Localization of diuretic effects along the loop of Henle: an in vivo microperfusion study in rats

R. J. UNWIN*, S. J. WALTER†, G. GIEBISCH‡, G. CAPASSO§ and D. G. SHIRLEY*

*Centre for Nephrology, Royal Free and University College Medical School, Institute of Urology and Nephrology, Middlesex Hospital, London W1N 8AA, U.K., †Division of Biomedical Sciences, Imperial College School of Medicine, Charing Cross Hospital, London W6 8RF, U.K., ‡Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520, U.S.A., and §Chair of Nephrology, Second University of Naples, Naples, Italy

ABSTRACT

In order to clarify the effects on sodium reabsorption in the loop of Henle of methazolamide (a carbonic anhydrase inhibitor), chlorothiazide and the loop diuretics frusemide and bumetanide, superficial loops were perfused in vivo in anaesthetized rats and the individual diuretics were included in the perfusate. Differentiation between effects in the pars recta and in the thick ascending limb of Henle (TALH) was achieved by comparing responses to the diuretics when using a standard perfusate, designed to mimic native late proximal tubular fluid, and a low-sodium perfusate, designed to block net sodium reabsorption in the pars recta. With the standard perfusate, methazolamide caused decreases in sodium reabsorption (\(J_{Na}\)) and water reabsorption (\(J_V\)); with the low-sodium perfusate, a modest effect on \(J_{Na}\) persisted, suggesting that carbonic anhydrase inhibition reduces sodium reabsorption in both the pars recta and the TALH. The effects of chlorothiazide were very similar to those of methazolamide with both the standard and low-sodium perfusates, suggesting that chlorothiazide also inhibits sodium reabsorption in the pars recta and TALH, perhaps through inhibition of carbonic anhydrase. With the standard perfusate, both frusemide and bumetanide produced the expected large decreases in \(J_{Na}\), but \(J_V\) was also lowered. With the low-sodium perfusate, the inhibitory effects of the loop diuretics, particularly those of frusemide, were substantially reduced, while net potassium secretion was found. These observations indicate that a significant component of the effect of frusemide (and possibly of bumetanide) on overall sodium reabsorption is located in the pars recta, and that loop diuretics induce potassium secretion in the TALH.

INTRODUCTION

The anatomical loop of Henle, which comprises the pars recta (proximal straight tubule), the thin descending and ascending limbs, and the thick ascending limb of Henle (TALH), reabsorbs approximately 40% of the filtered load of sodium and is an important target site for diuretic action. Although the mechanism of sodium reabsorption in the pars recta is not fully understood, it is generally assumed that fluid is reabsorbed iso-osmotically, as in the pars convoluta [1]. Very little is known about sodium handling in the thin descending limb of Henle. No convincing evidence for active transport has been obtained, but in some species the descending limb wall...
has a finite permeability to sodium [2], and there is some evidence for net sodium reabsorption down a concentration gradient established by the osmotic reabsorption of water [3]. However, any such reabsorption is only likely to be quantitatively significant in the short-looped juxtamedullary nephrons. The thin ascending limb of Henle (present only in juxtamedullary nephrons) has also proved resistant to experimental demonstration of active transport. The sodium reabsorption that takes place in this segment is generally believed to be mediated by passive processes [4]. Only in the TALH has the mechanism of sodium reabsorption been well defined: sodium enters cells via a luminal Na+/K+ co-transporter and is expelled across the basolateral membrane by the Na+/K+-ATPase, while the transepithelial potential difference drives additional, paracellular, reabsorption [5].

The so-called loop diuretics work principally by blocking the luminal co-transporter in the TALH. However, in a number of studies in which loops of Henle of superficial nephrons have been perfused in vivo, intraluminal frusemide has been shown to reduce not only sodium reabsorption but also water reabsorption [6,7]. On the presumption that the ascending limb of the loop of Henle is water impermeable, this observation suggests inhibition of fluid reabsorption in the pars recta. (It is unlikely that water reabsorption in the short thin descending limb of superficial nephrons would be inhibited, since the osmotic gradient in the outer medullary interstitium should not be disrupted by the presence of frusemide in an individual loop.) However, although evidence exists that frusemide can inhibit fluid reabsorption in the pars convoluta of the proximal tubule [8–10], an effect that is generally considered to result from the diuretic’s carbonic anhydrase inhibitory activity [11], no previous study has investigated the possible action of loop diuretics on reabsorption in the pars recta.

The effect of thiazide diuretics on sodium and fluid reabsorption in loops of Henle has yet to be defined. The only previous study found a small, non-significant reduction in sodium reabsorption when loops were perfused with chlorothiazide [12], but only six loops were tested. As with loop diuretics, several members of the thiazide group, notably chlorothiazide, can inhibit reabsorption in the pars convoluta of the proximal tubule [13], and again this is usually attributed to inhibition of carbonic anhydrase [14]. Whether this action extends to the pars recta is unknown.

The purpose of the present investigation was to clarify the actions of chlorothiazide and loop diuretics on the loop of Henle in vivo. For this, we used two approaches. First, the effects of the diuretics on reabsorption in perfused loops when using a standard perfusate were compared with those observed when using a low-sodium perfusate designed to suppress reabsorption in the pars recta [15]. Secondly, the role of carbonic anhydrase inhibition in mediating the observed effects was assessed by comparing the effects of the diuretics with those of the potent carbonic anhydrase inhibitor, methazolamide.

**METHODS**

Male Sprague-Dawley rats (n = 60; body weight 200–240 g) were used in all experiments. They were anaesthetized intraperitoneally with 5-ethyl-5-[1-methyl-1-propyl]-2-thiobarbiturate (Inactin; 120 mg kg⁻¹ body weight; Byk Gulden, Konstanz, Germany) or with 5-ethyl-5-[1-methyl-butyl]-2-thiobarbiturate (Trapanal; 110 mg kg⁻¹; Byk Gulden). All animals were prepared surgically for micropuncture as described previously [16], the left kidney being exposed through a flank incision, freed of perirenal fat and immobilized in a Perspex dish with 3% agar in 0.9% saline.

After a 2-h equilibration period, superficial loops of Henle were perfused at 20 nl min⁻¹ using methods described in detail in previous publications [6,16]. The perfusion micropipette was positioned in a final surface proximal convolution and the collection micropipette in the first surface distal loop. Two perfusion solutions were used, as follows.

Series 1: the solution used was designed to mimic native late proximal tubular fluid, and had a composition as described previously [6], i.e. (in mmol l⁻¹) 128 NaCl, 12 NaHCO₃, 3.6 KCl, 1 MgCl₂, 0.38 NaH₂PO₄ and 1.62 Na₂HPO₄, together with FD&C blue dye (0.07%) and [¹⁴C]inulin (12.5 µCi ml⁻¹; Amersham International, Aylesbury, U.K.).

Series 2: the solution proposed by Peterson et al. [15] was used in order to minimize the contribution of the pars recta to sodium reabsorption. The perfusate sodium concentration is lower in order to approximate the equilibrium concentration in the proximal tubule, while the total osmolality is maintained by addition of mannitol. The composition of the perfusate was as follows (in mmol l⁻¹): 93 NaCl, 10 NaHCO₃, 3.8 KCl, 1 MgSO₄, 1 NaH₂PO₄, 1 CaCl₂, 4 urea, 6 sodium gluconate and 61 mannitol, together with FD&C blue dye (0.07%) and [¹⁴C]inulin (12.5 µCi ml⁻¹). It has been shown that the use of this perfusate almost abolishes fluid reabsorption by the perfused loop [15,17]. Peterson and colleagues also reported that the addition of frusemide to the low-sodium perfusate (to block the Na⁺/K⁺/2Cl⁻ co-transporter in the TALH) resulted in the abolition of chloride reabsorption; similarly, we have shown that sodium reabsorption is reduced almost to zero when bumetanide is added to the perfusate [17]. These findings are consistent with an absence of fluid reabsorption in the pars recta.

In selected experiments, the following inhibitors were included in the perfusates: methazolamide (0.2 mM), chlorothiazide (0.1 mM), frusemide (0.1 mM) or...
bumetanide (1 μM). In a given series, at least two inhibitors (plus control perfusions) were used in each rat. The doses were selected on the basis of previous investigations of the inhibitory effects of intraluminal application on the relevant target nephron segments: Capasso et al. [6] found that methazolamide at 0.2 mM blocked the bulk of bicarbonate reabsorption in the proximal convoluted tubule and inhibited loop bicarbonate reabsorption by 70%; Costanzo [18] showed that 0.1 mM chlorothiazide was the lowest dose to cause maximal inhibition of sodium reabsorption in the perfused distal convoluted tubule; and several in vitro studies using rat TALH have indicated that frusemide at a dose of 0.1 mM is sufficient to suppress almost completely both NaCl reabsorption and the transepithelial potential difference [19–21]. Bumetanide is a more potent loop diuretic than frusemide [22], and studies in our own laboratory have demonstrated that a concentration as low as 1 μM causes major suppression of sodium reabsorption in perfused loops [16,17].

**Analyses**

Fluid collections from perfused tubules were deposited under water-saturated paraffin oil prior to analysis. Their volumes were measured using calibrated constriction pipettes, and duplicate samples were taken for measurement of [14C]inulin activity and sodium and potassium concentrations. [14C]Inulin activities in perfusates and collected fluids were measured by β emission spectroscopy (model 2000 CA; Canberra Packard, Pangbourne, U.K.); sodium and potassium concentrations were measured by helium glow photometry (Aminco, Silver Spring, MD, U.S.A.) or by electrothermal atomic absorption spectroscopy (model 3110; Perkin-Elmer, Beaconsfield, U.K.).

**Calculations**

The microperfusion pump (Hampel, Neu-Isenburg, Germany) was calibrated by direct measurement of volumes delivered under oil over timed periods. When set at the nominal rate of 20 nl min⁻¹, the actual pump rate was 20.1 ± 0.2 nl min⁻¹ (mean ± S.E.M.; n = 8). The in vivo perfusion rate during microperfusion was calculated as the rate of fluid collection at the early distal site multiplied by the collectate/perfusate concentration ratio for [14C]inulin (C/P In). Collected samples were accepted only if the calculated pump rate in vivo was in the range 85–115% of the predetermined rate.

Net fluxes of water (Jw), sodium (JNa) and potassium (JK) were calculated as the differences between perfusion and collection rates. Values were expressed per individual loop. Fractional reabsorption was calculated as absolute reabsorption/perfused load.

**Statistics**

Values are presented as means ± S.E.M. Statistical comparisons were made using one-way analysis of variance, with calculated pump rate (i.e. delivered load) as covariate. Where significant differences were indicated, post hoc comparisons were made using the least significant difference test. A P value of < 0.05 was considered to be statistically significant.

**RESULTS**

**Standard perfusate (Table 1)**

Control values for Jw, JNa and JK were comparable with those reported previously in perfused loops [6,16,23]. Inclusion of methazolamide in the perfusate caused a small but significant reduction in JNa, and a small increase in collectate sodium concentration; Jw was also decreased slightly, as was JK, although the latter change failed to achieve statistical significance. Chlorothiazide caused modest but significant decreases in JNa, Jw and JK; the collectate sodium concentration was raised slightly. None of the values during perfusion with chlorothiazide differed significantly from those observed during perfusion with methazolamide.

As would be anticipated, both frusemide and bumetanide caused marked decreases in JNa and greatly increased the sodium concentration of the collectate; Jw was also lowered significantly. At the drug concentrations used, the effects of frusemide were significantly greater than those of bumetanide. This was also seen with respect to potassium transport: bumetanide reduced

---

**Table 1  Effects of various diuretics on loop of Henle function in series 1 experiments (standard perfusate)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of tubules</th>
<th>Perfusion rate (nl min⁻¹)</th>
<th>C/P In</th>
<th>Jw (µmol min⁻¹)</th>
<th>JNa (µmol min⁻¹)</th>
<th>Collectate [Na⁺] (mM)</th>
<th>Collectate [K⁺] (µmol min⁻¹)</th>
<th>JK (µmol min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71</td>
<td>19.6 ± 0.2</td>
<td>1.83 ± 0.04</td>
<td>8.6 ± 0.2</td>
<td>64 ± 2</td>
<td>1975 ± 40</td>
<td>2.4 ± 0.1</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>35</td>
<td>19.6 ± 0.3</td>
<td>1.66 ± 0.04 *</td>
<td>7.6 ± 0.3 **</td>
<td>72 ± 3 **</td>
<td>1791 ± 48 **</td>
<td>2.6 ± 0.1</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>Chlorothiazide</td>
<td>21</td>
<td>19.5 ± 0.3</td>
<td>1.60 ± 0.05 **</td>
<td>7.1 ± 0.4 *</td>
<td>71 ± 3 *</td>
<td>1729 ± 52 *</td>
<td>2.8 ± 0.2 *</td>
<td>39 ± 3 *</td>
</tr>
<tr>
<td>Frusemide</td>
<td>29</td>
<td>19.7 ± 0.2</td>
<td>1.40 ± 0.02 ***</td>
<td>5.5 ± 0.3 ***</td>
<td>150 ± 2 ***</td>
<td>528 ± 51 ***</td>
<td>5.9 ± 0.3 ***</td>
<td>−10 ± 4 ***</td>
</tr>
<tr>
<td>Bumetanide</td>
<td>25</td>
<td>20.2 ± 0.3</td>
<td>1.48 ± 0.03 ***</td>
<td>6.4 ± 0.3 ***</td>
<td>136 ± 3 ***</td>
<td>891 ± 50 ***</td>
<td>5.6 ± 0.2 ***</td>
<td>0 ± 3 ***</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. The drug doses used were: methazolamide, 0.2 mM; chlorothiazide, 0.1 mM; frusemide, 0.1 mM; bumetanide, 1 μM. Significance of differences: *P < 0.05, **P < 0.01, ***P < 0.001 compared with control; †P < 0.05, ††P < 0.001 for bumetanide compared with frusemide.

© 2000 The Biochemical Society and the Medical Research Society
potassium reabsorption to zero, while frusemide caused net potassium secretion.

Since the calculated perfusion rates (and therefore delivered loads) were similar for all perfusates, the observed changes in absolute $J_{Na}$ and $J_{K}$ were reflected by similar changes in fractional reabsorptive or secretory rates for sodium and potassium ($FR_{Na}$ and $FR_{K}$ respectively) (Figure 1).

Low-sodium perfusate (Table 2)

As shown previously in this laboratory [17], when using the low-sodium perfusate the control value for $J_{Na}$ was decreased to approximately half that found with the standard perfusate. $J_{K}$ was also reduced markedly, although remaining slightly greater than zero, as reflected by a $C/P$ In ratio exceeding unity. $J_{R}$ was also lower than that seen with the standard perfusate.

Table 2  Effects of various diuretics on loop of Henle function in series 2 experiments (low-sodium perfusate)

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of tubules</th>
<th>Perfusion rate (nl·min⁻¹)</th>
<th>$C/P$ In</th>
<th>$J_{Na}$ (pmol·min⁻¹)</th>
<th>Collectate In (mM)</th>
<th>$J_{R}$ (pmol·min⁻¹)</th>
<th>Collectate K (mM)</th>
<th>$J_{K}$ (pmol·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63</td>
<td>20.0 ± 0.2</td>
<td>1.06 ± 0.01</td>
<td>1.0 ± 0.1</td>
<td>72 ± 1</td>
<td>833 ± 30</td>
<td>2.3 ± 0.1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>28</td>
<td>19.9 ± 0.3</td>
<td>1.04 ± 0.01</td>
<td>0.8 ± 0.2</td>
<td>77 ± 2*</td>
<td>740 ± 27*</td>
<td>2.1 ± 0.1</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>Chlorothiazide</td>
<td>26</td>
<td>19.6 ± 0.2</td>
<td>1.05 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>79 ± 1**</td>
<td>698 ± 29**</td>
<td>2.3 ± 0.1</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Frusemide</td>
<td>18</td>
<td>19.5 ± 0.3</td>
<td>1.00 ± 0.01</td>
<td>0.2 ± 0.2**</td>
<td>101 ± 3***</td>
<td>123 ± 48***</td>
<td>5.1 ± 0.2**</td>
<td>-26 ± 4**</td>
</tr>
<tr>
<td>Bumetanide</td>
<td>34</td>
<td>19.9 ± 0.3</td>
<td>1.01 ± 0.01</td>
<td>0.1 ± 0.2**</td>
<td>108 ± 1***</td>
<td>70 ± 28**</td>
<td>4.9 ± 0.2**</td>
<td>-22 ± 3***</td>
</tr>
</tbody>
</table>
In this setting, methazolamide had a smaller inhibitory effect on \( J_{Na} \) than was observed with the standard perfusate, with the increase in collectate sodium concentration being barely discernible, while \( J_{Fr} \) and \( J_{K} \) were completely unaffected. Values obtained during perfusion with chlorothiazide were again indistinguishable from those observed with methazolamide. Thus only a small inhibitory effect of chlorothiazide on \( J_{Na} \) remained, while the inhibitory effects on \( J_{Fr} \) and \( J_{K} \) were abolished.

Furosemide and bumetanide caused severe decreases in \( J_{Na} \) and markedly increased the sodium concentration of the collectate. In absolute terms, the decreases in \( J_{Na} \) were considerably smaller than those observed when using the standard perfusate. In further contrast with the findings when using the standard perfusate, the effect of furosemide on \( J_{Na} \) was, on average, slightly less than that of bumetanide (difference not significant). Somewhat surprisingly, \( J_{Fr} \) and \( C/P \) were also reduced significantly with both drugs, as compared with control values, while the direction of net potassium transport was reversed, with reabsorption being replaced by net secretion.

Figure 2 shows \( FR_{Na} \) and \( FR_{K} \) when using the low-sodium perfusate. Figure 2 confirms that both methazolamide and chlorothiazide modestly inhibited \( FR_{Na} \), without affecting \( FR_{K} \), and that furosemide and bumetanide inhibited \( FR_{Na} \) and caused net potassium secretion.

**DISCUSSION**

A major advantage of microperfusion of loops of Henle *in vivo* is that, unlike *in vitro* studies, the loop segments remain in the renal interstitium of the intact animal, thereby allowing assessment of loop function in the integrated system. Additionally, because all diuretics act luminally, the microperfusion method was particularly suited to the present study, as the concentration of diuretic in the perfusate could be controlled, whereas free-flow micropuncture would require intravenous administration with attendant systemic changes and unknown intraluminal concentrations. Nevertheless, the problem remains that the perfused loop is a heterogeneous structure in which superficial nephrons include the pars recta, the thin descending limb, the TALH and a very small section of distal convoluted tubule. This makes it difficult to attribute any observed functional changes to a specific locus.

In the present study, in order to achieve some functional differentiation, the contribution of the pars recta was minimized by means of a low-sodium perfusate to which mannitol had been added to maintain approximate isotonicity [15,24]. In agreement with the results of Peterson and colleagues [15,24], \( J_{K} \) was greatly reduced with this perfusate, the residual value (~ 1 nl·min⁻¹) being presumed to represent reabsorption in the thin descending limb of superficial nephrons [25]. Moreover, frusemide and bumetanide, inhibitors of the \( Na^+/K^+/2Cl^- \) co-transporter in the TALH, lowered \( J_{Na} \) to values only slightly greater than zero. These results confirm that the contribution of the pars recta segment to overall sodium reabsorption in the loop is greatly reduced, if not completely abolished, when using the low-sodium perfusate. Although the standard and low-sodium perfusates also differed with respect to calcium and gluconate content, we have shown recently that the addition of 1 mM calcium or of 6 mM gluconate to the standard perfusate has no discernible effect on \( J_{Na}, J_{Fr} \) or \( J_{K} \) (R. J. Unwin, S. J. Walter and D. G. Shirley, unpublished work). Thus it is unlikely that these subtle differences in the compositions of the two perfusates will have had an impact on baseline reabsorptive rates.

**Methazolamide**

The carbonic anhydrase inhibitor methazolamide has been shown to reduce bicarbonate reabsorption in perfused loops by ~ 70% [6]. In the present study, when using the standard perfusate (series 1), the same dose of methazolamide caused modest decreases in \( J_{Na}, J_{Fr} \) and \( J_{K} \) (although the last of these was not statistically significant). When the low-sodium perfusate was used (series 2), methazolamide had a reduced effect on \( J_{Na} \) and none on \( J_{Fr} \) or \( J_{K} \). These results suggest that inhibition of carbonic anhydrase has small inhibitory effects on sodium reabsorption in both the pars recta (hence the reduced \( J_{K} \)) and the TALH. When reabsorption in the pars recta was suppressed by means of the low-sodium perfusate, only the effect on the TALH was seen (therefore unaccompanied by a decrease in \( J_{K} \)).

\( H^+ \) secretion in the pars recta and the TALH is believed to be mediated by a combination of \( Na^+/H^+ \) exchange and \( H^+-ATPase \) [17,26–28], with both mechanisms being ultimately dependent on carbonic anhydrase. Since methazolamide can cross cell membranes [29], it should have gained access not only to luminal carbonic anhydrase (type IV) but also to the intracellular isoform (type II). Although type IV carbonic anhydrase is absent from the \( S_2 \) segment of the pars recta, it is present in the \( S_2 \) segment and in the TALH, while type II is present throughout the pars recta and TALH [27,30]. In view of this wide scope for the action of methazolamide, it might seem surprising that such limited effects on sodium reabsorption were seen. However, it should be borne in mind that, in absolute terms, sodium bicarbonate reabsorption in the loop constitutes only a small fraction of total sodium reabsorption, and the methazolamide-induced inhibition of sodium reabsorption that we observed is consistent with the previously reported inhibition of bicarbonate reabsorption [6].

© 2000 The Biochemical Society and the Medical Research Society
Chlorothiazide

Using the standard perfusate, chlorothiazide caused small decreases in \( J_{\text{Na}} \) and \( J_{\text{K}} \). When proximal tubular reabsorption was blocked by means of the low-sodium perfusate, the inhibitory effects of chlorothiazide on \( J_{\text{K}} \) and \( J_{\text{Na}} \) were abolished, while that on \( J_{\text{Na}} \) was attenuated. This suggests that some, but not all, of chlorothiazide’s action in the loop is through an effect on the pars recta. An effect of chlorothiazide on reabsorption in the proximal convoluted tubule, generally attributed to its carbonic anhydrase inhibitory activity, is well documented \([13,14]\). The close similarity between the effects of chlorothiazide and methazolamide in the present study is consistent with the proposal that the effect seen on the pars recta also resulted largely from carbonic anhydrase inhibition. The present findings do not allow a definitive explanation for the residual inhibitory effect of chlorothiazide on \( J_{\text{Na}} \) when using the low-sodium perfusate. Although the effect may have resulted partly from carbonic anhydrase inhibition in the TALH, it should be remembered that the perfused loop includes a very small section of the distal convoluted tubule, the principal thiazide-sensitive segment. (Indeed, close inspection of the data shows that, with each perfusate, the inhibitory effect of chlorothiazide on \( J_{\text{Na}} \) slightly exceeded that of methazolamide, although the difference was not statistically significant.)

Loop diuretics

Addition of frusemide or bumetanide to the standard perfusate caused the expected decrease in \( J_{\text{Na}} \), but also a significant decrease in \( J_{\text{K}} \). Previous studies have also reported an inhibitory effect of loop diuretics on \( J_{\text{K}} \) \([6,7]\). If it is assumed that the TALH is impermeable to water, this suggests that the diuretics inhibit reabsorption at a more proximal site. We tested the hypothesis that the pars recta is sensitive to loop diuretics by using the low-sodium perfusate. In this setting, the inhibitory effects of these agents on \( J_{\text{Na}} \) (when measured in absolute terms) were indeed attenuated, thus providing support for the hypothesis. Alternatively, it could be argued that the lower sodium load delivered to the loop when using the low-sodium perfusate might have resulted in less sodium reaching the TALH, and that the baseline \( J_{\text{Na}} \) in the TALH would therefore be lower; if so, this could account for the lesser effects of loop diuretics when using the low-sodium perfusate. However, estimates of baseline TALH sodium transport in the two situations suggest otherwise. If it is assumed that (i) the difference in baseline \( J_{\text{K}} \) between the two perfusates is the result of fluid reabsorption in the pars recta, which occurred only when using the standard perfusate, (ii) fluid reabsorption in the pars recta is iso-osmotic, and (iii) little or no sodium is reabsorbed in the thin descending limb of superficial nephrons, it can be calculated that sodium reabsorption in the TALH during loop perfusion with the standard perfusate averaged 888 pmol min\(^{-1}\) \([\text{total } J_{\text{Na}} - (\text{pars recta water reabsorption} \times \text{perfusate Na}^+ \text{concentration})]\), compared with a value of 833 pmol min\(^{-1}\) with the low-sodium perfusate. Thus the frusemide-induced reduction in \( J_{\text{Na}} \) when using the standard perfusate \((A J_{\text{Na}} = 1447 \text{ pmol min}^{-1})\) greatly exceeded the calculated TALH baseline \( J_{\text{Na}} \). Support for the view that frusemide affected reabsorption in the pars recta comes from the finding that with the standard perfusate, but not with the low-sodium perfusate, the inhibitory effect of frusemide significantly exceeded that of bumetanide. Since with the low-sodium perfusate both loop diuretics reduced \( J_{\text{Na}} \) to values close to zero, it must be presumed that they had virtually abolished sodium reabsorption in the TALH; the greater effect of frusemide when using the standard perfusate therefore points to frusemide-sensitive reabsorption in the pars recta.

Comparison of the effects of bumetanide on \( J_{\text{Na}} \) with the two perfusates suggests that this diuretic also inhibits sodium reabsorption in the pars recta, although in this case the evidence is less strong \((A J_{\text{Na}} = 1084 \text{ pmol min}^{-1})\) with the standard perfusate, and 763 pmol min\(^{-1}\) with the low-sodium perfusate). The bumetanide-induced reduction in \( J_{\text{Na}} \) with the standard perfusate exceeded the calculated TALH baseline \( J_{\text{Na}} \) by \(\sim 200\) pmol min\(^{-1}\). Thus it appears that, at this dose, the effect of bumetanide on the pars recta is relatively minor. A contributory factor to the greater potency of frusemide in inhibiting pars recta sodium reabsorption may be its greater carbonic anhydrase inhibitory properties \([11,31]\), although this cannot be the complete explanation, given the relatively small effect on \( J_{\text{Na}} \) of the powerful carbonic anhydrase inhibitor methazolamide. Whatever the mechanism(s) involved, the conclusion that a significant fraction of the effect of loop diuretics on overall sodium transport is located in the pars recta strengthens previous suggestions that the natriuretic properties of these agents depend to some extent on reduced proximal reabsorption, coupled with suppression of compensatory sodium reabsorption by the TALH \([16]\).

A striking finding in the present study was that the already low \( J_{\text{K}} \) observed when using the low-sodium perfusate was reduced further when frusemide or bumetanide was present. On the face of it, this suggests that a small fraction of the effect of the diuretics on overall water reabsorption in the loop was located beyond the pars recta. It seems unlikely that water reabsorption in the thin descending limb will have been affected, because this is driven by the (small) osmotic gradient in the outer medullary interstitium, and there is no reason to believe that the intruluminal presence of frusemide or bumetanide in a single loop would disrupt this gradient. Although the TALH is considered to be impermeable to water (a prerequisite for the efficient operation of the ‘single effect’ in the ascending limb), it
remains to be demonstrated in vivo that this impermeability is absolute. Indeed, a recent in vitro study found that the water permeabilities of rat medullary TALH apical and basolateral membranes, although very low, exceeded zero [32]. If there were any water reabsorption in the TALH (however small), blockade of the Na⁺/K⁺/2Cl⁻ co-transporter by loop diuretics should abolish it. A possible alternative explanation for the effect of the loop diuretics on Jₚ during suppression of pars recta reabsorption is reduced water reabsorption in the small segment of distal convoluted tubule that is included in the perfused loop, owing to the raised osmolality of the fluid emerging from the TALH during loop diuretic treatment [33]. However, the extremely low water permeability of the distal convoluted tubule [18,25], together with the very short length of this segment included within the perfused loop, argues strongly against this hypothesis.

The effects of the loop diuretics on potassium transport deserve comment. When using the standard perfusate, bumetanide abolished potassium reabsorption. The effect of frusemide was even more striking, with net potassium secretion being observed. Potassium reabsorption in the TALH is believed to depend partly on the apical Na⁺/K⁺/2Cl⁻ co-transporter and partly on paracellular flux, driven by the lumen-positive transepithelial potential difference [34]. By inhibiting the Na⁺/K⁺/2Cl⁻ co-transporter, loop diuretics abolish both transport mechanisms and thereby potassium reabsorption. At the same time, potassium efflux from cell to lumen may persist through the apical potassium channels in the TALH [35], and/or the direction of paracellular potassium transport may be reversed. Therefore net potassium transport in the loop as a whole during loop diuretic treatment may depend on two opposing fluxes: reabsorption in the pars recta versus secretion in the TALH. This would explain why frusemide caused net potassium secretion even with the standard perfusate, because this diuretic had the greater inhibitory action on reabsorption in the pars recta. When reabsorption in the pars recta was blocked by using the low-sodium perfusate, net potassium secretion was seen with both loop diuretics.

Conclusions
In summary, in the microperfused loop of Henle in vivo, we have provided evidence that (i) methazolamide, a carbonic anhydrase inhibitor, causes slight decreases in sodium reabsorption in the pars recta and TALH; (ii) chlorothiazide inhibits loop sodium reabsorption partly through an effect on the pars recta; (iii) a significant component of the inhibitory effect of frusemide, and possibly of bumetanide, on sodium reabsorption is also located in the pars recta; and (iv) loop diuretics reverse the direction of net potassium transport in the TALH.

ACKNOWLEDGMENTS
We thank the Wellcome Trust for financial support, and J. Skinner and E. J. Folkerd for technical assistance.

REFERENCES


© 2000 The Biochemical Society and the Medical Research Society

Received 3 December 1999; accepted 10 January 2000