Inhibition of Calcium Transport in Mitochondria by β-Receptor Blocking Substances and its Reactivation by Phospholipids

It is well established now that the extent of the myoplasmatic calcium level is an important factor for the regulation of the excitation-contraction mechanism of cardiac muscle. It is supposed that several cardioactive drugs will act by changing the rate of intracellular calcium flux or the degree of free calcium. Besides the sarcoplasmatic reticulum, phosphorylating mitochondria are capable of performing energy-linked transport of calcium and accumulating large quantities of calcium[1]. Thus they might participate in regulating the intracellular calcium level[2,3]. In previous reports[4,5] we have shown that a variety of cardioactive drugs: quinidine, alprenolol, propranolol, oxyfedrin and tetracain – that means especially β-adrenergic receptor blocking agents – are able to decrease or to block the rate of energy-dependent calcium and adeninenucleotide uptake in mitochondria isolated from rat liver and rabbit heart without altering coupling and efficiency of oxidative phosphorylation. It is remarkable that three β-receptor blocking agents, LB 46, ICI 50 172 and Kö 592, were ineffective even at concentrations as high as $2 \times 10^{-3} M$. In these experiments we examined the stoichiometric uptake of small amounts of ADP and Ca^{++} measuring the changes in oxygen consumption, the ratio Ca^{++}:O and other related parameters by means of atomic adsorption spectroscopy, the microglass electrode and the polarographic method of CHANCE[6]. The results showed that the degree of inhibition with heart mitochondria was 2- to 3-fold higher than with liver mitochondria, ADP uptake being more effected than calcium accumulation. These experiments supported our conception, which agrees with that of several other authors, that on the molecular level there is a competition of the drugs with phospholipids of the inner mitochondrial membrane which are participating in calcium and ADP transport mechanism. BLAUSTEIN[7], FEINSTEIN[8], NAYLER[9], PICCININI et al.[10], and other authors observed that phospholipids bind inorganic cations and facilitate their transport from water into organic solvents in which they normally are not soluble.

We therefore extracted the lipids from rabbit heart mitochondrial protein in a chloroform/methanol phase in order to investigate the effects of the drugs cited above on lipid-facilitated transport of radioactive calcium. Incubating 2 volumes of lipid-containing chloroform with 1 volume of Ringer's solution containing ^{45}Ca, according to the method of NAYLER[9], we got the data which are depicted in Figure 1. The addition of an aqueous solution of D- and L-alprenolol and DL-propranolol to the chloroform extracts of rabbit heart mitochondrial fractions resulted in a consistent inhibition of the transport of calcium from the Ringer's solution into the chloroform phase. The D- and L-configuration of alprenolol was equieffective.

In the table a series of β-receptor blocking agents and related compounds are compiled which perform a 50% inhibition of the lipid-facilitated transport of calcium ions. The ability of these substances to impair the transport of ionized calcium across an aqueous lipid-solvent interface corresponds to their potency to inhibit the energy-dependent calcium transport in mitochondria,

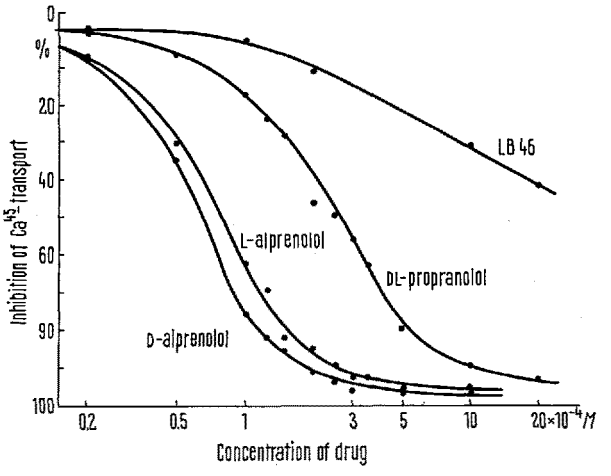

Fig. 1. Inhibition of the lipid-facilitated transport of calcium from an aqueous phase into chloroform by D-alprenolol, L-alprenolol, DL-propranolol and LB 46. Lipids were extracted from the mitochondrial fraction of rabbit hearts and dissolved (1 mg/ml) in the chloroform/methanol (2:1) phase. The reaction mixture contained 0.2 μC of $^{45}Ca^{++}$ per ml. The radioactivity of aliquots of the chloroform phase was determined by using a liquid scintillation counter.

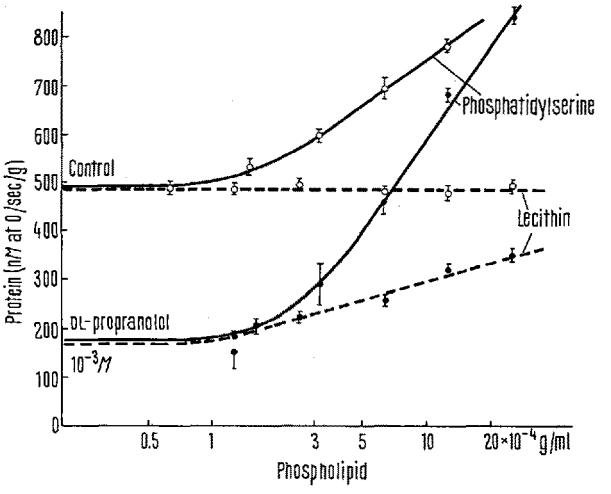

Fig. 2. Reactivation of the DL-propranolol-inhibited rate of Ca^{++}-transport by phospholipids calculated from the extent of extra oxygen consumption induced by small amounts of calcium (500 nM) in rabbit heart mitochondria (2 mg mitochondrial protein per ml). Incubation medium: sodium glutamate, 10 mM, sodium fumarate, 2.5 mM, $MgCl_2$, 10 mM, inorganic phosphate, 2.5 mM, mannitol, 200 mM, sucrose, 66 mM and *Tris*-HCl, 4.4 mM. pH 7.40, temperature 25 °C, final volume 2.5 ml. Polarographical measurement. Each point represents the mean ± S.E.M.

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as described in previous papers[4,5]. Kö 592, LB 46 and ICI 50 172, which did not have any influence on the active transport of calcium into mitochondria, were less effective here, too. Only very high concentrations of these drugs ($\leqq 1.5 \times 10^{-3} M$) produced an inhibition.

In order to test the affinity of these drugs to the phospholipids of the mitochondrial membrane, we investigated whether the delay of calcium uptake in isolated mitochondria, which is induced by the drugs, is reactivated by adding special phospholipids. Figure 2 shows our results. The calcium transport of rat liver mitochondria was inhibited by $10^{-3} M$ propranolol. On adding rising amounts of phosphatidylserine or lecithin, there was a reactivation of the uptake velocity, phosphatidylserine being much more effective than lecithin. While phosphatidylserine slightly increased the rate of uptake also in the controls, lecithin had no influence. Phosphatidylaethanolamine or phospholipid fragments, such as serinphosphate or serine, had no reactivating capacity in a final concentration from $0.5–20 \times 10^{-4}$ g/ml at all. In addition to these findings in isolated mitochondria, the slowing of the velocity of ADP uptake induced by $10^{-3} M$ propranolol could be restituted by adding phosphatidylserine and lecithin, too. These results indicate that the drugs tested may react in a similar way with phospholipids of the mitochondrial membrane in vivo.

β-receptor blocking agents and some related cardioactive compounds (quinidine, tetracain) have a pronounced negative inotropic effect on the myocardium. This effect might be brought about by the reaction of the drug in a cationic form with anionic binding sites of specific phospholipids in competition with cations occupying the carriers for translocating calcium, potassium or hydrogen ions. On the basis of this kinetic pattern, an interaction between the phospholipids of the mitochondrial membrane and the effective drug might inhibit or impede the intracellular transport of calcium and adeninenucleotides. With respect to β-receptor blocking agents, this would result in a depression of the cardiac activity and metabolism which, in accordance with FLECKENSTEIN et al.[11], is not correlated to the specific β-blocking activity.

50% inhibition of lipid-facilitated calcium transport

Drug	$\times 10^{-4} M$
D-alprenolol	0.69
L-alprenolol	0.70
DL-propranolol	2.55
Quinidine	3.45
DL-oxyfedrin	2.60
L-oxyfedrin	3.10
Kö 592	15.0
LB 46	30.0
ICI 50 172	> 50.0

Zusammenfassung. β-Rezeptorenblocker hemmen den aktiven Kalziumtransport an isolierten Herzmitochondrien und den phospholipidvermittelten Kalziumtransport in Ausschüttelungsversuchen. Die Hemmung des mitochondrialen Kalziumtransportes lässt sich durch Phosphatidylserin und weniger stark durch Lecithin ($\geqq 10^{-4}$ g/ml) aufheben, während andere Phospholipide und deren Bausteine wirkungslos sind.

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Chronic Urea Intoxication in Dogs

Chronic toxicity of urea has as yet still to be properly defined and consequently its role in causing uremic symptoms is not clear. Many experiments aimed at shedding light on this problem are based on the acute administration of urea to animals (JAVID et al.[1], STEVENSON et al.[2]) or to volunteers (PERKOFF et al.[3], EKNOYAN et al.[4]), while others have been based on the study of uremic animals (GROLLMANN et al.[5]) and of patients with renal failure (MERRILL et al.[6], HUTCHINGS et al.[7]) dialyzed with urea in the dialyzate.

Neither of these experimental schedules allow clear-cut conclusions on chronic toxicity of urea. This may be assessed only by studying animals in which the only blood chemical abnormality is a high blood urea level maintained for long periods of time. The study of dogs in this condition is the subject of the present paper.

Materials and methods. After a control period of 10–15 days, 12 mongrel dogs, in which 1 kidney had been previously removed, were injected s.c. every 8 h with a 10% urea solution in each single dose of 30–40 ml/kg body wt. The fluid injected was rapidly absorbed, did not cause local side effects and induced plasma urea levels ranging from 600–700 mg/100 ml (20–30 min after the injection) to 200–350 mg/100 ml immediately before the subsequent administration).

In 4 of the dogs studied the spontaneous movements were continuously recorded for 3 days during the control period, and for 3 days during intoxication. This was done by connecting the cages containing each of the 4 animals with an electrokimograph. In 2 other animals permanent electrodes were implanted for registration of the EEG.

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