 μmol/l), which is also known to block SACs (19). In this group, only the perfusion step was performed. The role of inhibition of L-type Ca2+ channels by Gd3+ was investigated in a fifth and sixth group of papillary muscles. The fifth group (n = 6) was incubated with the L-type Ca2+ channel blocker verapamil (0.1 μmol/l) and again only a perfusion step was performed. The sixth group (n = 6) was loaded with the cell-permeant acetoxymethyl ester form of the fluorescent intracellular Ca2+-indicator fura 2 (Molecular Probes F1221, final concentration 10 μmol/l) and basal force development was measured. The ratio of fura 2 fluorescence at 520 nm after excitation at wavelengths 340 and 380 nm was collected with a photomultiplier (model MPS2021, Zeiss) before and after addition of 10 μmol/l Gd3+, with acquisition rate of 66 Hz. In all experiments, the fura 2 signal was at least five times above background level (autofluorescence).

**Data analysis.** Force (mN/mm2) was normalized by dividing measured force by muscle cross-sectional area (mm2). The peak 340-to-380-nm ratio for each muscle in control conditions, i.e., before addition of 10 μmol/l Gd3+, was normalized with respect to this value (normalized 340-to-380-nm ratio). The fluorescence signal was not converted to intracellular [Ca2+]i ([Ca2+]i) values with an in vivo calibration procedure because accurate reproducible calibration procedures without compartmentalization problems are difficult (38) and our primary interest was in the relative changes of [Ca\text{\text{\textit{2}}}+]. Statistical differences within each group of muscles were tested with a one- or two-way repeated-measures analysis of variance, followed by a Bonferroni or Dunnett post hoc test; P < 0.05 was considered significant. All data are expressed as means ± SE.

**RESULTS**

**Perfusion step and perfusion pulse.** Raw data tracings of the effects induced by a perfusion pulse (A) and perfusion step (B) are presented in Fig. 1. A perfusion pulse results in an immediate increase in force, which slowly decreases and returns to basal values after ~50–60 s. A perfusion step increases force during the first 7–9 contractions; after 40 s, the force slowly starts to decrease, reaching a steady state after ~125 s (25 contractions).

Figure 2 shows the averaged absolute increase in F\text{\text{\textit{dev}}} after a perfusion pulse (Fig. 2A) and perfusion step (Fig. 2B) before (closed circles) and after (open circles) SAC blockade with 10 μmol/l Gd3+ (n = 7, Fig. 2, top), NOS inhibition with 100 μmol/l L-NNA (n = 10, Fig. 2, middle) or NO donation with 10 μmol/l SNP (n = 6, Fig. 2, bottom). In control conditions, i.e., before addition of the pharmacological agents, a perfusion pulse results in an immediate increase in F\text{\text{\textit{dev}}}, with a maximum increase of 3.0 ± 1.1 mN/mm² for the Gd3+ group, 2.7 ± 0.9 mN/mm² for the L-NNA group and 3.3 ± 0.9 mN/mm² for the SNP group. Hereafter, the F\text{\text{\textit{dev}}} slowly returns to basal values after ~50 s. In the Gd3+ group, F\text{\text{\textit{pass}}} is significantly increased in the first contraction after the P-pulse, 0.36 ± 0.07 mN/mm² (P < 0.05), whereas in the second contraction (10 s) after the P-pulse, the F\text{\text{\textit{pass}}} value returns to basal values (0.1 ± 0.04 mN/mm², P > 0.05). Also, the muscle diameter (Fig. 1) is related to vascular filling and emptying (I) and returns to basal values within 10 s. This indicates that a perfusion pulse induces a process that results in a transient increase of F\text{\text{\textit{dev}}}.

The perfusion-pulse-induced increase in F\text{\text{\textit{dev}}} is completely blunted by SAC blockade (maximum increase of F\text{\text{\textit{dev}}} 0.2 ± 0.3 mN/mm²), whereas NOS inhibition or NO donation has no effect (maximum increase of F\text{\text{\textit{dev}}} 3.1 ± 0.63 and 3.4 ± 0.9 mN/mm², respectively). In the Gd3+ group, the increase in F\text{\text{\textit{pass}}} in the first contraction after the P-pulse (0.30 ± 0.27 mN/mm², P < 0.05) and...
the $F_{\text{pas}}$ value in the second contraction following P-pulse ($0.09 \pm 0.05 \, \text{mN/mm}^2, P > 0.05$) are not affected by Gd$^{3+}$. Similar results for $F_{\text{pas}}$ are found for the L-NNA and SNP group (data not shown).

A perfusion step results in an immediate increase in $F_{\text{dev}}$ that is not different from the perfusion-pulse-induced increase in $F_{\text{dev}}$ and reaches a maximum increase ($\Delta F_{\text{max dev}}$) after 40 s (Table 1 and Fig. 2). Hereafter, the $F_{\text{dev}}$ starts to decline but remains significantly increased in the steady state ($\Delta F_{\text{SSdev}}$) (Table 1 and Fig. 2). The time constant of the increase in $F_{\text{dev}}$ was not different for the three control groups ($8.9 \pm 6.0$ s for Gd$^{3+}$ group, $10.9 \pm 8.3$ s for the L-NNA group and $16.2 \pm 14.4$ for the SNP group).

The perfusion step-induced increase in $F_{\text{dev}}$ is completely blunted by SAC blockade with $10 \, \mu\text{mol/l Gd}^{3+}$ ($\Delta F_{\text{SSdev}}: 0.8 \pm 1.3 \, \text{mN/mm}^2$, Table 1) and is dose dependently inhibited by streptomycin, another SAC blocker ($2.8 \pm 1.0$ and $1.3 \pm 0.9 \, \text{mN/mm}^2$ at 40 and 100 $\mu\text{mol/l}$, respectively, Table 1). NOS inhibition, NO donation, or L-type Ca$^{2+}$ channel blockade has no effect on the steady-state increase in $F_{\text{dev}}$ to perfusion step ($5.7 \pm 3.2$, $9.1 \pm 4.1$, and $6.9 \pm 1.7 \, \text{mN/mm}^2$, respectively, Table 1). In the steady state, the perfusion step...
results in a significant but small increase in $F_{\text{pas}}$ ($\Delta F_{\text{SSpas}}$, Table 1), which is not affected by the addition of any of the pharmacological agents (Table 1).

**Repetitive perfusion pulses.** The absolute increase in $F_{\text{dev}}$ of 10 consecutive perfusion pulses (the repetitive perfusion pulses, $n = 4$) on $F_{\text{dev}}$ is shown in Fig. 3. Repetitive perfusion pulses result in an immediate increase in $F_{\text{dev}}$ of 1.5 ± 0.6 mN/mm$^2$, which is not significantly different from the passive pulse or step-induced immediate increase in $F_{\text{dev}}$. After 40 s, when two pulses still are to be applied, the increase in $F_{\text{dev}}$ already starts to decrease. The maximal increase in $F_{\text{dev}}$ of the repetitive perfusion pulses is 7.8 ± 2.2 mN/mm$^2$ with a time constant of 24.5 ± 16.3 s. Both values are not significantly different from the perfusion step-induced values. SAC blockade with Gd$^{3+}$ completely blunts the repetitive perfusion pulse-induced response. With Gd$^{3+}$, the maximal increase in $F_{\text{dev}}$ is 1.0 ± 1.1 mN/mm$^2$, which is not significant from basal values.

**Basal muscle properties, characteristics, and Ca$^{2+}$ transient.** Table 1 shows that the averaged optimal muscle length ($L_{\text{max}}$) and the averaged cross-sectional area of the muscles are statistically not different between the experimental groups. In basal conditions, i.e., low perfusion (10 cmH$^2$O) and at 95% $L_{\text{max}}$, $F_{\text{pas}}$ (see Table 1) is not different between the groups and not affected by the addition of one of the pharmacological agents. The absolute values of $F_{\text{dev}}$ at basal conditions (see Table 1) are not affected by addition of Gd$^{3+}$, L-NNA, SNP, or streptomycin, whereas addition of verapamil and Gd$^{3+}$ under HEPES conditions result in a decrease of basal $F_{\text{dev}}$ of 56.0 ± 4.5% and 43.1 ± 8.5%, respectively (Table 1).

The last column in Table 1 shows that the absolute values of coronary flow at high perfusion (80 cmH$^2$O) are not affected by the addition of Gd$^{3+}$ or SNP, whereas L-NNA significantly reduced coronary flow. In the streptomycin, verapamil, or HEPES experiments, the relative flow values were not different between the control group and after addition of a pharmacological agent (data not shown); unfortunately, technical problems did not allow us to obtain accurate absolute flow measurements in these experiments. Figure 4 shows, from a separate series of experiments, that the addition of Gd$^{3+}$ in basal conditions (low perfusion) does not affect the passive or peak [Ca$^{2+}$]i transient.

**DISCUSSION**

Our study shows that a short-lived, a repetitive, and a sustained increase in coronary perfusion pressure result in an increase in $F_{\text{dev}}$. Similar immediate increases in $F_{\text{dev}}$ are seen with all three perfusion protocols; the peak value and the time constant of the increase in $F_{\text{dev}}$, in response to the repetitive and
Fig. 4. Intracellular Ca\(^{2+}\) transient, as normalized 340-to-380-m ratio, of basal (low perfusion) isometric contraction before and after addition of 10 \(\mu\)mol/l Gd\(^{3+}\) (n = 6). Intracellular Ca\(^{2+}\) transient (passive value, peak value, and surface area) was not different before or after SAC blockade with Gd\(^{3+}\), indicating that Gd\(^{3+}\) does not inhibit L-type Ca\(^{2+}\) channels. Each line represents the mean.

sustained increase in perfusion, are not different. All three perfusion-induced increases in F\(_{\text{dev}}\) are completely blunted by SAC blockade (Gd\(^{3+}\)), but not affected by NOS inhibition (L-NNA) or NO donation (SNP). Another SAC blocker, streptomycin, dose dependently inhibited the perfusion step-induced increase in F\(_{\text{dev}}\), whereas blockade of L-type Ca\(^{2+}\) channels with verapamil did not affect the perfusion-induced increase in F\(_{\text{dev}}\). We conclude that brief, repetitive, or sustained increases in coronary perfusion augment cardiac contractility through activation of stretch-activated ion channels, whereas endothelial NO release and L-type Ca\(^{2+}\) channels are not involved.

Perfusion-induced changes. In our isolated perfused papillary muscles, where autoregulation is restricted due to adenosine addition, an increase in coronary perfusion pressure results in an increase in F\(_{\text{dev}}\), the Gregg effect, and is in line with earlier findings (13, 16, 36). From the literature it is known that in perfused papillary muscles an increase in oxygen delivery is not responsible (17, 36). It has also been suggested (2) that the perfusion-induced response is related to a change in sarcomere length ("garden hose" effect). Our perfusion experiments show that at the second contraction after a perfusion pulse the increase in F\(_{\text{pas}}\) and muscle diameter (Fig. 1) have returned to basal values, whereas F\(_{\text{dev}}\) remains increased. Moreover, SAC blockade by either Gd\(^{3+}\) or streptomycin inhibits the perfusion-induced increase in developed tension with no effect on the small perfusion-induced increase in passive tension (Table 1), indicating that the perfusion-induced changes are not related to changes in myofilament overlap. Thus our experimental results are in line with literature (25) showing that the perfusion-induced response is not related to a change in sarcomere length.

In our experiments, NOS inhibition by L-NNA or NO donation by SNP did not affect the responses to a perfusion change, indicating that the increase in cardiac contractility to a perfusion change is not related to shear stress-induced NO release by endothelial cells. Coronary flow at high coronary perfusion was reduced by L-NNA, indicating that NOS inhibition by L-NNA was active (Table 1).

The verapamil experiments show that L-type Ca\(^{2+}\) channel inhibition does not affect the perfusion-induced increase in F\(_{\text{dev}}\) (Table 1), which indicates that the increase in F\(_{\text{dev}}\) to a perfusion step. The Gregg effect, therefore, involves activation of SACs. Cardiac myocytes exhibit activation of SACs, which is inhibited by Gd\(^{3+}\) (24, 37, 40). Activation of SACs in heart cells can lead to changes in fluxes of Ca\(^{2+}\), Na\(^{+}\), or K\(^+\) cations (7, 8, 35, 37, 39, 40), which may affect the myocardial contractile state.

The involvement of SACs in the perfusion-induced increase in cardiac contractility is confirmed by comparable properties of SACs activation and perfusion-induced responses. An increase in perfusion results in an immediate (5 s) increase in F\(_{\text{dev}}\) of similar magnitude for all three perfusion protocols. Similarly, the stretch-induced inward cation current via SACs appeared within 10 ms in rat ventricular myocytes (5, 40), indicating that SACs activation is fast enough to account for the perfusion-induced response. The perfusion-induced increase in F\(_{\text{dev}}\) immediately starts to decrease when the perfusion pressure is lowered (data not shown), which is in accordance with the results that the SACs cation currents disappear when the stimulus is released (4, 40).

The mechanisms by which the mechanosensitive activation of SACs is linked to the perfusion-induced increase in cardiac contractility are still speculative; they do not only involve changes in calcium availability and calcium sensitivity, but also cytoskeletal changes. Glogauer et al. (21) showed, in human gingival fibroblasts, that a single 1-s stretching force application resulted in a SACs-mediated increase of [Ca\(^{2+}\)]\(_i\), which lasted 150 s. A similar mechanism may underlie the transient increase in F\(_{\text{dev}}\) by the P-pulse (Figs. 1 and 2) because F\(_{\text{dev}}\) remained increased for several contractions, whereas muscle diameter quickly returned to basal values. In chicken cardiomyocytes, a mechanical stimulus by pressing the membrane elicited an inward cation current, which slowly inactivated during the plateau phase, probably due to changes in the cytoskeleton (4), a process that is also seen at sustained perfusion pressures resulting in adaptation of SACs (34). In another study, Glogauer et al. (20) showed that repetitive force application progressively inhibited the amplitude of the force-induced [Ca\(^{2+}\)]\(_i\) increase, which is compatible with our observation that maximum value and time constant of the increase in F\(_{\text{dev}}\) are not different between repetitive perfusion pulses and perfusion step.
From the present results we propose a likely mechanism for the Gregg effect. Increased coronary perfusion via hoop (circumferential) stress mechanically deforms (by stretching or changing shape) the membrane of cardiomyocytes. A basis for the deformation of the membrane of the cardiomyocytes by increased perfusion pressures is provided by the findings of Heslinga et al. (23), who showed in isolated perfused papillary muscles that an increase in coronary perfusion resulted in an increase in intramyocardial pressure. The membrane deformation changes ion fluxes through SACs, resulting in increased cardiac contractility. This hypothesis is supported by the findings that the Gregg response was related to capillary perfusion (13, 15) and changes in coronary vascular volume (3), but was not related to arterial endothelium or its released inotropic substances (12, 14, 32, 33). The identity of the coronary perfusion-induced increased cation influx is not yet clear and needs further experiments.

Gadolinium. The use of Gd$^{3+}$ to block stretch-activated ion channels has been questioned in literature (28, 9). Lacampagne et al. (28) showed that Gd$^{3+}$ blocks L-type Ca$^{2+}$ channels and Caldwell et al. (9) noted that most of the Gd$^{3+}$ in the presence of bicarbonate is bound to bicarbonate due to the equilibrium dissociation constants for Gd$^{3+}$. However, Caldwell et al.'s study (9) noted that it is a presumption that only free Gd$^{3+}$ can block SACs and that the assumption that Gd$^{3+}$ anion complexes, such as bicarbonate-Gd$^{3+}$, can block SACs, cannot be excluded.

The results of our Gd$^{3+}$ experiments, i.e., inhibiting the Gregg effect (Fig. 2 and Table 1), in bicarbonate conditions, show that Gd$^{3+}$ does block SACs in these conditions. Our experiments with another type of SAC blocker, streptomycin (19), show a dose-dependent inhibition of the perfusion-induced increase in F$\text{dev}$ (Table 1), confirming that SACs underlie the Gregg effect. These results also make it unlikely that the inhibition of the Gregg effect by Gd$^{3+}$ is a nonspecific side effect, because two different types of SAC blockers, Gd$^{3+}$ and streptomycin, would then have a similar nonspecific side effect. The findings of Nicolosi et al. (31) support the fact that Gd$^{3+}$ blocks SACs in bicarbonate conditions: Gd$^{3+}$ was able to eliminate the adverse effects of overstretching in isolated guinea pig papillary muscles using a solution with bicarbonate without affecting basal force development.

The experiments with the L-type Ca$^{2+}$ channel blocker verapamil (Table 1), which resulted in a decrease of basal force of 56.0 ± 4.5%, did not affect the perfusion-induced increase in F$\text{dev}$. These results, together with the absence of a decrease of basal force development (Table 1) and no change in basal [Ca$^{2+}$], transient (Fig. 4) by the addition of Gd$^{3+}$ in the presence of bicarbonate, indicate that L-type Ca$^{2+}$ channel blockade by Gd$^{3+}$ does not occur in bicarbonate conditions. Boland et al. (6) also showed that Gd$^{3+}$ did not inhibit voltage-activated Ca$^{2+}$ channels in the presence of bicarbonate. Our results and literature thus show clearly that Gd$^{3+}$, in the presence of bicarbonate, does not block L-type Ca$^{2+}$ channels.

Gd$^{3+}$ also reduces basal force development and inhibits the perfusion-induced increase in F$\text{dev}$ in the HEPES experiments (Table 1). This indicates that in bicarbonate free conditions, “free Gd$^{3+}$” inhibits not only SACs but also L-type Ca$^{2+}$ channels, as mentioned by Lacampagne et al. (28). This suggests that the inhibition of L-type Ca$^{2+}$ channels by Gd$^{3+}$ depend on its conformation: the “free” form inhibits L-type Ca$^{2+}$ channels whereas the “bicarbonate-bound” form does not.

Therefore, our results and other studies (6, 31) show clearly that Gd$^{3+}$, in the presence of bicarbonate, does block SACs and that the perfusion-induced increase in F$\text{dev}$ (Gregg effect) is related to activation of SACs and not to L-type Ca$^{2+}$ channel blockade or a nonspecific side effect of Gd$^{3+}$.

Implications. Dankelman et al. (10, 11) clearly showed that the Gregg effect is present under normal physiological conditions; however, it is not observable due to the speed of autoregulation. The Gregg effect will be uncovered during ineffective autoregulation (16), which can occur in pathological situations, such as the onset of hibernation or distal from a stenosis, where autoregulation is restricted. When coronary perfusion increases, stretch of the membranes of cardiomyocytes will activate SACs, and cardiac contractility and oxygen consumption will increase. When coronary perfusion decreases, reduced deformation of the membranes of cardiomyocytes will inactivate SACs, and cardiac contraction and oxygen consumption will be reduced. Therefore, the Gregg effect may constitute an immediate protective mechanism in preventing a mismatch between cardiac contractility and oxygen delivery. In low coronary perfusion conditions, when autoregulation is ineffective, the Gregg effect may constitute an immediate mechanism that increases the pump function of the heart after increased coronary perfusion. A similar statement was made by Merkus et al. (29), who found a shorter diastolic time fraction (earlier onset of relaxation) at lower perfusion pressures in anesthetized open-chest dogs. Changes in interstitial volume and changes in buffer capacity for ions were put forward as mechanisms, whereas the involvement of oxygen shortage or a NO pathway was excluded. These findings are similar for the Gregg effect, as mentioned in this study. Unfortunately, the role of SACs was not investigated by Merkus et al. (29), which would have provided more information on a common mechanism for the effect of perfusion changes on cardiac contraction. We conclude that brief, repetitive, or sustained increases in coronary perfusion augment cardiac contractility through activation of stretch-activated ion channels, whereas endothelial NO release and L-type Ca$^{2+}$ channels are not involved.

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