OPPOSITE EFFECTS OF BONGKREKIC ACID AND ATRACTYLOSIDE ON THE ADENINE NUCLEOTIDES INDUCED MITOCHONDRIAL VOLUME CHANGES AND ON THE EFFLUX OF ADENINE NUCLEOTIDES

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1. Introduction

Both bongkrekic acid (BA) and atractyloside (ATR), have been shown to be specific inhibitors of the adenine nucleotide (ANP) translocation in mitochondria [1–3]. This inhibition, however, is obtained by opposite effects of these antibiotics on the ANP carrier: whereas ATR prevents binding of ANP to the carrier sites, BA prevents dissociation of the ANP from the carrier sites [4].

In the present communication two further examples for opposite effects of BA and ATR on mitochondrial functions related to the ANP carrier will be described.

2. Results and discussion

2.1. *Effect of bongkrekic acid on the ADP induced contraction of mitochondria*

It has been shown by Stoner and Sirak [5] and later also by Weber and Blair [6] that the mitochondrial contraction induced by ADP or ATP is independent of the function of ADP and ATP in oxidative phosphorylation since it is not inhibited by oligomycin or uncouplers, wuch as FCCP. On the other hand ATR abolishes ADP induced contraction. The effect requires low concentrations of ADP or ATP (K_M = 0.5 μM) [1] which make the participation of ADP and ATP in energy transfer improbable. These properties are similar to those which characterize the specific binding of ADP and ATP to the mitochondrial membranes which are supposed to reflect binding to the carrier [7]. It has therefore been suggested by us in personal discussions with Dr. Stoner that these changes in the mitochondrial volume are a consequence of the binding of ADP and ATP to the carrier sites, possibly induced by a conformational change of the carrier protein.

The application of BA should permit, in contrast to ATR, to differentiate whether the observed volume changes require only binding of ANP to the ANP carrier (increase of the change by BA) or the entrance of ANP into the mitochondria followed possibly by an intramitochondrial reaction which elicits the change.

The corresponding experiments with beef heart mitochondria are shown in fig. 1. Addition of ADP (trace a) causes an increase in the absorbance corresponding to increased turbidity reflecting a contraction of the mitochondria. Subsequent addition of BA further increases the absorbance. The following addition of ATR is unable to reverse the absorbance increase. With BA alone (trace b) a small effect is seen and the subsequent addition of ADP causes a rapid maximum increase which is also insensitive to ATR. The control experiment (trace c) shows that ATR can completely reverse the ADP induced absorbance increase in the absence of BA. Subsequently, BA can induce again a slow absorbance increase.

All these absorbance changes of the mitochondria are analogous to the influence of BA on the specific ADP binding to mitochondria [4, 7]. The carrier is partially saturated with ADP (about 60%) in the presence of 10 μM ADP [8]. Under the influence of BA the binding becomes saturated and is then insensitive to ATR.

In another experiment volume changes of the mito-

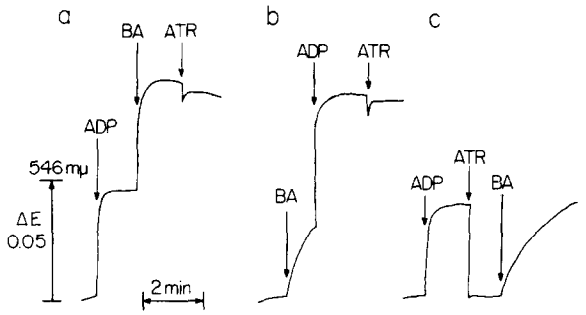

Fig. 1. Influence of BA on ANP induced shrinkage of mitochondria. Beef heart mitochondria (0.45 mg protein/ml) incubated in 0.25 M sucrose, 20 mM TRA, 2 mM EDTA, pH 6.5, 25°. Additions of each 10 μM BA, ATR, ADP as indicated. Recording of absorbance at 546 mμ in an Eppendorf photometer with a 5 mm path cuvette. Total absorbance 0.75 cm^{-1}.

chondria were followedsas induced by an ATP-analogue, adenosine disphospho-imido-phosphate, ADPNP, which recently has been developed by R.G. Yount (Biochemistry, in print). With this analogue the possibility of an energy transfer is also excluded, since the NH-bond between P_β and P_γ is not split by the mitochondrial ATPase. As seen in fig. 2, ADPNP alone induces a smaller effect as ATP, in agreement with their about 60% lower translocation activity. Under the influence of BA the contraction is strongly increased to the same extent as with ATP. When first BA is added only a slow increase is observed andsthe subsequent addition of ADPNP again increases the absorbance. The changes induced by ADPNP are slower than those caused by ATP, similar to an about 60% reduced translocation activity as compared to ATP.

These results strongly support the hypothesis that the observed ADP induced contractions reflect binding of ADP to the ANP carrier, possibly caused by a conformational change of the carrier in the membrane. It might be added that these effects are observed only with intact mitochondria and are largely abolished in depleted mitochondria which, however, still have the capaicity for the specific binding of ADP. The observed turbidity changes represent a propagation of the membrane effect on the matrix containing densely packed proteins and influence possibly its osmotic state. These aspects require further investigation. The observed effects can also be utilized as a convenient assay for the mutual interaction of ANP and BA with the carrier.

2.2. *Inhibition of adenine nucleotide efflux from mitochondria by bongkrekic acid*

The transport of ANP through the mitochondrial membrane may be divided up into two forms: (a) The coupled exchange between exogenous and endogenous ANP and (b) the uncompensated efflux of endogenous ANP. The efflux of ANP is 100 to 1000-fold slower than the exchange [9–11]. There are several reasons to assume that the efflux occurs also by the ANP carrier similar as the exchange: Mitochondria can loose endogenous ANP before they loose other components,

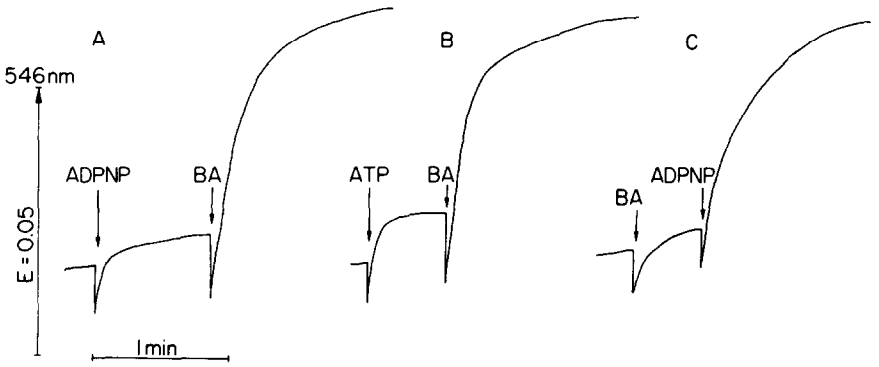

Fig. 2. Influence of BA on mitochondrial shrinkage as induced by the ATP-analogue ADP-imido-phosphate (ADPNP). Rat heart mitochondria (0.25 mg protein/ml) incubated in 0.30 M sucrose, 20 mM TRA and 2 mM EDTA, pH 6.5, 25°. Other conditions of the legend of fig. 1. Total absorbance 0.9 cm^{-1}.

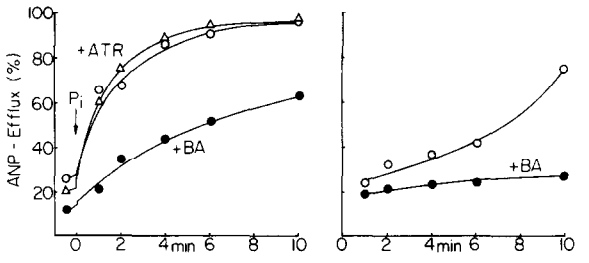

Fig. 3. Inhibition of adenine nucleotide efflux from mitochondria by bongkrekic acid. Rat liver mitochondria, loaded with ^{14}C-ANP by preincubation with ^{14}C-ADP for 50 min and washing according to [10], incubated at 0.94 mg protein/ml in a medium containing 0.25 M surcose, 2 mM EDTA, 10 mM TRA, 5 mM $MgSO_4$, 2 mM glutamate, 2 mM malate, O_2 saturated, pH 7.0 at 30°. Preincubation of mitochondria for 2 min ±20 μM BA, 20 μM ATR. Addition at t = 0 min of 10 mM Pi in exp. A. At the time indicated 1 ml samples are withdrawn and centrifuged and the supernatants assayed ^{14}C-ANP.

such as endogenous NAD and NADP, which do not possess a transmembrane carrier [12]. The efflux occurs through apparently intact membranes since intramitochondrial soluble enzymes remain confined [12]. The efflux has a similar specificity as the exchange such that ADP and ATP but not AMP participate in the efflux [9].

In apparent contradiction to this theory the specific efflux is insensitive to ATR [9]. The ineffectiveness of ATR was explained by assuming that ATR does not penetrate to the carrier sites on the inside of the membranes which catalyze the efflux. It was, therefore, of particular interest whether BA inhibits the

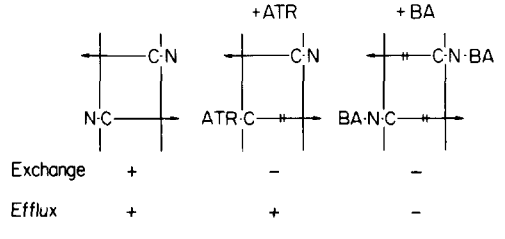

Fig. 4.

efflux since BA can be assumed due to its lipophilic nature to penetrate the membrane and therefore also to block inside oriented carrier sites.

In fig. 3 the efflux of endogenous ANP, as induced by the addition of phosphate, is followed under the influence both of BA and ATR. The efflux is considerably inhibited by BA but not influenced or even slightly increased by ATR. The half time of ANP leakage $t_{1/2}$ = 1 min increases with BA to $t_{1/2}$ = 8 min. In the absence of Pi the efflux is considerably slower and accelerates only after a certain lag period. Also this low efflux is strongly inhibited by BA.

The opposite effects of ATR and BA on the ANP efflux are not interpreted on the basis of opposite effects of these inhibitions on the binding but rather to be caused by their different permeability through the mitochondrial membranes: ATR is assumed not to be able to penetrate the membranes whereas BA can reach also the inner sites. The mechanism is explained by the dimer carrier model (cf. [11]) for the ANP carrier where the efflux is catalyzed by the inside localised carrier site, with or without an exchange. ATR does not reach the inner site, whereas BA blocks inner and outer site as the ANP-carrier-BA-complex (C–N–BA) (cf. fig. 4).

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