Stereoselective inhibition of renal organic cation transport in human kidney

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Amantadine was found to be concentrated by human cortical slices, with apparent K_m and V_{max} values of $187 \pm 11 \mu\text{M}$ and $1.37 \pm 0.28 \text{ nmol mg}^{-1} \text{ min}^{-1}$ respectively (mean $\pm$ s.e. mean, $n = 4$). Addition of quinine (8S, 9R-(–)-isomer) or quinidine (8R, 9S-(+)-isomer) competitively inhibited this accumulation (apparent K_i values of 261 ± 44 ($n = 4$) and $586 \pm 68 \mu\text{M}$ ($n = 3$), respectively). The stereoselectivity of this inhibition is the reverse of that seen with respect to the renal clearances of the diastereoisomers.

Keywords amantadine quinine quinidine

Introduction

Quinine and quinidine are cationic diastereoisomers, quinine being the 8S, 9R-(–)-isomer and quinidine the 8R, 9S-(+)-isomer. Notterman *et al.* (1986) reported a three- to four-fold higher renal clearance in humans for quinidine compared with quinine. In contrast, we observed that quinine was more potent than quinidine as an inhibitor of the accumulation of the achiral organic cation, amantadine, in rat renal cortical slices and isolated proximal tubules (Wong *et al.*, 1990). This apparent inversion in stereoselectivity *in vitro* was postulated to be due to predominant amantadine transport by quinine-selective transport sites, a greater stereoselective competition for luminal reabsorptive influx by quinine compared with quinidine, and/or a potential species difference in the renal transport of organic cations (Grantham & Chonko, 1991). In the present study, the availability of fresh human renal tissue allowed us to examine potential species differences in renal organic cation handling between human and rat.

Methods

Renal cortical slice preparation

Renal cortex was obtained from uninvolved portions of human kidneys from eight patients with renal cell carcinoma (six males and two females, age 42–77 years; mean $\pm$ s.e. mean = 65 ± 4 years). Surgical uninephrectomy was performed under general anaesthesia induced by fentanyl, sufentanil or morphine, in conjunction with atropine, droperidol or ephedrine. Disease-free samples of renal cortex were verified by a pathologist and were

dissected in ice-cold oxygenated pH 7.4 phosphate buffered Ringer's buffer (Wong *et al.*, 1990, 1992). Sagittal slices of the cortex (approximately 0.5 mm) were washed thoroughly in 50 ml buffer three times to minimise potential drug contamination of tissue. The interval between excision of each kidney and experimental utilisation of the slices did not exceed 1 h. The study was approved by the Faculty Committee on the Use of Human Subjects in Research at the University of Manitoba.

Transport studies

Each slice (20–40 mg) was incubated in oxygenated buffer with 10–400 μM [^3H]-amantadine (Amersham International Limited, Buckinghamshire, UK (350 mCi mmol^{-1})), in the absence or presence of 100–400 μM quinine or quinidine (Sigma Chemical Co., St Louis, MO, USA), at 25° C for 30 s. Accumulated radioactivity was assayed by liquid scintillation with automated quench correction. Non-specific uptake of amantadine was assessed with 2,4-dinitrophenol (5 μM)-pretreated tissue and twice freeze-thawed tissue. The proximal tubule protein content of the cortical slices was estimated as described previously (Wong *et al.*, 1990, 1992).

Expression and analysis of accumulation

[^3H]-amantadine accumulation was expressed as the slice (pmol g^{-1} slice wet weight) to medium (pmol ml^{-1} medium) ratio, and as the uptake velocity (pmol mg^{-1} estimated tubule protein min^{-1}). Non-specific amantadine uptake ($\approx 25\%$ of total uptake) was subtracted prior to all calculations. Michaelis-Menten

parameters were calculated using a weighted Lineweaver-Burk analysis (Lineweaver & Burk, 1934; Wilkinson, 1961), with coefficients of determination (r^2) exceeding 0.96 in all cases. The apparent inhibition constant (K_i) was used as an index of inhibitory potency (Dixon, 1953). Statistical significance between inhibition constants for quinine and quinidine was determined by the unpaired Student's t -test with the level of significance at $P < 0.05$. The statistical power for this comparison was 91% (Dawson-Saunders & Trapp, 1990).

Results

Control amantadine uptake

Amantadine accumulation in human cortical slices was linear for up to 3 min and thereafter approached saturation. The slice/medium ratio for a 30 s incubation with 10 μM amantadine was 0.6 ± 0.1 (mean, $\pm$ s.e. mean $n = 4$). Lineweaver-Burk transformation of uptake at varying initial amantadine concentrations (10–400 μM) yielded apparent K_m and $V_{\max}$ values of $187 \pm 11 \mu\text{M}$, and $0.23 \pm 0.05 \text{ nmol mg}^{-1} \text{ slice wet weight min}^{-1}$, respectively. Conversion of slice wet weight to estimated proximal tubule protein correspondingly modified control $V_{\max}$ to $1.37 \pm 0.29 \text{ nmol mg}^{-1} \text{ estimated proximal tubule protein min}^{-1}$. Kinetic parameters from individual experiments are listed in Table 1.

Inhibition of uptake

The incorporation of either quinine or quinidine into the medium inhibited amantadine accumulation by the cortical slices. Inhibition was observed to be competitive as illustrated in Figures 1a and b. Thus, the intersection of the two regression lines for the two amantadine concentrations was above the abscissa. Down-extrapolation of the intersection point onto the abscissa provided inhibition constant (K_i) values for quinine and quinidine of 301 and 543 μM , respectively. These data

Table 1 Michaelis-Menten constants describing [^3H]-amantadine accumulation by human renal cortical slices using a 30 s incubation, and inhibition constants for quinine and quinidine. $V_{\max}$ values are expressed in $\text{nmol amantadine mg}^{-1} \text{ estimated proximal tubule protein min}^{-1}$. Amantadine concentrations used in the inhibition experiments were 10 and 80 μM . ND = not determined owing to limited tissue availability. Data for subject 8 are omitted as the tissue was used only to examine the time-course of amantadine uptake

Subject	K_m (μM)	$V_{\max}$ ($\text{nmol mg}^{-1} \text{ min}^{-1}$)	K_i (μM)	
			Quinine	Quinidine
1	165	1.08	232	495
2	170	1.17	154	ND
3	213	2.22	358	ND
4	ND	ND	ND	543
5	198	0.99	ND	ND
6	ND	ND	301	ND
7	ND	ND	ND	720
Mean	187 ± 11	1.4 ± 0.3	$261 \pm 44^*$	$586 \pm 68^*$
$\pm$ s.e. mean				

*Significant difference at $P < 0.05$.

suggest an approximate two-fold greater inhibitory potency for the (–) isomer, quinine, over the (+) isomer, quinidine. All K_i values calculated for individual experiments are shown in Table 1.

Discussion

Previous systematic studies using human tissues, including renal proximal tubule cells, have demonstrated tissue viability for up to 2 h at body temperature, and for 24 h when storage temperature was reduced (Trump & Harris, 1979).

The achiral organic cation, amantadine, is concentrated by human renal cortical slices. Uptake is saturable and inhibited by pretreatment of the tissue with the metabolic inhibitor, 2,4-dinitrophenol, suggesting an energy-dependent, carrier-mediated transport process (Grantham & Chonko, 1991). In the present study the

Figure 1 (a) and (b) Representative Dixon plots for inhibition of [^3H]-amantadine uptake by renal proximal tubules by quinine (subject 6) and quinidine (subject 4). Amantadine concentrations were (■) 10 μM and (●) 80 μM . The velocity of accumulation (V) is expressed in $\text{pmol amantadine mg}^{-1} \text{ estimated proximal tubule protein min}^{-1}$.

values of the apparent affinity ($K_m = 187 \pm 11 \mu\text{M}$) and maximal capacity ($V_{\max} = 1.37 \pm 0.29 \text{ nmol mg min}^{-1}$) for the transport sites involved were found to be similar to those documented previously with rat cortical slices ($K_m = 94 \pm 5 \mu\text{M}$, $V_{\max} = 1.3 \pm 0.1 \text{ nmol mg min}^{-1}$; Wong *et al.*, 1990). Furthermore, the results with human tissue confirm the greater inhibitory potency of quinine relative to quinidine as observed previously in the rat. Thus, the stereoselectivity of this inhibition is the reverse of that observed in the renal clearances of the diastereoisomers in man (Notterman *et al.*, 1986). Others have also observed a dissociation between the rate of tubular secretion of a competitive organic cation transport inhibitor, cyanine dye no. 863, and its ability to produce inhibition (Rennick *et al.*, 1956). Clearly, a greater inhibitory potency of quinine on the renal transport of amantadine does not reflect a correspondingly higher

tubular secretory rate for this isomer.

In conclusion, we have shown that stereoselective inhibition of renal amantadine transport by quinine and quinidine is similar in rat and human cortical slices. This supports the use of rat tissue for examining chiral aspects of renal organic cation transport.

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