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pH-dependent transport of procainamide in cultured renal epithelial monolayers of OK cells: consistent with nonionic diffusion

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1 Previous studies suggest that procainamide is a substrate for organic cation/proton antiport. In order to study the coupling between procainamide flux and proton flux in greater detail we investigated the effects of extracellular procainamide addition upon intracellular pH in cultured monolayers of renal OK cells. Intracellular pH was monitored by use of BCECF as a probe.

2 Apical addition of procainamide (10 mM) caused a significant alkalinisation of intracellular pH. Basolateral addition of procainamide was equally effective in raising intracellular pH. A similar alkalinisation was found in two other renal cell lines: MDCK strain 1 and $LLCPK_1$.

3 In contrast, both tetraethylammonium and N-methylnicotinamide, archetypal substrates for organic cation/proton antiport were without effect upon intracellular pH.

4 At physiological pH values, procainamide exists as a neutral weak base (B) and its conjugate weak acid (BH^+). To test which species of procainamide was responsible for the alkalinisation, experiments in which [B] was kept constant whilst [BH^+] was varied from 1.15 mM to 7.25 mM were performed. The results suggested that the neutral weak base (B) was the permeant species.

5 Procainamide efflux from procainamide-loaded cell monolayers resulted in a significant acidification of intracellular pH. As with procainamide uptake, this result could be ascribed to the movement of neutral weak base.

6 These effects of procainamide upon intracellular pH are consistent with nonionic diffusion of procainamide rather than an interaction of procainamide with the organic cation/proton antiporter. In addition, the results suggest that organic cation/proton antiport is not highly expressed in OK cells.

Keywords: Organic cation transport; OK cells; intracellular pH

Introduction

A wide range of organic cations, including many xenobiotics, are actively secreted across the renal proximal tubular epithelium (Holohan & Ross, 1981; Rennick, 1981). Over the last decade, the transport mechanisms underlying this secretion have been the subject of intense scrutiny. Organic cation secretion consists of two discrete transport steps; the uptake of organic cations across the basolateral membrane into the cell and the exit of these substrates across the apical membrane. The major route of organic cation uptake at the basolateral membrane is driven by the transmembrane potential difference (Schäli *et al.*, 1983; Montrose-Rafizadeh *et al.*, 1989; Dantzler *et al.*, 1991; Ullrich, 1994) and may be mediated by the recently reported OCT1 transport protein (Gründeman *et al.*, 1994). Using tetraethylammonium (TEA), as a probe of organic cation secretion across the apical membrane, an electroneutral organic cation/proton antiport (OC/H^+) in which exit of organic cations across the apical membrane is tightly coupled to the entry of protons has been widely documented (Pritchard & Miller, 1993). Indeed both proton-gradient driven uptake of TEA and its converse organic cation-gradient driven proton flux have now been demonstrated in isolated membrane vesicles (Holohan & Ross, 1981; Takano *et al.*, 1983; Hsyu *et al.*, 1987; Solkol *et al.*, 1988; Ott *et al.*, 1991) and cultured renal epithelial cell lines (Innui *et al.*, 1985; Yuan *et al.*, 1991; Saito *et al.*, 1992). Indirect evidence suggests that in addition to TEA, OC/H^+ antiport accepts a wide range of substrates, many of which are weak bases (McKinney & Speeg, 1982; McKinney & Kunneman, 1985; Boom *et al.*, 1992; Takano *et al.*, 1992). However, much of this evidence is inconclusive since it has proved difficult to differentiate between transport mediated by OC/H^+ antiport and nonionic diffusion.

In order to try and resolve this issue we have studied the putative coupling between organic cation and proton flux in greater detail. To do this we have loaded cell monolayers, grown upon permeable filter supports, with the pH-sensitive dye, 2',7'-*bis*(carboxyethyl)-5-6-carboxyfluorescein (BCECF) and measured the effects of procainamide and other organic cations upon intracellular pH. This novel approach has two main advantages: Firstly it allows direct assessment of substrates not commercially available in radiolabelled form, but perhaps more importantly, it allows the contribution of neutral weak base (B) or conjugate weak acid (BH^+) to overall weak base transport to be assessed. The major finding of this study was that both procainamide uptake and secretion across the apical membrane of OK cells was consistent with nonionic diffusion of unionised procainamide in response to transmembrane pH gradients. Additionally the lack of effect of the archetypal substrates TEA and NMN upon intracellular pH suggested that OC/H^+ antiport is not highly expressed in the apical membrane of OK cell monolayers.

Methods

Cell cultures

OK cells were cultured in Eagle's minimum essential medium (EMEM) with 10% foetal calf serum (FCS), 1% non-essential amino acids, 2 mM glutamine and 50 $\mu g\ ml^{-1}$ gentomycin. $LLC\text{-}PK_1$ cells were grown in Medium 199 supplemented with 3% FCS and gentomycin antibiotic (50 $\mu g\ ml^{-1}$). MDCK-1 cells were cultured in EMEM with 10% FCS, 2% non-essential amino acids, 2 mM glutamine and 10 $\mu g\ ml^{-1}$ kanomycin an-

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tibiotic. Cell monolayers were prepared by seeding at high cell densities ($4-5 \times 10^5$ cells cm^2) onto tissue culture inserts (Transwell polycarbonate filters; Costar). Cell monolayers were maintained at 37°C in a humidified 5% CO_2 in air atmosphere. Monolayer confluence was estimated by both microscopy and measurement of transepithelial electrical resistance.

Intracellular pH measurements

Intracellular pH (pH_i) was measured as described previously (Thwaites *et al.*, 1993). Briefly, epithelial monolayers grown on 12 mm diameter permeable filter supports were loaded by incubation with BCECF-AM (5 μM) in both the apical and basolateral chamber for 40 min at 37°C in culture media. After loading, the inserts were placed in a 24 mm diameter perfusion chamber mounted on the stage of an inverted fluorescence microscope (Nikon Diaphot). The apical and basolateral cell aspects were perfused independently using a compressed air driven system (flow rate 5 ml min^{-1}) that allowed any combination of 6 apical and basolateral solutions. Apical and basolateral bath volumes were 0.5 ml and 1 ml respectively. All experiments were performed at 37°C. Intracellular H^+ concentration was quantified by fluorescence (excitation at 440/490 nm, emission at 520 nm) from a small group of cells (5–10) using a photon counting system (Newcastle Photometrics). Intracellular BCECF fluorescence was converted to pH_i by comparison with values from an intracellular calibration curve using nigericin (10 μM) and high K^+ solutions (Thomas *et al.*, 1979). The response of the BCECF to changes in intracellular pH in OK cell monolayers was linear over the range 6.8–8.0 at 37°C (data not shown).

Solutions

Monolayers were perfused with a modified Krebs solutions (mmol l^{-1}): NaCl 140, KCl 5.4, $CaCl_2$ 2.0, $MgSO_4$ 1.2, NaH_2PO_4 0.3, KH_2PO_4 0.3, HEPES 10, glucose 5 (buffered to pH 7.4 or 8.4 at 37°C with Tris base). Where the apical pH was held at pH 6.0, 10 mM MES was used in place of 10 mM HEPES.

Statistical analysis

Data are expressed as means ± s.e.mean. Statistical comparison of mean values was made with Student's *t* test (2-tailed solution) for paired or unpaired data as appropriate. For multiple comparisons, one way analysis of variance (ANOVA) was used and significance assigned by either a Dunnet or a Bonferroni post test.

Materials

Cell culture media, supplements and tissue culture plastics were from Life Technologies (Paisley, Scotland). Tissue culture inserts were from Costar U.K. (High Wycombe, Bucks U.K.). BCECF-AM was purchased from Calbiochem (Nottingham). All other biochemicals were supplied by either Sigma (Poole, Dorset) or BDH (Poole, Dorset).

Results

Effect of procainamide upon intracellular pH

To mimic the conditions used by other investigators to demonstrate OC/H^+ antiport in renal cells, the effects of procainamide upon intracellular pH (pH_i) were measured in the presence of an outwardly-directed pH gradient ($pH_o = 8.4$) and an inwardly directed procainamide gradient (10 mM). Figure 1a shows the results of a typical experiment. The initial intracellular pH of a representative cell monolayer perfused across both the apical and basolateral surfaces with Krebs solution at pH 7.4 was 7.46 (mean 7.49 ± 0.06, $n = 3$). As can be seen from Figure 1, imposition of an outwardly directed H^+ gradient across the apical membrane (apical perfusate pH 8.4, basolateral perfusate pH 7.4) had no significant effect upon intracellular pH (mean 7.56 ± 0.07, $n = 3$, $P > 0.05$). However addition of procainamide (10 mM) to the apical perfusate at pH 8.4 led to a marked alkalinisation of pH_i (mean results; from 7.56 ± 0.07 at pH 8.4 to 7.83 ± 0.03 at pH 8.4+ procainamide $n = 3$, $P < 0.02$). Removal of the procainamide pulse with the perfusate pH held at 8.4 resulted in a rapid restoration of pH_i towards the initial value, with a complete recovery of pH_i to control levels upon reperfusion with pH 7.4 buffer (pH_i 7.48 ± 0.05, $n = 3$). Repetition of the experimental protocol resulted in an identical response. Surprisingly, as shown in Figure 1b, procainamide was equally effective when applied to the basolateral membrane (apical pH 7.4, basolateral pH 8.4) with an alkalinisation of pH_i from 7.49 ± 0.06 at pH 8.4 to 7.88 ± 0.03 on addition of procainamide ($P < 0.005$, $n = 3$). As with the apical membrane, perfusion of the basolateral membrane with pH 8.4 alone was without effect on intracellular pH. The magnitude of the procainamide-induced change in pH_i was dependent upon the extracellular pH (Figure 2). Even in the absence of significant pH gradient (apical and basolateral pH 7.4) apical addition of procainamide (10 mM) resulted in a significant intracellular alkalinisation (mean results; 7.39 ± 0.06 to 7.56 ± 0.05, $P < 0.05$, $n = 8$). When

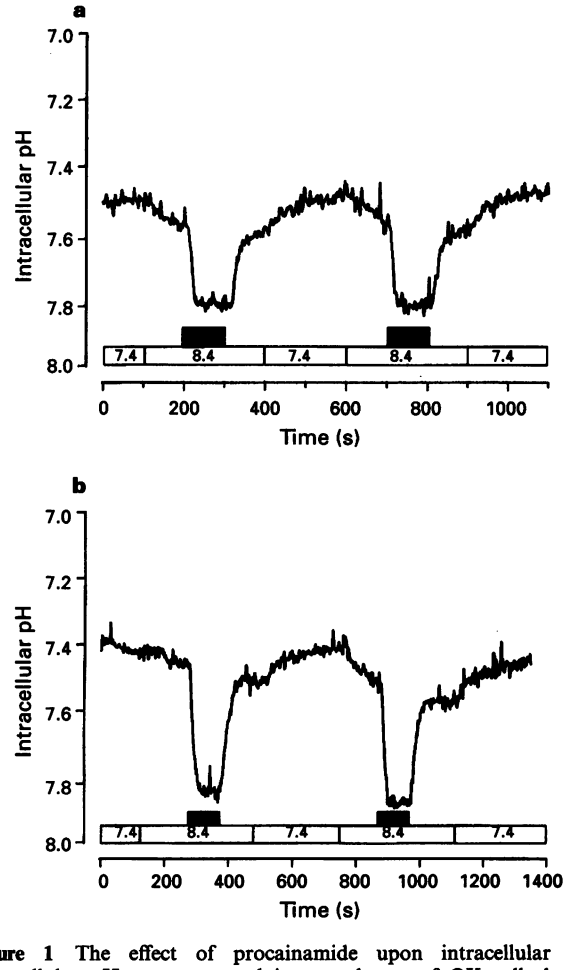

Figure 1 The effect of procainamide upon intracellular pH. Intracellular pH was measured in monolayers of OK cells loaded with the pH-sensitive fluoroprobe BCECF. (a) The effect of apical addition of 10 mM procainamide at an apical perfusion pH of 8.4 (at solid bar); basolateral perfusion pH was constant at pH 7.4. A single trace representative of 3 separate experiments. (b) The effect of basolateral addition of 10 mM procainamide at pH 8.4 (at solid bar), apical perfusion pH was pH 7.4; a single trace representative of 3 separate experiments.

the apical perfusate pH was 8.4, apical procainamide addition caused a significantly larger shift in pH_i from pH 7.38 ± 0.05 to pH 7.76 ± 0.04 ($P<0.001$, $n=8$) than was seen at pH 7.4. In contrast, application of an inwardly directed proton gradient across the apical membrane ($pH_o=6.0$) resulted in a small acidification of pH_i from 7.39 ± 0.03 to 7.26 ± 0.04 ($P<0.05$, $n=8$). Subsequent addition of procainamide to the apical surface at pH 6.0 did not produce a further change in pH_i (7.26 ± 0.04 to 7.19 ± 0.09, $P>0.2$, $n=8$).

Is the ionisation state of procainamide important?

Procainamide exists in two forms; an unionised weak base (B) and its conjugate weak acid (BH^+). Transport of either form could result in an intracellular alkalinisation; the neutral weak base as intracellular protons are buffered, the conjugate weak acid as protons are exported via OC/H^+ antiport. To differentiate between these two possibilities, an experiment was designed in which pH_i was monitored in cells exposed to pulses of procainamide in which the concentration of unionised procainamide was constant at 100 μM but, by manipulation of external pH, the concentration of ionised procainamide varied over a 6 fold range. A typical trace of one such experiment is shown in Figure 3. Initially monolayers were perfused across both membranes with pH 7.4 Krebs solution. The mean pH_i in this batch of cells was 7.28 ± 0.03 ($n=3$); addition of 7.35 mM procainamide at pH 7.4 ($BH^+=7.25$ mM, $B=100$ μM) to the apical membrane resulted in a significant alkalinisation (7.28 ± 0.03 to 7.42 ± 0.05, $P<0.02$, $n=3$) which was reversed upon removal of procainamide. Identical changes in pH_i were found when the monolayer was perfused with either 3.0 mM procainamide ($BH^+=2.90$ mM, $B=100$ μM) at pH 7.8 (7.26 ± 0.04 to 7.42 ± 0.06, $n=3$) or 1.25 mM procainamide ($BH^+=1.15$ mM, $B=100$ μM) at pH 8.2 (7.28 ± 0.03 to 7.41 ± 0.04, $n=3$). These results suggest that the neutral weak base (B) rather than its conjugate weak acid (BH^+) is the principal transported species. Indeed, OK cells may be impermeable to protonated procainamide (BH^+) since, in contrast to NH_3/NH_4^+ entry (Boyarsky *et al.*, 1988), there was no slow acidification of the plateau phase upon prolonged exposure of cells to procainamide nor any hint of an overshoot of pH_i to a value more acid than the control value upon removal of extracellular procainamide.

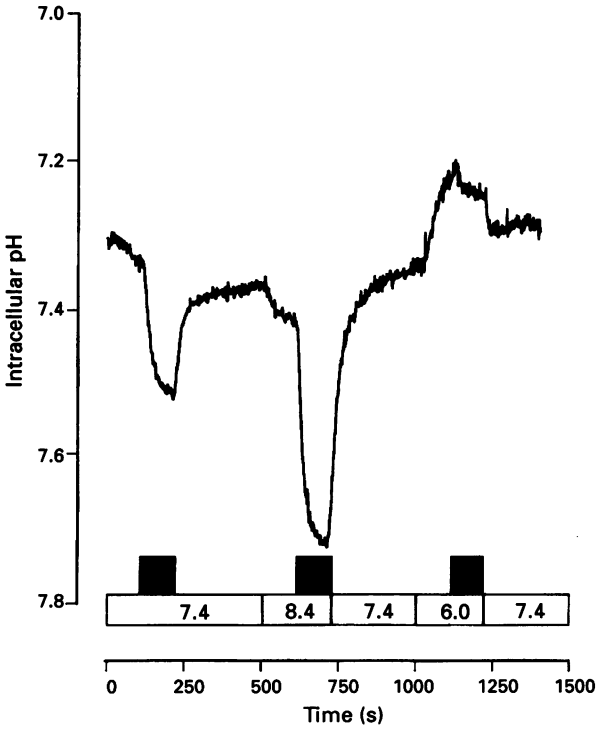

Figure 2 The effect of apical perfusate pH upon the magnitude of procainamide-induced intracellular alkalinisation. The effect of apical addition of 10 mM procainamide (indicated by solid bar) was measured at 3 apical perfusate pH values; pH 6.0, pH 7.4 and pH 8.4. Basolateral perfusate pH was constant at pH 7.4. A single representative trace typical of 7 others.

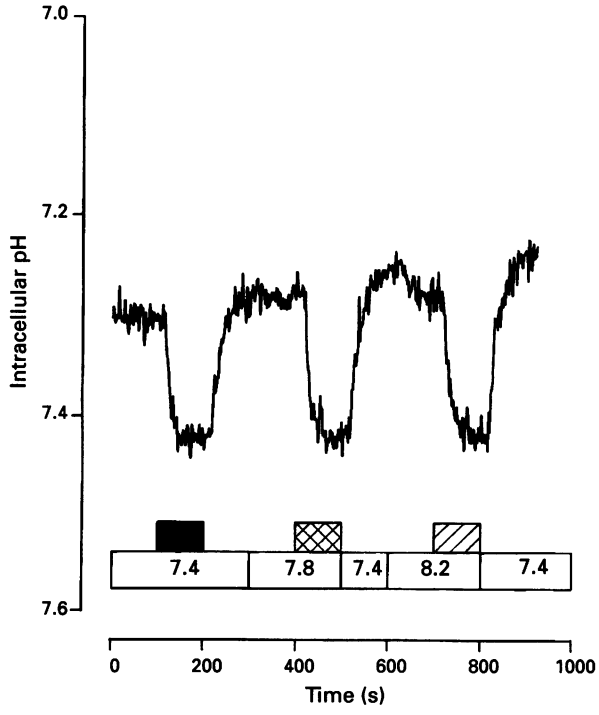

Figure 3 To test the relative contribution of the neutral weak base (B) and conjugate weak acid (BH^+) forms of procainamide, cells were exposed to a pulse of procainamide with a constant [B] of 100 μM but with $[BH^+]$ varied from 1.15 mM to 7.25 mM. At a perfusate pH of 7.4, $[BH^+]$ was 7.25 mM (solid bar). At pH 7.8 $[BH^+]$ was 2.9 mM (cross-hatched bar). At pH 8.2 $[BH^+]$ was 1.15 mM (hatched bar). A single trace representative of 3 separate determinations.

Procainamide efflux studies

To investigate the coupling between procainamide efflux across the apical membrane and proton flux, OK cell monolayers were loaded with both BCECF-AM and procainamide (7.35 mM) for 30 min at 37°C and pH 7.4. At steady state (assuming $B_i=B_o$ at 100 μM) and 7.35 mM procainamide (100 μM B) in both apical and basolateral perfusates, the mean intracellular pH was 7.65 ± 0.03 ($n=3$) (Figure 4). Imposing an outwardly directed neutral weak base (B) gradient (at a constant BH^+ concentration) by dropping the apical perfusate pH to pH 7.0 ($[B_o]=40$ μM) resulted in a marked acidification of pH_i from pH 7.69 ± 0.03 to pH 7.53 ± 0.04, ($P<0.006$, $n=3$) which was reversed upon return to pH 7.4 Krebs containing procainamide. In contrast, perfusion of the membrane with pH 7.0 Krebs in the absence of a neutral weak base gradient ($B_o=B_i$) had no significant effect upon pH_i. A larger acidification was seen if the apical membrane was perfused at pH 7.4 with a procainamide-free Krebs solution ($[B_o]=0$).

The effects of tetramethylammonium (TEA) and N-methylnicotinamide (NMN) on pH_i

TEA, a quaternary ammonium compound, remains ionised across the entire pH range. Since TEA can cross the membrane only as a cation and is a prime substrate for OC/H^+ antiport (Pritchard & Miller, 1993), an inwardly-directed gradient of TEA might be expected to cause an intracellular alkalinisation

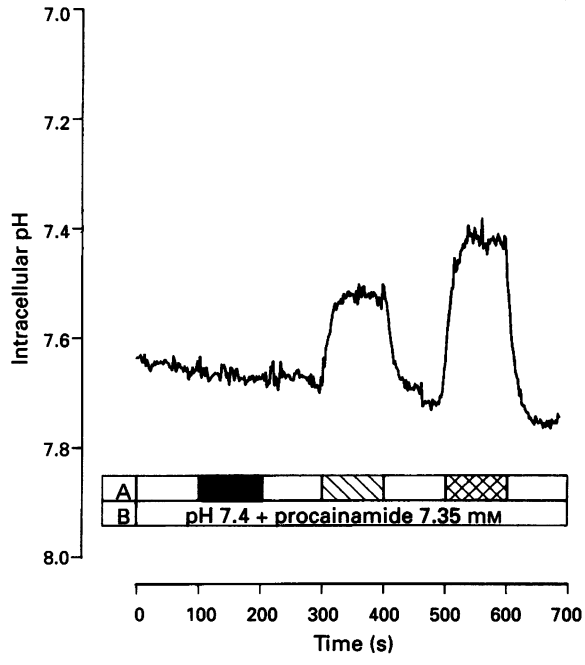

Figure 4 The effect of procainamide efflux across the apical membrane upon intracellular pH was measured in procainamide-loaded cell monolayers. Cell monolayers were loaded with procainamide (7.35 mM, pH 7.4) for 30 min. Intracellular pH was measured under 4 experimental conditions: (1) (open bar) no pH or procainamide gradient across the apical membrane; (2) (solid bar) an inwardly directed $[BH^+]$ gradient with $[B_o]=[B_i]$, pH 7.0, procainamide 18.3 mM; (3) (hatched bar) an outwardly directed $[B_o]$ gradient with $[BH^+_o]=[BH^+_i]$ pH 7.0, procainamide 7.35 mM, (4) (cross hatched bar) an outwardly directed [B] gradient with $[BH^+_o]$ set at zero, pH 7.4, no procainamide. A single trace representative of 3 separate experiments.

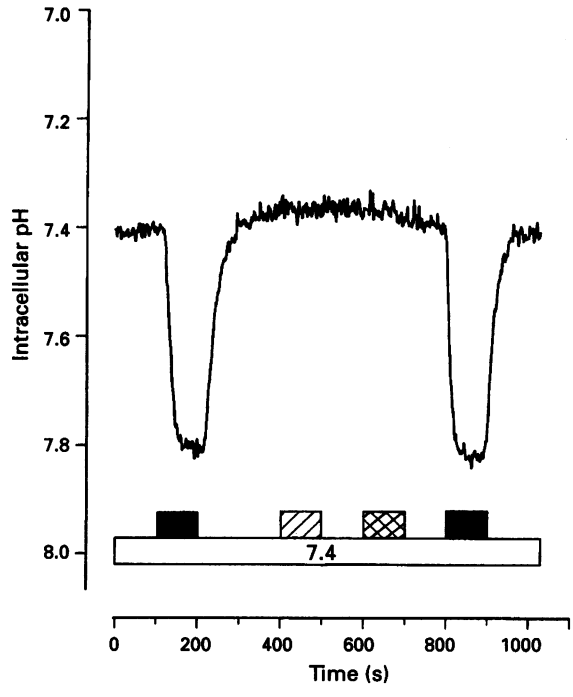

Figure 5 A comparison of the effects of procainamide, tetraethylammonium (TEA) and N-methylnicotinamide (NMN) upon intracellular pH. Procainamide 7.35 mM, (solid bar), TEA 10 mM (hatched bar) or NMN 10 mM, (cross hatched bar) were included in the apical perfusate at pH 7.4. The basolateral perfusate was pH 7.4. A single trace representative of 4 others.

as a result of OC/H^+ antiport. To investigate whether this was the case, the effects of both TEA and N-methyl nicotinamide (NMN), another prototypic substrate for the OC/H^+ exchanger, upon pH_i were measured. The results of a typical experiment are shown in Figure 5. Initially the monolayers were perfused across both surfaces with Krebs solution at pH 7.4. The mean pH_i of 5 monolayers was 7.45 ± 0.06. Addition of procainamide (7.35 mM) to the apical perfusate at pH 7.4 resulted in a marked intracellular alkalinisation (7.45 ± 0.06 to 7.8 ± 0.02, $P<0.001$, $n=5$). In contrast, perfusion of the apical membrane with either 10 mM TEA or 10 mM NMN had no significant effect upon pH_i ($P>0.5$, $n=5$ for both conditions). The integrity of the monolayer was checked with a second procainamide pulse which caused an identical change in pH_i to the first. Similarly in a second series of experiments, TEA at 20 mM had no effect upon the magnitude of alkalinisation caused by 0.5 mM procainamide (0.26 ± 0.03 v 0.3 ± 0.06 pH units, $P>0.5$, $n=5$). Taken together these results suggest that procainamide has a different mode of action from TEA or NMN and may suggest that OC/H^+ antiport is not highly expressed in the apical membrane of OK cells. Indeed, addition of 10 μM 1,1' diethyl2,2'-cyanine (decynium 22) a novel potent inhibitor of renal OC/H^+ antiport (Schömig *et al.*, 1993) to the apical perfusate had no effect on procainamide-induced alkalinisation in OK cell monolayers.

The effects of organic cations on pH_i in other renal epithelial cell lines

In light of the above results we went on to test the effects of procainamide and TEA upon pH_i in other renal cell lines (Figure 6). In LLC-PK1 cell monolayers, addition of procainamide to the apical perfusate at pH 8.4 (Figure 6a) resulted in a marked alkalinisation of intracellular pH from a mean of 7.65 ± 0.03 to 7.83 ± 0.02, ($P<0.001$, $n=6$). Basolateral addition of procainamide (Figure 6b) had a similar effect on pH_i (pH 7.38 ± 0.02 to 7.67 ± 0.03, $P<0.001$, $n=4$). In MDCK (strain 1) cell monolayers apical addition of procainamide (Figure 6c) increased pH_i from pH 7.56 ± 0.02 to 7.75 ± 0.02, ($P<0.001$, $n=4$) as did basolateral addition (pH 7.60 ± 0.02 to 7.88 ± 0.03, $P<0.001$, $n=4$) (Figure 6d). As found with OK cell monolayers, addition of TEA (10 mM) to the apical membrane of either LLC-PK1 or MDCK (strain 1) was without effect upon pH_i (data not shown).

Change in pH_i to procainamide exhibits saturation kinetics

A range of procainamide concentrations (0.156 to 10 mM total base, 18.9–1213 μM [B]) was tested to establish the concentration-dependence of the change in pH_i to procainamide. Procainamide was added to the apical perfusate at pH 8.4, the basolateral perfusate was pH 7.4. Initial pH of the monolayers perfused with pH 7.4 was 7.22 ± 0.03 ($n=3$). Expressing the results as change in pH_i against concentration of neutral weak base (B_o) a saturable relationship between $[B_o]$ and the magnitude of intracellular alkalinisation was found (Figure 7). A similar relationship between $[B_o]$ and the magnitude of alkalinisation has been reported for NH_3 (Boron, 1992). The non linear relationship between $[B_o]$ and the magnitude of alkalinisation at high $[B_o]$ is a consequence of the progressive increase in pH_i as neutral weak base enters the cell and the pH-dependence of protonation of the weak base (pK_a-pH_i) once inside the cell.

Discussion

In contrast to the clear evidence in favour of TEA/H^+ antiport in the apical membrane of renal proximal tubule cells, the mechanism underlying secretion of organic cations which are also weak bases has always been open to interpretation. In this study we have sought to clarify this ambiguity by measuring the effect of procainamide upon pH_i. Three pieces of experimental evidence strongly suggest that procainamide uptake

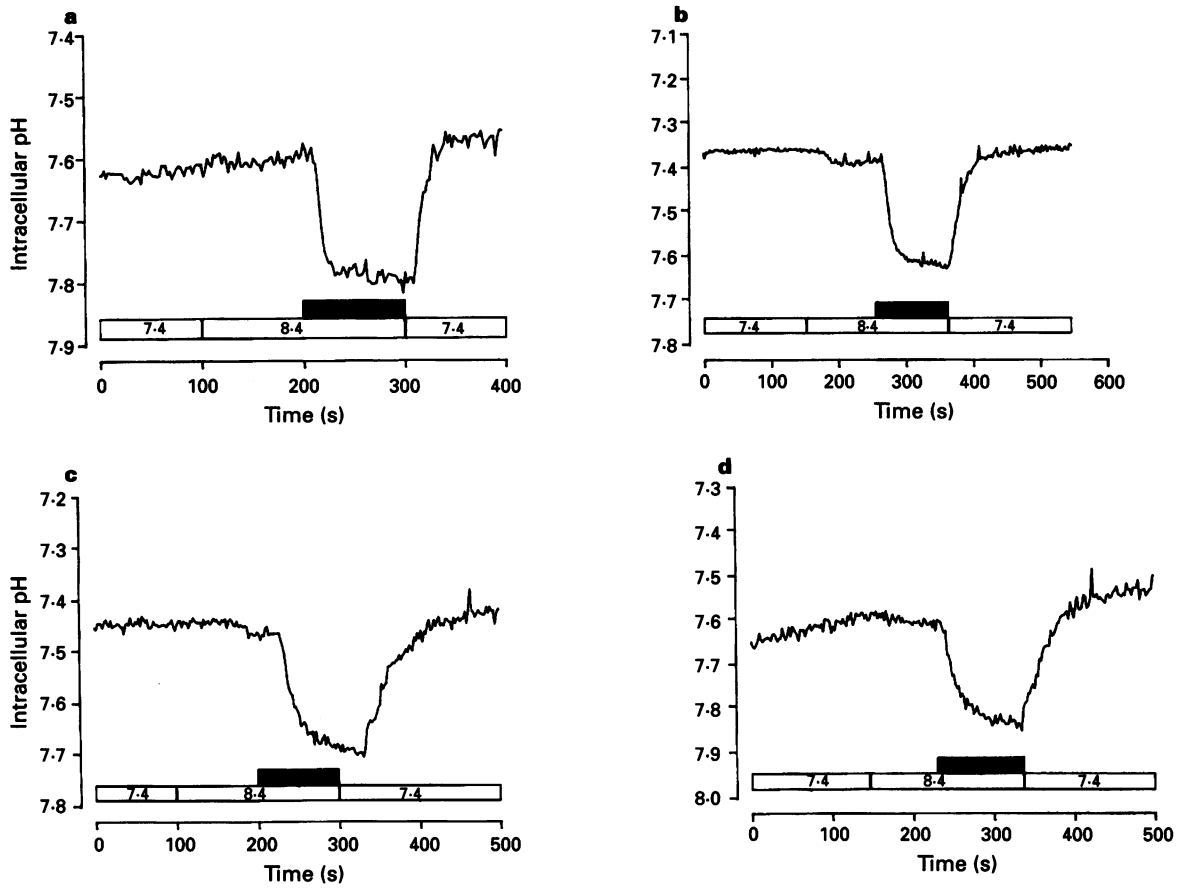

Figure 6 The effect of procainamide upon intracellular pH in LLC-PK$_1$ and MDCK strain 1 cell monolayers. Procainamide, 10 mM (indicated by solid bar below trace) was added to the apical perfusate of (a) LLC-PK$_1$ cells (c) MDCK strain 1 cells or to the basolateral perfusate of LLC-PK$_1$ (b) or MDCK strain 1 cells (d). The traces represent single traces typical of either 6 (LLC-PK$_1$) or 4 (MDCK) separate determinations.

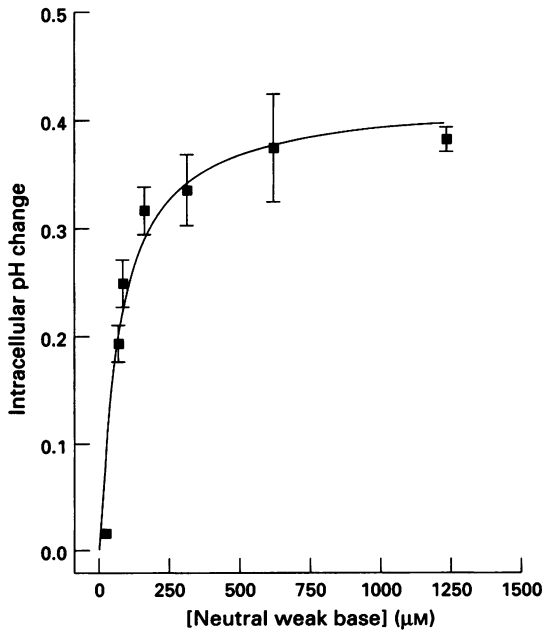

Figure 7 Apparent dose-response curve of the change in intracellular pH as a function of procainamide concentration. Procainamide was perfused across the apical membrane at pH 8.4, basolateral pH was pH 7.4. Procainamide concentration is expressed as neutral weak base concentration [B]. The results are expressed as the mean ± s.e.mean of 4 separate determinations.

into OK cells is mediated primarily by nonionic diffusion and not by OC/H$^+$ antiport: (1) the magnitude of alkalinisation seen upon addition of procainamide to the apical membrane was dependent upon the gradient of unionised procainamide (Figure 2) and was independent of either the concentration of ionised procainamide or the magnitude and direction of the imposed pH gradient; (2) there was no alkalinization in the presence of similar gradients of TEA or NMN (Figure 3) archetypal substrates for OC/H$^+$ antiport; (3) procainamide caused an alkalinisation at both apical and basolateral membranes faces and in cells derived from both proximal and distal nephron segments, whereas OC/H$^+$ antiport is found only at the apical membrane and is restricted to the proximal tubule.

Although it has been proposed that OC/H$^+$ plays a central role in the secretion of a wide range of hydrophobic weak bases, there is little direct evidence to support this view. For example, the evidence to suggest that the weak base, procainamide, is a substrate for OC/H$^+$ antiport is derived mainly from *cis*-inhibition studies of the effect of procainamide upon TEA/H$^+$ antiport. In these studies inhibition of proton gradient driven [^{14}C]-TEA uptake by external procainamide has been interpreted as competition between TEA and the cationic form of procainamide for the exchanger. Given our observations that procainamide crosses the membranes of a number of cultured renal cell lines predominantly by nonionic diffusion and that procainamide uptake is associated with a significant change in intracellular pH, it is equally feasible that the *cis*-inhibition of TEA uptake is the result of a procainamide-induced collapse of the imposed proton gradient rather than from a direct interaction between procainamide and TEA at the level of the TEA/H$^+$ exchanger. A similar argument can be

applied to the observations of McKinney & Kunneman (1985) that *cis*-inhibition of [^{3}H]-procainamide uptake by the quaternary ammonium, tetramethylammonium (TMA), implies a direct interaction between procainamide and TMA rather than an indirect coupling via pH, since activation of TMA/H^+ antiport would result in an alkalinisation of intracellular pH and reduce the 'pH trapping' of unionised procainamide with the cell. Direct measurement of procainamide uptake across the apical membrane of LLC-PK_1 cells (Takano *et al.*, 1992) has shown that procainamide uptake is greater at an alkaline external pH than at an acid pH results which, although compatible with OC/H^+ antiport, are in complete agreement with our observations of the pH-dependent effects of external procainamide upon intracellular pH. However, with the measurement of intracellular pH we can differentiate between OC/H^+ antiport and the nonionic diffusion of procainamide across the apical membrane of OK cell monolayers and clearly demonstrate that only unionised procainamide (B) has a significant permeability. Indeed taken together there is no compelling evidence in the literature to link weak base secretion to a direct interaction with TEA/H^+ antiport. Further highlighting the differences between procainamide and TEA handling in OK cells is the observation that, in contrast to the marked effects of procainamide upon pH_i, we were unable to demonstrate any significant effects of TEA or NMN upon intracellular pH. This may relate to the low apparent expression of OC/H^+ antiport in OK cells. In agreement with this, Yuan *et al.* (1991) recently reported the existence of TEA/TEA self antiport in OK cell monolayers grown on plastic but found only a small (30%) stimulation of TEA efflux by a proton gradient. Similar data suggesting a low expression of TEA/H^+ antiport has been presented for intact LLC-PK_1 cells (McKinney *et al.*, 1988; Saito *et al.*, 1992).

The current model of renal organic cation secretion in the proximal tubule involves electrogenic uptake of organic cation across the basolateral membrane followed by secretion across the apical membrane via OC/H^+ antiport. The driving force across the apical membrane being the organic cation gradient and the acid lumen of the tubule. Equally it is possible to model the secretion of organic cations which are weak bases without invoking the presence of an apical OC/H^+ exchanger (see Figure 4): in this case, exit across the apical membrane is mediated by nonionic diffusion with the magnitude of secretion being governed by the p*K*a of the compound and the pH gradient across the apical membrane. Indeed this could be a more effective secretory mechanism for a compound like procainamide than OC/H^+ antiport. Since the acidic pH generated in the proximal tubular lumen would not only favour procainamide secretion across the apical membrane but also mean that the filtered load of procainamide would be predominantly in the impermeant ionised form (BH^+).

In conclusion we have presented evidence to suggest that procainamide uptake and secretion across the apical membrane of cultured renal OK cells is consistent with nonionic diffusion rather than OC/H^+ antiport. Clearly further studies are necessary to investigate whether nonionic diffusion plays an important role in organic weak base secretion by the proximal tubule. Similarly the substrate specificity of TEA/H^+ antiport needs to be urgently reassessed.

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