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CALCIUM ANTAGONISTS AND BAY K8644 PROMOTE DEPOLARIZATION OF THE RAT HEART MITOCHONDRIAL MEMBRANE POTENTIAL

FURTHER EVIDENCE FOR A ROLE IN ALTERATION OF OXIDATIVE METABOLISM

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Abstract—Studies were carried out using a tetraphenylphosphonium (TPP^+)-selective electrode to monitor the effect of selected calcium (Ca^{2+}) antagonists and the dihydropyridine Ca^{2+} agonist Bay K8644 on membrane potential (Ψ) associated with isolated rat heart mitochondria. Verapamil and diltiazem (10–500 μM), standard Ca^{2+} antagonists, produced a depolarization of both liver and heart mitochondria at concentrations >150 μM. In contrast, nitrendipine (10–200 μM), a dihydropyridine compound, produced a concentration-related inhibition of Ψ in mitochondria from both sources, effects which were statistically significant at concentrations >50 μM. Cinnarizine (10–100 μM) and bepridil (10–100 μM) also produced inhibition of heart Ψ, these effects being particularly noted in the presence of bepridil, where depolarization of the membrane was statistically significant with only 10 μM drug. The results indicate the complexity of action of these drugs at the mitochondrial level. In general, drug actions on Ψ appear to be correlated with previously reported effects on Ca^{2+} transportation rather than oxidative phosphorylation associated with rat heart mitochondria. The findings also illustrate that the mitochondrial actions of cardiovascular compounds may be of relevance *in situ*, particularly during ischaemia/reperfusion when mitochondria become loaded with Ca^{2+}.

Calcium (Ca^{2+}) antagonists are a heterogeneous group of compounds with diverse structural, clinical and pharmacological profiles [1]. These compounds are commonly used clinically in a number of aspects of cardiovascular disease, such as hypertension and cardiac arrhythmia. In pathological states associated with excessive influx of Ca^{2+}, such as irreversible ischaemia, reperfusion or conditions related to the Ca^{2+} paradox [2–4], direct prevention of Ca^{2+} overload by Ca^{2+} antagonists may be of benefit to the heart as a result of better maintenance of ionic homeostasis and membrane integrity [5, 6].

While Ca^{2+} antagonists have been shown to have a primary site of action of L-type Ca^{2+} channels on the plasma membrane of the heart, intracellular accumulation of these compounds may also occur [7, 8]. The evidence supporting accumulation is by no means conclusive, and it may depend on the physico-chemical properties of each individual compound. However, the effects of Ca^{2+} antagonists cannot be fully explained by their action on L-type Ca^{2+} channels [9]. Recent research has shown that there is a high capacity, low affinity binding site for Ca^{2+} antagonists associated with the inner mitochondrial membrane, a site distinct from α_1 subunit of the L-type Ca^{2+} channel [10]. Zernig and Glossman [10] have estimated that 28% of mitochondrial intracellular binding sites could become occupied with a plasma concentration of 70 μg/mL.

It is well established that movements of Ca^{2+} occur across the mitochondrial membrane [11]. In cardiac mitochondria, Ca^{2+} is transported across the inner mitochondrial membrane by specific, unidirectional carrier systems [12], the electrophoretic influx of Ca^{2+} involving diffusion of the ion down an electrochemical gradient generated as a result of the transfer of protons along the respiratory chain. This proton electrochemical gradient (ΔH^+†) is composed of both a membrane potential (Ψ) and a pH gradient (ΔpH) [13].

Several Ca^{2+} antagonists have previously been shown to alter both mitochondrial oxidative phosphorylation [14, 15] and mitochondrial Ca^{2+} fluxes [16–18]. Since Ca^{2+} movements occur along or against electrochemical gradients to ions like Ca^{2+}, Na^+ and H^+, alterations in calcium flux may be the result of changes in membrane potential. In order to elucidate further the mechanisms of drug-induced changes in Ca^{2+} fluxes associated with mitochondria, the effects of Ca^{2+} antagonists and related compounds on Ψ were investigated by monitoring the distribution of tetraphenylphosphonium ions (TPP^+) across the mitochondrial membrane, using an ion-selective electrode.

MATERIALS AND METHODS

Isolation of mitochondria. Tightly coupled rat heart mitochondria were prepared from male Wistar rats (250–550 g) according to the method of Vercesi

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† Abbreviations: TPP^+, tetraphenylphosphonium; Ψ, membrane potential; ΔpH, pH gradient; ΔH^+, proton electrochemical gradient.

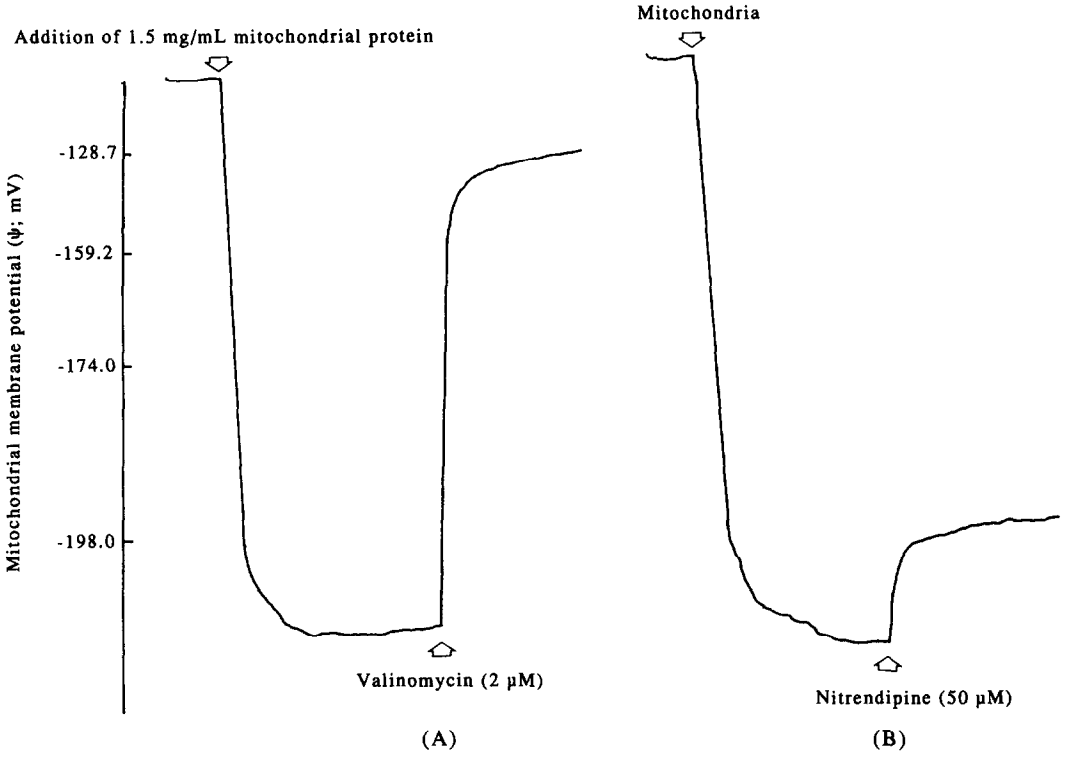

Fig. 1. The action of a known inhibitor of mitochondrial energy metabolism, valinomycin, on rat heart mitochondrial membrane potential. Valinomycin (2 μM) was added to the incubation medium 1 min after maximal development of membrane potential (A). For comparison, (B) shows a trace depicting the effect of 50 μM nitrendipine added in a similar manner. Valinomycin produced a collapse of Ψ. The incubation medium contained 1250 μmol sucrose, 17 μmol Tris–HCl, 17 μmol dihydrogen orthophosphate (potassium salt), 0.5 μmol EDTA, 10 μmol magnesium chloride, 25 μmol Tris-succinate and 50 μmol TPP^+. Addition of 1.5 mg/mL mitochondrial protein to the medium resulted in uptake of TPP^+ according to the magnitude of the mitochondrial membrane potential. Once uptake was complete a stable base line enabled subsequent drug additions.

et al. [19]. The rats were stunned by a blow to the head, bled and the hearts rapidly removed into ice-cold isolation medium containing 210 mM mannitol, 70 mM sucrose, 5 mM Tris–HCl buffer (pH 7.4) and 1 mM EGTA. The hearts were minced, washed and incubated with Nagarse (1 mg/g tissue wet weight in 1 mL/g buffer) for 10 min at 4°. After incubation, excess Nagarse was washed off and the tissue homogenized in 100 mL of isolation medium using a glass "Uniform" type H homogenizing tube and three strokes of a Potter–Elvehjem homogenizer set at low speed. The resulting homogenate was centrifuged at 500 *g* for 10 min in a Beckman high speed JS-21 centrifuge at 4°. The resulting supernatant was decanted and re-centrifuged at 10,000 *g* for 7 min to obtain the mitochondrial pellet. The pellet was washed with ice-cold buffer containing 210 mM mannitol, 70 mM sucrose and 5 mM Tris–HCl (pH 7.4), mitochondria were resuspended and recentrifuged at 10,000 *g* for 7 min and the purified fraction was resuspended in EGTA-free buffer to give a final protein concentration of 40–50 mg/mL.

Measurement of mitochondrial membrane potential. A TPP^+ electrode was prepared according to the method of Kamo *et al.* [20]. A solution containing 250 mM sucrose, 3.4 mM Tris–HCl, 3.4 mM dihydrogen orthophosphate (K^+ salt), 0.1 mM EDTA, 2 mM $MgCl_2$, 5 mM Tris-succinate and either 10 or 30 μM TPP^+ Cl^- was pipetted into a 10 mL jacketed water bath maintained at 37° to give a final volume of 5 mL. The TPP^+-selective electrode and a standard calomel reference electrode were placed in the solution and attached to read millivolts through a Petracourt PHM 10 meter connected to a BBC SE120 flat bed recorder. For studies where mitochondrial membrane potential was visualized before drug addition, 1.5 mg/L mitochondrial protein was added to the reaction medium initiating the development of a membrane potential with drug additions being made after its full development. Preincubation of mitochondria with drug formed a separate set of experiments; incubation was maintained for varying lengths of time, before transfer to the reaction medium and measurement of membrane potential. Membrane potential was calculated using the equation as described previously [20], with an assumed mitochondrial matrix volume of 1 μL/mg [21]. Changes in Ψ may reflect changes in mitochondrial volume although the presence of a sucrose-based incubation (as opposed to KCl) and

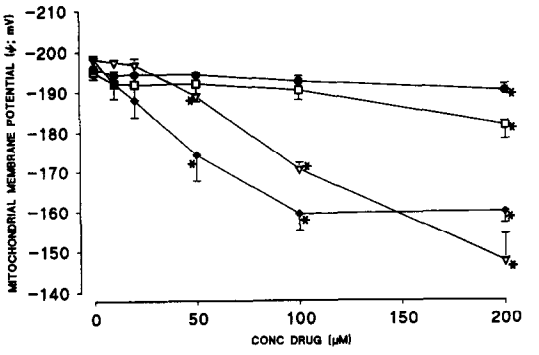

Fig. 2. The effect of the calcium antagonists verapamil, diltiazem and nitrendipine, along with the dihydropyridine calcium agonist BAY K8644 on rat heart mitochondrial membrane potential. Verapamil (□) and diltiazem (●) produced a slight depolarization of Ψ which was only slightly significant at high concentrations (200 μM: $P < 0.05$). The dihydropyridines nitrendipine (▽) and BAY K8644 (◆) produced a concentration-dependent inhibition of Ψ which was significantly reduced in the presence of 50 μM of either drug ($P < 0.05$). Each bar is mean ± SE of four separate experiments. Drug additions were made 1 min after the full development of membrane potential as described in the legend to Fig. 1. Incubation medium in this instance contained 30 μM TPP^+.

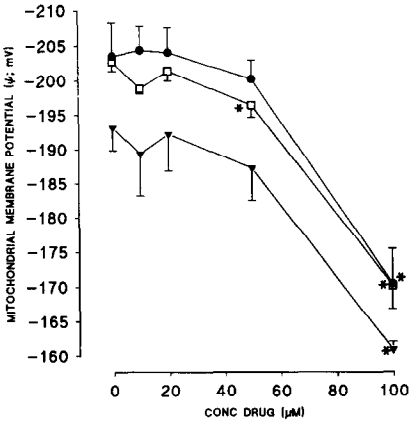

Fig. 3. The actions of nitrendipine's stereoisomers on rat heart mitochondrial membrane potential. The laevo (▽) and dextro (●) isomers of nitrendipine (□) mirrored the actions of the latter parent compound as had been demonstrated previously on rat heart Ψ. The depolarization of Ψ was not sufficiently reduced, however, in the case of either stereoisomer, to be significant at 50 μM, unlike racemic nitrendipine. Experiments were carried out in incubation media as noted in the legend to Fig. 1; TPP^+ was maintained at 10 μM. Each bar is mean ± SE of four separate experiments. * Statistical significance at $P < 0.05$.

of EDTA may alleviate these problems [14, 22]. Indeed, verapamil and diltiazem have previously been shown to protect against phosphate-induced swelling [14].

Nitrendipine, BAY K8644 (Ca^{2+} agonist [15]), verapamil or diltiazem (all 10–200 μM) was added to mitochondria that had already developed a membrane potential in the presence of 30 μM TPP^+. In other studies (10–100 μM) nitrendipine, (−) nitrendipine, (+) nitrendipine, cinnarizine or bepridil was added to mitochondria that had already developed a membrane potential in the presence of 10 μM TPP^+. Positive controls were performed using 2 μM valinomycin. Incubation studies were conducted using concentrations of 10–100 μM for all drugs except valinomycin (2 μM). None of the aforementioned drugs interfered with the ion-selective membrane of the TPP^+ electrode *per se*.

Measurement of oxidative phosphorylation and Ca^{2+} *transport.* Oxidative phosphorylation and calcium transport studies were carried out as described previously [15]. Only cinnarizine (10–100 μM) and bepridil (10–100 μM) were studied in these systems.

Statistical analysis. Data presented are the means ± SEM of at least four different preparations; statistical significance was calculated using a Student's unpaired *t*-test.

Materials. Valinomycin, verapamil hydrochloride, diltiazem hydrochloride, cinnarizine, bepridil, tetrahydrofuran, polyvinyl chloride and sodium tetraphenylboron were purchased from the Sigma Chemical Co. (Poole, U.K.). Tetraphenylphosphonium chloride was purchased from Lancaster Synthesis, (Morcambe, U.K.). Nitrendipine, its stereoisomers and Nagarse were gifts from ICI Pharmaceuticals (Alderley Park, U.K.). Bay K8644 was donated by Bayer AG (Pharmaceutical Centre, F.R.G.). All other analytical grade chemicals biochemicals were purchased from British Drug Houses (Poole, U.K.).

All water-soluble compounds were dissolved in distilled deionized water at room temperature. Water-insoluble compounds were dissolved in dimethyl sulphoxide, with the final concentration of the solvent in the reaction medium not exceeding 1% (v/v).

RESULTS

Membrane potential

Measurement of heart mitochondrial Ψ produced values within the range of -190 to -210 mV, comparable with those reported by other workers for liver mitochondria [13, 23]. The addition of valinomycin (2 μM) caused the mitochondrial Ψ to collapse (Fig. 1).

At concentrations <100 μM, verapamil produced no statistically significant effect on the Ψ of rat heart mitochondria ($P < 0.06$; $N = 4$; Fig. 2). A 200 μM concentration of verapamil however produced a significant decrease in Ψ, reducing the value from -194.7 ± 1.6 to -181.4 ± 3.5 mV ($P < 0.05$; $N = 4$; Fig. 2). Similar effects were observed with diltiazem, a concentration of 200 μM reducing the value from -195.8 ± 1.2 to -189.9 ± 1.6 mV ($P < 0.05$; $N = 4$; Fig. 2).

Replacement of the Ca^{2+} antagonists with the Ca^{2+} agonist, BAY K8644, produced marked effects on Ψ. BAY 8644 (10–100 μM) produced a

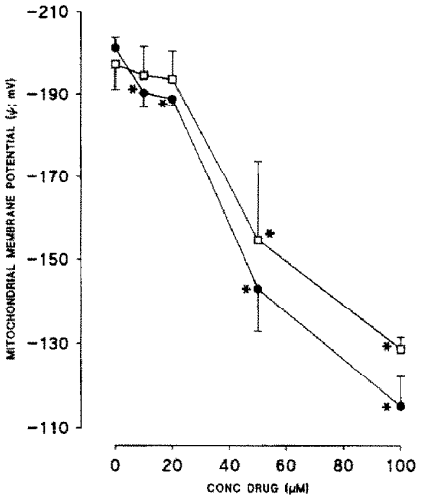

Fig. 4. The effect of two non-selective calcium antagonists, cinnarizine and bepridil, on rat heart mitochondrial membrane potential. Cinnarizine (□) and bepridil (●) both caused a concentration-dependent depolarization of Ψ which, for the former, was significant at 50 μM ($P < 0.05$; $N = 5$) and, for the latter, at 10 μM ($P < 0.05$; $N = 6$). Experiments were carried out in incubation media as noted in the legend of Fig. 1: TPP^+ concentration in buffer was 10 μM. The results are the means of N different experiments ± SEM.

concentration-dependent reduction in Ψ from −198.1 ± 2.2 to −159.2 ± 4.2 mV at 100 μM ($P < 0.001$; N = Fig. 3). Similar effects were seen with the Ca^{2+} antagonist, nitrendipine. This Ca^{2+} antagonist (10–200 μM) produced a concentration-dependent decrease in mitochondrial Ψ from −198.0 ± 1.0 to −147.4 ± 6.6 mV at 200 μM ($P < 0.01$; $N = 4$; Fig. 3). Substitution of the racemer for its stereoisomers, (+) and (−) nitrendipine, resulted in similar effects (Fig. 3). Cinnarizine (10–100 μM), a Ca^{2+} antagonist with no known action on the slow Ca^{2+} inward current [24], also produced significant reductions in Ψ. Cinnarizine (100 μM) reduced the membrane potential from −196.2 ± 6.1 to −129.5 ± 1.8 mV ($P < 0.001$; $N = 4$; Fig. 4). Replacement of cinnarizine with bepridil (10–100 μM), another non-selective Ca^{2+} antagonist [25], reduced Ψ in a concentration-dependent manner. A low concentration (10 μM) of bepridil reduced Ψ from −201.1 ± 2.8 to −190.2 ± 3.4 mV ($P < 0.05$; $N = 6$; Fig. 4) and further, at 100 μM, to −115.0 ± 7.2 mV ($P < 0.001$; $N = 5$; Fig. 4).

Oxidative phosphorylation

In order to relate the above changes in Ψ induced by cinnarizine and bepridil to previous studies on mitochondrial function [15, 16] studies on ATP synthesis plus cation transport were carried out. Studies on oxidative phosphorylation confirmed that both compounds were capable of increasing endogenous or pre-State 4 respiratory activity; with respect to cinnarizine (10–100 μM) this effect was found to be concentration dependent. In the presence of 100 μM cinnarizine, the rate increased from 37.5 ± 5.8 to 65.1 ± 13.4 ng atoms oxygen consumed/min/mg protein ($N = 6$; $P < 0.05$). A similar increase was observed in the presence of bepridil (<20 μM). For example, at a bepridil concentration of 20 μM the endogenous rate increased from 34.4 ± 4.7 to 62.0 ± 11.1 ng atoms oxygen consumed/min/mg protein ($N = 6$; $P < 0.05$). This increase was not maintained at concentrations of bepridil >40 μM. Both compounds (10–100 μM) were found to stimulate State 4 (oxygen and substrate in excess, ADP absent) while inhibiting State 3 (oxygen and substrate in excess, ADP present) respiration, resulting in a loss of respiratory control.

Ca^{2+} *transport*

In studies using an ion-selective electrode, both bepridil (10–40 μM) and cinnarizine (10–100 μM) were found to inhibit Ca^{2+} uptake into rat heart mitochondria. The introduction of 40 μM bepridil into the external medium prior to the addition of mitochondria resulted in the rate of influx being reduced from 129.5 ± 19.6 to 52.1 ± 14.3 mmol Ca^{2+}/min/mg protein ($N = 4$; $P < 0.05$), while the amount of Ca^{2+} taken up was reduced from 184.0 ± 26.2 to 55.3 ± 9.1 nmol Ca^{2+}/mg protein ($N = 4$; $P > 0.050$). The addition of bepridil (10–100 μM) to mitochondria pre-loaded with Ca^{2+} stimulated a significant ($P < 0.01$) release of the cation from the matrix into the external medium. At concentrations >40 μM, the release was exponential in nature producing an EC_{50} value of 46.6 ± 0.9 μM ($N = 4$). The effects of release of Ca^{2+} from rat heart mitochondria have also been described by Schneider *et al.* [26].

Studies involving cinnarizine (10–100 μM) confirmed that this compound had no significant effect on the rate of Ca^{2+} influx or Ca^{2+} release from preloaded mitochondria. In contrast, however, total mitochondria uptake was found to be inhibited in a concentration-dependent manner with 100 μM cinnarizine reducing the amount taken up from 164.1 ± 23.2 to 38.3 ± 2.1 nmol Ca^{2+}/mg protein ($N = 4$; $P < 0.01$), resulting in an IC_{50} value of 37.0 ± 3.9 μM.

Pre-incubation studies on Ψ

In a series of experiments mitochondria were pre-incubated for either 1 or 5 min with a number of selected drugs, which resulted in changes in Ψ (Table 1; $N = 4$). In the presence of 10 μM diltiazem a 1 min pre-incubation resulted in the Ψ being reduced from −187.9 ± 1.4 to −182.2 ± 1.0 mV ($P < 0.05$), while increasing the period to 5 min caused a further reduction to −176.6 ± 3.6 mV (Table 1; $P < 0.05$). Verapamil produced a similar reduction in Ψ to that seen with diltiazem; however, the pattern of events was more clearly established after a 5 min pre-incubation, with 10 μM verapamil reducing the Ψ from −192.3 ± 1.3 to −175.1 ± 5.1 mV (Table 1; $N = 4$; $P < 0.05$). Neither BAY K8644, nitrendipine, cinnarizine nor bepridil (all 10–100 μM) produced a statistically significant reduction in Ψ after 1 of 5 min preincubation.

DISCUSSION

The results reported here demonstrate that

Table 1. The effect of drug pre-incubation on rat heart mitochondrial membrane potential

Compound (10 μM)	Ψ (mV) 0	1 min	5 min
Diltiazem	−187.9 ± 1.4	−182.2 ± 1.0*	−176.6 ± 3.6*
Verapamil	−192.2 ± 1.3	−187.2 ± 1.0*	−175.1 ± 5.1*
Nitrendipine	−182.1 ± 5.9	−180.1 ± 4.4	−162.0 ± 8.1
BAY K8644	−186.7 ± 3.0	−182.6 ± 1.8	−177.5 ± 2.4

Drugs (50 nmol) were pre-incubated for 1 or 5 min with 1.5 mg mitochondrial protein, before addition to the reaction medium (as described in the legend to Fig. 1) and initiation of Ψ.

Results are means ± SEM (N = 4).

* Statistical significance at $P < 0.05$.

modifiers of Ca^{2+} fluxes in mitochondria are capable of producing a reduction in the Ψ of rat heart mitochondria. Sustained reductions in Ψ will ultimately disrupt the functioning of the whole heart, since the protonmotive force will subside, leading to a decrease in the production of ATP. In myocardial cells, mitochondrial oxidative phosphorylation competes with mitochondrial calcium ion transport, both processes being influenced by the Ψ. Alterations in mitochondrial Ψ will thus compromise both physiological processes and the effects of the Ca^{2+} antagonists at this level may be a feature of possible toxicity in drug treatment.

The Ca^{2+} modifying agents used in this study produced a range of effects on Ψ which may be related to actions of Ca^{2+} transport and oxidative phosphorylation. Verapamil and diltiazem had little effect on the mitochondrial Ψ, except at high concentrations (200 μM); however, both these compounds have been shown to reduce respiratory control indices in oxidative phosphorylation [14, 15], albeit at high concentrations (verapamil >200 μM; diltiazem >400 μM). In Ca^{2+} transport studies these drugs produced an inhibition of Ca^{2+} uptake (verapamil, IC_{50} 19.5 ± 2.0 μM; diltiazem, IC_{50} 93.8 ± 8.3 μM) [15]. At high values of Ψ, a small reduction would not influence uniporter activity provided that Ψ was always maintained above −110 mV [27]. Indeed Ψ appears to be affected by verapamil and diltiazem only once oxidative phosphorylation is affected. Thus, it is more likely that both drug actions inhibit Ca^{2+} uptake via actual changes in calcium carrier kinetics. Verapamil is therefore most likely to have a primary mechanism of action at the level of the uniporter, whilst diltiazem has already been shown to be a potent inhibitor of the Na^+/Ca^{2+} antiporter [18]. Ca^{2+} efflux through the latter mechanism is again unlikely to be influenced by Ψ [27].

Pre-incubation of heart mitochondria with both diltiazem and verapamil increased the sensitivity of the membrane to depolarization when exposed to the Ψ probe, TPP^+. Such effects may relate to a drug action, mediated through an adverse effect on the mitochondrial ATPase, such that the introduction of substrate does not enable the mitochondria to generate a Ψ [28].

The dihydropyridine Ca^{2+} antagonist nitrendipine produced a concentration-dependent reduction in Ψ, an effect made apparent at concentrations lower than those required to produce any significant action on mitochondrial oxidative phosphorylation. Furthermore, Baydoun *et al.* [16, 17] have shown previously that Ca^{2+} influx is inhibited over the concentration range 10–100 μM nitrendipine, whereas pre-stimulated Ca^{2+} release occurs over a concentration range of 40–60 μM. The nature of nitrendipine-induced Ca^{2+} release suggests that this drug is a compound that induces mitochondrial membrane permeability transition, since release was observed in the absence of sodium and occurred at a rate greater than 1 nmol/mg/min [29]. Such non-specific permeability of the membrane to Ca^{2+} is also associated with depolarization of the membrane, swelling, uncoupling of oxidative phosphorylation and oxidation of pyridine nucleotides [30]. The depolarization of rat heart mitochondria is therefore secondary to another action which causes changes in membrane permeability.

Permeability transition occurs in calcium-loaded mitochondria in response to an inducing agent, which is either a drug or even calcium ions *per se*. Despite the preparation of mitochondria in the presence of EGTA, endogenous Ca^{2+} will be present in the intramitochondrial matrix, although the presence of Mg^{2+} and EDTA during Ψ measurements will remove the possibility of transient depolarizations as well as offering some protection against phosphate-induced swelling [31, 32], without chelating calcium to the extent that depolarization of the membrane occurs [11].

The stereoisomers of nitrendipine produced a concentration-dependent reduction in mitochondrial Ψ which was no different from that of their parent. Thus, no stereoselectivity was observed, as noted in other studies [15].

BAY K8644 is another dihydropyridine which unlike nitrendipine has Ca^{2+} agonistic rather than antagonistic activity on voltage-operated channels [33]. However, BAY K8644 also produced a reduction in mitochondrial Ψ, an effect which was maximal at 100 μM. Baydoun *et al.* [15] concluded that the effects of BAY K8644 were to increase mitochondrial Ca^{2+} by inhibition of the Na^+/Ca^{2+}

antiporter without disturbing Ψ. This could not be confirmed by our studies, but Na^+-independent Ca^{2+} efflux is influenced by Ψ [27], suggesting that depolarization of the membrane occurs as a result of BAY K8644-mediated inhibition of Ca^{2+} efflux. Inhibition of this antiporter would result in an increased intramitochondrial Ca^{2+} concentration with possible concomitant stimulation of respiration, as has been noted previously with this Ca^{2+} agonist [15].

The mitochondrial membrane depolarization induced by cinnarizine was not consistent with an action at the level of Ca^{2+} uniporter, since cation uptake was inhibited but influx kinetics remained unchanged. These effects suggest that cinnarizine either has an effect on Ψ *per se*, or that its depolarization action is mediated through a stimulation of Ca^{2+} efflux, in a similar manner to the actions of BAY K8644. The stimulation of respiratory activity noted with cinnarizine is probably related to the depolarization induced either directly or indirectly by this drug.

Bepridil produced the most significant effect of all Ca^{2+}-modifying compounds studied. This non-selective Ca^{2+} antagonist also has effects on Ca^{2+} transport whereby Ca^{2+} influx and uptake are decreased significantly at concentrations below 40 μM [34], whereas Ca^{2+} efflux is stimulated at concentrations above 10 μM [26]. Younes and Schneider [34] further found that, at concentrations below 10 μM, bepridil increased Ca^{2+} uptake in the presence of succinate, but when ATP was used to promote Ca^{2+} influx, bepridil completely abolished the process. This suggests that bepridil is an inhibitor of ATPase-linked reactions acting at the level of the enzyme itself [26, 34] leading to depolarization of the mitochondrial membrane. Thus, the actions of bepridil target the ATPase activity of heart mitochondria initially causing Ψ to fall, along with a stimulation of respiration and inhibition of Ca^{2+} transport. The Ca^{2+} releasing action of bepridil is similar to that of nitrendipine in that release occurs very quickly. However, the higher concentrations of bepridil required to induce pre-stimulated Ca^{2+} release make the actions of this drug on other parameters such as Ψ more relevant.

The results demonstrate a range of findings which relate to the specific nature of each group. It has been deemed that the pharmacological [5, 6] and biochemical [14, 15] inactivity of diltiazem and verapamil with respect to Ψ is beneficial. In contrast, the dihydropyridines produced a depolarization of Ψ related to a multitude of actions occurring simultaneously. There is now much indirect evidence to suggest that calcium antagonists could accumulate to intracellular concentrations within the micromolar range [35, 36]. Clinically, the possible toxicity of bepridil is highlighted by its potent inhibition of Ψ. These considerations require further investigation, but may explain the anti-ischaemic actions of diltiazem and verapamil, and the toxicity of others.

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