Effect of (±)-propranolol on the recovery of urinary concentration process after frusemide, in the rat

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Frusemide eliminates the intrarenal sodium concentration gradient. When its effect wears off, the recovery of urinary concentration process can be followed. In anaesthetized dogs, propranolol delays this recovery (Imbs, Schmidt, Belhadj-Mostefa & Schwartz, 1975). We have observed the effect of propranolol on the recovery of the sodium cortico-papillary gradient after frusemide, in conscious male Wistar rats. (a) Propranolol does not modify the diuretic action of frusemide but keeps urinary osmolality down: 579 ± 40 (s.e. mean, $n = 20$) instead of 901 ± 78 ($P < 0.001$), and 1236 ± 105 instead of 1671 ± 60 m osmol/kg ($P < 0.005$) respectively 4 h and 8 h after frusemide injection. (b) Papillary and cortical sodium concentrations were measured according to Atherton, Hai & Thomas (1968), 30 min, 1, 2, or 3 h after injecting 4 groups of 20 rats with frusemide (20 mg/kg). Half these rats had been treated with propranolol (6 mg/kg). Ratio of these two concentrations, obtained from dry tissue weight, served as an index to intrarenal sodium gradient.

Propranolol inhibits the recovery of intrarenal sodium concentration gradient (Figure 1). Cortical sodium concentration remained constant. Medullary hypertonicity remained lower much longer in propranolol-treated animals. Three hours after frusemide injection, the papillary Na-cortical Na ratio was 3.6 ± 0.2 ($n = 10$) instead of 4.4 ± 0.2 ($P < 0.05$) without propranolol.

Our grateful thanks to ICI for the (±)-propranolol hydrochloride.

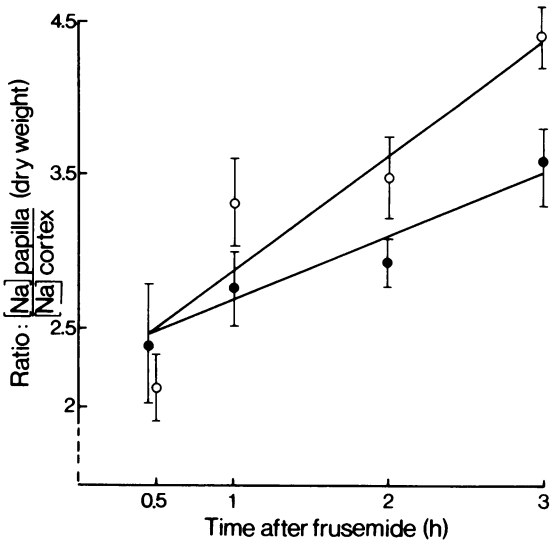

Figure 1 Regression curves for the papillary Na/cortical Na ratio expressed as a function of time between frusemide injection and kidneys removal. The equation for the curve is $Y = 0.797\ X + 2.039$ ($r = 0.6819$, $n = 40$) after frusemide (○), and $Y = 0.479\ X + 2.184$ ($r = 0.5233$, $n = 40$) after frusemide combined with propranolol (●). The slopes of these 2 curves are significantly different ($P < 0.05$). Points represent the mean of 10 measurements, vertical bars, s.e. mean.

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The role of potassium in the inhibition by cardiac glycosides of (Na^+-K^+)-ATPase prepared from human heart

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We have prepared an enriched fraction of human heart (Na^+-K^+)-ATPase from homogenates treated by NaI (De Pover & Godfraind, 1976). The activity of this preparation was equal to 10–15 μmol Pi mg protein^{-1} h^{-1} at 37°C in a medium containing (mM): NaCl 100, KCl 3, $MgCl_2$ 3, Tris-ATP 2.5, EGTA 1, Tris-maleate 20 (pH 7.4) (final volume 1 ml). The ATPase reaction was started by the addition of enzyme preparation (10 μg) and stopped by 0.1 ml of 50% trichloroacetic acid. The preparation contained 5% of residual Mg-ATPase.

The inhibition of (Na^+-K^+)-ATPase by digoxin, digitoxin, gitoxin and ouabain has been studied. The presence of the sugar chain and of the unsaturated lactone ring was required for full activity as reported

Table 1 Concentrations of cardiac glycosides producing 50% inhibition of human heart (Na^+-K^+)-ATPase

Cardiac glycosides	*IC_{50} without preincubation in the absence of K^+ (μM)*	*IC_{50} with preincubation in the absence of K^+ (μM)*
Digoxigenin	0.36 (0.08)	0.32 (0.03)
Digoxigenin digitoxoside	0.066 (0.006)	0.013 (0.001)
Digoxigenin *bis*-digitoxoside	0.10 (0.01)	0.020 (0.003)
Digoxin	0.19 (0.02)	0.036 (0.003)
Desacetyl lanatoside C	0.30 (0.05)	0.042 (0.004)
Dihydrodigoxin	2.1 (0.3)	2.6 (0.6)

The activity of (Na^+-K^+)-ATPase was estimated by measuring the release of Pi after 1 h incubation at 37°C. The incubation medium contained (mM) Na 100, KCl 10, $MgCl_2$ 3, Tris-ATP 2.5, EGTA 1, Tris-maleate 20 (pH 7.4) and various concentrations of cardiac glycosides.

In the experiments quoted without preincubation, the ATPase reaction was started by the addition of the enzyme to the incubation medium. In those quoted with preincubation, the enzyme preparation was preincubated for 15 min in the incubation medium where KCl was absent; the ATPase reaction was started by the addition of KCl to reach 10 mM.

IC_{50} were estimated from dose effect curves. The standard error, given between brackets, was the estimated residual standard error of the straight line drawn by regression analysis with three concentrations ($n = 6$) which evoked an inhibition of between 20 and 80 per cent.

by Portius & Repke (1964) for guinea-pig heart (Na^+-K^+)-ATPase. As shown for the digoxin series (Table 1), the number of sugars was also important, the monoside being the most active compound as demonstrated with enzymes from animal species (Erdmann & Schöner, 1974).

In the presence of KCl concentrations varying from 0.5 to 20 mM, the dose-effect curve of digoxin was displaced to the right. Hunter-Downs plot analysis indicated competitive inhibition at potassium concentrations up to 5 mM and non-competitive inhibition above that concentration.

The concentrations of cardiac glycosides producing 50% inhibition of (Na^+-K^+)-ATPase (IC_{50}) were estimated with or without a preincubation in the absence of KCl (Table 1). As far as concerned digoxin and other heterosides, IC_{50} measured with preincubation were 5 to 7 times lower than without preincubation ($P < 0.001$). This was not the case for digoxigenin nor for dihydrodigoxin; this indicates that their binding and the binding of the heteroside to the enzyme were dissimilar.

It has been shown that, within the range of free concentrations found in the blood of patients treated for heart failure (10^{-9} M to 5.10^{-9} M), digitoxin stimulated the sodium pump in human heart slices and inhibited isolated (Na^+-K^+)-ATPase only by about 5 to 10% (Godfraind, 1972). Within this therapeutic range of concentrations, the cardiac glycosides here examined did not evoke an inhibition of (Na^+-K^+)-ATPase much different from that achieved by digitoxin.

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