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Inhibition of the sodium pump by cardioactive DPI 201-106

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The effects of DPI 201-106 were examined on contractions of papillary muscles from the right ventricle of feline heart, on [^{3}H]-ouabain binding and Na^+, K^+-ATPase activity in feline ventricular membrane particles and on ouabain-sensitive ^{86}Rb uptake in human erythrocytes. DPI 201-106 partially inhibited the Na^+ pump at concentrations that caused pronounced positive inotropic effects.

Introduction

DPI 201-106 (4[3-(4-diphenylmethyl-1-piperazinyl)-2-hydroxypropyl]-1H-indole-2-carbonitrile) was recently introduced as a cardiotonic agent that does not inhibit the Na^+ pump (Scholtysik *et al.*, 1985). We now describe conditions under which DPI 201-106 partially inhibits the Na^+ pump. We also compare the potency of DPI 201-106 as inhibitor of the Na^+ pump with its inotropic potency.

Methods

All experiments were carried out at 37°C. Cats (0.8–2.6 kg), pretreated with reserpine 3 mg kg^{-1} s.c. 20 h before the experiment, were anaesthetized with halothane and exsanguinated. The hearts were rapidly removed and washed free of blood in an oxygenated solution containing (mmol l^{-1}): Na^+ 140, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 0.5, Cl^- 98.5, SO^{2-} 0.5, HCO_3^- 34, fumarate 5, pyruvate 5, L-glutamate 5 and glucose 10, equilibrated with 95% O_2 and 5% CO_2 in deionized and twice distilled water. Right ventricular papillary muscles (width <0.8 mm) were mounted in an apparatus (Blinks, 1965) containing the above solution. The muscles were attached to strain gauge transducers, driven at a frequency of 12 min^{-1} with pulses of 5 ms duration and stretched to a length at which maximal contractile force developed (Kaumann, 1972).

Ventricular membrane particles were prepared by the method of the Pitts & Schwartz (1975). ATPase activity was determined by the method of Brown (1982). The incubation medium contained (mmol l^{-1}): imidazole 25(pH 7.4), NaCl 90, KCl 20, $MgCl_2$ 3, EGTA 0.1 and ATP ([γ^{32}P] ATP + unlabelled ATP) 3. Membranes were preincubated for 10 min, with or without DPI 201-106 followed by a 10 min incubation after the addition of ATP.

For binding experiments the membrane suspension was incubated for 105 min with the indicated concentrations of DPI 201-106 and 25 nmol l^{-1} [^{3}H]-ouabain (Amersham, specific activity 1.55 GBq mmol^{-1}) in a final volume of 200 µl incubation buffer containing (mmol l^{-1}): Tris phosphate 3, $MgCl_2$ 3 and imidazole 50 (pH 7.4). The binding observed in the presence of 2.5 µM unlabelled ouabain was considered to be non-specific. The reaction was stopped by the addition of 2 ml of ice-cold incubation buffer. The membrane particles were collected by vacuum on Whatman GF/C glass fibre filters and washed 3 times with 3 ml ice-cold incubation buffer. The radioactivity was counted in 8 ml of PCS (Amersham).

Ouabain-sensitive ^{86}Rb uptake into human red blood cells was measured essentially as described by Young & Ellory (1982), in a medium containing (mmol l^{-1}): NaCl 150, glucose 5, ^{86}RbCl 10, MOPS 15, pH7.4. Cells were preincubated with DPI 201-106 for 30 min at 37°C before the start of the experiment.

All biochemical assays were carried out in the presence of 5% dimethyl sulphoxide (DMSO). DPI 201-106 was dissolved in DMSO to a stock concentration of 20 mM. The maximum concentration of DMSO used, 0.3%, did not modify the contractile force of 8 papillary muscles. Concentrations greater than 0.3 mM DPI 201-106 appeared to precipitate in the incubation buffers.

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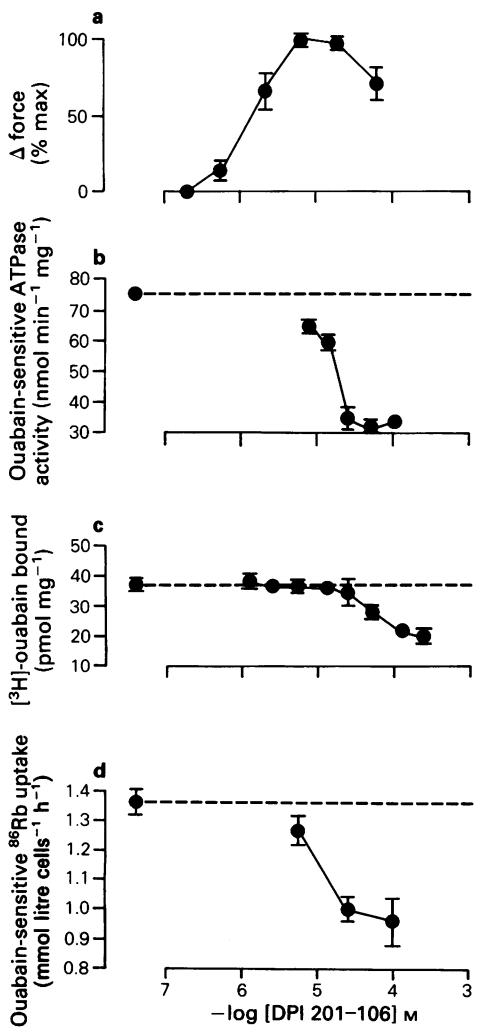
Results

DPI 201-106 reduced in a concentration-dependent manner the ATPase activity (Figure 1b) and [^{3}H]-ouabain binding (Figure 1c) in ventricular membrane particles and ^{86}Rb uptake into erythrocytes (Figure 1d). These effects are consistent with an inhibition of the Na^+-pump. Threshold inhibition of the Na^+-pump occurred at concentrations of DPI 201-106 (6–8 μM) that increase maximally contractile force in papillary muscles (Figure 1a). DPI 201-106 60 μM caused only 70% of the maximum positive inotropic effect (observed at 6 μM), but significantly inhibited the Na^+ pump (Figure 1).



Discussion

Three mechanisms may contribute to the positive inotropic effects of DPI 201-106; (i) Delay of the inactivation of the tetrodotoxin (TTX)-sensitive inward current (Buggisch *et al.*, 1985) consistent with a persistent open state of the Na^+ channel (Kohlhardt *et al.*, 1986); (ii) Sensitization of myocardial contractile proteins to Ca^{2+} (Scholtysik *et al.*, 1985); and (iii) Inhibition of the Na^+ pump (this paper). Binding of DPI 201-106 to the ventricular Na^+ channel prolongs the action potential (Buggisch *et al.*, 1985) with a concentration-dependence (Scholtysik *et al.*, 1986) similar to that found by us for the positive inotropic effects, suggesting a close link. TTX blocks both the prolongation of the action potential and the positive effects of DPI 201-106 making unlikely a contribution of sensitization of contractile proteins to Ca^{2+} (Buggish *et al.* 1985). The inhibition of the Na^+ pump is observed only at concentrations of DPI 201-106 that cause pronounced inotropic effects. It is therefore conceivable that an expected gain of intracellular Na^+, due to partial inhibition of the Na^+ pump, only influences the inotropic effects of high DPI 201-106 concentrations.

Although DPI 201-106 has some antiarrhythmic properties it does not protect against ouabain-induced arrhythmias (Scholtysik & Williams, 1986). These authors suggested that Na^+-loading of cardiac cells (Buggisch *et al.*, 1985) enhances Na^+/K^+-ATPase activity which in turn leads to enhanced glycoside binding. Our evidence is not consistent with the

Figure 1 Relationship between inotropic effects and inhibition of the Na^+-pump by DPI 201-106. All data are expressed as mean ± s.d. (s.d. not shown if smaller than symbol). (a) Cumulative concentration-effect curve for DPI 201-106 on papillary muscles ($n = 12$). Contractile force was 6.3 ± 20 mN mm^{-2} in the absence and 16.8 ± 4.9 mN mm^{-2} in the presence of DPI 201-106 (6 μM). (b) Partial inhibition of the Na^+, K^+-ATPase. Each symbol represents triplicate determinations. Another experiment yielded similar results. In a separate assay, DPI 201-106 (2 μM) caused only marginal inhibition. Ouabain (10 μM) reduced the control ATPase activity to 5%. (c) Partial inhibition of [^{3}H]-ouabain binding. Each symbol represents triplicate determinations. Two additional experiments yielded similar results. In the presence of ouabain (2.5 μM), 98% of bound ouabain was removed with a K_D of 7.7 nM. The K_D for [^{3}H]-ouabain estimated from a saturation experiment was 6.9 nM. (d) Partial inhibition of ^{86}Rb uptake. Each symbol represents quintuplicate determinations. In the presence of ouabain (10 μM) control ^{86}Rb uptake was reduced by 70%.
The dotted lines represent basal values shown on the left hand of (b), (c) and (d).

suggestion of Scholtysik & Williams (1986) because DPI 201-106 can actually inhibit the Na^+/K^+-ATPase.

The S-enantoimer of DPI 201-106 was reported to be more active than the R-enantiomer with regard to both prolongation of the action potential and increase in contractile force (Scholtysik *et al.*, 1985). We used racemic DPI 201-106. Work with the enantiomers should clarify the question of stereoselectivity of the Na^+ pump inhibition and its relevance for the inotropic effects of DPI 201-106.

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