Effects of the anaesthetic propofol on the calcium-induced permeability transition of rat heart mitochondria: direct pore inhibition and shift of the gating potential

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Abstract Mitochondrial calcium exchanges are involved in intracellular calcium homeostasis and in the contraction-relaxation process in myocytes. The calcium-induced permeability transition of the heart mitochondria inner membrane appears to be an important calcium efflux mechanism involved in some physiological and pathological situations. The negative inotropic effect of the anaesthetic propofol results in part from a decrease in intracellular calcium availability. Thus, this study evaluates the effects of propofol on calcium transport and permeability transition of heart mitochondria. The propofol-inhibition of the permeability transition of liver mitochondria was previously investigated [Eriksson, O. (1991) FEBS Lett. 279, 45–48] in such conditions that its uncoupling effect was not taken into account. We show here that propofol uncoupling results in a decrease in calcium uptake rate which could in part explain the decreased permeability transition rate. However, comparison of equipotent uncoupling concentrations of propofol and carbonylcyanide *m*-chlorophenylhydrazone reveals that beyond this uncoupling effect, propofol has a direct inhibitory action on the permeability transition pore, concomittant with a shift of its gating potential.

Key words: Calcium; Anaesthetic; Propofol; Mitochondria (rat heart); Permeability transition pore; Cyclosporin A

1. Introduction

The short-acting intravenous anaesthetic propofol (2,6 diisopropylphenol) is widely used. Unfortunately, induction of anaesthesia with propofol can result in a significant decrease in arterial blood pressure, systemic vascular resistance and cardiac output. Some studies have demonstrated an intrinsic negative inotropic effect of propofol [1,2]. Recently, it was suggested that this effect on myocardial contractility results from a decrease in intracellular Ca^{2+} availability [3].

Mitochondria seem to play an important role in Ca^{2+} homeostasis in both non-excitable and excitable cells; moreover, mitochondria are Ca^{2+}-excitable organelles which appear to be involved in the transduction of cell Ca^{2+} signals [4]. The mechanisms by which mitochondria transport Ca^{2+} are complex and numerous [5]. Ca^{2+} influx into energized mitochondria is potential-dependent and occurs through the ruthenium red-sensitive Ca^{2+} uniport. Two separate mechanisms are known to account for Ca^{2+} efflux from mitochondria: (i) a Ca^{2+}/Na^{2+} exchanger and (ii) a Ca^{2+}-induced increase in membrane permeability. The biochemical mechanisms mediating this permeability transition of the mitochondrial inner membrane have not been entirely elucidated. However, there is now considerable evidence that the mitochondrial membrane contains a latent pore: the permeability transition pore (PTP) or megachannel [6–13]. The PTP is a Ca^{2+}-dependent and cyclosporin A (CsA)-sensitive. An increase in matrix Ca^{2+} or in matrix pH, or a drop of membrane potential favour the opening of the pore [11–13]. The resulting permeability transition is accompanied by a decrease in membrane potential, a loss of matrix ions including Ca^{2+}, and mitochondrial swelling. Very low concentrations (1 μM) of CsA inhibit the permeability transition.

Ca^{2+} interactions in heart mitochondria are involved in some physiological or pathological processes [14–16]. In cardiac myocytes, Ca^{2+} is a physiological regulator of mitochondrial metabolism and ATP synthesis [14]. Mitochondrial Ca^{2+} cycling might affect the sarcoplasmic reticulum Ca^{2+} content in the heart [17]. In myocytes, the PTP contributes to mitochondrial Ca^{2+} cycling and provides a pathway for mitochondrial Ca^{2+} extrusion [18].

Propofol and other substituted phenols are known to affect oxidative phosphorylation in mitochondria [19,20]. Propofol acts as a mild protonophore-like uncoupler and reduces the mitochondrial membrane potential. In liver mitochondria, propofol has been found to induce an apparent uptake of Ca^{2+} and to stabilize the mitochondrial membrane by interaction with the PTP [21]. The aim of this study was to investigate the effects of propofol on the PTP in heart mitochondria. Mitochondrial swelling was used to monitor the Ca^{2+}-induced permeability transition [22], and the mitochondrial Ca^{2+} uptake and release were investigated by measuring the external Ca^{2+} concentration with a Ca^{2+}-sensitive electrode. The current study showed that propofol decreased both uptake and release by two different mechanisms. Comparison of the effects of propofol and the uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP) at an equipotent uncoupling concentration demonstrated that propofol inhibits the mitochondrial PTP and shifts its gating potential.

2. Material and methods

2.1. Mitochondrial isolation

Care of the animals conformed to the recommendations of the Institutional Animal Care Committee and the French Ministry of Agriculture. Because all anaesthetic agents used could potentially alter mito-

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Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; CsA, cyclosporin A; HM, heart mitochondria; PTP, permeability transition pore; RR, Ruthenium red.

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chondrial function after isolation, adult male Wistar rats (weight 350–400 g) were killed by cervical dislocation. The hearts were quickly removed and put into an ice-cold medium containing 70 mM sucrose, 210 mM mannitol, 10 mM EDTA and 50 mM Tris-HCl (pH 7.4). Mitochondria were isolated by differential centrifugation as previously described [23]. Protein concentration of the mitochondrial suspension was determined by the Biuret method. All the experiments were carried out in a resin (Chelex-100)-treated buffer containing: 210 mM mannitol, 70 mM sucrose, 5 mM HEPES-KOH (pH 7.4) and 0.2 mM KH_2PO_4.

2.2. Mitochondrial swelling

Changes in volume of the heart mitochondria were monitored at 540 nm in a Perkin-Elmer spectrophotometer. Mitochondria (0.5 mg protein/ml) were preincubated at 30°C in the presence of rotenone (1 μM) for 1 min prior to the addition of Ca^{2+} (200 nmol). Mitochondria were energized 1 min later by succinate (5 mM). The swelling rate was expressed as ΔA_{540}/min/mg.

2.3. Measurement of oxygen consumption and external free calcium

Measurements were made at 30°C in the 2 ml chamber of an ESON-6 computerized analyzer (Mutual Data Inc., Moscow). Oxygen and free Ca^{2+} concentrations were measured in the medium with calibrated mini-electrodes [4].

2.4. Mitochondrial membrane potential

Changes in membrane potential were measured by following fluorescence of rhodamine 123 [24]. Mitochondria suspended in the same medium (0.25 mg/ml) were loaded with rhodamine 123 (0.1 μg/ml). Fluorescence was recorded in a fluorimeter (SFM125, Kontron) at the 485–535 nm excitation-emission pair. Results were expressed in relative fluorescence intensity (ΔF, in %). ΔF_0 corresponded to the high membrane potential of energized mitochondria in the absence of adenosine diphosphate.

2.5. Chemicals

Cyclosporin A was a gift from Sandoz (Basle, Switzerland). All the other chemicals used were from Sigma. Propofol (2,6-diisopropylphenol) was dissolved in ethanol at 50 mM concentration.

2.6. Data analysis

Data were expressed as mean ± standard deviation. The traces shown are representative of at least three experiments. Data were plotted and analyzed using KaleidaGraph (Synergy Software, Reading, PA). Concentration-reponse curves were fitted to the equation $y = \text{Min} + (\text{Max} - \text{Min})/(1 + (C_{50}/C)^H)$, where C_{50} is the concentration producing half-maximal response and H is the Hill coefficient. Values for IC_{50} were obtained from this curve fitting and are reported as ± 95% confidence limits. Statistical analysis was performed using the unpaired Student's t test. Differences at $P < 0.05$ were considered significant.

3. Results

3.1. Ca^{2+}-induced permeability transition in heart mitochondria

As previously reported [22], Ca^{2+} induced swelling of rat heart mitochondria (ΔA_{540}/min/mg = 0.42 ± 0.07, n = 5). Cyclosporin A (2 μM), a specific inhibitor of the PTP, inhibited swelling. Ruthenium red (1 μM) prevented Ca^{2+} accumulation by mitochondria and consequently inhibited opening of the pore and swelling (Fig. 1).

3.2. Propofol inhibition of the mitochondrial permeability transition

Propofol, added before energization of mitochondria by succinate, produced a dose-dependent inhibition of swelling (Fig. 2). The concentration of propofol yielding 50% of the maximal inhibition (IC_{50}) was 129 ± 9 μM.

Parallel measurements of Ca^{2+} concentration in the medium showed two different effects of propofol: a decrease in Ca^{2+} uptake and an inhibition of Ca^{2+} release which corresponded to the swelling inhibition (Fig. 3). The uncoupling effect of propofol on oxidative phosphorylation was responsible for a decrease in the Ca^{2+} accumulation rate by a decline in the mitochondrial membrane potential. This effect could explain in part the apparent inhibition of the PTP.

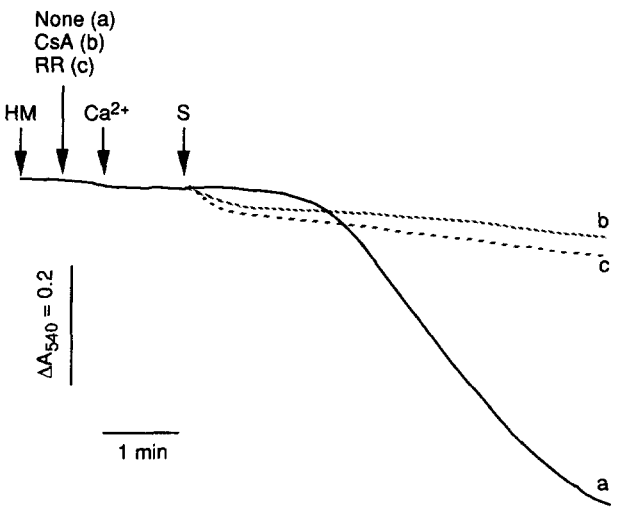

Fig. 1. Heart mitochondrial swelling. Ca^{2+}-induced permeability transition was monitored by the changes in volumes of mitochondria. Swelling was measured by the decrease in light scattering at 540 nm (A_{540}). Heart mitochondria (HM), 0.5 mg/ml, were incubated at 30°C in a medium of 210 mM mannitol, 70 mM sucrose, 5 mM HEPES-KOH (pH 7.4), 0.2 mM KH_2PO_4 and rotenone 1 μM. 200 nmol of Ca^{2+} and 5 mM succinate (S) were added as indicated. Trace a: Ca^{2+}-induced swelling. Traces b and c: inhibition of the swelling by 2 μM cyclosporin A (CsA) and 1 μM Ruthenium red (RR), respectively.

3.3. Comparison propofol versus CCCP

To prove a direct interaction between propofol and the PTP, we compared the effects of propofol and the classic uncoupler CCCP. An equipotent uncoupling concentration was found for both molecules by studying their effects on mitochondrial respiration in the presence of succinate and on the membrane potential (not shown). Propofol at 200 μM stimulated basal respiration (+57 ± 15 nmol O/min/mg, n = 5) and decreased the membrane potential (−41 ± 8% of ΔF_0, n = 5). CCCP at 0.12 μM had no statistically different effects: +60 ± 8 nmol O/min/mg (n = 5) and −45 ± 9% of ΔF_0 (n = 5).

At this concentration, CCCP inhibited Ca^{2+} uptake and swelling like propofol (Fig. 3). Inhibition of swelling by CCCP was not due to an interaction with the PTP, but could be explained by the effect on Ca^{2+} uptake. Indeed, CCCP was not able to block the PTP after Ca^{2+} accumulation (Fig. 4). In contrast, when propofol (200 μM) was added at the end of Ca^{2+} uptake just before the opening of the PTP, neither Ca^{2+} release nor swelling occurred (Fig. 4). These differences between CCCP and propofol could be explained by a direct inhibition of the PTP by propofol.

4. Discussion

Propofol is an intravenous anaesthetic agent with significant cardiovascular effects. In vitro studies have demonstrated the negative inotropic effect of propofol [1–3]. Cook et al. showed

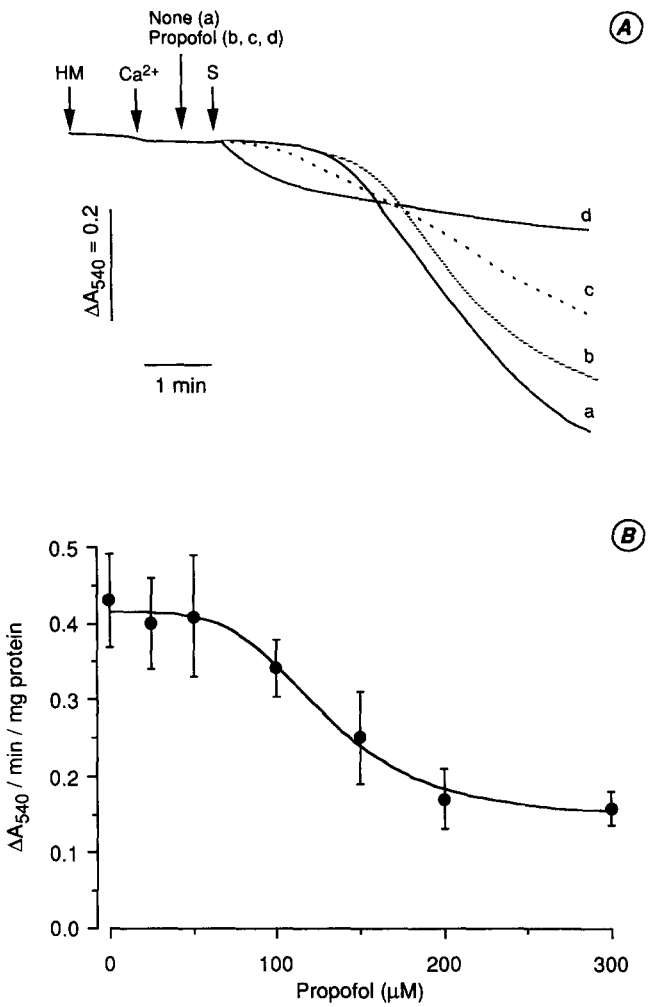

Fig. 2. Inhibition of Ca^{2+}-induced swelling by propofol. (A) Swelling was followed as in Fig. 1. Propofol inhibited swelling in a concentration-dependent manner. Trace a, Control; Traces b, c, d, Propofol 50 μM, 100 μM and 200 μM, respectively. (B) Dose-dependent inhibition by propofol of the swelling rate, quantified as ΔA_{540}/min/mg. Data are presented as mean ± S.D. The data sets (n = 3–5 for each point) are fitted to the Hill equation with an IC_{50} of 129 ± 9 μM and a Hill coefficient of 4.

in isolated ferret ventricular myocardium that propofol (30–300 μM concentration range) profoundly affected intracellular Ca^{2+} availability by interfering with transsarcolemnal Ca^{2+} influx [3]. The role played by mitochondria in intracellular Ca^{2+} homeostasis has not been entirely elucidated. However, the discovery of the permeability transition pore (PTP) in mitochondria has opened up a new area of research [5]. Mitochondria now appear to be Ca^{2+}-excitable organelles involved in the transduction of cell Ca^{2+} signals [4]. Altschuld et al. have suggested that the opening and closing of the PTP in cardiomyocytes contribute to the process of Ca^{2+} release from mitochondria, and that the PTP may be a normal pathway in functional heart cells [18].

The mitochondrial PTP is activated by Ca^{2+} and inhibited by H^+, Mg^{2+} and adenine nucleotides [8,13]. The specific inhibition by cyclosporin A characterizes the pore [7]. The open–closed transition of the PTP appears to be modulated by the mitochondrial membrane potential (membrane depolarization favours pore opening), the matrix pH and divalent cations [12,13]. This complexity of pore modulation means that an effector could have a direct or an indirect effect on the PTP. Propofol, like other substituted phenols, interacts with mitochondrial oxidative phosphorylation. Propofol affects the coupling between the respiratory chain and ATP synthesis by acting as a mild protonophore [19,20]. This uncoupling effect explains the decrease in transmembrane potential observed in the presence of propofol. Thus, the inhibition of the PTP by propofol could be due to different effects: (i) a direct interaction with the PTP, or (ii) an indirect action due to the uncoupling effect of this molecule resulting in a decreased rate of Ca^{2+} accumulation. Unlike CCCP, propofol was able to inhibit both swelling and Ca^{2+} release after addition at the end of Ca^{2+} accumulation into the mitochondria (Fig. 4). These results indicate that propofol is a direct inhibitor of the PTP opening.

Moreover, comparison of equipotent uncoupling concentrations of propofol and CCCP, in such conditions that mitochondrial calcium load and matrix pH are identical (Fig. 4), shows that the voltage gating of the pore is shifted to lower values (i.e. for an equivalent loss of potential, CCCP increases the rate of the transition while propofol has an opposite effect).

The clinical relevance of this study is debatable, since the concentrations of propofol used here are higher than those normally encountered during anaesthesia. The concentrations of propofol at which significant PTP inhibition is observed (50

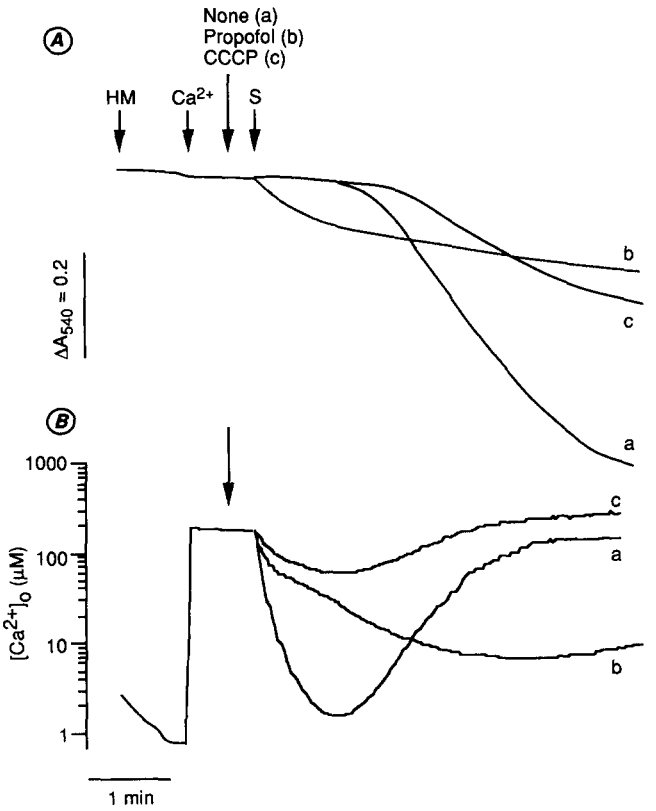

Fig. 3. Parallel measurements of mitochondrial swelling (A) and Ca^{2+} distribution (B). Swelling was monitored as in Fig. 1. Ca^{2+} concentration in the medium ($[Ca^{2+}]_0$) was monitored simultaneously using a Ca^{2+}-sensitive electrode. Trace a: after energization with succinate, Ca^{2+} was accumulated into mitochondria and then released. Ca^{2+} release was accompanied by swelling. Trace b: 200 μM propofol decreased the rate of Ca^{2+} uptake and inhibited swelling. Trace c: 0.12 μM carbonylcyanide m-chlorophenylhydrazone (CCCP) inhibited Ca^{2+} accumulation and swelling.

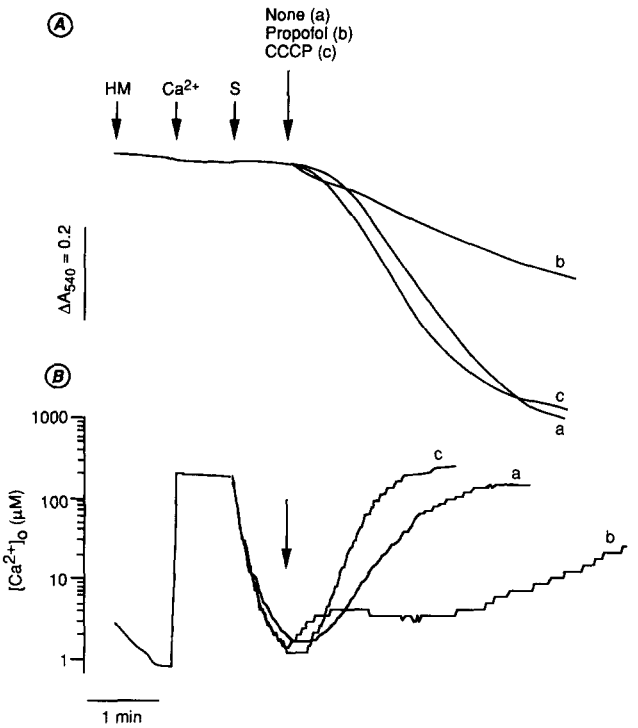

Fig. 4. Comparison of the activity of propofol and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) on the mitochondrial permeability transition pore (PTP). Experimental conditions were the same as in Fig. 3. To eliminate the effect of these molecules on Ca^{2+} uptake and to concentrate only on the PTP, propofol and CCCP at an equipotent uncoupling concentration were added after energization by succinate and Ca^{2+} accumulation, just before the onset of the swelling. Trace a: control. Trace b: propofol (200 μM) inhibited swelling (A) and Ca^{2+} release (B) after Ca^{2+} accumulation into the mitochondria. Trace c: CCCP (0.12 μM) had no inhibitory effect on the PTP opening. The inhibition of swelling by CCCP observed in Fig. 3 is thus explained by the sole decrease in Ca^{2+} accumulation.

μM) are at least tenfold the free plasma concentration detected after an intravenous bolus injection of propofol. However, the real cytosolic concentrations of propofol in cardiomyocytes are unknown, and cumulative effects of propofol after sustained intravenous administration have been reported [25]. The involvement of a propofol-PTP interaction in the cardiotoxicity of this anaesthetic remains to be evaluated. Thus, further studies will be necessary to investigate the effects of propofol on mitochondria in situ using isolated cardiomyocytes. Altschuld et al. have shown that cyclosporin A inhibits the mitochondrial PTP in myocytes and leads to the accumulation of Ca^{2+} by mitochondria [18]. These results could in part explain the effects of cyclosporin A on cardiac muscle [26]. In the case of propofol, the mitochondrial functions could be disturbed both by an uncoupling effect with a decrease in energy production and by a change in mitochondrial Ca^{2+} transport.

In summary, this study reports a new effect of the anaesthetic propofol on mitochondrial Ca^{2+} transport. The inhibition of the Ca^{2+}-induced permeability transition pore could play a role in the changes in intracellular Ca^{2+} availability observed in the presence of propofol.

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