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Effect of Reducing Agents and Uncouplers on the Electrical Potential Generated by Mitochondrial ATPase Activity

I. Encío*, C. de Miguel*, N. López-Moratalla and E. Santiago**

Departamento de Bioquímica
Universidad de Navarra
31008 Pamplona (Spain)

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Beef heart submitochondrial particles bound to phospholipids impregnated filters generated an electrical potential upon the addition of ATP. The magnitude of the electrical potential reached depended on the phospholipid mixture composition used for filter impregnation, phosphatidylethanolamine being the active component for the electrical potential generation. Uncoupler FCCP (p-trifluoromethoxy carbonyl cyanide phenylhydrazone) inhibited the transmembrane electrical potential generation by diminishing the electrical resistance of the system as a result of its protonophoric action. However, uncouplers 2, 4-dinitrophenol and dicoumarol did not provoke large modifications of the electrical resistance under the conditions of pH and concentration used, and their action varied with the time elapsed after the submitochondrial particles purification, favouring the idea of the uncoupler interaction with a specific site on the membrane. Addition of sodium dithionite resulted in a higher plateau value for the electrical potential consistent with the promoted increase in ATPase activity. The effect of this agent was reversed by the 2,6-dichlorophenol-indophenol added at equivalent concentrations.

Key words:ATPase and membrane potential, Uncouplers, ATPase and reducing agents.

A method of $\Delta\Psi$ monitoring, based on the association of membranous vesicles to lipid impregnated filters, allowing the measurement of electrical potential differences across membranes has been developed by SKULACHEV's group (3, 5, 25), and extensively applied to reconstituted proteoliposomes in which the embedded protein has electrogenic activity (3, 4, 6, 13, 24). Proteoliposomes reconstituted from oligomycin-sensitive H^+-ATPase were shown to generate an electrical potential upon ATP hydrolysis (4). Translo-

* Present address: Section on Clinical Genetics. Clinical Neurogenetics Branch, NIMH, Bethesda, Mar. 20205 (USA).

** To whom all correspondence should be addressed.

cation of protons into the vesicles associated-filter during the ATPase catalyzed reaction was proposed as responsible for the potential generation (4). The method has been extended to non-reconstituted systems and electrogenic activity of the SMP associated-phospholipid filter upon ATP addition has also been found (14, 19). The present paper describes the close dependence of the electrogenic activity on the composition of the phospholipid mixture used for filter impregnation; the action of some uncouplers, ATPase inhibitors and other reagents such as dithionite and 2,6-dichlorophenol-indophenol on the electrical signal is also reported, when using from beef heart mitochondria.

Materials and Methods

All reagents were prepared with deionized double distilled water. Microsomal phospholipids were extracted from rat liver (23) and phosphatidylethanolamine and phosphatidylcholine from microsomal phospholipids (17). The chloroformic phospholipid extracts were concentrated to dryness under nitrogen current and phospholipids redissolved in n-hexadecane (70-100 mg/ml).

Beef heart mithochondria were prepared according to LÖW and VALLIN (15) and stored at -20°C. Submitochondrial particles (SMP) were prepared by sonication of the thawed mitochondria in the presence of PPi (21). To include different compounds into the particles, when required, they were added to the formation medium of the particles at the appropriate concentrations before sonication. Proteins were determined by the Biuret method in the presence of 4 % deoxycholate using bovine serum albumin as standard (11). ATPase activity was determined by the Pi released (20). Aliquots of the SMP were preincubated for 5 min. at 30°C in 0.8 ml of a medium containing 50 µmoles of Tris-acetate, pH 7.4. The reaction was initiated by the addition of substrate Mg-ATP at the appropriate concentration. The incubation was continued for 5 min stopped by the addition of 0.1 ml of 50 % (w/v) trichloroacetic acid and Pi was determined. Reagent and enzyme blanks were determined in each experiment.

Ag/AgCl electrodes were prepared according to FERRIS (7) chloridizing a silver electrode by anodizing in a dilute solution of HCl.

The electrical measurements were performed by the method described by DRACHEV *et al.* (5) and SKULACHEV (25) in a chamber consisting of two 3.5 ml compartments connecting via a 1 cm diameter aperture in the separating wall. The aperture was closed with a Mitex filter, LSWP type, previously soaked in a solution of phospholipids in n-hexadecane (70-100 mg/ml), the lipid excess having been removed by capilarity. The two helf-cells were filled with 3 ml of the appropriate medium. The electrical potential difference across the filter was registered with a pair of Ag/AgCl electrodes fed into a Keithley electrometer, Model 642, with an actual input impedance higher than 10^{12} connected to a strip-chart recorder. SMP (approximately 0.1 mg protein/ml) were added to the grounded compartment only, and the electrical responses of the filter-associated SMP were initiated with the addition of ATP delivered to both compartments of the chamber, unless otherwise specified. The electrical resistance of the system was routinely checked before and after recording the SMP-induced electrical responses. The electrical time-constant of the systems was approximately 20 s.

Abbreviations: DNP, 2,5-dinitrophenol; FCCP, p-trifluoromethoxy carbonyl cyanide phenylhydrazone; $\Delta\Psi$, membrane potential. SMP, beef heart submitochondrial particles prepared by sonication in the presence of PPi; SMP-DNP, SMP-dicoumarol, SMP-FCCP, SMP-azide and SMP-KSCN, beef heart submitochondrial particles prepared by sonication in the presence of these reagents.

Results

Figure 1 shows the electrical potential difference across the filter, negative in the SMP containing compartment; it increased as a result of the addition of ATP to the microsomal impregnated filter associated SMP system. The resultant electrical potential reached a plateau of 18-22 mV, that is smaller than the 80-100 mV plateau value found by others (14, 19). The half time of the potential generation was 3-7 min at 30° C corresponding to an initial rate of increase of the potential of 3-6 mV per minute.

No differences in the plateau value were observed, when different amounts of Mg^{2+} were added, an indication that this cation was not required for the association of the particles to the filter. Therefore, the smaller value of the plateau cannot be ascribed to difference in concentration of this cation.

Experiments were performed, in which the filters used had been impregnated with different mixtures of phosphatidylcholine and phosphatidylethanolamine in n-hexadecane, and a proton gradient generated across the filter, in the absence of SMP, simply by the addition of HCl to one of the compartments. The electrical potential obtained versus the pH gradient is given in figure 2A. The dashed line shows the value of the potential predicted by the Nernst law for protons. The signal obtained with mixtures containing 30 % or more of phosphatidylethanolamine had an amplitude proportional to the pH gradient with a slope of 50 mV/pH unit, 9 mV lower than expected according to the Nernst law. This difference was even more pronounced at higher pH gradients. However, when an uncoupler (200 μM DNP, 5 μM dicoumarol or 1 μM FCCP) was added to the system, the potential reached a new proton distribution as predicted by the Nernst law. The same behaviour could be observed with mixtures with a lower content of phosphatidylethanolamine, althoug in these cases, when the uncoupler was absent, the electrical potential observed was smaller. The dependence of the electrical potential on the phosphatidylethanolamine content is also shown in figure 2B, where the maximal potential differences generated by ATP hydrolysis using mixtures of different phospholipid composition are given.

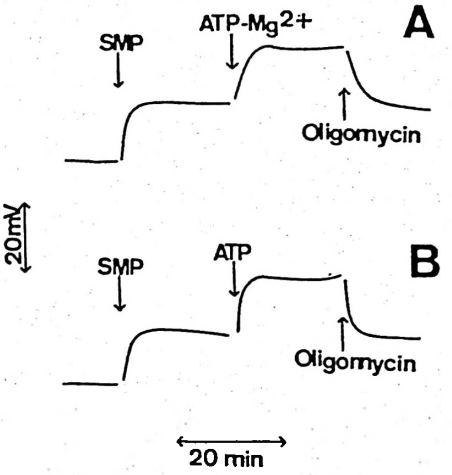


Fig. 1. *Electrical signal generated by ATP hydrolysis.*
The reaction mixture contained: A, 250 mM sucrose, 50 mM Tris-HCl pH 7.5; B, 250 mM sucrose, 50 mM Tris-HCl pH 7.5, 2.5 mM $MgCl_2$. Additions were: 0.1 mg SMP protein /ml and 1 mM oligomycin in both cases; 1 mM Mg^{2+}-ATP in A 1 mM ATP in B. The electrical resistance of the membrane was within the range 0.2-0.6× 10^9 Ω.

The sensitivity of the electrogenic activity of the filter associated SMP to uncouplers DNP (200 μM), dicoumarol (5 μM) and FCCP (1 μM) has also been studied. At the concentrations used, DNP, dicoumarol and FCCP were found to lower the resistance of the filter-associated SMP by a factor 4, 5 and 50 respectively. As shown in figure 3, DNP (200 μM) had a different effect on the signal which was found to be dependent on the time elapsed after the isolation of the SMP. The different behaviour

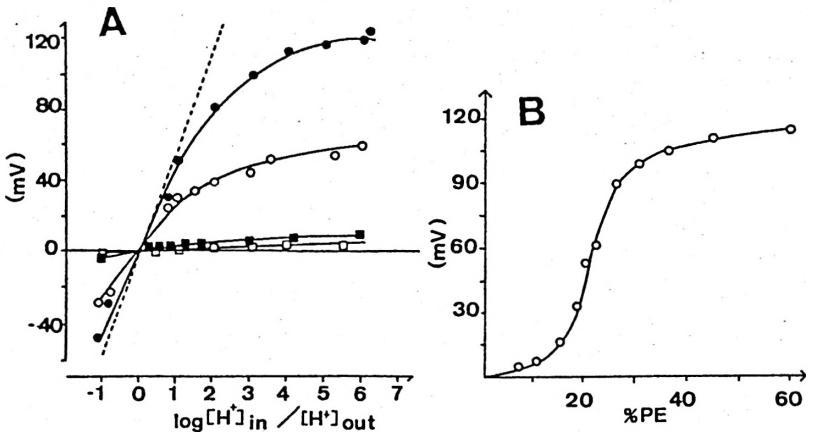


Fig. 2. *Effect of membrane phospholipid content on the electrical potential.*

A, Electrical potential generated by pH gradients, obtained by addition of HCl to the reference compartment only. The dashed line represents the expected value of the potential according to the Nerns law for protons. H^+ in represents the proton concentrations in the compartment connected to ground, to which HCl was added. H^+ out represents the proton concentration in the compartment free of HCl. Phosphatidylethanolamine (PE) content in the phospholipid mixture used for filter impregnation: □, 7 %; ■, 14 %, ○, microsomal phospholipids (22 %); ●, 30-100 %. B, Maximal value of the electrical potential registered in the ATP hydrolysis experiments versus the phosphatidylethanolamine (PE) content in the phospholipid mixture used for filter impregnation. The electrical resistance of the system was as in figure 1.

Table I. *Effect of dithionite, oligomycin and uncouplers DNP, FCCP and dicoumarol on the ATPase activity of the SMP at 3 mM Mg^{2+}-ATP concentration.*

SMP, SMP-DNP, SMP-FCCP and SMP-dicoumarol represent submitochondrial particles, prepared by sonication in the presence of these reagents and stored frozen for 2-3 days. SMP*, SMP-DNP*, SMP-FCCP* and SMP-dicoumarol* represent the same particles stored frozen for periods longer than a week. Experiments were carried out at a protein concentration of 0.1 mg/ml.

	Activity (μM Pi/min × mg)	Dithionite (35 μM)	Oligomycin (50 μM)	DNP (200 μM)	Dicoumarol (5 μM)	FCCP (1 μM)
SMP	1.13 ± 0.05	1.58 ± 0.12	0.17 ± 0.01	1.40 ± 0.06	1.20 ± 0.03	1.21 ± 0.03
SMP*	1.12 ± 0.06	1.50 ± 0.07	0.18 ± 0.01	1.16 ± 0.12	1.22 ± 0.02	1.22 ± 0.01
SMP-DNP	1.99 ± 0.15	2.66 ± 0.20	0.24 ± 0.02	2.18 ± 0.11	—	—
SMP-DNP*	1.86 ± 0.15	2.53 ± 0.10	0.34 ± 0.03	2.33 ± 0.12	—	—
SMP-Dicoumarol	1.32 ± 0.11	1.59 ± 0.11	0.19 ± 0.02	—	—	—
SMP-Dicoumarol*	1.30 ± 0.10	1.93 ± 0.13	0.19 ± 0.02	—	—	—
SMP-FCCP	1.46 ± 0.05	1.77 ± 0.16	0.19 ± 0.02	—	—	—
SMP-FCCP*	1.42 ± 0.05	1.87 ± 0.02	0.19 ± 0.02	—	—	—

of the uncoupler with that described above, when potential was generated by simply adding HCl to one of the chambers of the cell, has been interpreted as a result of a change in the participation of the component, $\Delta\Psi$ or Δ pH, of the electrical potential responsible for the electric current generation in the actual system (19). If SMP had been kept frozen for periods no longer than 2 to 3 days DNP always raised the plateau value of the response (fig. 3A); when SMP stored frozen for periods of 5-7 days were used, this effect of DNP was not observed (fig. 3B); finally, if the storage period had been prolonged for over a week, DNP even caused the potential plateau already reached with ATP to decrease (fig. 3C). As shown in tables I and II storage time of the particles did not substantially affect their synthetic and hydrolytic activities, and their coupling degree. However (table II), the effect of DNP on the ATPase hydrolytic activity varied with the time elapsed during the storage of the SMP; if SMP had been stored for 2-3 days, then DNP increased the ATPase hydrolytic activity of the SMP; this increase did not take place if the SMP had been stored for 5-7 days. It should be noticed that the native hydrolytic activity of the SMP, or their sensitivity to dithionite remained unchanged. In order to see if these changes in the response to DNP could be due to a progressive difficulty for the uncoupler to bind to the corresponding sites on the membrane (9, 12), experiments were carried out, where the uncoupler was added to the SMP in the formation medium, prior to sonication. Table II shows that, under these conditions, DNP had a greater effect on the hydrolytic activity of the

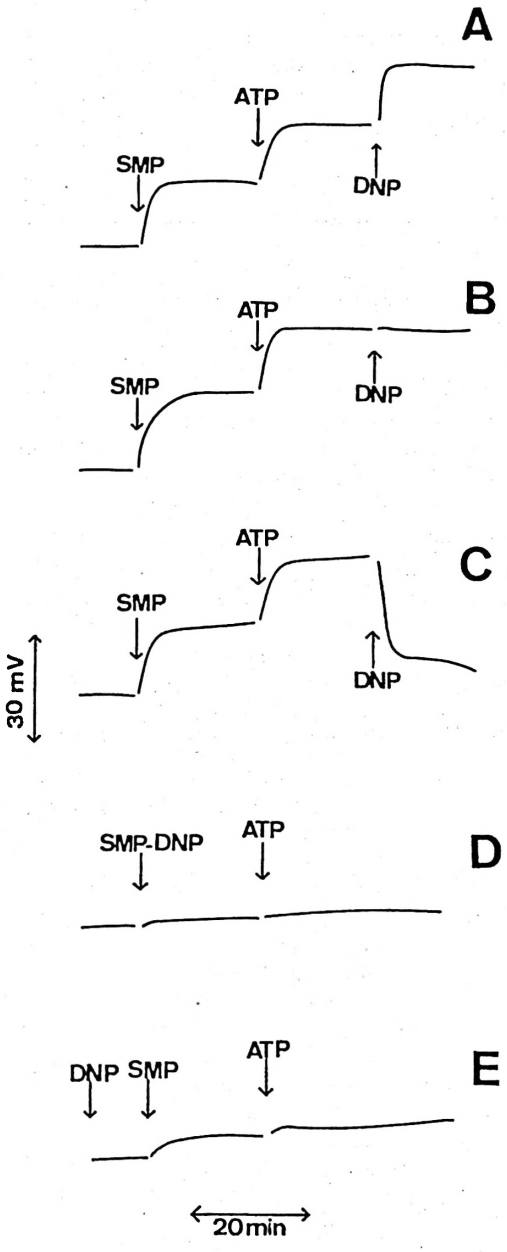
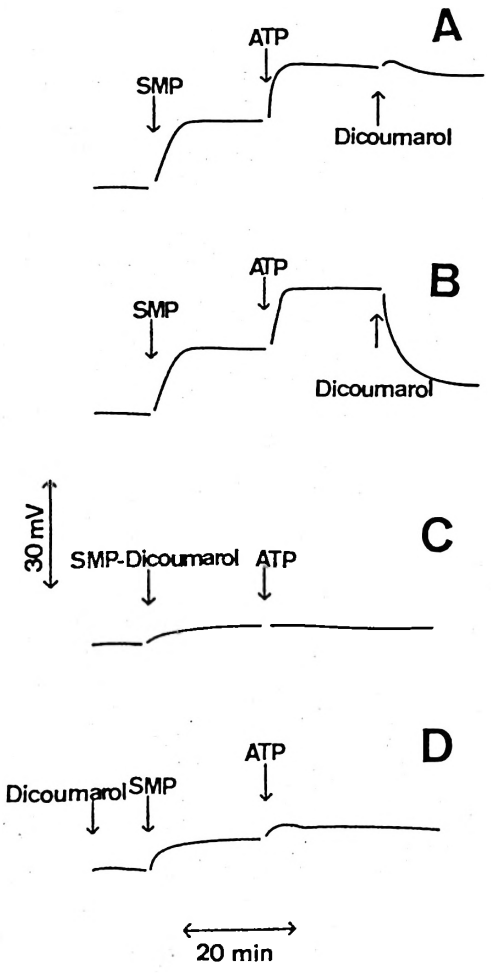


Fig. 3. *Effect of 2,4-dinitrophenol (DNP) on the electrical signal generated by ATP hydrolysis.* The reaction mixture was the same as in Fig. 1. Additions were: 0.5 mg SMP protein/ml (A-D) or 0.05 mg SMP-DNP protein/ml (E); 1 mM ATP and 0.2 mM DNP. A, B and C differ on the time elapsed after the SMP isolation (see text for details). The electrical resistance of the membrane was within the ranges 0.1-0.5 × 10^9 and 0.25-1.35 × 10^8, before and after DNP addition respectively.

Table II. *Effect of dithionite, 2,6-dichlorophenol-indophenol (DCPIP), azide, KSCN and oligomycin on the ATPase activity of the SMP at 3 mM Mg^{2+}-ATP concentration.*

SMP, SMP-acide and SMP-KSCN represent submitochondrial particles, prepared by sonication in the presence of these reagents and stored frozen for 2-3 days. SMP*, SMP-azide* and SMP-KSCN* represent the same particles stored frozen for periods longer than a week. Experiments were carried out at a protein concentration of 0.1 mg/ml.

	Activity (µM Pi/min × mg)	Dithionite (35 µM)	DCPIP (35 µM)	Azide (1 mM)	KSCN (2 mM)	Oligomycin (50 µM)
SMP	1.13 ± 0.02	1.63 ± 0.09	1.12 ± 0.02	0.25 ± 0.03	0.34 ± 0.03	0.16 ± 0.02
SMP*	1.13 ± 0.07	1.53 ± 0.14	1.08 ± 0.02	0.31 ± 0.03	0.41 ± 0.02	0.18 ± 0.02
SMP-azide	0.78 ± 0.04	1.24 ± 0.07	—	—	—	0.18 ± 0.01
SMP-azide*	1.04 ± 0.04	1.58 ± 0.07	—	—	—	0.23 ± 0.02
SMP-KSCN	0.35 ± 0.03	0.48 ± 0.05	—	—	—	0.16 ± 0.02
SMP-KSCN*	1.13 ± 0.10	1.41 ± 0.10	—	—	—	0.16 ± 0.03



particles, and also that this enhanced effect did not disappear during time of storage. Therefore, it seems that sonication facilitates the interaction of the uncoupler with its corresponding sites, whereas freezing could progressively turn this interaction more difficult. However, it was not possible to register an electrical potential with these particles (fig. 3). Similarly, no potential could be registered either with control particles, if DNP had been incorporated to the «thick membrane system» before the SMP addition (fig. 3).

Similar results were obtained with the uncoupler dicoumarol at 5 µM concentration (fig. 4, table I), except that in this case the increase in the electrical potential observed with SMP stored for 2-3 days did not take place and the increase in the hy-

Fig. 4. *Effect of dicoumarol (Dic) on the electrical signal generated by ATP hydrolysis.*
The reaction mixture was the same as in Fig. 1. Additions were: 0.05 mg SMP protein/ml (A-C) or 0.05 mg SMP-Dic protein/ml (D); 1 mM ATP and 5 µN dicoumarol. A and B differ on the time elapsed after the SMP isolation (see text for details). The electrical resistance of the membrane was within the ranges 0.2-0.6 × 10^9 and 0.4-1.5 × 10^8, before and after dicoumarol addition respectively.

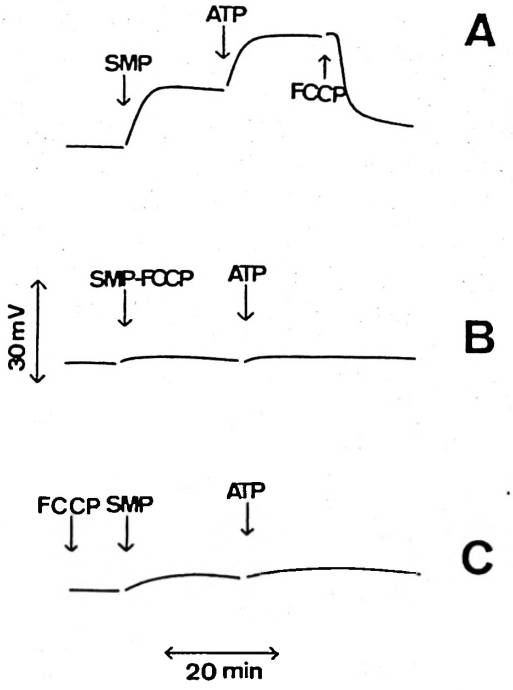


Fig. 5. *Effect of FCCP on the electrical signal generated by ATP hydrolysis.*
The reaction mixture was the same as in Fig. 1. Additions were: 0.05 mg SMP protein/ml (A-B) or 0.05 mg SMP-FCCP protein/ml (C), 1 mM ATP and 1 μM FCCP. The electrical resistance of the membrane was within the ranges 0.2-0.4 × 10^9 and 0.4-0.8 × 10^7, before and after FCCP addition respectively.

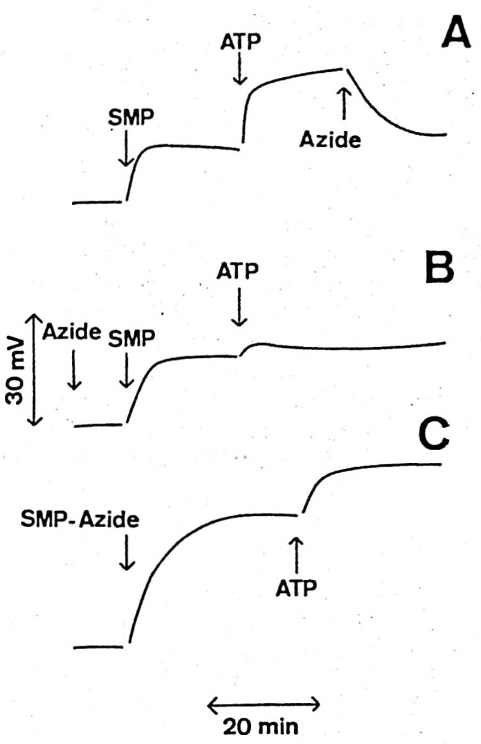


Fig. 6. *Effect of azide on the electrical signal generated by ATP hydrolysis.*
The reaction mixture was the same as in Fig. 1. Additions were: 0.10 mg SMP protein/ml (A-B) or 0.10 mg SMP-Azide protein/ml (C); 1 mM ATP and 1 mM azide. The electrical resistance of the membrane was as in figure 1.

drolytic activity of the particles did not dissapear with time.

When 1 μM FCCP was used, only the inhibitory effect on the electrical signal was observed (fig. 5). With regard to the effect on the ATPase activity of SMP, FCCP behaved similarly to dicoumarol (table I).

ATPase inhibitors such as oligomycin, azide, and KSCN (table II) prevented, or even reversed, the generation of the electrical potential (figs. 1, 6 and 7). If these inhibitors had been added to the medium before SMP, or if they had been included inside the particles, they inhibited the increase of the potential; once the electrical potential had been generated, their addition resulted in a decay of that potential.

Figure 8 show that 35 μM dithionite caused a 40 to 45 % increase in the plateau value of the electrical potential generated by ATP hydrolysis, as a consequence of the increase in the ATP hydrolytic activity of the particles promoted by reduction (table II). The addition of 2,6-dichlorophenol-indophenol reversed the dithionite effect if added at equivalent concentrations (fig. 8).

The results of similar experiments, in which the oxidizing agent had been added before dithionite (figure 8).

Discussion

Data reported in this paper confirm the electrogenic activity of the SMP associated with a phospholipid impregnated filter upon the addition of ATP (fig. 2), in agreement with KONSTANTINOV *et al.* (14), and PFISTER and POUGEOIS (19). It has been proposed that the potential generation is linked to the translocation of protons into the particles associated with the ATPase reaction. The absence of electrical potential generation when ADP and Pi were added directly to the SMP compartment simulating an ATP hydrolysis (data no shown), and the inhibitory

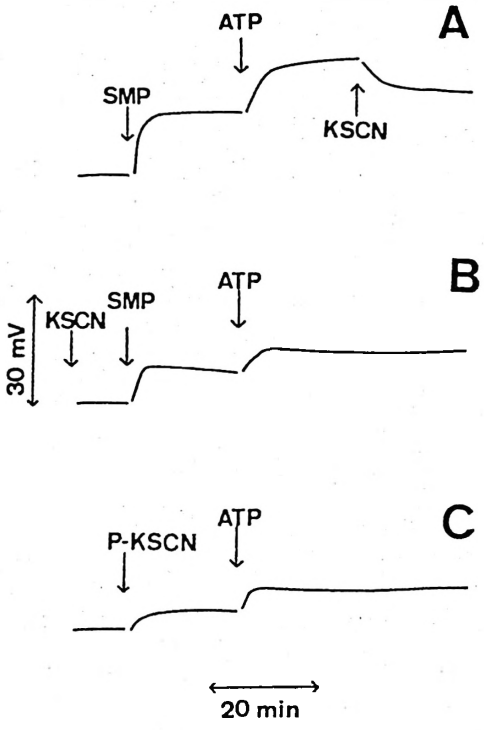


Fig. 7. *Effect of KSCN on the electrical signal generated by ATP hydrolysis.*
The reaction mixture was the same as in Fig. 1. Additions were: 0.10 mg SMP protein /ml (A-B) or 0.10 mg SMP-KSCN protein/ml (C); 1 mM ATP and 2 mM KSCN. The electrical resistance of the membrane was as in figure 1.

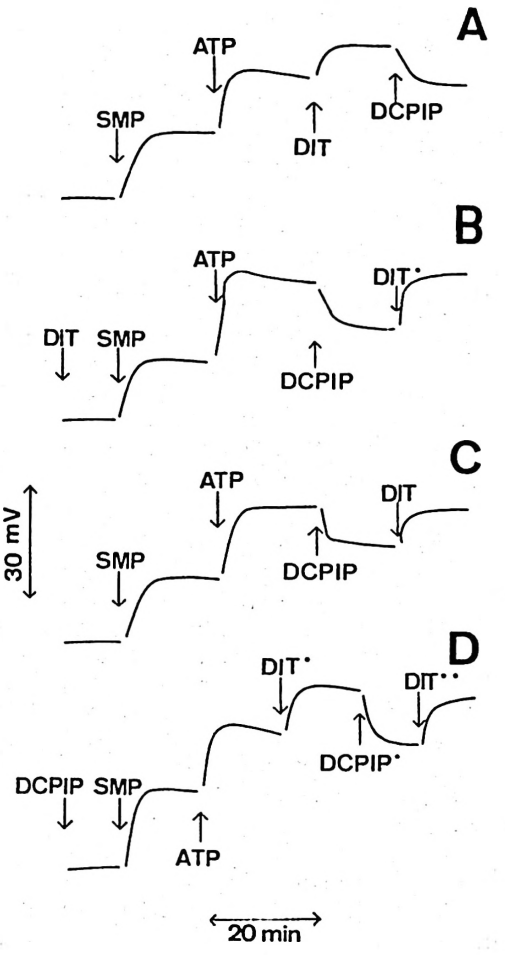


Fig. 8. *Effect of dithionite (DIT) and 2,6-dichlorophenolindophenol (DCPIP) on the electrical signal generated by ATP hydrolysis.*
The reaction mixture was the same as in Fig. 1. Additions were: 0.10 mg SMP protein/ml; 1 mM ATP; 35 µM DIT; 35 µM DCPIP; DIT* and DCPIP** represent additions of these reagents to bring their concentration to 70 µM; DIT** represents additions of that reagent to bring its concentration to 105 µM. The electrical resistance of the membrane was as in figure 1.

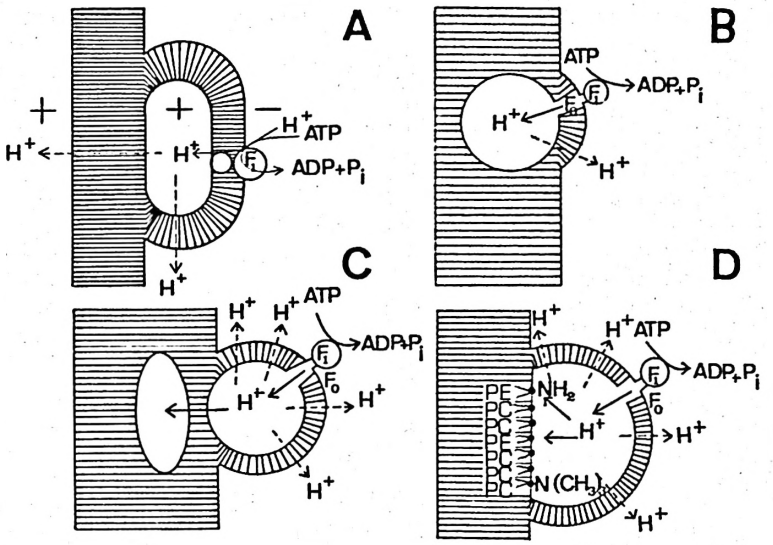


Fig. 9. *Hipothetical schemes illustrating the possible mechanisms underlying the electrical current generation.*

A, scheme proposed by DRACHEV et al. (4) invoking translocation of protons inside the vesicle and across the filter; B, C and D, schemes proposed by PFISTER and POUGEOIS (19) showing the possibilities of: (B) SMP penetration in the thick membrane, (C) trapping of protons in membrane aqueous compartments and (D) proton interaction with the membrane phospholipids.

effect of the potential that ATPase inhibitors provoked (figs. 6 and 7), support the proposal that the potential generation was due to the actual hydrolytic reaction. Figure 9 shows the different schemes proposed illustrating the possible mechanisms underlying the electric current generation (4, 19). They invoke translocation of protons into the particles and across the filter (4), or the possibility that the vesicles penetrate into the thick membrane (fig. 9A,B); figure 9C suggests the trapping of protons in aqueous compartments within the membrane, whereas figure 9D suggests a direct interaction of protons with the phospholipids of the thick membrane. The phosphatidylethanolamine requirement in the phospholipid mixture for the electric potential generation (figure 3) would fit into the scheme represented in figure 9D. In addition to this, if transport of protons across the filter were the cause of the electrical potential generation, the time constant of the signal would be the electrical time constant of the thick membrane (20 s), a value far too short as compared with those found in all the experiments here described (3-7 min).

«Classical uncouplers» are all moderately weak acids that increase the conductivity of phospholipid bilayers acting as proton uncouplers (10). Since MITCHELL (16) postulated that the effect of uncouplers is based on its short-circuiting effect on the H^+ current through the mitochondrial membrane, their protonophoric action has been considered by many investigators as a support for a chemiosmotic mechanism of oxidative phosphorylation. However, binding of uncouplers to a specific protein on the membrane has also been described (2, 9, 12, 27), and sometimes proposed as essential for their uncoupling activity. The existence of the un-

coupler binding protein has also been regarded as a support for mechanisms other than chemiosmosis (9).

The results of experiments described in this paper show the expected increase in the conductivity of the thick membrane induced by uncouplers. At the concentrations used FCCP, dicoumarol and DNP increased the conductivity of the system by a factor of 50, 5 and 4 respectively. The inhibitory effect on the potential generation always found with FCCP (fig. 6), could be explained through a protonophoric action; a similar explanation would be valid in the case of the inhibitory effect found with dicoumarol and DNP, when the uncoupler was added before the SMP or in the sonication medium of the particles (figs. 4 and 5). However, the results obtained with DNP and dicoumarol showing that their effect on the electrical potential disappeared or was even reversed when SMP had been kept frozen for periods longer than 7 or more days (figs. 3 and 4), would be difficult to reconcile with that kind of interpretation; at least in these cases there is no correlation between their behaviour as uncouplers and their protonophoric activity. This lack of correlation, already observed by others (1, 8, 28), seems to contradict an exclusively chemiosmotic mechanism for the uncoupling action as a consequence of their protonophoric action, thus favouring the idea of the interaction of these uncouplers with a specific site in the membrane. It has been proposed that an interaction site for DNP would be located on F_1 -ATPase. Its binding would lead to an enhancement of the hydrolytic activity (2). This activation would explain the increase in membrane potential as a result of the elevation of the hydrolysis rate. Storage could turn the binding progressively more difficult, and the uncoupler protonophoric action would become more prominent. According to this suggestion the uncoupler action would not be simple but composite. FCCP would exhibit only a protonophoric action as a result of its high solubility in the membrane thus strongly affecting membrane conductivity (27).

The possibility of redox reversible modifications of soluble and membrane ATPase was previously reported (22). Results of table II show that dithionite increased the ATPase activity of the SMP. Figure 8 also shows that the addition of dithionite resulted in a 40-45 % increase of the plateau value of the electrical potential. The reversal of the dithionite effect both on the electrical potential and on the ATPase activity by 2,6-dichlorophenolindophenol (table II, fig. 8) suggests a mechanism implying a redox reaction of H^+-ATPase.

The idea of considering H^+-ATPase as a redox proton pump should not be discarded as something unreasonable. This proposal would offer an alternative to the suggestion of by SLATER *et al.* (26) according to which a collision between an independent redox protein and ATPase would be necesary for the energy transfer.

The second independent way to synthesize ATP envisaged by PADAN and ROTTENBERG (18), besides that of an electrochemical proton gradient, could be through a direct energy transfer in a redox process, as now suggested.

Acknowledgements

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Resumen

Las partículas submitocondriales de corazón bovino adheridas a filtros impregnados con fosfolípidos generan un potencial eléctrico tras la adición de ATP. La magnitud alcanzada por el potencial eléctrico depende de la composición de la mezcla de fosfolípidos que se emplean para la impregnación del filtro. La fosfatidiletanolamina es el componente activo para la generación del potencial. El desacoplador FCCP (cianuro de p-trifluorometoxicarbonilo) inhibe la ge-

neración del potencial eléctrico al disminuir la resistencia eléctrica del sistema como resultado de su acción protonofórica. Sin embargo los desacopladores 2,4-dinitrofenol y dicumarol no provocan grandes alteraciones de la resistencia eléctrica en las condiciones de pH y de concentraciones utilizadas, y su acción varía con el tiempo transcurrido después de la purificación de las partículas, lo que favorece la idea de una posible interacción del desacoplador con su sitio específico en la membrana. La adición de ditionito de sodio da lugar a una meseta más elevada para los valores del potencial coherente con el aumento producido sobre la actividad de la ATPasa. El efecto de este agente se anula con la adición de 2,6-diclorofenolindofenol.

Palabras clave: ATPasa y potencial de membrana, Desacopladores, ATPasa y agentes redox.

References

1. Bakker, E. P., Van der Henvel, E. J., Wiechman, A.H.C.A. and Van Dam, K.: *Biochim. Biophys. Acta*, 292, 78-87, 1973.
2. Copeland, L., Deutsch, C. J., Tu, S. I. and Wang, J. H.: *Arch. Biochem. Biophys.*, 160, 471-457, 1974.
3. Drachev, L. A., Jasaitis, A. A., Kaulen, A. D. *et al.: Nature*, 249, 321-324, 1974.
4. Drachev, L. A., Jasaitis, A. A., Mikelsar, H. *et al.: J. Biol. Chem.*, 251, 7077-7082, 1976.
5. Drachev, L. A., Kaulen, A. D., Semenov, A. Yu, Severina, I. I. and Skulachev, V. P.: *Anal. Biochem.*, 96, 250-262, 1979.
6. Drachev, L. A., Kondrashin, A. A., Semenov, A. Yu and Skulachev, V. P.: *Eur. J. Biochem.*, 113, 213-217, 1980.
7. Ferris, C. D.: In: «Introduction to Bioelectrodes». Plenum Press, New York, 1974, pp. 90-100.
8. Hanstein, W. G. and Hatefi, Y.: *J. Biol. Chem.*. 249, 1356-1362, 1974.
9. Hanstein, W. G.: *Biochim. Biophys. Acta*, 456, 129-148, 1976.
10. Heytler, P. G.: In «Methods in Enzymology» (S. P. Collowick and N. O. Kaplan, eds.) Academic Press, New York, 1979, Vol. 55 F, pp. 462-472.
11. Jacobs, E. E., Jacob, M., Sanadi, D. R. and Bradley, L. B.: *J. Biol. Chem.*, 223, 147-156, 1956.
12. Katre, N. V. and Wilson, D.: *Arch. Biochem. Biophys.*, 191, 647-656, 1978.
13. Kondrashin, A. A., Remennikov, V. G., Samuilov, V. D. and Skulachev, V. P.: *Eur. J. Biochem.*, 113, 219-222, 1980.
14. Konstantinov, A., Skulachev, V. P. and Smirnova, I. A.: *FEBS Lett.*, 114, 302-306, 1980.
15. Löw, H. and Vallin, I.: *Biochim. Biophys. Acta*, 69, 361-374, 1963.
16. Mitchell, P.: *Biol. Rev.*, 41, 445-502, 1966.
17. Neskovik, N. M. and Kostik, D. M.: *J. Chromatogr.*, 35, 297-300, 1968.
18. Padan, E. and Rottenberg, H.: *Eur. J. Biochem.*, 40, 431-437, 1973.
19. Pfister, C. and Pougeois, R.: *Biochim. Biophys. Acta*, 589, 201-216, 1980.
20. Pullman, M. E., Penefsky, H. S., Datta, A. and Racker, E.: *J. Biol. Chem.*, 235, 3322-3329, 1960.
21. Racker, E.: *Proc. Natl. Acad. Sci. Usa*, 48, 1659-1663, 1962.
22. Santiago, E. and López-Moratalla, N.: *Rev. esp. Fisiol.*, 34, 481-490, 1978.
23. Santiago, E., Mule, S., Redman, C.R., Hokin, M. R. and Hokin, L. E.: *Biochim. Biophys. Acta*, 84, 550-562, 1964.
24. Skulachev, V. P.: *FEBS Lett.*, 64, 23-25, 1976.
25. Skulachev, V. P.: In «Methods in Enzymology» (S. P. Collowick and N. O. Kaplan, eds.). Academic Press, New York, 1979, Vol. 55F, pp. 586-603.
26. Slater, E. C., Berden, J. A. and Herweiger, M. A.: *Biochim. Biophys. Acta*, 811, 217-231, 1985.
27. Terada, H.: *Biochim. Biophys. Acta*, 639, 225-242, 1981.
28. Ting, H. P., Wilson, D. F. and Chance, B.: *Arch. Biochem. Biophys.*, 141, 141-146, 1970.