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Effects of the general anaesthetic Propofol on the Ca^{2+}-induced permeabilization of rat liver mitochondria

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The molecular mechanism of the Ca^{2+}-induced permeabilization of rat liver mitochondria was evaluated by studying a new effect of the commonly used general anaesthetic Propofol (2,6-diisopropylphenol). The compound was found to induce an apparent uptake of Ca^{2+} at steady-state in the Ca^{2+}-distribution between the medium and the mitochondria, and to inhibit swelling and release of accumulated Ca^{2+} induced by inorganic phosphate, *t*-butyl hydroperoxide, diamide or FCCP *plus* Ruthenium red. The compound did not stimulate the activity of the Ca^{2+}-uniporter and it is concluded that the effects seen are due to the inhibition of the Ca^{2+}-dependent, unspecific permeability increase. The results suggest two mechanisms whereby Propofol stabilizes the mitochondrial membrane in the presence of Ca^{2+}: (i) by interaction with the putative pore, thus causing its closure; and (ii) by scavenging of free radicals thus inhibiting its opening during oxidative stress.

Antioxidant; Calcium; Mitochondria; Nonspecific permeability; Propofol; Swelling

1. INTRODUCTION

Mitochondria are able to accumulate Ca^{2+} from the surrounding medium in an energy-dependent manner [1]. The uptake of Ca^{2+} by the mitochondria will, if a certain threshold of internal Ca^{2+} is exceeded, lead to a dramatic increase in the general permeability of the inner mitochondrial membrane with release of Ca^{2+}, a fall in $\Delta\Psi$, and swelling of the mitochondria [1]. A number of physiological and pharmacological agents of great chemical diversity have been shown to modulate this threshold value. Adenine nucleotides and Mg^{2+} raise the threshold and could thus be said to stabilize the mitochondrial membrane whereas so-called Ca^{2+}-releasing agents, such as P_i, fatty acids, SH-agents and peroxides make the mitochondria more sensitive to Ca^{2+}.

Various mechanisms have been proposed to explain the Ca^{2+}-induced permeabilization. According to one hypothesis [1], the permeabilization is due to a shift in the equilibrium between hydrolysis and reacylation of membrane phospholipids at the *sn*-2 position towards hydrolysis leading to accumulation of lysophospholipids and fatty acids. It has recently been proposed that the effects of Ca^{2+} and Ca^{2+}-releasing agents may converge to a common ultimate step, the production of oxygen radicals and peroxidized lipids [2] which would cause an increased general permeability of the inner mitochondrial membrane, swelling and possibly the accumulation of lysophospholipids [1]. The permeabilization has also been interpreted as being due to the reversible opening of a pore protein [3]. These mechanisms may, however, be operating simultaneously [4].

This study on the effects of the general anaesthetic Propofol on the Ca^{2+}-induced permeabilization was performed in order to obtain more information on the mechanism of the permeabilization. The results indicate that Propofol causes inhibition of the permeabilization by interacting with the pore-forming component, and by scavenging radicals, thereby indirectly inhibiting its opening during oxidative stress. Similar effects have been described the structurally related compound BHT [2].

2. MATERIALS AND METHODS

Liver mitochondria were prepared from male rats as described in [5]. The measurements were performed in a medium containing 250 mM sucrose, 10 mM Hepes-Tris, pH 7.40, at a mitochondrial concentration of 1 mg protein/ml and at room temperature. All media were run through a Chelex (Bio-Rad Laboratories, Richmond, USA) cation exchange column equilibrated with NaCl. The medium was purged with oxygen for 30 min prior to use when indicated. Propofol was purchased from Lancaster (Strasbourg, France), distilled twice at a pressure of 15 mm Hg and recrystallized in methanol/H_2O (3:1). Crystals were dried and dissolved in ethanol at a concentration of 100

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Abbreviations: BHT, butylated hydroxytoluene; tBHP, *t*-butylhydroperoxide; DMNTA, 2,2-dimethylnitrilotriacetate; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; PLA_2, phosphatidyl 2-acylhydrolase (EC 3.1.1.4); P_i, inorganic phosphate, Propofol, 2,6-di-isopropylphenol; RLM, rat liver mitochondria; $\Delta\Psi$, mitochondrial membrane potential

mM. Cyclosporin A was kindly provided as a gift by Dr P. Juutilainen (Sandoz Oy, Helsinki).

Mitochondrial swelling was measured by following the decrease in light scattering at 540 nm with a Shimadzu UV 3000 spectrophotometer (Shimadzu Corp., Japan). The oxygen consumption was measured polarographically using a Clark-type electrode. The distribution of Ca^{2+} between the solution and the mitochondria was measured with a Ca^{2+}-selective electrode [6] or with the arsenazo III method [7]. The activity of the uniporter was measured according to the method described in [8]. DMNTA was kindly provided by Dr Hans Kröner (University of Düsseldorf). The activity of the mitochondrial phospholipase A_2 was measured by HPLC [9].

3. RESULTS AND DISCUSSION

3.1. *Ca^{2+}-distribution and swelling during steady-state conditions*

RLM that had been suspended in sucrose medium containing 1 mM P_i were able to take up and retain 60 nmol Ca^{2+}/mg protein (Fig. 1, solid line). The steady-state external pCa^{2+} stayed at about 5.7 until anoxia when the intramitochondrial Ca^{2+} was released. A slow continuous swelling could be observed during the steady-state phase (Fig. 1, solid line), probably indicating an ongoing process where some of the RLM became permeabilized, released their Ca^{2+} and accumulated sucrose [3]. When Propofol (80 nmol/mg protein) was added, there was an apparent uptake of Ca^{2+} (Fig. 1, broken line) and the steady-state concentration decreased in the medium. This could be due to either the activation of Ca^{2+} influx of the intact mitochondria or to the inhibition of Ca^{2+} efflux. The question was addressed by measuring the activity of the Ca^{2+} uniporter in the presence of propofol using the method of Kröner [8]. Propofol failed to exhibit any effect on the uniporter (not shown) indicating that the compound caused a decrease in the steady-state Ca^{2+} concentration by inhibiting the Ca^{2+} efflux. This interpretation was further supported by the observation that the slow continuous swelling observed during the steady-state phase was partly inhibited by Propofol (Fig. 1, broken line). This probably indicates that Propofol causes the inhibition of Ca^{2+} release by inhibiting the Ca^{2+}-induced increase in the general permeability of the inner mitochondrial membrane. This interpretation was further tested by comparing the effects of Propofol with those of cyclosporin A which is known to specifically inhibit the Ca^{2+}-induced permeabilization [1]; the effect of cyclosporin A (0.5 nmol/mg protein) on Ca^{2+} distribution and swelling (Fig. 1, dotted lines) were clearly similar to the effects of propofol.

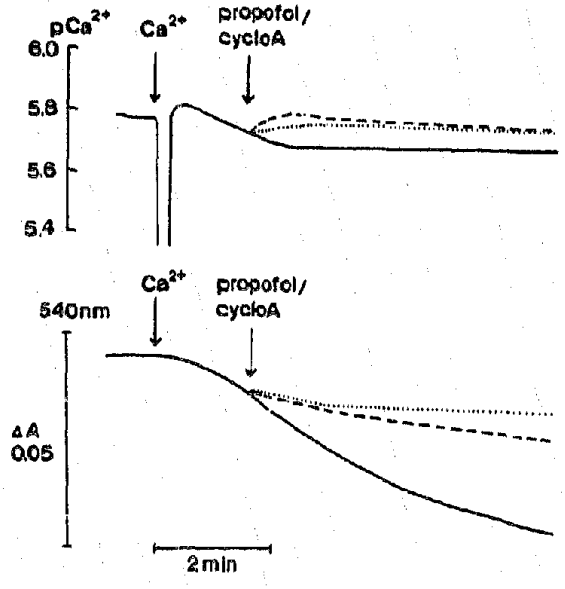

Fig. 1. Parallel measurements of Ca^{2+}-distribution (top) and mitochondrial swelling (bottom). Control experiment (solid line); Propofol (80 nmol/mg) (broken line) and cyclosporin A (0.5 nmol/mg) (dotted line) were added as indicated.

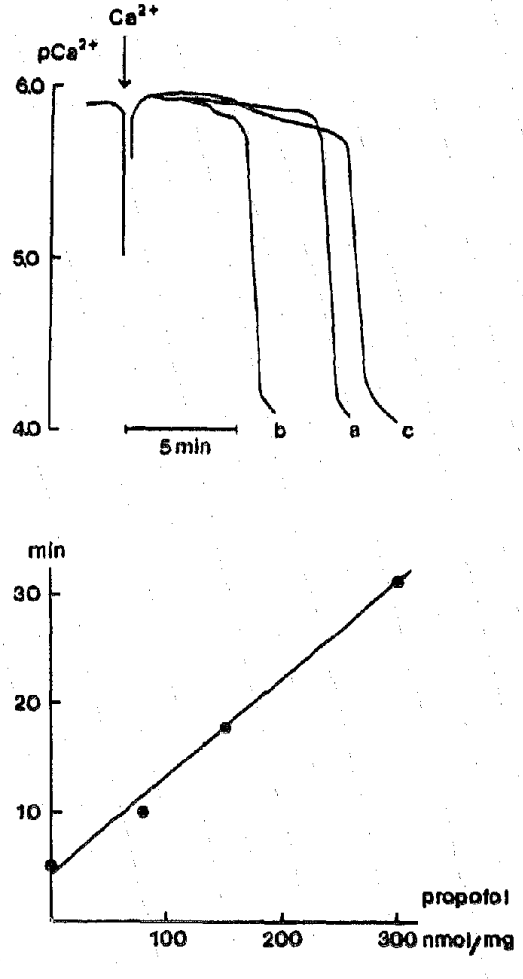

Fig. 2. The efflux of Ca^{2+} induced by 10 mM P_i: (a) under air, (b) under 100% O_2, (c) under 100% O_2, 80 nmol Propofol/mg protein added. Bottom: shows Ca^{2+} retention time under 100% O_2 as a function of the amount of Propofol added.

3.2. *Inhibition of Ca^{2+}-efflux and swelling in the presence of P_i tBHP, diamide or FCCP* plus *Ruthenium red*

In order to assess the potency of Propofol as an inhibitor of the Ca^{2+}-induced permeabilization the effects of the compound were studied under various conditions when the permeabilization is known to be activated, i.e. in the presence of P_i tBHP, diamide or FCCP *plus* Ruthenium red and also under hyperoxic conditions where an enhanced radical production has been shown to take place in cultured cells [10].

This was done by measuring the Ca^{2+} retention and the swelling of RLM suspended in sucrose medium containing 10 mM P_i or in the same medium purged with 100% O_2. In the control experiment (P_i not added) RLM were able to take up and retain Ca^{2+} (60 nmol/mg protein) as well under air as under 100% O_2 (not shown); increased O_2 partial pressure did not affect Ca^{2+} retention. When 10 mM P_i was included in the medium the accumulated Ca^{2+} was released after 9 min under air (Fig. 2 top, trace a) and after 5 min under 100% O_2 (Fig. 2 top, trace b). The Ca^{2+} release was accompanied by swelling (not shown) which was more rapid under 100% O_2; it is clear that the RLM were more sensitive to Ca^{2+} and P_i under 100% O_2 which might be attributable to an enhanced production of free radicals during these conditions. When Propofol (80 nmol/mg protein) was added to the medium (purged with 100% O_2), the Ca^{2+} release was inhibited (Fig. 2 top, trace c). The inhibition was concentration-dependent with a maximal effect seen at 300 nmol Propofol/mg protein when the inhibitory effect persisted for more than 30 min (Fig. 2, bottom) - higher concentrations appeared to cause perturbation of the mitochondrial membrane.

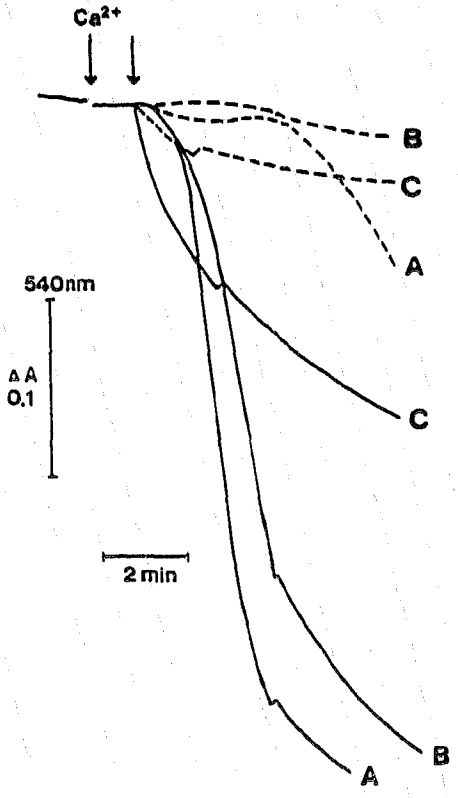

Fig. 3. Swelling induced by Ca^{2+} in the presence of (A) tBHP, (B) diamide or (C) FCCP *plus* Ruthenium red. Solid lines, control experiments; broken lines, in the presence of Propofol (80 nmol/mg). The second arrow indicates the addition of the experimental agent.

The effect of Propofol on swelling was also studied in the presence of either tBHP (100 nmol/mg), diamide (100 nmol/mg) or FCCP *plus* Ruthenium red (5 resp. 3 nmol/mg). RLM suspended in sucrose medium were allowed to accumulate Ca^{2+} (60 nmol/mg) whereupon the releasing agent was added. All the agents induced rapid swelling (Fig. 3, traces A-C, solid lines); a short lag phase could be observed when tBHP or diamide was present whereas in the presence of FCCP *plus* Ruthenium red swelling took place without any lag phase. Propofol (80 nmol/mg) caused inhibition of the swelling in all these cases (Fig. 3, traces A-C, broken lines).

These studies with the general anaesthetic Propofol clearly show that the compound is an inhibitor of the Ca^{2+}-induced permeabilization of RLM. It is well documented that oxidative stress contributes to the induction of increased inner membrane permeability; the effect of Propofol may thus be due to its ability to form a stable radical [11], and consequently to inhibit the propagation of radical reactions. However, Propofol also caused the inhibition of permeabilization under conditions of seemingly low oxidative stress, i.e. in the presence of P_i (under air) or when the permeabilization was induced by uncoupler *plus* Ruthenium red. It is thus conceivable that Propofol interacts with the same inner membrane component as cyclosporin A. It might be argued that Propofol inhibits permeabilization by inhibiting the mitochondrial PLA_2 [1]. However, Propofol had no effect on the activity of the enzyme at concentrations where it caused the inhibition of permeabilization, i.e. 80-300 nmol/mg protein (not shown). At even higher concentrations of Propofol a small activation of PLA_2 could be observed; that, however, could be due to perturbation of the membrane.

These studies also indicate that the generation of the Ca^{2+}-induced increase in the general permeability of the mitochondrial membrane is closely related to the interaction between radicals or oxidized molecules and Ca^{2+} and that these are among the factors that contribute to pore-formation in the inner mitochondrial membrane.

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