The effect of carbidopa and indomethacin on the renal response to γ -L-glutamyl-L-dopa in normal man

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1 The renal response to γ -L-glutamyl-L-dopa (gludopa, $25 \mu\text{g kg}^{-1} \text{min}^{-1}$) was investigated in seven normal male volunteers. The effects of oral carbidopa (100 mg) and indomethacin (100 mg) on the response to gludopa were studied in the same group.

2 Gludopa at this dose level produced a 900-fold increase in urine dopamine excretion and caused a natriuresis and suppression of plasma renin activity with only minor effects on pulse rate and blood pressure.

3 Carbidopa inhibited the increase in dopamine excretion by 97% and abolished the renal actions of gludopa.

4 The increase in urine dopamine produced by gludopa was not altered by indomethacin and the urine sodium output was similar to that caused by gludopa alone.

5 Gludopa is an effective renal dopamine prodrug whose activity can be blocked by the dopa decarboxylase inhibitor carbidopa. The results with indomethacin suggest that dopamine and the prostaglandins form separate natriuretic systems in the kidney.

Keywords renin natriuresis prostaglandins dopa decarboxylase

Introduction

The mechanisms of renal salt handling in man are complex. Both the renal dopaminergic (Lee, 1986) and prostaglandin (Lote & Haylor, 1986) systems have been suggested to play a physiological role in natriuresis although the relationship between them has not been defined. γ -L-glutamyl-L-dopa (gludopa) is a relatively renally selective dopamine prodrug which has been shown in man to increase renal blood flow (RBF), glomerular filtration rate (GFR), and salt excretion at a dose of $12.5 \mu\text{g kg}^{-1} \text{min}^{-1}$ without changing pulse or blood pressure (Worth *et al.*, 1985). Diuretic and vasodilator compounds, by their hypotensive actions, may produce compensatory stimulation of the renin-angiotensin and sympathetic nervous systems with a tendency to promote salt retention and offset the desired therapeutic effect (Cannella *et al.*, 1983; Freis *et al.*, 1953). A renally specific drug such as gludopa may thus have potential in the treatment of salt-retaining states and renal insufficiency. It also provides a useful tool to

investigate natriuretic mechanisms without the confounding effects of changes in the cardiovascular system. In the present study the effect of carbidopa, a peripheral dopa decarboxylase inhibitor, on the responses to gludopa, was assessed to further define its mode of action, and indomethacin, a cyclooxygenase inhibitor, was used to study the interactions of gludopa with the prostaglandin and other hormonal systems in the kidney.

Methods

Gludopa was synthesised by UCB Bioproducts (Rue Berkendael 68, 1060 Brussels, Belgium) and the preparation contained 92% pure peptide. It was prepared for use in 50 ml 0.9% sodium chloride with 0.5% human albumin as carrier. The study consisted of two parts involving seven healthy male volunteers aged 19–39 years. Subjects were excluded on the basis of

previous drug reactions, allergies, concomitant illness or drug therapy. All gave informed consent and the study was approved by the Ethics Committee of the University of Edinburgh. In the first part, six subjects were studied on two occasions separated by at least 7 days. All refrained from xanthine-containing drinks from 22.00 h on the previous evening and from alcohol for 24 h. Dietary advice was given to ensure only moderate sodium intake over the previous 48 h and a light breakfast was allowed on the morning of study. At 08.00 h 500 ml of water was taken before subjects arrived at the investigation suite, and 200 ml was then given hourly. At 09.00 h (0h), the bladder was emptied and urine discarded, and intravenous Venflon cannulae inserted into an antecubital vein in each arm. Thereafter subjects remained supine except to pass urine. Each study day consisted of three 2 h periods, during the first (control) and third of which 50 ml physiological saline were infused by Imed 960 Volumetric infusion pump. Gludopa 25 $\mu\text{g kg}^{-1} \text{ min}^{-1}$ in 50 ml saline was infused during the second period starting at 11.00 (2 h). At 10.00 (1 h) either placebo, or carbidopa (100 mg), was given by mouth. Supine blood sampling was followed by voiding of the bladder and collection of urine samples at 1, 2, 2.5, 3, 4, 5 and 6 h. Blood pressure and pulse were measured in duplicate, both supine and erect, at regular intervals, by automatic inflating cuff (Critikon Dinamap). Mean blood pressure was calculated from the systolic and diastolic pressures.

The second part of the study involved six subjects and was identical to the first except that placebo, or indomethacin (100 mg), was taken at 10.00 h (1h). Blood for PRA was taken into pre-cooled EDTA tubes, placed immediately on ice, centrifuged, and the plasma separated and stored at -40°C . PRA was measured by radio-immunoassay of angiotensin I generated under standard conditions (Haber *et al.*, 1969). The intra- and inter-assay coefficients of variation were 4% and 6% respectively. Urine samples were measured and samples aliquoted. Urine sodium and potassium were measured by flame photometry. An aliquot (20 ml) of each urine sample was frozen in a tube containing 5M HCl to prevent oxidation of dopamine. Urine dopamine was then extracted onto alumina and eluted with acetic acid by the method of Anton & Sayre (1964). The recovery from the extraction process was $69.7 \pm 0.5\%$ s.d. Dopamine was measured by high performance liquid chromatography using a Waters 4 μ Novapak column under radial compression. Epinine was added to all samples as an internal standard.

Eluates were injected into the column using a solvent system (pH 5.2) containing citric acid (5.75 g l^{-1}), sodium acetate (6.8 g l^{-1}), sodium hydroxide (2.4 g l^{-1}), acetic acid (1.0 g l^{-1}), disodium EDTA (0.1 g l^{-1}) and 1-octane sulphonic acid (0.1 g l^{-1}) in distilled water. Methanol 10% was added before use. Catecholamine concentration was determined by a Waters M460 electrochemical detector, set at a potential of 0.4 volts. The results were passed to a Shimadzu CR3A computing integrator. All sample runs contained six standard paired concentrations and two quality control samples, and test samples were analysed in duplicate. Intra- and interassay coefficients of variation were 3.3% and 2.1% respectively. The sensitivity of the assay was 5 ng ml^{-1} . Urine kallikrein activity was measured by the method of Amunsden *et al.* (1979), in which the enzyme degrades a chromogenic substrate. Results are in enzymatic units h^{-1} .

Results are expressed as means $\pm$ s.e. mean. Data were analysed by Student's *t*-test for paired observations. Values for PRA were log-transformed before analysis. Results of $P < 0.05$ were accepted as statistically significant.

Results

Effect of carbidopa on responses to gludopa

There were no significant differences in the control results between the 2 days. Table 1 shows the urine dopamine responses to gludopa. Excretion increased on average nearly 900-fold by the second hour of infusion with output continuing at high levels 2 h after the end of the infusion. Over 4 h mean total dopamine excretion was 18.7% of the gludopa administered on a molar basis. However carbidopa markedly attenuated the conversion of gludopa and over the 4 h period the percentage conversion to dopamine was only 0.7% of the dipeptide infused. Sodium output (Figure 1) increased from 0.12 ± 0.02 to $0.34 \pm 0.04 \text{ mmol min}^{-1}$ ($P < 0.01$) during the second hour of gludopa infusion and excretion was still elevated above baseline at the end of the study period. PRA fell sharply after the onset of gludopa from 1.3 ± 0.24 to $0.82 \pm 0.19 \text{ ng AI ml}^{-1} \text{ h}^{-1}$ at 30 min ($P < 0.01$) and levels remained suppressed after the infusion. Gludopa caused a short-lived, non-significant increase in urine kallikrein but levels had returned to baseline by 1 h. Table 2 shows that mean BP tended to fall with gludopa and at 4 h the supine value was significantly lower than that in the presence of carbidopa. In addition

Table 1 Urine dopamine output (nmol h⁻¹) before, during, and after gludopa (25 µg kg⁻¹ min⁻¹) with or without carbidopa (100 mg) or indomethacin (100 mg). (Values are means ± s.e. mean).

	Time (h)					
	1	2	3	4	5	6
Gludopa (n = 6)	79 ±9	61 ±5	29218 ±2567	52804 ±2765	39318 ±3347	15964 ±2760
Gludopa + carbidopa (n = 6)	77 ±14	73 ±5	709 ±109	1457 ±279	1390 ±345	829 ±137
Gludopa (n = 6)	81 ±7	63 ±5	29797 ±2563	53175 ±2894	35771 ±2579	12766 ±883
Gludopa + indomethacin (n = 6)	81 ±8	78 ±8	30443 ±1710	59048 ±4183	35613 ±2861	11663 ±670

Table 2 Systemic responses to gludopa (25 µg kg⁻¹ min⁻¹) with or without carbidopa (100 mg) or indomethacin (100 mg) by mouth. Values are means ± s.e. mean (n = 6)

	Time (h)	Mean BP (mm Hg)	Pulse rate (beats min ⁻¹)		Time (h)	Mean BP (mm Hg)	Pulse rate (beats min ⁻¹)
<i>Erect</i>				<i>Supine</i>			
Gludopa	2	87 ± 5	76 ± 4	Gludopa	2	84 ± 4	76 ± 4
	4	84 ± 4	+*85 ± 4		4	80 ± 3	+*85 ± 5
Gludopa + carbidopa	2	83 ± 4	72 ± 3	Gludopa +	2	88 ± 3	74 ± 4
	4	84 ± 4	74 ± 2	indomethacin	4	84 ± 2	70 ± 3
Gludopa	2	87 ± 5	67 ± 3	Gludopa	2	85 ± 3	67 ± 3
	4	+81 ± 4	67 ± 3		4	80 ± 3	67 ± 3
Gludopa + carbidopa	2	89 ± 4	64 ± 2	Gludopa +	2	91 ± 2	62 ± 4
	4	91 ± 3	62 ± 2	indomethacin	4	89 ± 3	59 ± 3

* $P < 0.05$ compared with 2 h control period; + $P < 0.05$ for comparisons between gludopa alone, and gludopa with carbidopa or indomethacin).

there was a small significant increase in mean erect pulse rate at 4 h.

By contrast in the presence of carbidopa the effects of gludopa were abolished. Sodium excretion and kallikrein output did not increase above baseline, and a small fall in PRA occurred that was not significant. There were no significant changes in pulse and mean BP, compared with the control period.

Effect of indomethacin on responses to gludopa

As in the first study gludopa significantly increased sodium excretion and lowered plasma renin activity (Figure 2). Indomethacin did not alter the conversion of gludopa to dopamine (Table 1) and the total output in the 4 h after gludopa was similar to that following gludopa alone. The natriuresis was not significantly altered by prior administration of indomethacin and the total sodium outputs were similar

(61.2 ± 13.0 vs 76.4 ± 13.6 mmol, NS). PRA sharply declined after indomethacin, a known effect, and levels continued to fall during, and after, gludopa. In the presence of indomethacin, urine kallikrein output did not change throughout the study. Mean BP tended to be higher 1 h after indomethacin and remained so throughout the study although the increase was not significant. The mean pulse rate did not increase after gludopa with indomethacin but showed a small decline both lying and standing.

Discussion

Our results confirm that gludopa is a highly efficient source for urinary, and presumably renal tissue, dopamine. At a dose of 25 µg kg⁻¹ min⁻¹ it produced a 900-fold increase in urine dopamine levels with output continuing at a high level 2 h after the end of the infusion. Previous

Figure 1 Responses of urine sodium, urine kallikrein and plasma renin activity to gludopa ($25 \mu\text{g kg}^{-1} \text{min}^{-1}$) with (●) or without (○) carbidopa (100 mg) by mouth. Values are means $\pm$ s.e. mean. ($n = 6$, ** $P < 0.01$ compared with 2 h control value).

Figure 2 Responses of urine sodium, urine kallikrein and plasma renin activity to gludopa ($25 \mu\text{g kg}^{-1} \text{min}^{-1}$) with (●) or without (○) indomethacin (100 mg) by mouth. Values are means $\pm$ s.e. mean. ($n = 6$, * $P < 0.05$, ** $P < 0.01$ compared with 2 h control value).

work by our group has shown that plasma dopamine levels increase only 4 and 25-fold with doses of gludopa at 12.5 and 100 $\mu\text{g kg}^{-1} \text{min}^{-1}$ respectively (Worth *et al.*, 1985) demonstrating the marked preferential renal accumulation of dopamine. The basis of the renal specificity lies in the sequential transformation of the dipeptide to dopamine under the actions of γ -glutamyl-transferase (EC 2.3.2.2) and dopa decarboxylase (L-amino-acid decarboxylase; EC 4.1.1.26) both of which are highly concentrated in the proximal tubular cells of the kidney (Albert *et al.*, 1961; Goldstein *et al.*, 1972). There is a great deal of evidence, that under physiological conditions, renal dopamine is largely synthesised extra-neuronally in the proximal tubule from circulating L-dopa and that the suggested dopaminergic nerves make little significant contribution to urine dopamine levels (Baines & Chan, 1980; Brown & Allison, 1981; Lee, 1982, 1986).

Gludopa was natriuretic with sodium excretion more than doubling and the response persisting beyond the infusion period, confirming our previous work in man. Dopamine produces renal vasodilatation by means of an effect on specific dopamine receptors (Goldberg *et al.*, 1984), and the increased GFR exerts a natriuretic action. In addition dopamine receptors have been identified on proximal tubular cells (Felder *et al.*, 1984) and, *in vitro*, dopamine exerts a tubular effect in inhibiting proximal sodium reabsorption (Bello-Reus *et al.*, 1982). It is of note that PRA was acutely inhibited by gludopa in agreement with previous work (Worth *et al.*, 1985). This finding is of potential therapeutic importance as an increase in renin release is the usual compensatory response to natriuretic agents, such as benzothiadiazine and loop diuretics. The effect of gludopa on renin release may reflect either a response to the increased tubular sodium load at the macula densa or may be a direct inhibitory action at the juxtaglomerular cells but this question has not yet been resolved. The small and short-lived increase in kallikrein excretion after gludopa does not support the hypothesis of Mills (1982) who suggested the concept of a natriuretic cascade with dopamine leading kallikrein. From our results any interaction between dopamine and kallikrein does not appear to be important.

Gludopa at a dose of 25 $\mu\text{g kg}^{-1} \text{min}^{-1}$ exerted a small but significant systemic effect on pulse and blood pressure. Worth *et al.* (1985) have previously reported that an infusion rate of 12.5 $\mu\text{g kg}^{-1} \text{min}^{-1}$ was without effect on BP and pulse but at 100 $\mu\text{g kg}^{-1} \text{min}^{-1}$ there was a definite reduction in diastolic pressure. The optimum dose, which balances effective natriuresis and

renin-suppression without producing marked systemic effects, is important to define if the drug is to have therapeutic application. The present dose of 25 $\mu\text{g kg}^{-1} \text{min}^{-1}$ may be reaching the upper limit of the renally-selective range. The fall in blood pressure and rise in pulse rate is likely to be the result of a reduction in peripheral resistance. This may be due to renal vasodilatation *per se*, as 20% of the cardiac output goes to the kidneys, or there may be effects on dopamine receptors in the systemic vasculature (Lokhandwala & Barrett, 1982) from the modest increase in plasma dopamine levels.

The effectiveness of carbidopa in blocking dopa decarboxylase was demonstrated by the reduction in urine dopamine output in response to gludopa by on average 97%. In the presence of carbidopa, gludopa had no effects on sodium excretion, renin and kallikrein responses or on the systemic parameters, pulse and blood pressure. The results also suggest that gludopa itself is inactive pharmacologically, and that it requires conversion to dopamine for renal activity. In this context we have recently shown that the dopamine receptor antagonist (+)-sulpiride attenuates the renal hemodynamic, natriuretic and renin-suppressive effects of gludopa (MacDonald *et al.*, 1987), demonstrating that formed catecholamine acts on specific dopamine receptors.

Prostaglandins are synthesised at various sites in the kidney including vascular endothelial cells, medullary interstitial cells and collecting tubules but there is little evidence for synthesis in the proximal tubules (Smith & Bell, 1978). On infusion arachidonic acid and prostaglandins are vasodilator and natriuretic (Lote & Haylor, 1986), effects similar to those of dopamine. In addition prostaglandins mediate, at least in part, the vasodilator and natriuretic actions of loop diuretics (Mackay *et al.*, 1984). It was of interest therefore to determine whether dopamine and the prostaglandins interact in the human kidney and whether the effects of dopamine could be mediated at least in part by the prostaglandins. There have been relatively few studies addressing these questions. In the dog and rat the renal vasodilator action of dopamine was not antagonised by indomethacin (Dressler *et al.*, 1975; Robertson *et al.*, 1980). In addition in healthy women, urine dopamine excretion was not affected by 7 days administration of indomethacin (Gullner *et al.*, 1982). In the present study a dose of indomethacin, 100 mg, was chosen which inhibits urinary prostaglandins by about 80% (Mackay *et al.*, 1984). Dopamine synthesis after gludopa was not affected by indomethacin, excluding an interaction at

enzyme level. The results presented indicate that gludopa remains actively natriuretic in the presence of indomethacin and the response was not significantly different from that of gludopa alone. There is evidence of an important role for the prostaglandins in distal tubular events both in salt handling, and in the interaction with the antidiuretic hormone arginine vasopressin. It is recognised that the prostaglandin system assumes greater importance in renal haemodynamics and sodium handling under conditions of salt depletion or reduced perfusion pressure (Lote & Haylor, 1986). In the salt replete subjects of this study, indomethacin did not alter baseline sodium excretion. It is conceivable that under certain circumstances, an effect of prostaglandin inhibition on baseline and gludopa stimulated sodium excretion could be demonstrated, due to opposing but independent effects on the tubule. However, it seems clear that the prostaglandins are not prerequisite for the natriuretic potential of gludopa, and a physiological interaction of any importance appears unlikely.

Indomethacin caused a marked decline in PRA which is in keeping with studies demon-

strating the prostaglandin dependence of renin secretion and its inhibition by cyclooxygenase inhibitors (Rumpf *et al.*, 1975). A stimulatory effect of prostaglandins on urine kallikrein has been demonstrated previously (Mills & Obika, 1977). However in the present study indomethacin did not alter control kallikrein values and after gludopa there was no change from baseline.

In summary intravenous gludopa has marked effects on normal renal function whilst exerting little systemic influence. A prerequisite for its activity is its selective conversion to dopamine in the kidney under the action of dopa decarboxylase, a process which can be effectively inhibited by carbidopa. The dopa/dopamine conversion forms a separate natriuretic system which seems to be independent of the prostaglandins.

Our thanks are due to J. Brown and N. Johnston for technical assistance. Carbidopa was generously donated by Merck Sharp and Dohme Ltd, Hoddesdon. R.F.J. is a Wellcome Research Fellow and T.M.M. is a Wellcome Lecturer.

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(Received 1 May 1987,
accepted 24 September 1987)