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# The differential effect of propofol on contractility of isolated myocardial trabeculae of rat and guinea-pig

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**1** The effects of propofol on myocardial contractility were studied in rat, in which the contractile activation mainly depends on calcium derived from the sarcoplasmic reticulum (SR), and guinea-pig, in which transsarcolemmal influx of calcium plays a major role.

**2** Intact and chemically skinned trabeculae from the right ventricle were studied. Intact trabeculae were electrically stimulated and force development during steady state and post rest contractions was measured. In saponin skinned trabeculae $Ca^{2+}$ uptake and release by the SR was studied. In Triton skinned trabeculae the influence of propofol on calcium sensitivity of the myofilaments was studied.

**3** In intact rat trabeculae propofol in concentrations of 28, 112 and 280 $\mu$M did not change peak force development nor the pattern of post rest contraction. In guinea-pig trabeculae propofol significantly reduced peak force to respectively 64, 40 and 23% of control values and the post rest contractions were potentiated. In skinned trabeculae propofol did not affect $Ca^{2+}$ handling by the SR, nor did it change force production and $Ca^{2+}$ sensitivity of the myofilaments.

**4** This study shows that, in contrast to rat, in guinea-pig propofol directly depresses myocardial contractility, probably by decreasing transsarcolemmal $Ca^{2+}$ influx. There is no significant influence of propofol on $Ca^{2+}$ handling by the SR, nor on the contractile proteins.
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**Keywords:** Contractility; propofol; sarcoplasmic reticulum; trabeculae

**Abbreviations:** BDM, butanedionemonoxime; BES, N,N-bis(hydroxylethyl)-2-aminoethanesulphonic acid; C, caffeine; EGTA, ethylene glycol-bis($\beta$-aminoethylether)-N,N,N′,N′-tetraacetic acid; HDTA,1,6-diaminohexane-N,N,N′,N′-tetraacetic acid; s.e.e., standard error of the estimate; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid

## Introduction

The influence of propofol on myocardial contractility has been studied in different animal models and isolated heart preparations (Brussel *et al*., 1989; Coetzee *et al*., 1989; De Hert *et al*., 1990; Goodchild & Serrao, 1989; Ismail *et al*., 1992; Mayer *et al*., 1993; Pagel & Warltier, 1993; Puttick *et al*., 1992; Stowe *et al*., 1992). Depending on study design and studied species the conclusions differ from no effect of propofol on contractility to a significant depression in contractility. Studies on electrically stimulated isolated papillary muscles of the rat (Riou *et al*., 1992) and the guinea-pig (Park & Lynch, 1992; Puttick & Terrar, 1993) showed a slight to moderate reduction in intrinsic myocardial contractility and a decrease in isotonic relaxation. The mechanism of this reduction is suggested to be an impaired transsarcolemmal $Ca^{2+}$ influx, probably in combination with a slight decrease in calcium uptake by the sarcoplasmic reticulum (SR). Studies on isolated myocardial cells of rat and guinea-pig showed that propofol induces a lowering of free cytosolic calcium (Li *et al*., 1997) and that it inhibits L-type calcium channels (Sakai *et al*., 1996; Yang *et al*., 1996; Zhou *et al*., 1997). Recent studies on $Ca^{2+}$ transients in rat cardiomyocytes and intact guinea-pig hearts using fluorescence methods suggest an impairment of SR function and not of $Ca^{2+}$ L-type current by propofol (Guenoun *et al*., 2000; Nakae *et al*., 2000). The direct influence of propofol on the SR and on calcium sensitivity of the myofilaments nevertheless remains unclear. The aim of our study was to assess if there is an influence of propofol on the function of the SR that can explain the impaired relaxation rate or on the contractile proteins in rat and guinea-pig. In intact electrically stimulated cardiac trabeculae, SR function was studied by recording post rest contractions in which a transient change in contractility occurs after a short period of rest, a phenomenon influenced by $Ca^{2+}$ uptake and release by the SR. The influence of propofol on SR $Ca^{2+}$ uptake, storage and release was studied directly in saponin skinned trabeculae. Saponin (50 $\mu$g ml$^{-1}$) permeabilizes the sarcolemma, but leaves the intracellular organelles intact (Endo & Iino, 1980) making the SR directly accessable for investigation (Herland *et al*., 1992). The influence of propofol on the contractile proteins was investigated in Triton skinned trabeculae in which sarcolemma and SR membranes are solubilized while the contractile apparatus remains intact. Theoretically, propofol can influence the maximum force producing capacity or change the $Ca^{2+}$ sensitivity of force production by a direct effect on the contractile proteins.

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Therefore we measured the influence of propofol on force production at different $Ca^{2+}$ concentrations.

## Methods

### *Preparations*

The present experimental procedures were performed after approval from our institutional animal care committee. Rats and guinea-pigs were handled identically. Male Wistar rats weighing 300–460 g and female guinea-pigs (Dunkin Hartley) weighing 350–500 g were anaesthetized by an intraperitoneal injection of pentobarbital (50 mg $kg^{-1}$ body weight). The hearts were excised, the aorta cannulated and perfused according to Langendorff at room temperature with a saline solution containing (mM): NaCl 128, KCl 4.7, $MgCl_2$ 1.0, $NaH_2PO_4$ 1.4, $NaHCO_3$ 20, $CaCl_2$ 1.4, and glucose 11.1. The solution was gassed with 95% $O_2$ and 5% $CO_2$. Then 25 mM 2,3 butanedione monoxime (BDM) was added to the saline solution to stop the heart beating and to inhibit cell damage during dissection (Mulieri *et al*., 1989). Right ventricular trabeculae with a diameter of 80–250 $\mu$m and a length of 1.5–2 mm were carefully dissected and kept intact or skinned chemically. Skinning was carried out during 30 min. by either adding 1% (vol $vol^{-1}$) Triton X-100 or 50 $\mu$g $ml^{-1}$ Saponin to the solution. The diameter of the skinned trabeculae was measured at 50 × magnification in two perpendicular directions and cross-sectional area was calculated assuming an elliptical cross-section. In the skinned rat trabeculae cross-sectional area ranged from 0.006 to 0.033 $mm^2$ with a mean of $0.022 \pm 0.004$ $mm^2$ (mean ± s.e.mean) and in the skinned guinea-pig trabeculae it ranged from 0.010 to 0.051 $mm^2$ (mean $0.038 \pm 0.006$ $mm^2$). In the experimental set-up of the intact trabeculae it was only possible to measure the diameter of the trabeculae from above, therefore the cross-sectional area was measured assuming a cylindrical form. In the rat mean value was $0.038 \pm 0.010$ $mm^2$ and in the guinea-pig $0.031 \pm 0.008$ $mm^2$. The differences between rat and guinea-pig in cross-sectional area were not significant.

### *Intact trabeculae*

The preparations were suspended horizontally by means of aluminium T-clips in a muscle bath of 150 $\mu$l between a force transducer (SensoNor AE801, Norway) and a length adjustment device and continuously superfused with a Tyrode solution containing (mM): NaCl 144, KCl 6, $MgSO_4$ 1, $Na_2HPO_4$ 1, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES) 10, $CaCl_2$ 2, glucose 20 and pyruvate 2 at a rate of 2.5 ml $min^{-1}$ 2. The solution was equilibrated with 100% $O_2$, the pH was 7.5 and temperature was kept at 37°C. By means of a two-way switch it was possible to make a quick change in superfusate with and without propofol. Trabeculae were stimulated by two platinum electrodes, which ran longitudinal to the preparation, at a rate of 2 Hz and with a 5 ms stimulus duration at 120% of threshold intensity. The length of the preparation was adjusted to the passive length at which twitch force was maximal. After a stabilization period of 1 h the preparations were alternately superfused with Tyrode with and without propofol 28, 112 and 280 $\mu$M. When the force was stable at a new level, which lasted between 15 and 30 min, a post rest contraction test was performed by interrupting the stimulation for 10 s. Contractions were recorded on a chart recorder and after conversion to digital form on a personal computer at a sample rate of 200 Hz.

### *Skinned trabeculae*

*Apparatus* The experimental set-up consisted of a series of troughs of 80 $\mu$l. The trough in which the preparation was activated had a volume of 30 $\mu$l. This was continuously stirred by motor-driven vibration of a diaphragm at the bottom of the bath and the temperature was kept at $20 \pm 1$°C. The skinned trabeculae were mounted in the same way as the intact trabeculae. Force was recorded on a chart recorder and after A to D conversion on a computer at a sample rate of 5 Hz. Sarcomere length of the preparation was measured in relaxing solution by means of a 10 mW HeNe laser and adjusted to 2.2 $\mu$m.

*Solutions* All the solutions used in the experiments on skinned trabeculae contained 100 mM *N,N*-bis(Hydroxyethyl)-2-aminoethanesulphonic acid (BES, pH 7.1, adjusted with KOH), (mM): $Na_2ATP$ 5.81, free $Mg^{2+}$ 1, PCr 10 and Na-azide 5 to prevent $Ca^{2+}$ accumulation by mitochondria. K-propionate was added to adjust the ionic strength to 200 mM. Three types of solutions were used: relaxing solution with 20 mM Ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA, pCa = 9.0), pre-activating solution with 0.1 mM EGTA and 19.9 mM 1,6-diaminohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (HDTA), and activating solution with 20 mM CaEGTA (pCa = 4.3). pCa is defined as the negative logarithm of the free calcium concentration in the solution. The solutions were prepared using the equilibrium constants given by Fabiato (Fabiato & Fabiato, 1979). Solutions with lower free $Ca^{2+}$ concentrations were obtained by appropriate mixing of the relaxing and activating solutions, assuming an apparent stability constant for the Ca-EGTA complex of $10^{6.58}$. By adding 20 mM caffeine (C) to the relaxing and the pre-activating solutions relaxing-*C* and pre-activating-*C* solutions were obtained. Three concentrations of propofol were tested: 28 $\mu$M (5 $\mu$g $ml^{-1}$) 112 $\mu$M (20 $\mu$g $ml^{-1}$) and 280 $\mu$M (50 $\mu$g $ml^{-1}$). We used Diprivan® (AstraZeneca Pharma, Zoetermeer, The Netherlands) which consists of propofol dissolved in an emulsion of soya bean oil, glycerol and purified egg phosphatide. Diprivan® nor its solvent influenced the free $Ca^{2+}$ concentration in the solutions as measured with a Ca sensitive electrode (Radiometer, Copenhagen, Denmark).

### *Protocol to study SR function*

In saponin-skinned trabeculae the SR was depleted of $Ca^{2+}$ before each cycle of measurement by incubation in the relaxing-C solution (4 min). Then the preparations were transferred to the low EGTA pre-activating solution (30 s). The trabeculae were immersed for 5 min in the $Ca^{2+}$-loading solution (pCa = 6.3), which was prepared by appropriate mixing of the relaxing and the activating solutions. After loading the preparations were moved to the pre-activating solution (3 min) and then quickly transferred to pre-activating-C solution in the activation trough to release $Ca^{2+}$ from the SR. The calcium content present in the SR

was assessed from the area under the caffeine induced force transients (Endo, 1977). After contraction the preparations were returned to the relaxing-C solution to start the next activation cycle. After the first contraction the sarcomere length of the preparations was readjusted to 2.2 $\mu$m and the width and depth were measured. To measure its influence on SR $Ca^{2+}$ uptake, propofol was added to the loading solution. By adding propofol to the pre-activating solution and the pre-activating-C solution of the $Ca^{2+}$ release phase the influence on SR $Ca^{2+}$ storage and release was assessed.

### *Protocol to study Ca-sensitivity of force production*

Triton skinned preparations were consecutively incubated in relaxing solution (4 min), pre-activating solution (3 min) and activating solution (until steady force was reached) and then back in relaxing solution. The first activation-contraction cycle was carried out at saturating calcium concentration (pCa = 4.3). After this, the sarcomere length was readjusted if necessary and the width and depth of the preparation was measured. The force developed during the second maximal contraction served as a reference at pCa 4.3. The next contractions were carried out at different pCa values (>4.3), with and without propofol 28 and 112 $\mu$M in the solutions. At regular intervals control measurements at saturating pCa were performed and this procedure was continued until the full force-pCa curve was obtained or the force of a control measurement was less than 80% of the first reference measurement. To correct for the gradual deterioration of force development, the force measurements were normalized to an interpolation of control values.

### *Statistical analysis*

Data are expressed as mean ± s.e.mean. Parameters determined in the same trabecula without and with propofol were compared by a Students' *t*-test for paired observations. Differences are considered significant at $P<0.05$. The normalized force–pCa curve was fitted by a non-linear least square method using the Hill equation: $P(Ca^{2+}) = [Ca^{2+}]^{nH}/(K^{nH} + [Ca^{2+}]^{nH})$ by means of commercially available software (Kaleidagraph, Synergy Software, Reading, U.S.A.). In this equation nH represents the steepness of the relationship and K is the $Ca^{2+}$ concentration at which force P(K) is half maximal, i.e. the midpoint of the normalized force–pCa relation. Parameter values are given ± one standard error of the estimate (s.e.e.).

## Results

### *Intact trabeculae*

The protocol used to study steady-state force developments and the post rest contractions is illustrated in a trace of a single trabeculum in Figure 1. In the intact electrically stimulated trabecula of the rat propofol did not change peak force development at a stimulation frequency of 2 Hz (Figure 2). The first contraction after a rest interval of 10 s was potentiated compared to the last contraction before rest. Propofol 28 and 112 $\mu$M did not influence the normal pattern of post rest potentiation (Figure 1, upper part).

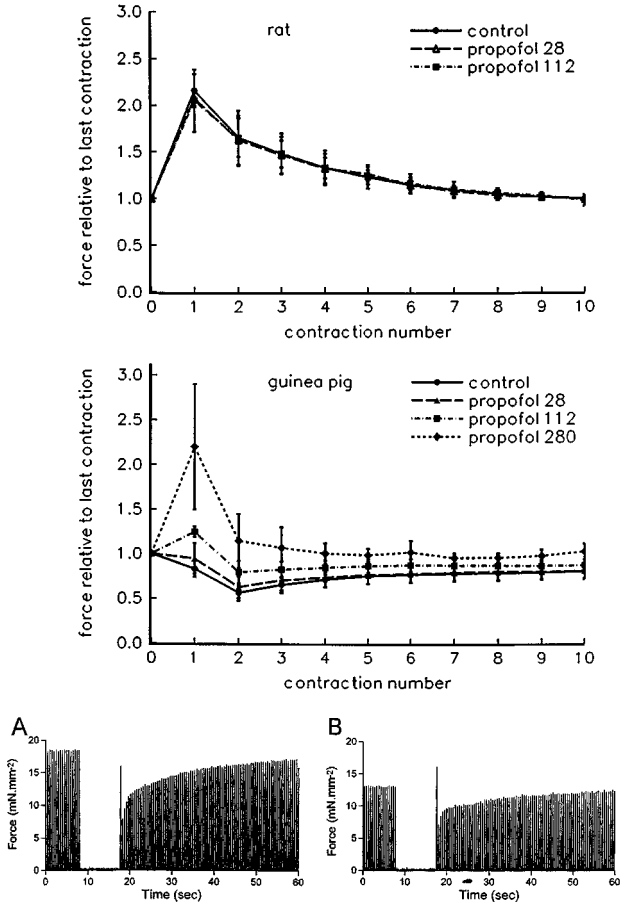


**Figure 1** The curves in the upper part show the effects of different concentrations (in $\mu$M) of propofol on the post rest contraction pattern in rat trabeculae ($n=5$) and in the middle part in guinea-pig trabeculae ($n=5$). The peak force of the first 10 contractions after interrupting the 2 Hz baseline stimulation for 10 s is expressed as a fraction of the force of the last contraction before the rest period. Data are mean ± s.e.mean. See also Figure 2: in guinea-pig contraction force decreases with increasing propofol concentration so at 280 $\mu$M propofol there is a 2.2 fold increase compared with a prerest level of 23% of control without propofol. In the lower part the protocol is illustrated by tracings of a single guinea-pig trabeculum. (A) Shows the results under control conditions and in (B) 112 $\mu$M propofol was added. Propofol causes a reduction in the steady state peak force and a relative potentiation of the first post rest contraction after 10 s of rest.

In the electrically stimulated trabeculae of the guinea-pig, however, propofol decreased peak force development (Figure 2). In concentrations of 28, 112 and 280 $\mu$M peak force was respectively reduced to 64 ± 11%, 40 ± 10% and 23 ± 9% of control values. The post rest contraction test without propofol showed a slight reduction in peak force of the first post rest contraction and a further decrease in peak force of the second contraction. From the third contraction on, peak force increased gradually, reaching the pre-rest level in 10–20 contractions (Figure 1, middle part). With 112 and 280 $\mu$M propofol in the superfusate a dose dependent potentiation of the first post rest contraction was seen. The relative peak forces of the following contractions normalised to the steady state peak force in the presence of propofol also showed a dose dependent increase. These observations were consistent in five guinea-pig trabeculae but one small trabeculae (cross sectional

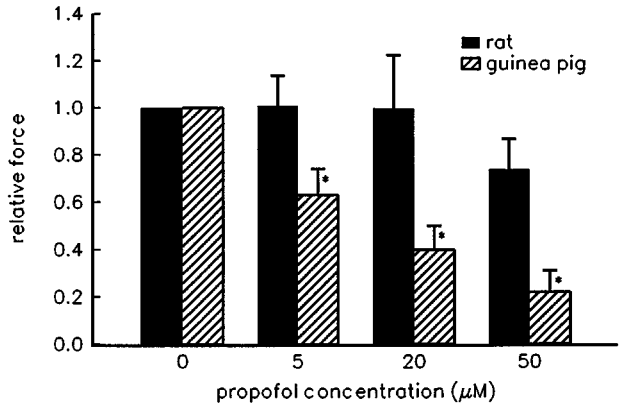


**Figure 2** Effects of propofol on peak force development of electrically stimulated trabeculae of rat and guinea-pig, expressed as a fraction of force development without propofol. Values are the means ± s.e.mean of five trabeculae of both species. *Significantly ($P<0.05$ different from control).

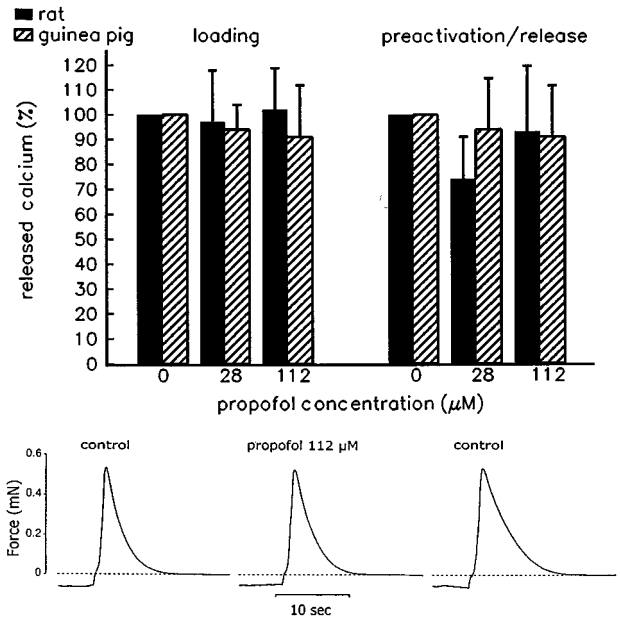


**Figure 3** The upper part shows the effects of different concentrations of propofol on the amount of released $Ca^{2+}$ from the SR of saponin skinned rat ($n=7$) and guinea-pig ($n=6$) trabeculae. On the left side the normalized force integrals are shown from rat and guinea-pig trabeculae in which 0, 28 and 112 $\mu$M propofol was added to the $Ca^{2+}$ loading solution. On the right side propofol of the same concentrations added to the preactivation solution, the preactivation-C solution and the caffeine containing solution in rat ($n=5$) and guinea-pig ($n=5$) trabeculae causing $Ca^{2+}$ release from the SR. Data are expressed as mean ± s.e.mean. The lower part is an example of force transients measured in a single saponin skinned rat trabeculum in which the SR is loaded with $Ca^{2+}$. Contraction is induced by immersing the preparation in a caffeine containing solution. Before the middle contraction the SR was loaded in a solution containing propofol 112 $\mu$M. The left and right contractions are controls (no propofol added). Dotted lines indicate the baseline of the force transducer output in the caffeine containing solutions. The negative level before the caffeine induced release was due to a difference in the offset of the force transducer in the chamber in which the preparation was immersed. The area under the curve is directly related to the amount of calcium released.

area 0.02 mm$^2$), harvested very close to the tricuspid valve, showed a relative stronger potentiation of the first post rest beat and a gradual decline in force during the following contractions in the presence of propofol, resulting in a pattern closely resembling that of post rest potentiation in the rat.

### *Skinned trabeculae*

In saponin-skinned trabeculae of the rat and the guinea-pig the influence of propofol on $Ca^{2+}$ loading, storage and release of the SR was assessed. In Figure 3 an example of force development is shown after loading and subsequent release of $Ca^{2+}$ from the SR in a single rat trabeculum. It was found that in rat trabeculae propofol added to the $Ca^{2+}$ loading solution did not significantly change the amount of $Ca^{2+}$ released by the SR, indicating that $Ca^{2+}$ loading of the SR was unaltered. After loading the SR in the absence of propofol, addition of propofol to the pre-incubation and release media did not change the amount of $Ca^{2+}$ released (Figure 3). This indicates that calcium leak during storage as well as caffeine-induced calcium release were not affected, because as shown below, propofol did not influence maximum force nor its calcium sensitivity. Similar results were obtained in trabeculae from guinea-pig, indicating that also in guinea-pig propofol had no influence on $Ca^{2+}$ handling of the SR (Figure 3).

By measuring isometric force in Triton skinned trabeculae at different $Ca^{2+}$ concentrations the influence of propofol on $Ca^{2+}$ sensitivity of the contractile proteins was compared with control measurements without propofol. An example of force development in a Triton skinned rat trabeculum after incubation in a $Ca^{2+}$ containing solution is shown in Figure 4. A control contraction at saturating $Ca^{2+}$ concentration (pCa 4.3) was followed by a contraction at pCa 5.8. Peak force and rate of force development decreased with increasing pCa. Figure 4 (upper part) illustrate the average normalized force – pCa relationships in rat trabeculae, in the absence and presence of propofol 112 $\mu$M. At none of the pCa values the difference in average force measured without and with propofol 112 $\mu$M was significant. These measurements with propofol 28 $\mu$M ($n=5$) showed comparable results. In Figure 4 (middle part) the same curve is shown for guinea-pig trabeculae obtained in the presence and absence on propofol 112 $\mu$M. The data were fitted to the Hill equation as described in Methods. It was found that the midpoint (pK) and the steepness (nH) of the relation did not differ significantly in the presence and absence of propofol. Hence also in guinea-pig trabeculae, no influence of propofol on the contractile proteins, nor on the $Ca^{2+}$ sensitivity of these proteins was observed.

We also tested the lipid emulsion in which propofol is dissolved (Intralipid 10%, Fresenius Kabi, The Netherlands). In agreement with previous studies (Kanaya *et al*., 1998; Park & Lynch, 1992; Puttick & Terrar, 1993) there was no effect of this emulsion on maximum force production and calcium sensitivity in Triton-skinned trabeculae from rat.

## Discussion

This is the first study on the effects of propofol on skinned trabeculae of two species. Different skinning methods permit

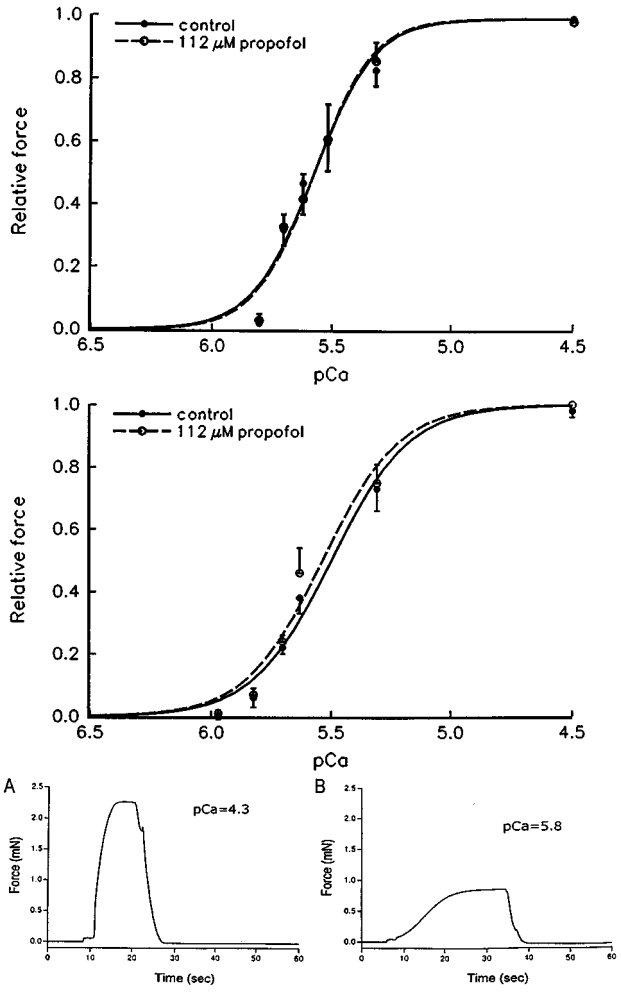


**Figure 4** Isometric force at different $Ca^{2+}$ concentrations in Triton skinned rat trabeculae ($n=5$, upper part) and guinea-pig trabeculae ($n=5$, middle part). The force for each preparation is normalized to the control force at saturating $Ca^{2+}$ concentration. The mean value of maximal force of all rat trabeculae was $50.0\pm4.8$ mN mm$^{-2}$ and $34.9\pm6.7$ mN mm$^{-2}$ ($\pm$s.e.mean) for all guinea-pig trabeculae. Propofol 112 $\mu$M was added to the activating solution and compared to control measurements. Mean values$\pm$s.e.mean are shown. The Hill curves were fitted as indicated in the text and the parameters$\pm$s.e.estimate were for propofol $P(K)=5.58\pm0.02$ (control $5.58\pm0.02$) and $nH=3.63\pm0.63$ (control $3.47\pm0.72$) in rat trabeculae and $P(K)=5.54\pm0.04$ (control $5.54\pm0.04$) and $nH=2.76\pm0.55$ (control $2.70\pm0.40$) in guinea-pig trabeculae. Propofol 28 $\mu$M showed comparable results in both species. The average data points at each pCa value were compared using a Student's *t*-test for unpaired observations. The lower part of the figure shows recordings of force development in a single Triton skinned rat trabeculum at pCa 4.3 (A) and pCa 5.8 (B). When the preparation is transferred from the preactivating into the activating solution isometric force rises rapidly until a maximum level is reached. Then the trabeculum is incubated in the relax solution, resulting in force relaxation. Guinea-pig Triton skinned trabeculae showed a similar contraction pattern.

direct access to the function of the SR and the contractile proteins. By using trabeculae of two different species in the same experimental set up it was possible to compare the effects of propofol on myocardial contractility in species with well established differences in the contraction process. Contractile activation in the myocardium of the rat mainly depends on calcium released from the SR (Mitchell *et al.*, 1987). In the guinea-pig calcium influx through the L-type calcium channels in the cell membrane is relatively more important. For this reason, the different responses of these species to propofol may be explained by its specific influence on either the SR calcium handling or transsarcolemmal calcium influx.

In electrically stimulated trabeculae of the rat we observed that propofol did not influence isometric force. These results are fairly similar to those of a previous study on rat ventricular papillary muscle in which propofol 5.6–66 $\mu$M induced only a small change on intrinsic myocardial contractility by decreasing isotonic relaxation, probably due to a slight decrease in calcium uptake by the SR (Riou *et al.*, 1992). In contrast, in guinea-pig trabeculae, we observed that propofol decreased isometric force, as previously reported (Azari & Cork, 1993; Park & Lynch, 1992; Puttick & Terrar, 1993). In view of the fact that propofol has been found to block L-type calcium channels (Sakai *et al.*, 1996; Yang *et al.*, 1996; Zhou *et al.*, 1997) it can be expected that contractility in guinea-pig is more sensitive to the effect of propofol than rat. The post rest contraction is the result of a brief rest period introduced during stimulation. Force development of the post rest contractions depends on the duration of the rest interval and on the studied species (Lewartowski & Pytkowsky, 1987). During repetitive stimulation, cyclic $Ca^{2+}$ release and uptake of the SR reaches a steady rate, which depends on the stimulus interval. When stimulation is interrupted initially there is a redistribution of $Ca^{2+}$ inside the SR or alternatively a change in the kinetics of calcium handling by the SR, leading to an enhanced $Ca^{2+}$ release from the SR at the first post-rest beat. In the rat this leads to an increased force during the first post-rest beat. In guinea-pig, however, no increase in force normally occurred with the stimulation protocol employed. Here, a period of rest causes a decrease in force during the first and second post rest beat which probably reflects a decrease in $Ca^{2+}$ released by the SR. In subsequent contractions force gradually increases to the prerest level. It should be noted that this is consistent with the species dependent differences in relative contribution of calcium release by the SR and of calcium influx through the sarcolemma. Studies on the mechanism of relaxation in rat and rabbit cardiac cells by measuring changes in intracellular calcium concentration showed that in both cell types SR $Ca^{2+}$-ATPase is more powerful than the sarcolemmal $Na^{+}$-$Ca^{2+}$ exchange, but the dominance is more marked in the rat (13 fold vs 2.5 fold in rabbit). The estimation was made that the fraction of $Ca^{2+}$ transported by the SR, transarcolemmal $Na^{+}$-$Ca^{2+}$ exchange and slow systems (mitochondria and sarcolemmal calcium pump) during a twitch are 70, 28 and 2% respectively in rabbit myocytes and 92, 7 and 1% respectively in rat myocytes (Bassani *et al.*, 1994; Puglisi *et al.*, 1996). In this aspect guinea-pig myocardium is comparable to that of the rabbit (Bers, 1985). The post rest contraction test in rat trabeculae showed the well known phenomenon of post rest potentiation (Lewartowski & Pytkowski, 1987). Propofol did not change this pattern suggesting that it did not interfere with the SR $Ca^{2+}$ handling. Our experiments in saponin skinned trabeculae of the rat confirm this notion. It was found that propofol did not affect uptake, storage and release of $Ca^{2+}$ by the SR. This implies that propofol did not cause an increase in $Ca^{2+}$ leak. In this aspect propofol differs from

halothane which causes a leak of $Ca^{2+}$ out of the SR, thus impairing force development (Herland *et al*., 1992).

In guinea-pig trabeculae propofol considerably depressed the steady state peak force and altered the pattern of post rest contraction. Overall it showed a dose-dependent increase in normalized post rest peak force. At concentrations of 112 and 280 $\mu$M it caused a potentiation of the first post rest contraction. Therefore, at these concentrations post rest contraction in the guinea-pig closely resembles that of the rat. It is known that the post rest contraction test not only differs between species but also when comparing different regions of the heart (Lewartowski & Pytkowski, 1987). In the guinea-pig, atrial tissue exhibits a large potentiation of the first post rest contraction. It is feasible that different parts of the ventricles demonstrate distinct patterns of post rest contraction. The fact that we found that one out of five guinea-pig trabeculae showed a different post rest contraction pattern may support this notion.

A surprising observation is that propofol potentiates post rest contractions in the guinea-pig, although the peak force during steady state contractions is decreased. For a complete explanation of this result detailed knowledge of intracellular $Ca^{2+}$ handling is required. We observed that propofol neither in saponin skinned trabeculae from the guinea-pig nor from the rat affected $Ca^{2+}$ handling by the SR. Therefore we favour the following tentative explanation. During steady state contractions, force in the guinea-pig mainly depends on transsarcolemmal $Ca^{2+}$ influx. Evidence from studies of the effects of propofol on action potentials and calcium channel currents in guinea-pig isolated ventricular myocytes suggests that this flux is depressed by propofol (Puttick & Terrar, 1992; Takahashi *et al*., 1994; Sakai *et al*., 1996; Yang *et al*., 1996). Our results indicate that SR function is not affected by propofol. But, if $Ca^{2+}$ influx decreases, $Ca^{2+}$ handling by the SR becomes relatively more important. For guinea-pig this may explain the post rest potentiation of contractions. Therefore in the presence of propofol the SR gains in relative importance and as a result contractions in guinea-pig myocardium start to resemble those in rat.

The influence of propofol on the contractile proteins was assessed in Triton skinned trabeculae from rat and guinea-pig. The force–pCa curve was not changed by propofol at any concentration. This indicates that propofol did not alter the contractile properties of the myofilaments nor did it affect $Ca^{2+}$ sensitivity of force production. Recent studies using fluorescence techniques in rat cardiomyocytes (Kanaya *et al*., 1998; Guenoun *et al*., 2000) and intact guinea-pig hearts (Nakae *et al*., 2000) showed a decrease in intracellular calcium transients by propofol without decreasing cardiac contraction. It is suggested that this may be due to a change in calcium handling by the SR and an enhancement of myofilament $Ca^{2+}$ sensitivity. The difference with our results can be explained by the fact that in these studies intact myocytes or even complete spontaneously beating hearts were used, while we directly studied the independent function of intracellular structures. Furthermore we believe that the role of the sarcolemmal Na-Ca exchanger in these studies is underestimated and should be investigated in more detail. The results of these studies may be, at least partly, explained by an effect of propofol on this exchanger. Further study on this aspect is needed.

Before relating our results to the clinical situation some points have to be mentioned. Firstly, we studied isolated trabeculae which can be considered, after the myocyte, as the smallest intact contractile unit in the heart. Our interest was to assess the influence of propofol on intrinsic myocardial contractility and on the SR and contractile filaments in particular. Secondly, the myocardial responses of two different species were compared, which exhibit known differences in calcium handling. In this aspect human myocardium is more comparable to that of guinea-pig than to that of rat (Bers, 1985; Sakai *et al*., 1996), although electrophysiological differences exist (Azari & Cork, 1993). Thirdly, we used propofol in concentrations of 28, 112 and 280 $\mu$M. The first concentration is considered to be in the clinical range but transiently higher concentrations may be expected. In blood, a large fraction of propofol is bound to serum proteins (98%) (Riou *et al*., 1992; Yang *et al*., 1996). Therefore, the propofol concentrations in our solutions may be considerably higher than the unbound fraction in blood. We used higher concentrations in order to resolve small effects of propofol which may be important with regard to the mechanism underlying its inotropic effects. In summary, our study indicates that: (1) Propofol depresses myocardial contractility in the guinea pig, not in the rat; (2) The depression of myocardial contractility by propofol can not be explained by an effect of propofol on SR calcium handling nor by changes in calcium sensitivity of the contractile filaments; (3) Negative inotropic effects in guinea-pig are best explained by inhibition of the L-type calcium channels, as has been proved in other studies. Possible involvement of the Na-Ca exchanger is yet unclear.

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