■ LABORATORY INVESTIGATIONS

Negative and Positive Inotropic Effects of Propofol via L-type Calcium Channels and the Sodium–Calcium Exchanger in Rat Cardiac Trabeculae

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Background: Conflicting opinions are present in the literature regarding the origin of the negative inotropic effect of propofol on the myocardium. This study aims to resolve these discrepancies by investigating the inotropic effects of propofol *via* the L-type calcium channels and the sodium–calcium exchanger (NCX).

Methods: The effect of 20 μg/ml propofol on force development was determined in rat cardiac trabeculae at different pacing frequencies and different extracellular calcium concentrations. Postrest potentiation, sodium withdrawal during quiescence, and the NCX inhibitor KB-R7943 were used to study changes in the activity of the reverse mode of the NCX by propofol.

Results: The effect of propofol on steady state peak force depended on pacing frequency and calcium concentration. A negative inotropic effect was observed at pacing frequencies greater than 0.5 Hz, but a positive inotropic effect was observed at 0.1 Hz and low calcium, which cannot be explained by an effect on the L-type calcium channel. Propofol enhanced postrest potentiation in a calcium-dependent manner. Sodium withdrawal during quiescence and the use of the specific NCX inhibitor KB-R7943 provided evidence for an enhancement of calcium influx by propofol *via* the reverse mode of the NCX.

Conclusions: The effects of propofol on the myocardium depend on pacing frequency and calcium concentration. The positive inotropic effect of propofol is associated with increased calcium influx *via* the reverse mode of the NCX. The authors conclude that the net inotropic effect of propofol is the result of its counteracting influence on the functioning of the L-type calcium channel and the NCX.

PROPOFOL is a widely used intravenous anesthetic with advantageous properties such as rapid emergence after cessation of infusion. However, it also has cardiovascular side effects, such as bradycardia, decreased systemic vascular resistance, hypotension, and decreased cardiac output.[1] In patients, negative inotropic effects on the left[2] and the right ventricle[3] were observed. *In vitro* studies revealed that propofol has L-type calcium channel-blocking properties,[4-6] which might explain the negative inotropic effects of propofol in the myocardi-

um.[7-13] It should be noted, however, that others did not observe this inotropic effect[14-16] or found it only at supraclinical concentrations.[17]

Studies on the effect of propofol on calcium handling by the sarcoplasmic reticulum (SR) yielded conflicting results. Propofol was found to prolong calcium transients[18,19] and to impair myocardial relaxation.[13,14] Recently, it was observed that propofol could decrease the availability of calcium and prolong intracellular calcium transients without having a negative inotropic effect.[19] However, possible interference due to changes in beating frequency complicate interpretation of this study. Others concluded that propofol did not influence SR calcium handling.[20] Direct measurement of the effect of propofol on calcium handling by the SR proved that the SR was not affected,[21] although a recent study by Guenoun *et al.*[18] showed a decreased calcium uptake by the SR-calcium pump.

The objective of this study was to find an explanation for these contradictory results and to characterize the origin of inotropic effects of propofol in the myocardium in more detail. Force depends on the amount of free calcium present in the myocyte and the calcium sensitivity of the myofilaments. The actions of the L-type calcium channel, the ryanodine receptor (calcium release channel), the SR-calcium pump, and the sarcolemmal sodium–calcium exchanger (NCX) determine the amount of free calcium, and their relative contributions depend on pacing frequency and calcium concentration.[22] In the literature, a range of both pacing frequency and calcium concentration was used in the study of the inotropic effects of propofol. Therefore, we assessed the effect of propofol on the force–frequency relation in isolated rat right ventricular trabeculae at two different extracellular calcium concentrations: 0.5 mM and 1.36 mM.

Our results demonstrate that pacing frequency and extracellular calcium concentration significantly influence the inotropic effects of propofol. Surprisingly, we found that propofol can also exert a positive inotropic effect during certain experimental conditions. Blockade of the L-type calcium channels cannot provide an explanation for this observation. We hypothesized that this positive inotropic effect could be explained by alterations in calcium handling *via* the NCX. By studying postrest potentiation of force (PRP), we tried to gain more insight in the influence of the NCX on SR calcium load. Moreover, using a sodium-free superfusate and the

NCX inhibitor KB-R7943, evidence was obtained that propofol facilitates calcium influx *via* the reverse mode of the NCX.

Materials and Methods

Preparation

After we obtained approval from the institutional animal care and use committee of VU University Medical Center, male LBN F1 rats (weight, 275–450 g) were anesthetized by an intraperitoneal injection of pentobarbital sodium (60 mg/kg). The hearts were rapidly excised and retrogradely perfused at room temperature in a Langendorff perfusion system with a saturated (95% O_2–5% CO_2) Tyrode solution (pH 7.4). The Tyrode solution contained 128.8 mM NaCl, 4.7 mM KCl, 0.5 or 1.36 mM $CaCl_2$, 1.05 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 20.2 mM $NaHCO_3$, and 11.1 mM glucose. During the initial phase of the experiment, 30 mM 2,3 butanedione monoxime was added to the solution to inhibit myocardial contraction and prevent heart muscle damage during dissection.[23]

The right ventricle was carefully opened, and suitable trabeculae (diameter, 60–210 μm; length, 2–4 mm) running from the atrioventricular ring to the free ventricle wall were dissected. After excision, the preparation was mounted between a force transducer (AE801; SensoNor, Horten, Norway) and a micromanipulator in a muscle bath and superfused with Tyrode solution without 2,3 butanedione monoxime. Two platinum electrodes, which ran parallel to the longitudinal axis of the preparation, were used for field stimulation (rectangular pulses, 5 ms in duration). Force was recorded on a chart recorder and stored on a personal computer at a sampling rate of 200 Hz.

Stabilization of at least 60 min was allowed for the trabeculae to reach steady state force of contraction. During the last phase of the stabilization period, optimal length (L_{max}; the muscle length at which active force development is maximal) was determined. Muscle diameter was measured through a binocular dissection microscope at 50× magnification using a calibrated reticule. Stimulation voltage was set at three times the stimulation threshold to ensure that stimulation was supramaximal also in the presence of propofol. Experimental temperature was set at 27 ± 0.1°C. For each preparation, maximum isometric force per cross-sectional area was calculated.

Dose–Response Curve

The effect on steady state force of contraction was determined at concentrations of propofol ranging from 1 to 50 μg/ml, in a cumulative fashion, in the presence of both 1.36 and 0.5 mM calcium. The preparations were paced at a frequency of 1 Hz. At the end of the protocol, a control measurement of developed force was performed at the appropriate calcium concentration in the absence of propofol. All absolute forces were normalized to time-corrected control forces, which were obtained through linear interpolation of the control values measured at the start and the end of the experimental series. In all other experiments, propofol was used in a final concentration of 20 μg/ml (112 μM).

Steady state Force–Frequency Relation

The effect of propofol on the steady state force of contraction was studied at pacing frequencies ranging from 0.1 to 2 Hz, in random order, in the absence and presence of propofol. Within one experiment, all peak forces were normalized to the force found at 1 Hz in the absence of propofol (F_o). The effect of propofol on the force-frequency relation was characterized at two different extracellular calcium concentrations, 0.5 mM and 1.36 mM, in separate experiments. The inotropic effect of propofol was calculated by expressing the force found in the presence of propofol to the force found at the same pacing frequency in the absence of propofol. In control experiments, trabeculae were superfused with a Tyrode solution containing the lipid vehicle (Intralipid 10%) in the same concentration as during the propofol experiments (0.02%). In control experiments, the specific blocker of the reverse mode of the NCX, KB-R7943, was used at a concentration of 5 μM[24] to assess the role of the reverse mode of the NCX in the force–frequency relation.

Postrest Potentiation of Force

In these experiments, at 0.5 mM Ca^{2+}, trabeculae were paced at 1 Hz. After obtaining steady state force of contraction, pacing was interrupted for a period ranging from 2 to 60 s, in random order. Following this rest interval, stimulation was resumed to study PRP. Relative potentiation was quantified by normalizing peak force of the first potentiated contraction (F_p) to the steady state force recorded immediately before the rest interval (F_{ss}): F_p/F_{ss}. This protocol was performed in the absence and presence of propofol. The relation between interval duration and PRP was studied when steady state force of contraction was titrated back to F_o by adding extra $CaCl_2$ (1 M stock solution) to the Tyrode solution, in the presence of propofol. This was done to assess whether the effect of propofol on PRP could be explained by a decrease in the amount of calcium held by the SR, due to its L-type calcium channel-blocking properties. In control experiments, the specific blocker of the reverse mode of the NCX, KB-R7943, was used at a concentration of 5 μM[24] to establish whether the effects are mediated by the reverse mode of the NCX or can be explained by inhibition of the L-type calcium channels alone.

Reverse Mode of the Sodium–Calcium Exchanger

Another group of experiments was designed to obtain more insight in the effects of propofol on the activity of the reverse mode of the NCX in 0.5 mM calcium. The protocol used was as follows. The trabeculae were paced at 1 Hz in 0.5 mM calcium until a steady state was reached. Then stimulation was stopped and superfusion was subsequently switched to a caffeine (20 mM)-containing Tyrode to deplete the SR of calcium. One brief caffeine pulse was sufficient to prevent force development, but 2–3 caffeine pulses were administered to ensure that all releasable SR calcium had been released. Thereafter, superfusion was switched to a Tyrode containing 128.8 mM tetramethylammonium chloride (TMA), which replaced sodium chloride in the normal Tyrode. This replacement changes the thermodynamic driving force of the NCX and promotes calcium influx[25] *via* the reverse mode of the NCX. This calcium influx caused contractile activation and force development. The trabeculae were superfused with TMA–Tyrode for a period of 30, 60, and 120 s, respectively. At the end of each period, superfusion was switched to normal Tyrode (128.8 mM Na^+) containing caffeine (20 mM). This caused release of calcium taken up by the SR during TMA incubation. The maximum force was used as an index of the amount of calcium taken up during TMA incubation. The preparation was kept in the caffeine solution until force relaxation was complete. Then superfusion was again switched to TMA–Tyrode, and the next superfusion period commenced. To assess time-dependent effects on force development as well as the effect of propofol administration and withdrawal, the trabeculae were paced at 1 Hz between control–TMA and propofol–TMA measurements. A steady state negative inotropic effect usually occurred within 10 min after the start of propofol superfusion. At this point, pacing was stopped and the TMA protocol was executed as described above, in the presence of propofol. In additional control experiments, the specific blocker of the reverse mode of the NCX, KB-R7943, was used at a concentration of 5 μM[24] to establish whether the studied effects are specific for the reverse mode of the NCX.

Statistical Analysis

Each preparation served as its own control. All control and treatment values were obtained in paired experiments. All data, except those from PRP experiments, were analyzed by paired Student *t* tests. Data from PRP experiments were analyzed using analysis of variance statistics for between-group comparisons and evaluated further by Tukey *post hoc* tests. Data are expressed as mean ± SEM of n trabeculae. Effects were considered significant at $P < 0.05$.

Chemicals

Propofol at 10 mg/ml (1% Diprivan®) was purchased from Zeneca (Zoetermeer, the Netherlands). Intralipid® 10% was purchased from Fresenius Kabi Nederland BV ('s-Hertogenbosch, the Netherlands). Pentobarbital sodium (Nembutal®) was obtained from Sanofi BV (Maassluis, the Netherlands). 2,3 Butanedione monoxime was obtained from Sigma Aldrich Chemicals BV, and TMA was purchased from Fluka Chemie (Zwijndrecht, the Netherlands). KB-R7943 was a gift from Kanebo LTD (Osaka, Japan). Other components of the Tyrode solution were obtained from Merck Netherlands BV (Amsterdam, the Netherlands).

Results

Force Measurements

An illustration of the force recordings is shown in figure 1. In figure 1A a force recording is shown of a preparation paced at 1 Hz, in the absence of propofol, at 1.36 mM Ca^{2+}. Mean steady state peak force reached during these control conditions amounted to 66.3 ± 5.4 mN/mm² (n = 7). Figure 1A also shows the effect of an interruption of stimulation for about 12 s. The first contraction after such a period of rest is potentiated. Peak force returned to the steady state value found before the period of rest in about 5 s. Figure 1B illustrates the effects of 20 μg/ml propofol during these conditions. It can be seen that the peak steady state force is depressed by about 25% and that the relative magnitude of the postrest contraction is increased.

Figures 1C and D show recordings of an experiment where a calcium concentration of 0.5 mM was used. Mean steady state peak force amounted to 33.2 ± 4.7 mN/mm² (n = 7), *i.e.*, about half of the force obtained at 1.36 mM Ca^{2+}. At this low calcium concentration, propofol depressed force more than at 1.36 mM Ca^{2+}. Also, the relative potentiation of the first postrest contraction was larger than at the higher calcium concentration.

Contraction duration was measured at 50% of peak force at a pacing frequency of 0.1 and 1 Hz. At 1 Hz and 1.36 mM Ca^{2+}, control contraction duration amounted to 0.21 ± 0.01 s. By adding propofol there was a small but significant decrease to 0.20 ± 0.01 s ($P < 0.05$). At 1.36 mM Ca^{2+}, 0.1 Hz, and at 0.5 mM Ca^{2+}, 0.1 and 1 Hz, no effect of propofol on contraction duration was observed.

Dose–Response Curve

In figure 2, the effect of propofol on force of contraction is shown (n = 7) at concentrations ranging from 1 to 50 μg/ml, and [Ca^{2+}] of 1.36 mM. Usually it took 10–15 min before a new steady state was obtained on a change in the concentration propofol. At concentrations
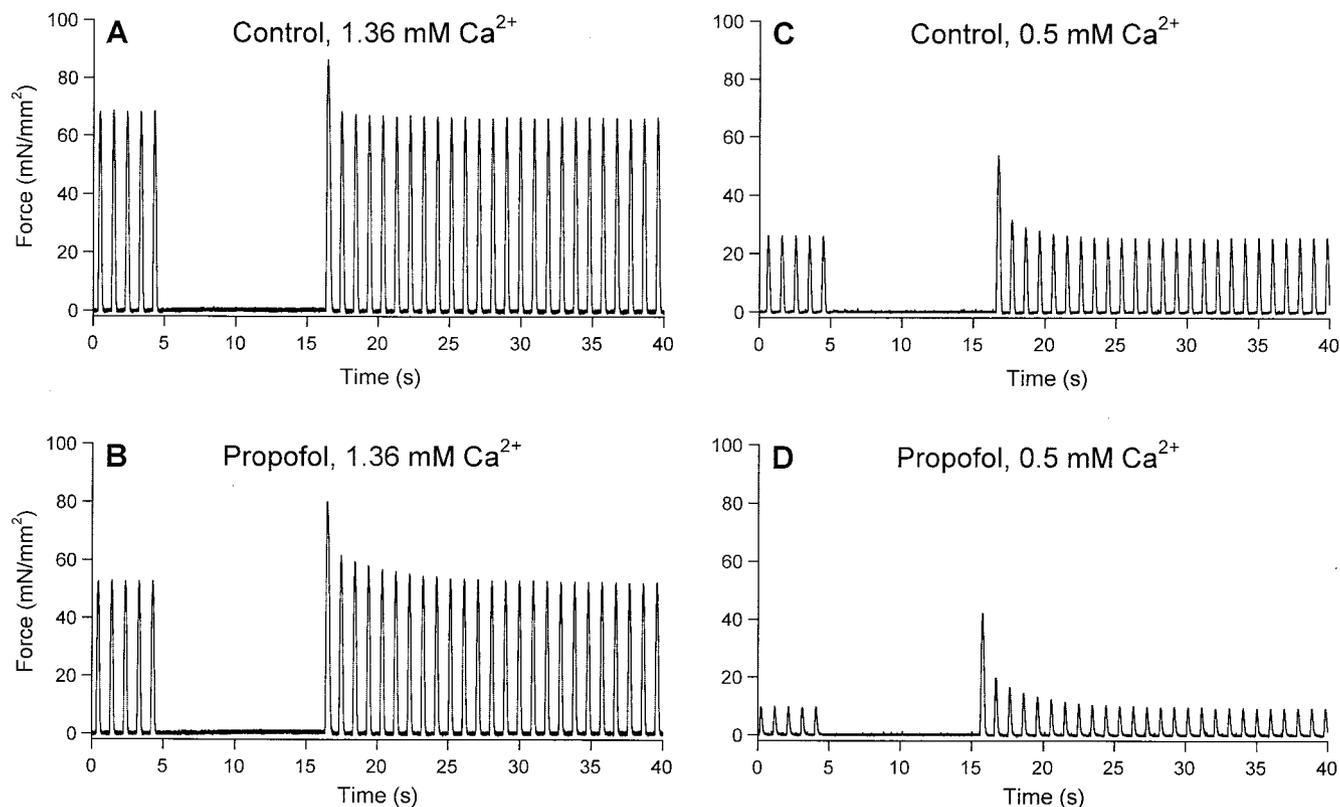
Fig. 1. Original tracing of force development of a preparation paced at 1 Hz during control conditions and in the presence of 20 μg/ml propofol at a [Ca^{2+}] of 1.36 mM (A and B) and 0.5 mM (C and D). Steady state contractions and postrest potentiation after a 12-s rest interval are shown. Lowering of [Ca^{2+}] increases the negative inotropic effect of propofol. However, propofol caused a relative increase of peak force of the first postrest contraction compared with steady state force.

from 5 μg/ml, a significant depression of contractile force was observed ($P < 0.01$). In four of these seven trabeculae, the dose-response curve was also determined at 0.5 mM calcium.

Force-Frequency Relation

The force-frequency relation was obtained by stimulating the trabeculae at frequencies ranging from 0.1 to 2 Hz. Usually it took about 10 min before a steady state was attained on a change in frequency. In figure 3A, the force-frequency relation at 1.36 mM Ca^{2+} is shown in the absence and presence of propofol (n = 7). During control conditions, an increase in stimulation frequency from 1 to 2 Hz caused a small, nonsignificant increase in peak force. At lower stimulation frequencies a more pronounced increase in peak force was observed. Relative force was maximal at 0.1 Hz and amounted to 1.25 ± 0.13 of F_o. Propofol caused a significant reduction in the steady state force both at 1 and 2 Hz, to 0.75 ± 0.02 and 0.82 ± 0.03 of F_o, respectively, which
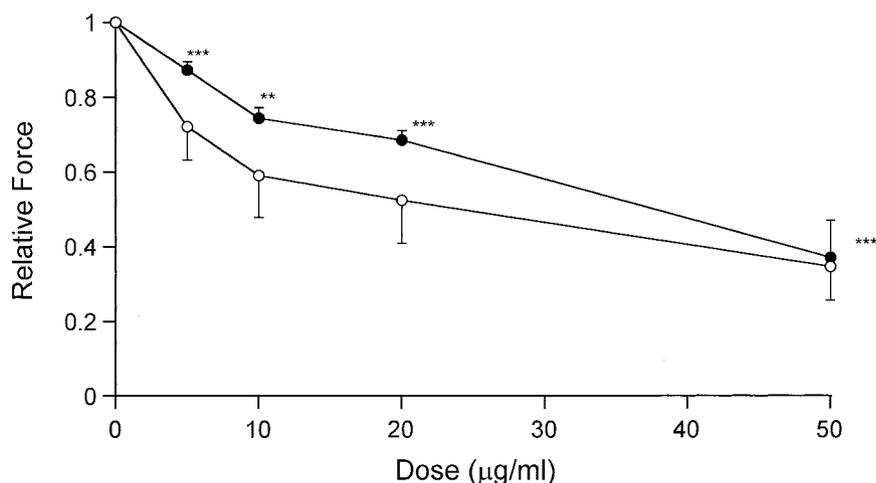
Fig. 2. Dose–response curve of propofol in concentrations ranging from 1 to 50 μg/ml, at a calcium concentration of 1.36 mM (n = 7, closed circles). At concentrations of 5 μg/ml and higher, propofol induced a significant depression of force development ($P < 0.01$; ***$P < 0.001$). The open circles represent the dose–response curve of propofol at an external calcium concentration of 0.5 mM.**
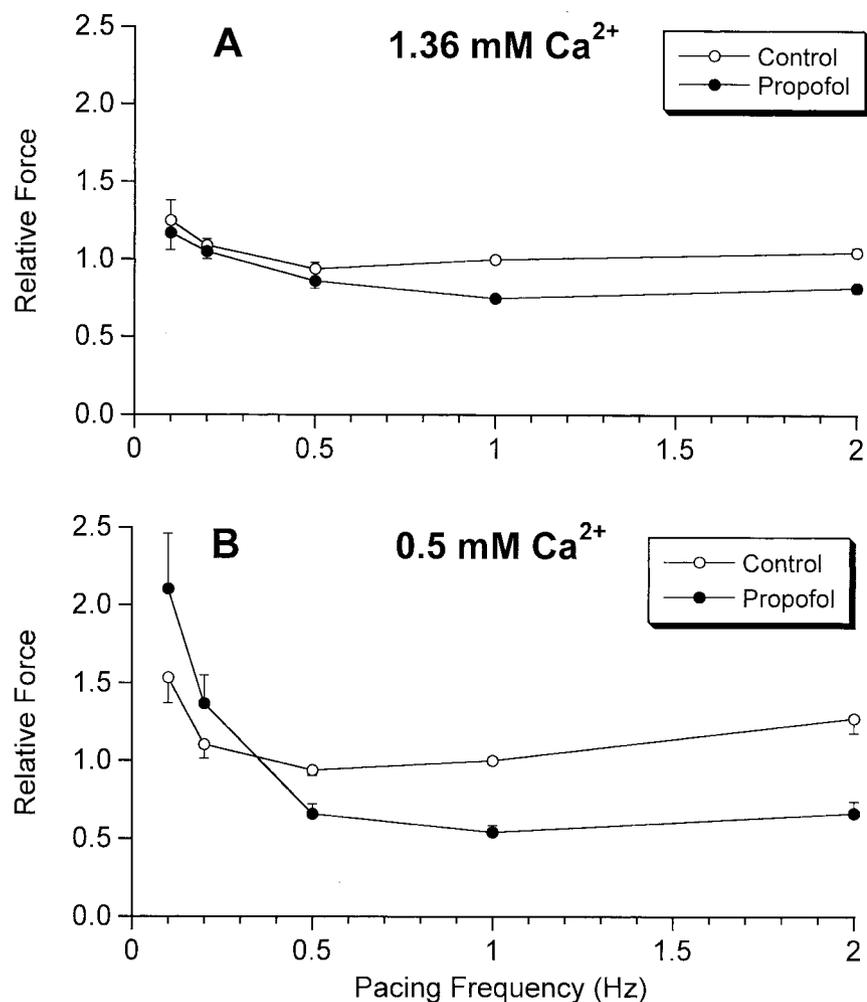
Fig. 3. Force–frequency relations of trabeculae at 1.36 mM (*A*) and 0.5 mM (*B*) Ca^{2+}. Steady state forces were normalized to control force at 1 Hz. Open circles represent relative force during control conditions. Filled circles represent relative forces in the presence of 20 μg/ml propofol. At 1.36 mM Ca^{2+}, propofol had a significant negative inotropic effect at 1 and 2 Hz but no effect at lower frequencies. At 0.5 mM Ca^{2+}, propofol had a significant negative inotropic effect at 0.5, 1, and 2 Hz but a significant positive inotropic effect at 0.1 Hz (mean ± SEM).

is consistent with the L-type calcium channel-blocking properties of propofol. The negative inotropic effect, expressed relative to the steady state forces obtained at the same frequency, was highly significant and amounted to 25 ± 2% at 1 Hz and to 22 ± 4% at 2 Hz ($P < 0.01$). However, the effect of propofol on force at frequencies less than 1 Hz was small or even absent. There was no significant inotropic effect of propofol at these pacing frequencies.

The effect of propofol on the force–frequency relation was also studied at 0.5 mM Ca^{2+} (fig. 3B). In general, it appeared that pacing frequency and propofol affected force of contraction more pronounced than at 1.36 mM Ca^{2+}. Remarkably, a positive inotropic effect of propofol was observed at pacing frequencies less than 0.5 Hz. Superimposed on the positive inotropic effect at 0.1 Hz in the control situation, propofol caused a further increase in steady state peak force of 36 ± 27% ($P < 0.05$) relative to force measured at 0.1 Hz in the absence of propofol. Control experiments (n = 3) showed that KB-R7943 caused an inhibition of this positive inotropic effect by 31 ± 12%. The negative inotropic effect of propofol, observed at 0.5–2 Hz, reached a maximal value

of 48 ± 7% at 2 Hz ($P < 0.01$). KB-R7943 did not influence this negative inotropic effect at 1Hz. Three experiments performed at 37°C, where the NCX is supposed to be more active, showed a similar trend. At the pacing frequency used (0.1 Hz), force in the presence of propofol increased by a factor of 2.0 ± 0.6. Inotropic effects of the lipid vehicle were tested at 0.1 and 2 Hz, at 1.36 and 0.5 mM of Ca^{2+} in each of four trabeculae. At 0.1 Hz, force increased by 2 ± 20% at high Ca^{2+} concentration and decreased by 16 ± 16% at low Ca^{2+} concentration (not significant). At 2 Hz, the increase amounted to 10 ± 13% and 29 ± 27%, respectively (not significant).

Postrest Potentiation of Force

As shown in figure 1, propofol appears to affect the magnitude of PRP, in particular at 0.5 mM of Ca^{2+}. These effects were studied in more detail at 0.5 mM Ca^{2+} by varying the duration of the pauses from 2 to 60 s, where saturation of PRP became evident (n = 5; fig. 4A). During control measurements, relative potentiation (F_p/F_{ss}) increased significantly with increasing duration of the rest intervals until a plateau was reached at 10-s intervals.

Fig. 4. Postrest potentiation (F_p/F_{ss}) of the first postrest contraction (F_p) relative to steady state force (F_{ss}), as a function of rest interval duration, at 0.5 mM Ca^{2+}. (*A*) Open circles = control; filled circles = 20 µg/ml propofol; filled triangles = 20 µg/ml propofol + extra calcium, to regain control steady state force. The significant effect of propofol on postrest potentiation was abolished after adding extra calcium (n = 5). (*B*) Open circles = control; filled circles = 20 µg/ml propofol; open squares = 5 µM KB-R7943; filled squares = 20 µg/ml propofol + 5 µM KB-R7943 (n = 6). KB-R7943 did not influence the effect of propofol on postrest potentiation of force (mean ± SEM).
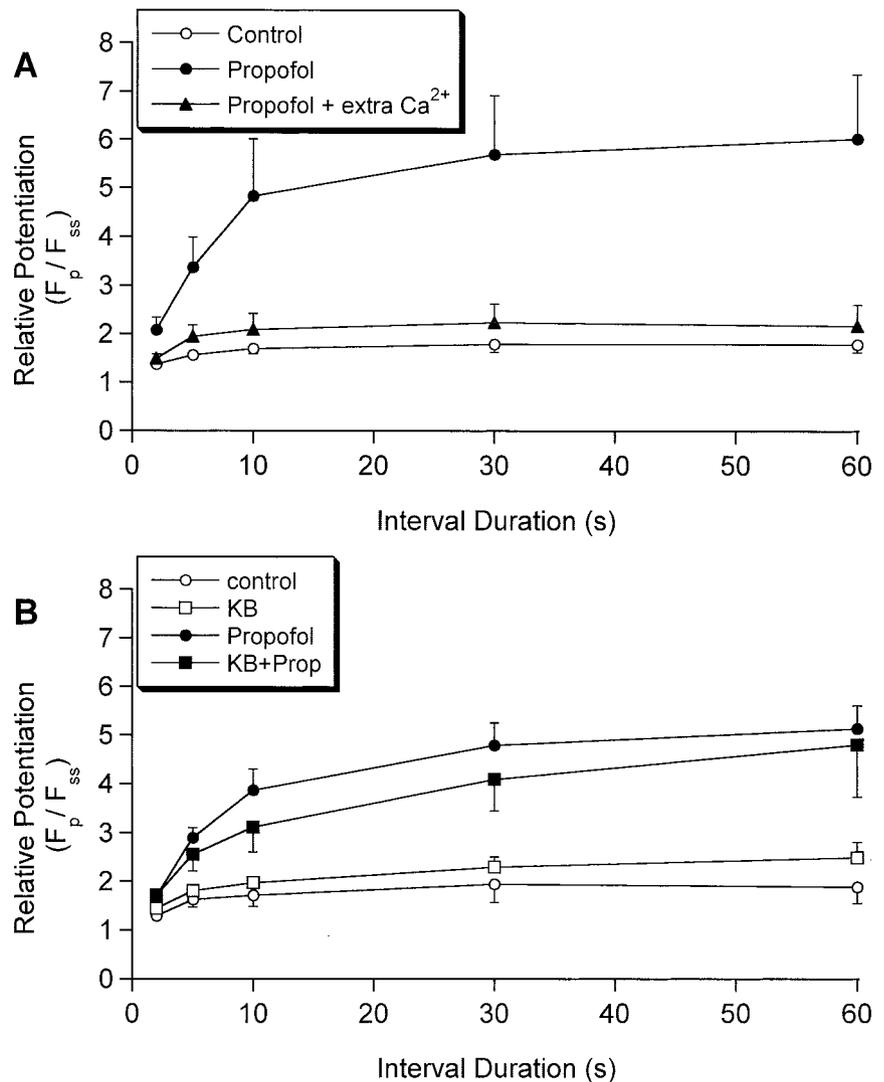
Relative potentiation ranged from 1.37 ± 0.06 at 2-s intervals to 1.79 ± 0.17 at 30- and 60-s intervals. It can also be seen in figure 4 that, compared with control, propofol caused a significant increase at 5-s intervals: F_p/F_{ss} amounted to 3.37 ± 0.62 (propofol) *versus* 1.56 ± 0.06 (control) (*P* < 0.05). PRP reached a maximum value of 6.03 ± 1.33 at 60-s intervals.

It should be noted that absolute force of the postrest contractions was depressed by propofol, presumably because of its L-type calcium channel-blocking properties. We investigated whether the increase in relative potentiation could be explained by a decrease of the preceding steady state force, due to the decrease in the amount of calcium held by the SR as a result of the action of propofol on the L-type calcium channels. To this end, the decreased steady state force, in the presence of propofol, was titrated back to the force, found in the absence of propofol, by adding extra $CaCl_2$ to the initial amount of calcium, 0.5 mM. On average, 0.37 ± 0.12 mM of extra $CaCl_2$ was required to regain control steady state force. It can be seen in figure 4A that after adding

calcium, the effect of propofol on the relative potentiation was markedly reduced. The magnitude of potentiation ranged from 1.49 ± 0.09 at 2-s intervals to maximally 2.23 ± 0.39 at 60-s intervals (not significant compared with controls). In separate experiments (n = 6), the influence of KB-R7943 on relative potentiation was assessed in the absence and presence of propofol (fig. 4B). Inhibition of the NCX by KB-R7943 had no effect on control values, nor did it affect the influence of propofol on relative potentiation.

Reverse Mode of the Sodium–Calcium Exchanger

Figure 5 illustrates the protocol used to study the reverse mode of the NCX. Here, TMA-induced contractures after 60 and 120 s of TMA incubation in the absence (fig. 5A) and presence (fig. 5B) of propofol are shown. At t = 0, superfusion was switched to the Tyrode where sodium chloride was replaced by TMA. The resulting calcium influx caused the development of a contracture. After 30, 60, and 120 s, respectively, superfusion was switched to normal Tyrode that contained
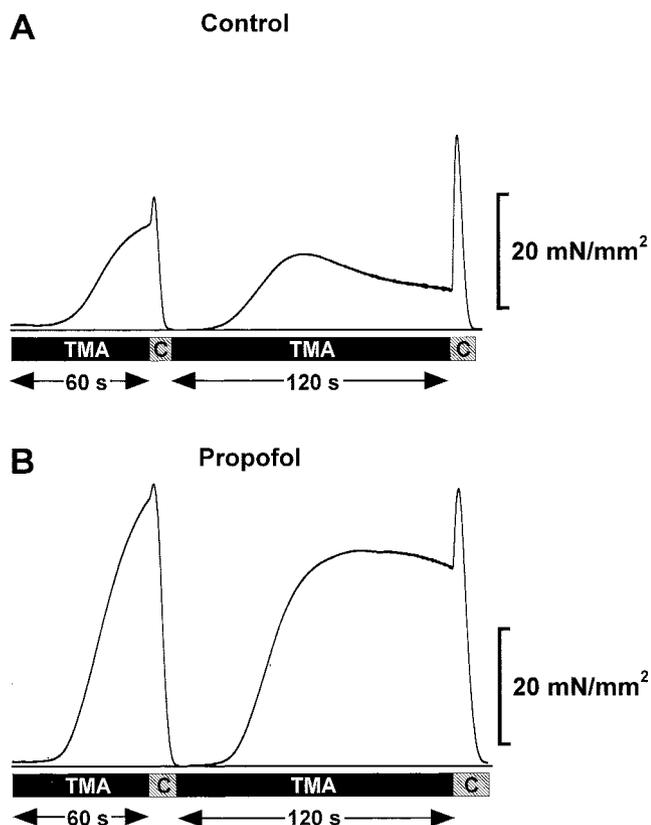
A   **Control**

20 mN/mm^2

TMA | C | TMA | C
◀—60 s—▶ ◀——120 s——▶

B   **Propofol**

20 mN/mm^2

TMA | C | TMA | C
◀—60 s—▶ ◀——120 s——▶

Fig. 5. Force development during tetramethylammonium chloride (TMA) incubations for a 60- and 120-s period. At t = 0, after emptying the sarcoplasmic reticulum (SR) with caffeine (20 mM), superfusion was switched from a NaCl-containing Tyrode to Tyrode containing 128.8 mM TMA to favor calcium influx *via* the reverse mode of the sodium calcium exchanger (indicated as TMA). Here, after 60 and 120 s of TMA incubation, superfusion was again switched to normal Tyrode containing caffeine (indicated as C) to release all SR calcium and to obtain maximum calcium activated force development.

caffeine to deplete the SR of calcium. Note the spike shaped force response, which is caused by the caffeine-induced release of calcium from the SR. Because of the presence of Na^+ in the Tyrode solution, calcium efflux

from the myocyte, through NCX, is facilitated, resulting in complete relaxation of force.

Figure 6 shows the mean caffeine-induced peak forces after 30, 60, and 120 s of TMA incubation in the absence and presence of propofol (n = 8). Peak force of the TMA-induced contractures in the absence of propofol increased from $16.5 \pm 4.8 \text{ mN/mm}^2$ after 30 s to $48.1 \pm 6.7 \text{ mN/mm}^2$ after 120 s of TMA superfusion. The presence of propofol during TMA incubation did not alter peak force after brief 30-s TMA superfusion ($13.3 \pm 3.9 \text{ mN/mm}^2$). However, propofol increased mean peak force of the contracture after prolonged incubation periods of 60 and 120 s. Mean peak force increased significantly from $36.2 \pm 7.6 \text{ mN/mm}^2$ (control) to $63.2 \pm 5.9 \text{ mN/mm}^2$ ($P < 0.01$). Also, after 120 s, TMA incubation peak force was increased from $48.1 \pm 6.7 \text{ mN/mm}^2$ to $66.3 \pm 6.3 \text{ mN/mm}^2$ ($P < 0.05$). Control experiments (n = 3) showed that KB-R7943 depressed the TMA-induced contractures in the presence of propofol by $89 \pm 7\%$, $81 \pm 9\%$, and $73 \pm 12\%$ at 30, 60, and 120 s, respectively. This indicates that the TMA-induced contractures are specific for the reverse mode of the NCX. In additional control experiments (n = 3) using TMA at $37°C$, the initial slope of the relation between force and the duration of TMA incubation was found to be about a factor 3 higher than at $27°C$. This corresponds with an increase in the speed of the NCX, with a $10°$ increase in temperature, by a factor of 3. In the presence of propofol, force of the contracture was maximally 3.79 ± 1.21 times higher than control values (120-s incubation).

Discussion

This study focuses on the origin of the inotropic effects of the intravenous anesthetic propofol on the myocardium. It is shown that the inotropic effect of propofol in rat myocardium depends on the extracellular calcium concentration and pacing frequency. At high pacing fre-
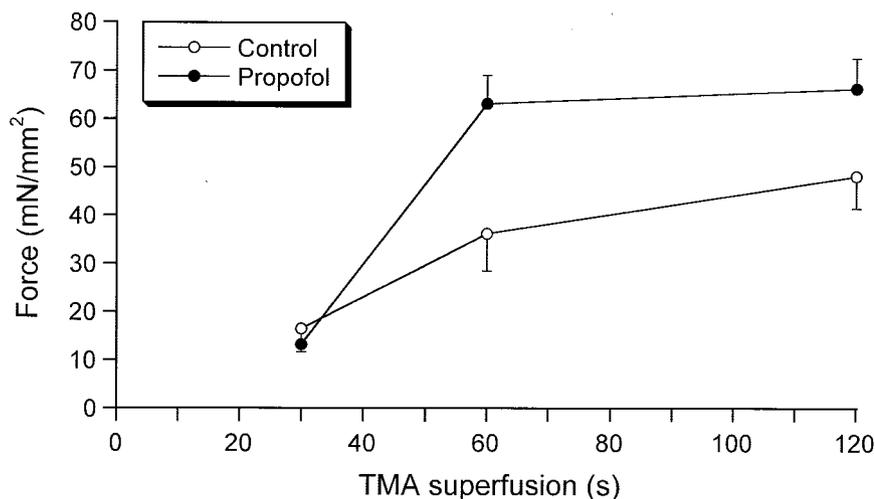

Fig. 6. Mean peak force of contraction after 30, 60, and 120 s of tetramethylammonium chloride (TMA) incubation and subsequent caffeine-induced release of SR calcium. After 60 and 120 s of TMA incubation, absolute force of the contractures increased significantly compared with control values (mean ± SEM).

quencies, a negative inotropic effect could be observed. However, at low pacing frequencies and 0.5 mM Ca^{2+}, a positive inotropic effect was observed. Inhibition of the L-type calcium channels by propofol cannot provide an explanation for this. Propofol affected PRP of force in a calcium concentration-dependent manner. Finally, it was shown that propofol enhanced the reverse mode of the NCX.

Force–Frequency Relation

In the literature[26] and in our experiments, heart muscle of rat is found to have a biphasic force–frequency relation: force decreases with increasing stimulation frequency to a certain minimum and increases again at higher pacing frequencies. Force development at low frequencies has been shown to depend on SR function.[26] The interaction between the SR and the sarcolemmal NCX, in determining cytosolic calcium concentrations and calcium load of the SR, has been established.[22,27] The increase of force at low pacing frequencies depends, in part, on intracellular sodium concentration and associated calcium influx *via* the reverse mode of the NCX during diastole.[28] The frequently observed potentiation of developed force at high pacing frequencies is induced by an increase of calcium influx *via* the sarcolemmal L-type calcium channels,[26] due to slow inactivation of these channels.[29] In our study, force development at 1.36 mM of calcium was affected by propofol in a pacing frequency-dependent manner. At 0.5 Hz and higher, a negative inotropic effect was observed, but at low pacing frequencies there was no significant inotropic effect. It has been shown that the inotropic effect of other anesthetics also decreases at low pacing frequencies.[30] The observed frequency dependence is consistent with the previous finding that propofol is an L-type calcium channel inhibitor.[26] This frequency dependence of the inotropic effect of propofol might partly explain the contradictory findings in the literature. In several studies, isolated myocytes and papillary muscles were paced at frequencies less than 0.5 Hz,[4,5,9,14–17,31] while in others higher pacing frequencies were used.[6,8,18,20] In addition, a recent study revealed that the negative inotropic effect of propofol was much more pronounced in guinea pig myocardium compared with that of rat.[21] This suggests that rat myocardium will be less sensitive to propofol than rabbit, guinea pig, and also human myocardium, where sarcolemmal calcium flux is more important compared with in the rat.[32–34]

The effect of propofol on force of contraction at 0.5 mM Ca^{2+} was distinctly different compared with its effect at 1.36 mM Ca^{2+}. The negative inotropic effect was more pronounced. This can be explained by the sigmoidal relation between calcium concentration and force development. At 1.36 mM of calcium, peak force development is nearly maximal, and the slope of the curve is small. At 0.5 mM of calcium, force development is submaximal and occurs in the steeper part of the calcium–force relation. Hence, a similar decrease in calcium influx, due to propofol, will have a more pronounced effect on force. This phenomenon can be used as a tool to detect changes in SR calcium load more clearly than would be possible at a high calcium concentration. In the literature, calcium concentrations ranging from 1 to 3.8 mM are used in the investigation of the inotropic effects of propofol. Our findings imply that the differences in magnitude of the observed inotropic effects among investigators can be explained, in part, by the differences in the extracellular calcium concentration. In addition, a positive inotropic effect was observed at low pacing frequencies and 0.5 mM calcium. Enhancement of contractile force at low pacing frequencies suggests an additional effect of propofol on calcium handling. Increased peak force at low pacing frequencies can be due to increased calcium content of the SR that is released during systole, or by an increased calcium sensitivity of the contractile proteins. The effect of propofol on the function of the SR remains unclear. Some investigators provide evidence for a decrease in calcium uptake, by the SR calcium pump, due to propofol.[14,18,19] Others could not establish this.[20,21] Direct measurements of calcium uptake showed that propofol did not affect SR calcium uptake, release, or storage.[21] A possible effect of propofol on calcium sensitivity of the contractile proteins was suggested in several studies[17,19] but ruled out by others.[9] Measurements of calcium sensitivity in Triton skinned trabeculae, in which the calcium concentration is heavily buffered, showed that indeed there was no direct effect.[21] However, a recent study revealed a possible indirect effect of propofol on the calcium sensitivity due to an increase in intracellular pH and the activation of protein kinase C.[35] The observed impairment of myocardial relaxation[13,14] may be due to these alterations of calcium sensitivity, but prolongation of calcium transients[18,19] cannot be explained in this way. An alternative explanation for the positive inotropic effect, and the aforementioned observations, might be found in a possible influence of propofol on calcium handling *via* the NCX. The attenuating effect of KB-R7943 on the positive inotropic effect of propofol is in support of this notion. Interestingly, volatile anesthetics[36] and the antibiotic Adriamycin[37] have been shown to inhibit the forward mode of the NCX. Adriamycin was found to have negative as well as positive inotropic effects on the myocardium,[38] similar to our current observations.

Postrest Potentiation

Postrest potentiation was used as a tool to obtain more insight in a possible shift in the interplay between the NCX and SR-calcium pump by propofol. Although PRP has been shown to represent an intrinsic property of the SR in rat,[39] evidence also suggests that the NCX plays a role in the calcium homeostasis[27,40,41] and may therefore contribute to PRP. We found that administration of

propofol only mildly depressed active force of the first postrest contraction but strongly increased the ratio between the force of the first contraction and the preceding steady state force (PRP). Adding extra calcium to compensate for the inotropic effect on steady state peak force abolished the effect of propofol on PRP. In addition, KB-R7943 had no effect on relative force during control measurements or in the presence of propofol. This shows that, at least in part, the effect of propofol on PRP of force can be explained by its effects on L-type calcium channels. This notion is in agreement with the finding that L-type calcium channel blockers have little effect on peak force of the first postrest contraction.[42]

Reverse Mode of the Sodium–Calcium Exchanger

The NCX is an electrogenic sarcolemmal antiporter linking calcium efflux from the cytosol to sodium influx (forward mode). However, the NCX can also reverse its action, thereby increasing calcium and decreasing sodium load of the myocyte (reverse mode). This bimodal action is determined by the concentration gradients of sodium and calcium across the sarcolemma.[40] Theoretically, the NCX could induce a positive inotropic effect by an inhibited forward mode or an enhanced reverse mode. Both will cause an increase in SR-calcium load. In the rat it has been shown that during diastole, calcium influx occurs *via* the reverse mode of the NCX, resulting in an increase of peak force at low pacing frequencies.[28,43] Possible effects of alterations in calcium handling *via* the NCX might become apparent at low pacing frequencies, because of the longer diastolic periods. As argued above, at this pacing frequency, force generation relies more on SR function rather than L-type calcium channels.

By replacing NaCl in normal Tyrode solution with TMA during quiescence, a reversal of the NCX was evoked, resulting in calcium influx and the development of a contracture. This calcium influx *via* the reverse mode was shown to be inhibited by the specific inhibitor KB-R7943 at 23 and 36°C.[25] To create a common starting point for control and propofol experiments with respect to SR-calcium load, the SR was emptied by means of caffeine. During TMA incubation SR-calcium pump activity was not inhibited. To enable us to link the amount of calcium influx to the development of force, caffeine was applied at the end of each TMA superfusion interval to release calcium from the SR. The significant increase of contractile force after propofol–TMA incubations of 60 and 120 s clearly demonstrates an increase in activity of the reverse mode of the NCX due to propofol. The absence of an effect at 30 s of TMA incubation most likely reflects the presence of diffusion delays inside the trabeculae. Enhanced calcium influx provides an explanation for the observed positive inotropic effect of propofol. It should be noted, however, that the increase in calcium load of the myocytes could either be a direct effect of propofol on the NCX or due to an indirect effect mediated through an increase of intracellular sodium concentration. In a recent study by Kanaya *et al.*,[35] evidence was obtained in support of an indirect effect. Propofol was found to enhance the Na^+–H^+ exchanger. The associated increase in intracellular sodium concentration may induce changes in NCX activity, leading to the positive inotropic effect observed in this study. Inhibition of calcium efflux *via* the NCX due to an increase in intracellular sodium concentration might also explain the prolongation of calcium transients[17–19] and possibly the impaired relaxation of the myocardium[14,17] observed in the presence of propofol.

Clinical Relevance

The propofol concentration used in this study is approximately 100 times the free propofol concentration in humans. This implies that caution should be exerted when assessing the clinical relevance of this study for humans. However, it should be noted that there are several complicating factors with regard to the upper limit of clinically relevant concentrations of propofol, as discussed in detail by Kanaya *et al.*[17] The specific goal of our study was to assess whether pacing frequency and calcium concentration modulate the inotropic effect of propofol, and the findings might serve as an explanation for some of the conflicting results in the literature. While varying these experimental variables, propofol concentration was kept constant. We consider it safe to assume that the effects found in this study will be smaller at clinically relevant concentrations of propofol. However, we expect that the underlying mechanism will be preserved. Further insight on the action of propofol on the NCX may be obtained by studying the concentration dependence of alterations in transmembrane ion fluxes. It should also be noted that the effects of propofol are species-dependent,[21] which warrants the use of a relatively high propofol concentration in experiments on rat tissue.

The absolute force values showed the same dependencies as those in the relative force values. We chose to present the data as relative effects to visualize relative changes of contractile force caused by alterations in pacing frequency or incubation with propofol, but without the variance introduced by differences in absolute contractile force or time-related decay of force. These are specific properties of the preparation.

Most experiments were performed at an ambient temperature of 27°C. Although evidence was obtained that the observed effects also occur at body temperature, the relative contribution of the different calcium handling structures could be altered. This does not influence the mechanistic alterations observed in the current study. The reverse mode of the NCX operates during every heartbeat. It is conceivable that both mechanisms, *i.e.*, depression of L-type calcium channels and stimulation of

the reverse mode of the NCX, operate at the same time. The negative inotropic (L-type channel) effect of propofol during physiologic conditions will be counteracted to some extent by the positive inotropic effect. In relation to the clinical practice, it should be noted that in human ventricular cardiac myocytes, the NCX is more important in determining cellular calcium load than in the rat.[33]

In conclusion, our results confirm the negative inotropic effect of propofol found by several other investigators. This effect, however, is modulated by pacing frequency and extracellular calcium concentration. A positive inotropic effect was observed at low pacing frequencies and low calcium concentration. Calcium influx *via* the reverse mode of the NCX was significantly enhanced by propofol. We conclude that the net inotropic effect of propofol may be the result of its counteracting actions on the L-type calcium channel and the NCX.

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