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Analysis of the natriuretic action of a loop diuretic, piretanide, in man

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1 The renal responses to a loop diuretic, piretanide, were investigated in a group of fourteen healthy volunteers. The effect of fluid replacement on the drug-response relationship was evaluated in the absence and in the presence of probenecid pretreatment following both oral and intravenous administration of piretanide.

2 Urinary excretion of piretanide was greater when volume losses were replaced than in the absence of volume replacement (i.v. dose: 3.32 ± 0.15 *vs* 2.55 ± 0.23 mg $6\ h^{-1}$, $P < 0.01$; oral dose: 2.57 ± 0.09 *vs* 1.87 ± 0.27 mg $6\ h^{-1}$, $P < 0.01$). With intravenous piretanide urinary excretion of sodium was likewise greater in the fluid replaced group (198 ± 4 *vs* 141 ± 10 mmol $6\ h^{-1}$, $P < 0.01$); these differences caused by fluid replacement did not however occur after oral dosing of piretanide (181 ± 12 *vs* 167 ± 14 mmol $6\ h^{-1}$).

3 Probenecid pretreatment significantly decreased the renal excretion of piretanide in all subjects and consistently decreased the natriuretic response with the exception of intravenous piretanide challenge in subjects not undergoing fluid replacement. In this situation, despite probenecid causing a decrease in the amount of drug excreted (2.55 ± 0.23 *vs* 1.63 ± 0.15 mg $6\ h^{-1}$, $P < 0.05$) the sodium output was unaltered (141 ± 10 *vs* 152 ± 16 mmol $6\ h^{-1}$, NS).

4 Complete replacement of the induced fluid losses resulted in the enhancement of the renal response, without affecting the shape of the diuretic response curve, of either the intravenous or orally administered piretanide. The natriuretic-response curve of intravenous piretanide alone was observed to be displaced to the right of that seen after oral administration. This implies a fundamental difference in pharmacokinetic-pharmacodynamic relationship of piretanide for the two routes of administration. Pretreatment with probenecid eliminated the observed differences in the drug-response curves, between intravenous and orally administered piretanide, such that the curves were now superimposable irrespective of the route of administration of the diuretic.

5 The differences between the drug-response curves of intravenous and orally administered piretanide shows that a substantial portion of drug delivered to the kidney, after intravenous dosing was wasted as it failed to participate in the pharmacological response. Probenecid prevented this 'wastage' and altered the profile of the drug-response relationship of intravenously administered diuretic. This suggests that caution is needed in interpreting the renal pharmacodynamic responses elicited when bolus doses of diuretics have been administered intravenously.

Keywords piretanide natriuretic effect route of administration fluid replacement probenecid interaction

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Introduction

The inter-relationship between the pharmacokinetics and pharmacodynamics of loop diuretics is of considerable interest, especially as these drugs are used extensively in the treatment of many acute and chronic cardiovascular diseases. Of particular importance is the development of acute tolerance, especially following single doses of diuretics administered intravenously (Cook & Smith, 1987; Hammarlund *et al.*, 1985, Noormohamed *et al.*, 1990a). The development of acute tolerance has been attributed to the activation of fluid conserving mechanisms following volume disturbances after diuretic challenge and can be partially or completely prevented by the provision of adequate fluid replacement (Cook & Smith, 1987; Hammarlund-Udenaes & Benet, 1989; Li *et al.*, 1986; Lu *et al.*, 1987; Noormohamed *et al.*, 1990a). There is however some debate as to whether fluid replacement alone, without the accompanying electrolytes, is sufficient to overcome the development of this form of acute tolerance (Li *et al.*, 1986). In addition there is some uncertainty as to the role of the proximal tubule in the development of diuretic tolerance.

One way of exploring proximal tubular influences has been to use an inhibitor of organic acid transport pathway, probenecid. However, even when probenecid has been used, anomalous findings have emerged. Thus for example the effect of frusemide has been found to be enhanced by probenecid pretreatment without any changes in the diuretic excretion (Chennavasin *et al.*, 1979). The reported increase in the sodium excretion without a concurrent increase in drug excretion has been attributed to alterations in the time course of delivery of frusemide following probenecid pretreatment (Kaojarern *et al.*, 1982). Our experience (Dixey *et al.*, 1988) and that of others (Odlind & Beermann, 1980; Odlind *et al.*, 1983) has shown the natriuretic effect of loop diuretics is always blunted in the presence of probenecid. Some of these conflicting observations may be explained by differences in experimental design, variations in experimental techniques, extent of fluid replaced, mode of administration of diuretic and the duration of probenecid priming.

In the present study we have sought to clarify some of these uncertainties that surround the phenomenon by analysing the dynamic aspects of the inter-relationship between the renal handling of a loop diuretic, piretanide, and the course of development of its natriuretic response. The associated systemic and urinary kinetics have been reported separately (Noormohamed *et al.*, 1990a).

Methods

Fourteen healthy male volunteers were recruited into the study after undergoing routine biochemical and haematological screening. All subjects gave informed consent and were counselled by a research dietician in order to stabilize their dietary intake of sodium (approximately 150 mmol day^{-1}). The study has been approved by Riverside Health District Ethics Committee. The subjects were asked to refrain from eating high salt foods, undertaking strenuous exercise, smoking, consuming caffeine or alcoholic beverages/snacks and from having any concurrent medication without prior approval.

Prior to assignment into one of the study groups and before each of the study periods, subjects undertook a 24 h urine collection to assess basal output of sodium. The first group (n = 6) did not undergo fluid replacement after diuretic challenge, but each subject was encouraged to drink 250 ml h^{-1} tap-water; the second group (n = 8) were fully hydrated orally by an initial load of 20 ml kg^{-1} water with subsequent replacement of all urinary and insensible losses, for 6 h post-diuretic dosing. Where possible each subject was studied on a minimum of two occasions, once with piretanide alone, and once with piretanide on the third day of probenecid pretreatment schedule (2 g daily for 3 days). Piretanide challenge was given as 6 mg intravenously or orally with the order of treatment randomized. Urinary volume, sodium and drug excretion were measured in samples collected at regular intervals for 6 h and over 6–24 h post dosing. We have previously demonstrated that neither the process of water loading nor pretreatment with probenecid had any effect on the basal excretion of sodium (Dixey *et al.*, 1988; Noormohamed *et al.*, 1990b).

Non-fluid replaced subjects

All six subjects received piretanide alone (6 mg orally) while four of the six subjects also received oral piretanide on the third day of probenecid pretreatment schedule. Additionally the same six subjects also received intravenous piretanide alone (6 mg) and again in the presence of probenecid pretreatment. Urine samples were collected every 2 h for 6 h post-dose and then over 6–24 h period.

Fluid replaced subjects

In the fluid replaced group of eight subjects, six out of the eight received oral piretanide, both alone and after probenecid pretreatment. Four of the eight subjects also received piretanide intravenously alone and after probenecid pretreatment. Urine samples were collected every 20 min for 6 h post-dose and then over 6–24 h period. All urinary fluid losses were replaced orally with an equivalent volume of water, plus 1 ml min^{-1} to allow for insensible losses. Oral replacement of fluid was generally complete within 5 min of voiding and collection of urine sample. The subjects were allowed to equilibrate for a period of 80 to 120 min, while collecting spontaneously voided urine samples at 20 min intervals and maintaining the maximally hydrated state, before being exposed to the diuretic.

Analyses

Drug analyses were performed by injecting urine samples directly (10 μl) on to a reverse phase (C_{18}) h.p.l.c. column, after addition of an internal standard,

and analysed for piretanide using fluorescence detection (220 nm excitation and 418 cut-off emission) as described previously (Dixey *et al.*, 1988). Urinary sodium analyses were performed using atomic absorption spectrophotometry (Perkin–Elmer model 603, Beaconsfield, U.K.).

All results are expressed as means or mean ± s.e. mean. The slopes and intercept of the linear drug-response curves, for each subject, were calculated using linear regression. Results were analysed using the appropriate non-parametric test for unpaired comparisons (Wilcoxon Rank Test) and paired *t*-tests. Differences were considered significant at $P < 0.05$ level.

Results

Basal output of sodium

There were no significant differences in the basal output of sodium within each of the designated groups prior to diuretic dosing. Results from each subject were therefore pooled to express a mean basal output for each treatment group. The mean basal urinary excretion of sodium in the non-hydrated group was (mean ± s.e. mean; $n = 6$) 47 ± 9 mmol 6 h^{-1} and 153 ± 23 mmol 24 h^{-1} in the absence and 48 ± 10 mmol 6 h^{-1} and 137 ± 16 mmol 24 h^{-1} in the presence of probenecid. During the equilibration phase of maximal hydration (80 to 120 min prior to diuretic dosing), the rate of sodium excretion (mean ± s.e. mean; $n = 8$) was 155 ± 24 μmol min^{-1} (= 56 ± 9 mmol 6 h^{-1}) in the absence and 110 ± 22 μmol min^{-1} (= 47 ± 9 mmol 6 h^{-1}) in the presence of probenecid. The mean basal urinary excretion of sodium, over the day preceding maximal hydration and diuretic exposure was 132 ± 22 mmol 24 h^{-1} in the absence and 109 ± 11 mmol 24 h^{-1} in the presence of probenecid.

Oral piretanide

The cumulative urinary excretion of piretanide following oral dosing was 1.93 ± 0.28 mg 24 h^{-1}, in the absence of any fluid replacement. This recovery rate rose to 2.78 ± 0.15 mg 24 h^{-1} ($P < 0.05$) when all the fluid losses had replaced orally. In the presence of probenecid pretreatment, urinary recovery of piretanide decreased significantly to 1.19 ± 0.08 mg 24 h^{-1} ($P < 0.001$) and 1.40 ± 0.04 mg 24 h^{-1} ($P < 0.01$) respectively when compared with piretanide exposure on its own, but with no significant difference between the two states of fluid replacement. The major portion of the urinary recovery of the drug was complete within the first 6 h post-dosing, regardless of experimental conditions employed (Table 1).

In the non-hydrated group, 24 h urinary excretion of sodium following oral dosing with piretanide was 217 ± 18 mmol which rose to 247 ± 14 mmol (NS) in the volume replaced group. In the presence of probenecid pretreatment, sodium excretion decreased to 172 mmol 24 h^{-1} ($P < 0.05$) and 175 ± 16 mmol 24 h^{-1} ($P < 0.01$) in the two groups respectively. The absolute natriuretic responses, over the first 6 h post-piretanide dosing, are shown in Table 1.

The composite relationship between the cumulative excretion of piretanide and the associated natriuretic responses over the 6 h period, under various experimental conditions is illustrated in Figure 1. After oral dosing the development of the natriuretic response followed a linear course. The magnitude of the final cumulative response depended on whether fluid

Table 1 Urinary excretion of piretanide and sodium in subjects in whom either no fluid losses were replaced or fully replaced, in absence and in presence of probenecid.

Treatment	*Non-fluid replaced* (n = 6)	*Fluid replaced* (n = 6)
	Urinary excretion of piretanide (mg 6 h^{-1})	
Piretanide (oral)	1.87 ± 0.27	2.57 ± 0.09[f]
Piretanide (oral) + probenecid	1.03 ± 0.07[bg]	1.13 ± 0.04[cf]
Piretanide (i.v.)	2.55 ± 0.23[d]	3.32 ± 0.15[dfg]
Piretanide (i.v.) + probenecid	1.63 ± 0.15[ae]	1.80 ± 0.16[beg]
	Urinary excretion of sodium (mmol 6 h^{-1})	
Piretanide (oral)	167 ± 14	181 ± 12[f]
Piretanide (oral) + probenecid	98 ± 16[bg]	98 ± 11[c]
Piretanide (i.v.)	141 ± 10[d]	198 ± 4[fg]
Piretanide (i.v.) + probenecid	152 ± 16	141 ± 10[aeg]

[a] $P < 0.05$ when compared with oral piretanide alone
[b] $P < 0.01$ when compared with oral piretanide alone
[c] $P < 0.001$ when compared with oral piretanide alone
[d] $P < 0.05$ oral piretanide alone *vs* i.v. piretanide alone
[e] $P < 0.001$ oral piretanide *vs* i.v. piretanide in the presence of probenecid
[f] $P < 0.05$ hydrated responses *vs* non-hydrated responses
[g] $n = 4$.

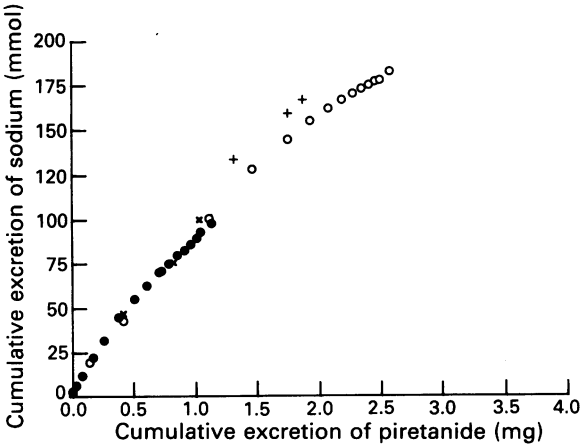

Figure 1 Relationship between the mean cumulative excretion of piretanide and mean cumulative excretion of sodium following single oral doses of piretanide (6 mg) in healthy volunteers. Non-fluid replaced group: piretanide alone (+); after probenecid pretreatment (×). Fluid replaced group: piretanide alone (○); after probenecid pretreatment (●).

replacement was undertaken or not (Figure 1). The greater natriuretic response seen in the fluid replaced group was also associated with a greater total delivery of the diuretic under these conditions (Table 1). The drug-response relationship in the presence of probenecid remained unaltered in the two states of hydration. The equations describing the linear cumulative drug-response curves for orally administered piretanide are shown in Table 2. The slopes (m) and the intercepts (c) of the drug-response curves, after piretanide alone, were similar in the two hydrated states. Addition of probenecid caused a significant increase in the slope [66 ± 5 (n = 12) *vs* 84 ± 6 (n = 10); $P < 0.01$] with the resulting intercept [36 ± 8 vs 9 ± 4; $P < 0.01$] much nearer to the origin.

Intravenous piretanide

Urinary recovery of piretanide was always greater after intravenous dosing than after oral piretanide under all the conditions investigated. The 24 h urinary recovery was 2.78 ± 0.15 mg ($P < 0.05$) when no fluid replacement was undertaken and 3.41 ± 0.16 mg ($P < 0.01$) in the fluid replaced group. Probenecid pretreatment decreased the drug recovery significantly to 1.70 ± 0.15 mg 24 h^{-1} ($P < 0.01$) and 1.94 ± 0.17 mg 24 h^{-1} ($P < 0.01$) in the two groups respectively. Irrespective of the presence of probenecid, fluid replacement was associated with greater urinary recovery of piretanide ($P < 0.05$) with the bulk of the total diuretic confined to the first 6 h post-dosing (Table 1).

Urinary excretion of sodium was 205 ± 8 mmol 24 h^{-1}, in absence of volume replacement, and 270 ± 25 mmol 24 h^{-1} ($P < 0.05$) in the volume replaced group. In the non-volume replaced group, pretreatment with probenecid did not result in any change in the sodium output (205 ± 8 *vs* 231 ± 20 mmol 24 h^{-1}; NS) despite a concomitant 39% decrease in the urinary excretion of piretanide (2.78 ± 0.15 *vs* 1.70 ± 0.15 mg 24 h^{-1}; $P < 0.01$). A significant decrease in sodium output was however observed (270 ± 25 *vs* 215 ± 23 mmol 24 h^{-1} ($P < 0.01$) in the volume replaced group. This was accompanied by a 43% decrease in drug excretion (3.41 ± 0.16 *vs* 1.94 ± 0.17 mg 24 h^{-1}; $P < 0.01$). All these trends were apparent within the first 6 h after dosing (Table 1).

Following probenecid pretreatment, the cumulative drug-response curve with intravenous piretanide (Figure 2) showed a distinct shift towards the left, irrespective of the state of fluid replacement. The resulting slopes of the cumulative drug-response curves, for all subjects, were not significantly different, irrespective of whether probenecid was given or not (89 ± 13 *vs* 72 ± 7; NS). The initial quantity of drug excreted, which was not associated with natriuresis, was determined by extrapolation of the regression curves to zero sodium response and calculated to be 0.47 ± 0.15 mg. This represented 17 ± 6% of the total amount piretanide excreted renally or 8 ± 3% of the administered dose. When the responses after piretanide alone were pooled, the drug-response curves after intravenous dosing remained distinct from those seen after oral dosing irrespective of the state of hydration. Probenecid pretreatment caused the drug-response curve following

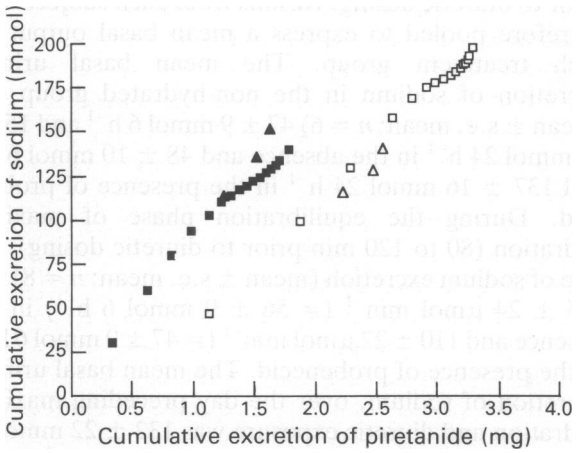

Figure 2 Relationship between the mean cumulative excretion of piretanide and mean cumulative excretion of sodium following single intravenous doses of piretanide (6 mg) in healthy volunteers. Non-fluid replaced group: piretanide alone (△); after probenecid pretreatment (▲). Fluid replaced group: piretanide alone (□); after probenecid pretreatment (■).

Table 2 Regression analysis (mean ± s.e. mean) of the linear drug-response relationship between cumulative urinary excretion of piretanide (x, mg) and cumulative excretion of sodium (y, mmol) in healthy subjects under different experimental states, expressed by a general equation $y = m\,x \pm c$ where m = slope (mmol Na/mg); c = intercept

	Oral piretanide		*Intravenous piretanide*	
Subjects	*Alone*	*With probenecid*	*Alone*	*With probenecid*
	m *c*	*m* *c*	*m* *c*	*m* *c*
Non-fluid replaced (n = 6)	$y = 65\,x + 52$ (± 9) (± 13)	$y = 83\,x + 11$*[a] (± 2) (± 11)	$y = 76\,x - 48$ (± 11) (± 23)	$y = 103\,x - 11$ (± 19) (± 34)
Fluid replaced (n = 6)	$y = 68\,x + 19$ (± 4) (± 2)	$y = 85\,x + 8$*† (± 10) (± 2)	$y = 67\,x - 24$[a] (± 4) (± 10)	$y = 69\,x + 19$††[a] (± 3) (± 6)
All subjects (pooled) (n = 12)	$y = 66\,x + 36$ (± 5) (± 8)	$y = 84\,x + 9$**††[b] (± 6) (± 4)	$y = 72\,x - 32$[b] (± 7) (± 14)	$y = 89\,x + 1$[b] (± 13) (± 21)

[a] n = 4 subjects; [b] n = 10 subjects

* $P < 0.05$; ** $P < 0.01$ when comparing slopes with and without probenecid
† $P < 0.05$; †† $P < 0.01$ when comparing intercepts with and without probenecid.

intravenous piretanide to shift to the left of the original curve obtained in the absence of probenecid (Figure 2), to such an extent that the curve was now superimposable on that obtained after oral administration of piretanide (Figure 3).

Discussion

A number of previous studies have defined the basic inter-relationship between natriuresis and the urinary excretion of diuretics. In order to explain some of the unexpected observations seen by various groups (Branch, 1983; Chennavasin *et al.*, 1979; Dixey *et al.*, 1988; Homeida *et al.*, 1977; Honari *et al.*, 1977), terms such as 'time course of delivery' of drug and the concept of 'maximally efficient dose' have been introduced as important determinants of the overall diuretic response (Kaojarern *et al.*, 1982). Using these terms it was possible to explain why there was a biphasic response, with an initially reduced sodium excretion followed by a subsequent increase; the result was a greater overall natriuretic response to intravenous frusemide, in the presence of probenecid. It was claimed that probenecid caused the frusemide excretion rate to be closer to the 'maximally efficient' rate for a longer period of time (Kaojarern *et al.*, 1982). If this hypothesis were generally applicable then an oral dose of any loop diuretic, would also be expected to be far more effective in the presence of probenecid, as the delivery of the drug to the kidney would be at the supposedly maximally efficient dose for a greater duration. A problem does arise however, in that the observed renal responses to oral and intravenous frusemide may be influenced by the known differences in bioavailability of this compound (Hammarlund-Udenaes & Benet, 1989). Piretanide, on the other hand, is more consistent and completely absorbed resulting in good oral bio-availability approaching 100% in both healthy subjects and cardiac failure patients (McNabb *et al.*, 1988; Noormohamed *et al.*, 1990a). In our present group of subjects the overall bioavailability of piretanide was 110 ± 12% ($n = 8$) in the absence, and 96 ± 7% ($n = 6$) in the presence of probenecid (unpublished observations). Piretanide was therefore a more suitable choice of drug with which to investigate the effect of probenecid on the renal pharmacodynamic response. Other variables that could have influenced the overall natriuretic response, even with this highly bioavailable drug, were route of administration and the state of hydration (Noormohamed *et al.*, 1990a).

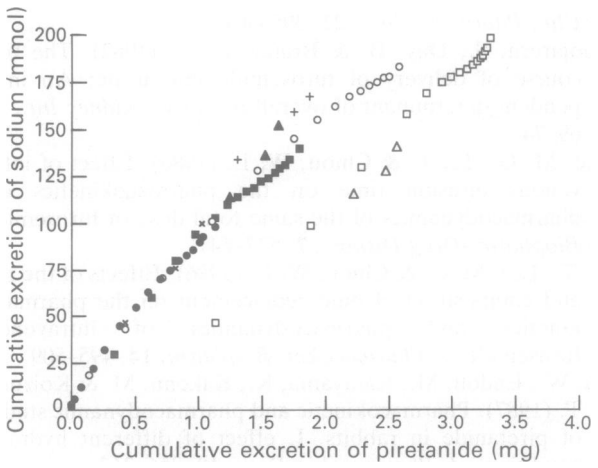

Figure 3 Cumulative drug-response curves obtained under all the various experimental procedures employed in this study. The response profile of intravenously administered piretanide alone (△, □) is clearly very distinct when compared with all other responses (see Figures 1 and 2 for explanation of individual symbols).

In the present study, the magnitude of the natriuresis was shown to be dependent on the state of hydration while the course of the development of the response was dependent on the route of administration of the diuretic. Fluid replacement altered the systemic disposition of piretanide such that a greater amount of the diuretic was delivered to the kidney. The kidney then responded with increased sodium output, over a given period of time, when compared with the fluid deprived state where the corresponding drug delivery was less. The present analysis clearly shows that the development of the natriuretic response follows the same course, irrespective of the state of hydration, for a chosen route of administration of the diuretic. The absence of fluid replacement only diminished the overall response as a result of a corresponding decrease in the delivery of the drug, without altering the basic drug-response relationship. When the diuretic was administered orally, the kidney responded immediately in an appropriate manner that was proportional to the amount of drug excreted. By contrast, the drug-response curve following intravenous piretanide showed a distinct shift to the right suggesting that in this case the kidney was less responsive to piretanide. The kidney only appeared to be refractory to the initial delivery of the drug which, though substantial, failed to produce an appropriate response. The extent of this refractoriness was not dependent on the state of hydration as the two drug-response curves, obtained under these two conditions, were essentially superimposable. The duration of the refractory phase must have been less than 20 min, as the displacement of the curve was practically complete by the first collection period. Any subsequent tubular drug delivery always produced the expected response which was proportional to the amount of drug excreted, as illustrated by the parallel drug-response curves (Figure 3). This initial "wastage", which constituted a significant fraction of the total renally excreted drug, has been noted by other workers but in practice has generally been ignored for the purposes of pharmacodynamic modelling except when calculating the 'efficiency' of the diuretic (Cook & Smith, 1987; Hammarlund *et al.*, 1985; Kaojarern *et al.*, 1982). Handling of data in such a way would inevitably result in the calculated values of efficiency being lower after intravenous dosing when compared with oral dosing. Our present findings show that the pretreatment with probenecid prevented this initial 'wastage' of diuretic characteristic of intravenous dosing of piretanide. This leads to the normalization of the responsiveness of the nephron to the amount of diuretic delivered. We believe this and not the convoluted concept of 'maximally efficient dose' to be the most likely mechanism involved in the apparent increase

in the efficiency of frusemide noted by Brater (1983).

The use of the cumulative drug-response curve allows the calculation of the fraction of dose 'wasted' which, in the case of piretanide, was approximately 8% of the intravenously administered dose or 17% of the renally excreted fraction. Why such a relatively large fraction of drug, supposedly at its site of action, fails to participate in the development of the natriuretic response still remains to be determined. Understanding of this phenomenon could eventually help to explain why the dose-response curve of loop diuretics becomes dissociated, such that increasing intravenous doses of frusemide failed to produce any additional response (Andreasen *et al.*, 1989). The present study does however help to answer some of the previous observations where equivalent amounts of diuretic infused continuously over an extended period of time produces a greater response then a bolus infusion of the same dose (Lee *et al.*, 1986; McInnes *et al.*, 1984).

We have shown that a major factor in the acute development of tolerance to the actions of a loop diuretic is the magnitude of the intralumenal delivery of the diuretic. This delivery of drug was largely determined by whether simultaneous fluid replacement had been undertaken or not. Why the state of fluid balance should affect the disposition of the drug is not known, though part of the answer may be altered renal blood flow, secondary to changes in extracellular fluid volume following diuretic challenge as implied by us previously (Noormohamed *et al.*, 1990a). Whether the development of acute tolerance can be prevented by just fluid or both fluid and electrolyte replacement is still a matter some debate. We have clearly shown that fluid replacement alone is sufficient to prevent the development of acute tolerance. Other workers (Li *et al.*, 1986) have shown that replacement of fluids alone was ineffective whilst replacement of both fluid and electrolyte losses led to a significant increase in the natriuretic response to frusemide.

We conclude that the use of intravenously administered bolus doses of loop diuretics should be avoided when investigating potential pharmacodynamic interactions as there are fundamental differences in the drug-response relationships obtained with orally and intravenously administered diuretics. The observed differences were the result of the refractoriness of the nephron to the high initial delivery rate of the diuretic characteristic of intravenous dosing. Failure to consider this feature may have contributed towards confused interpretation of some of the previously published data on pharmacodynamic interactions of loop diuretics, irrespective of the state fluid balance. It may be that the initial bolus delivery of the drug which is essentially 'wasted' after intravenous dosing, is in a form incapable of interacting at the relevant site in the nephron perhaps because of the presence of increased intrauabular albumin, following diuretic challenge (Ala-Houhala *et al.*, 1987; Pillay *et al.*, 1972). This possibility still remains to be investigated.

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