(+)-sulpiride antagonises the renal effects of γ -L-glutamyl-L-dopa in man

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- 1 γ -L-glutamyl-L-dopa (gludopa) was given by intravenous infusion to six healthy salt-replete men on two occasions, with and without pretreatment with (+)-sulpiride.
- 2 Gludopa increased sodium excretion, glomerular filtration rate and effective renal plasma flow whilst decreasing plasma renin activity.
- 3 (+)-sulpiride had no significant effect on baseline natriuresis, renal haemodynamics or plasma renin activity, but significantly attenuated the rise in sodium excretion, glomerular filtration rate and effective renal plasma flow produced by gludopa.
- 4 (+)-sulpiride abolished the acute fall in plasma renin activity seen with gludopa.
- 5 (+)-sulpiride raised serum prolactin concentration but did not affect the rise in urine dopamine excretion rate caused by gludopa.
- 6 Gludopa exerts its renal effects by stimulating specific dopamine receptors which are principally of the DA₁ subtype.

Keywords γ -L-glutamyl-L-dopa (gludopa) (+)-sulpiride kidney renin natriuresis

Introduction

Worth *et al.* (1985) have shown that γ -L-glutamyl-L-dopa (gludopa) is natriuretic, increases renal plasma flow (RPF), and glomerular filtration rate (GFR) and lowers plasma renin activity (PRA) when given intravenously to normal man at a dose of $12.5 \mu\text{g kg}^{-1} \text{min}^{-1}$. At this dose, it has relative renal specificity with no detectable cardiovascular effects. Wilk *et al.* (1978) have shown that gludopa is converted to dopamine (DA) in the kidney of the rat. It is likely therefore that the effects of gludopa are produced by stimulation of specific dopamine receptors within the kidney, but this has never been proven. Supporting this concept, we have shown recently (Jeffrey *et al.*, 1987) that glutytrosine, which is structurally similar to gludopa, does not alter sodium excretion and does not raise urinary dopamine excretion.

According to the classification of Goldberg & Kohli (1981), peripheral dopamine receptors are either of DA₁ or DA₂ subtypes. In the dog, the dopamine mediated increases in natriuresis and RPF are thought to be due to DA₁ receptor stimulation (Frederickson *et al.*, 1985) as they are blocked by SCH23390 a very specific DA₁-receptor antagonist. Evidence also exists suggesting that PRA is under direct dopaminergic control (Williams *et al.*, 1983). Worth *et al.* (1986) have shown that domperidone, a specific DA₂-receptor antagonist, did not attenuate the changes in renal haemodynamics or sodium excretion mediated by gludopa but domperidone did attenuate the gludopa induced fall in PRA, suggesting that gludopa, and therefore dopamine generated intrarenally, inhibits renin release by stimulating DA₂-receptors.

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We postulated that in man gludopa exerts its renal haemodynamic effects by stimulating dopamine receptors of the DA₁ sub-type. The most specific antagonist of renal DA₁ receptors available for use in man is d or (+)-sulpiride (Goldberg *et al.*, 1979; Goldberg & Kohli, 1981; Glock *et al.*, 1981; Kohli *et al.*, 1982; Shepperson *et al.*, 1982; Bass & Robie, 1984). The purpose of this study was to examine the effects of this compound on the natriuretic, renal haemodynamic and PRA responses caused by gludopa given to normal man.

A preliminary communication of our results was given to the British Pharmacological Society Meeting, London, December 1986 (MacDonald *et al.*, 1987).

Methods

Six healthy male volunteers, mean age 28 (range 23–35) years were studied on two occasions separated by at least 7 days. They ate an unrestricted diet but abstained from alcohol for 36 h prior to each study day and fasted from 22.00 h the previous evening. At 08.00 h (–60 min) they took a 200 mg capsule of (+)-sulpiride or a matched placebo in a single-blind balanced randomised fashion, along with 500 ml of tap water. Cannulae were placed in each antecubital fossae and at time zero bolus intravenous injections of polyfructosan (Inutest, Laevosan-Gesellschaft, Linz/Donau) 2.5 g in 30 ml followed by a continuous infusion of 19.5 mg min⁻¹ and *p*-aminohippurate (Merck Sharp and Dohme) 0.5 g in 2.5 ml followed by a constant infusion of 19.8 mg min⁻¹ were given. At the same time, (+)-sulpiride 1.5 mg kg⁻¹ (or placebo) corresponding to the previous oral dose, was given intravenously over 5 min followed by an infusion of 26 µg kg⁻¹ min⁻¹ for 55 min or placebo (the same volume of dextrose). At time 180 min, gludopa 25 µg kg⁻¹ min⁻¹ was given until time 300 min on both occasions. Blood samples were drawn at time 0, 60, 90, 120, 150, 180, 195, 210, 240, 270 and 300 min. From time 0, 200 ml tap water was taken by mouth every 30 min. Subjects emptied their bladders at time 60 min and thereafter urine was collected half hourly. Subjects remained supine throughout the study except when rising to pass urine. Pulse, systolic and diastolic blood pressure were measured every 15 min from time 0 using a Dinamap recorder (Critikon Ltd).

(+)-sulpiride was dissolved in 100 ml pyrogen free water containing 500 mg NaCl and the pH adjusted with 2 M sulphuric acid until dissolution was complete. It was sterilised by filtration, packed in ampoules and stored at –40° C.

Gludopa was synthesised by UCB Bioproducts (Rue Berkendael 68, 1060 Brussels, Belgium) and shown to contain 92% peptide by thin layer and high performance liquid chromatography, the balance being acetic acid carried over from freeze drying. It was dissolved in 0.9% sodium chloride solution with 0.5% human albumin as a carrier and made up, sterile and pyrogen free, in ampoules and stored at –40° C.

All subjects gave informed consent and the protocol was approved by the Ethics of Medical Research Sub-Committee for Medicine and Oncology.

Sample collection and analysis

Blood samples for measurement of plasma renin activity (PRA) were collected into tubes containing heparin and kept on ice. After centrifugation at 2000 *g* at 4° C for 10 min, the plasma was separated and stored at –40° C. Portions were obtained from the measured urine collections and stored at –40° C. Urine samples for dopamine assay were acidified with 5 M HCl to prevent oxidation.

Plasma and urine sodium and potassium were measured by flame photometry. *p*-aminohippurate and polyfructosan were measured by autoanalyser methods (Harvey & Brothers, 1962; Dawborn, 1965). PRA was measured by radioimmunoassay of angiotensin I generated under standard conditions (Haber *et al.*, 1969). The intra- and inter-assay coefficients of variation were 4% and 6% respectively. Serum sulpiride levels were measured by E. R. Squibb and Sons Ltd using the method of Nicolas *et al.* (1986).

Urine dopamine was extracted onto alumina and eluted with acetic acid by the method of Anton & Sayre (1964). The recovery from the extraction process was 69.7 ± 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 M 460 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 inter-assay

coefficients of variation were 3.3% and 2.1% respectively. The sensitivity of the assay was 5 ng ml^{-1} .

Serum prolactin was assayed by a method based on Huang *et al.* (1973) modified for use on the Kemtek 3300 automated radioimmunoassay system. It is a liquid-phase double antibody assay standardised against human pituitary prolactin preparation 84/500 (National Institute for Biological Standards and Controls, London). Intra-assay coefficient of variation was 10%, interassay 12%. Plasma aldosterone concentrations (PA) were determined by radioimmunoassay (Fraser *et al.*, 1973). Urine kallikrein activity (UKA) was measured by the method of Amundsen *et al.* (1979) in which the enzyme degrades a chromogenic substrate.

Calculation of results

Effective renal plasma flow (ERPF) was calculated as the renal clearance of PAH and glomerular filtration rate (GFR) was calculated as the renal clearance of polyfructosan. Filtration fraction was calculated as GFR divided by ERPF.

Mean arterial blood pressure (MAP) was calculated as diastolic blood pressure plus one third of pulse pressure. Results have been expressed as mean $\pm$ s.e. mean, $n = 6$ unless otherwise stated. The results of the individual collection periods before gludopa administration were cumulated to a single mean (baseline value) for each parameter measured. The individual collections after gludopa were similarly cumulated to a mean (mean gludopa value). Data were log transformed and statistical comparisons within and between groups were made using the Student's *t*-test, the null hypothesis being rejected when $P < 0.05$. When a significant difference was found between groups by this method, further analysis of log transformed data at each individual time point was performed.

Where baseline values were not closely matched between study days, then the percentage change in a parameter from mean baseline value was calculated for each time point after gludopa administration. When this was done, statistical analysis between groups was made using the signed-rank test (Wilcoxon, 1945).

Results

Response to gludopa with placebo pretreatment

Gludopa produced significant increases from baseline in sodium excretion (Figure 1) GFR and ERPF, but caused a fall in PRA (shown in Figures 1 and 2, and Tables 1 and 2). These

effects clearly increased with time being maximal in the last 30 min of administration. Gludopa caused a marked increase in urine free dopamine excretion (Figure 4), but had no effect on PA, UKA, MAP (Tables 1 and 2) or prolactin (Figure 5). Gludopa caused a small but significant rise in mean pulse rate (Table 1).

Response to gludopa with (+)-sulpiride pretreatment

In the presence of (+)-sulpiride pretreatment, gludopa was still natriuretic (Figure 1), but failed to increase ERPF and GFR, indeed GFR tended to fall after gludopa (Figure 2 and Table 1). In the presence of (+)-sulpiride gludopa produced little change in PRA acutely, but PRA did tend to fall towards the end of the infusion (Figure 2 and Table 2). Urine free dopamine excretion rose significantly (Figure 4) but there were no significant effects on PA, UKA or MAP (Tables 1 and 2). Serum prolactin concentrations were elevated with (+)-sulpiride pretreatment tending to fall with time. Gludopa administration did not seem to modify the rate of fall in prolactin concentration (Figure 5). Figure 6 shows the mean (+)-sulpiride concentrations achieved in this experiment. Gludopa caused a small but significant fall in mean pulse rate in the presence of (+)-sulpiride (Table 1).

Comparison of response to gludopa between pretreatment with placebo and (+)-sulpiride

Baseline sodium excretion tended to be lower with (+)-sulpiride pretreatment despite the tendency for both GFR and ERPF to be higher, the latter almost reaching statistical significance. Baseline PRA also tended to be higher on the (+)-sulpiride day, but this also failed to reach statistical significance. Despite these differences in baseline there were still significant differences between placebo and (+)-sulpiride days for mean natriuresis, GFR and PRA during gludopa infusion, but no significant difference in ERPF (Tables 1 and 2). To clarify the changes in these parameters GFR, ERPF and PRA were calculated as mean percentage change from baseline values and are shown in Figure 3. It is clear from this that (+)-sulpiride does significantly attenuate the effects of gludopa on renal haemodynamics. (+)-sulpiride also inhibits the acute fall in PRA caused by gludopa but this effect is less marked towards the end of the infusion.

There were no significant differences in urine free dopamine excretion, PA, UKA, MAP or PR between placebo and (+)-sulpiride days.

Figure 1 The effect of gludopa on sodium excretion rate with placebo pretreatment (○) and (+)-sulpiride pretreatment (△). * $P < 0.03$ when compared with (+)-sulpiride.

There were no adverse effects observed (or reported by the volunteers) with (+)-sulpiride treatment.

Discussion

Our results confirm the observations of Worth *et al.* (1985) who showed that gludopa increased sodium excretion, ERPF and GFR whilst causing a fall in FF and PRA. We found a small but significant rise in supine mean pulse rate with gludopa, $25 \mu\text{g kg}^{-1} \text{min}^{-1}$ which was probably due to a reflex response to vasodilation. This effect was abolished in the presence of (+)-sulpiride.

(+)-sulpiride has been reported to exert cardiovascular effects in the guinea pig (Horn *et al.*, 1982) and man (Agnoli *et al.*, 1984; Corvol *et al.*, 1973). Racemic sulpiride is a weak α_2 -adrenoceptor and 5-HT-receptor antagonist

(Kohli & Cripe, 1979; Spedding & Berg, 1981). However, we did not detect any significant difference in MAP or pulse rate between placebo and (+)-sulpiride pre-treated subjects with the dose and routes employed in this study.

The most specific DA_1 -receptor antagonist so far discovered is SCH23390 (Goldberg *et al.*, 1984). However, as this is not available for use in man, (+)-sulpiride represents the most specific DA_1 -receptor antagonist that can safely be administered to human subjects. It has been reported to be 30 times (Glock *et al.*, 1981), 130 times (Bass & Robie, 1984), and 100 times (Brown & O'Connor, 1980) less potent than the (-)-isomer on DA_2 receptors.

Nevertheless, (+)-sulpiride does elevate prolactin levels in rats and man (Scapagnini *et al.*, 1979), although to a lesser extent than (-)-sulpiride. As little as 4 mg (+)-sulpiride given intravenously has been shown to cause a prompt rise in prolactin in normal male volunteers

Figure 2 The effect of gludopa on GFR, ERPF and PRA with placebo pretreatment (●) and (+)-sulpiride pretreatment (▲).

Table 1 Haemodynamic and natriuretic effects of gludopa with placebo or (+)-sulpiride treatment

	<i>Placebo</i>	<i>(+)-sulpiride</i>	<i>Placebo vs (+)-sulpiride</i>
MAP (mm Hg)			
mean baseline	92.3 ± 4.0	91.9 ± 2.6	NS
mean gludopa	91.8 ± 3.6	90.1 ± 2.9	NS
PR (beats min ⁻¹)			
mean baseline	64.2 ± 2.4	68.7 ± 2.8	NS
mean gludopa	66.6 ± 2.2*	64.8 ± 1.8*	NS
ERPF (ml min ⁻¹)			
mean baseline	588 ± 69	650 ± 89	NS
mean gludopa	738 ± 49*	672 ± 69	NS
GFR (ml min ⁻¹)			
mean baseline	95.6 ± 5.5	104.8 ± 5.0	NS
mean gludopa	112.5 ± 2.8*	100.4 ± 3.9	<i>P</i> < 0.05
FF			
mean baseline	0.171 ± 0.015	0.167 ± 0.014	NS
mean gludopa	0.156 ± 0.011*	0.156 ± 0.015*	NS
UNa V (mmol min ⁻¹)			
mean baseline	0.19 ± 0.03	0.17 ± 0.02	NS
mean gludopa	0.52 ± 0.09**	0.28 ± 0.04*	<i>P</i> < 0.02

Significant difference when compared with mean baseline **P* < 0.05, ***P* < 0.005

Table 2 Hormonal effects of gludopa with placebo or (+)-sulpiride treatment

	<i>Placebo</i>	<i>(+)-sulpiride</i>	<i>Placebo vs (+)-sulpiride</i>
PRA (ng AI ml ⁻¹ h ⁻¹)			
mean baseline	1.25 ± 0.24	1.58 ± 0.15	NS
mean gludopa	0.78 ± 0.23**	1.46 ± 0.19	<i>P</i> < 0.01
PA (mg 100 ml ⁻¹)			
mean baseline	4.2 ± 1.4	5.8 ± 1.5	NS
mean gludopa	4.9 ± 2.4	5.0 ± 1.4	NS
UKA (Eu min ⁻¹ × 10 ⁻³)			
mean baseline	12.4 ± 3.3	10.9 ± 2.0	NS
mean gludopa	9.9 ± 2.5	9.9 ± 2.5	NS

Significant difference when compared with mean baseline ***P* < 0.005

Figure 3 Mean percentage change from mean baseline values in GFR, ERPF and PRA following gludopa administration with placebo (●) or (+)-sulpiride (▲) pretreatment. * $P < 0.05$ when compared with (+)-sulpiride.

Figure 4 The effect of gludopa on mean urine dopamine excretion rate with placebo treatment (□) or (+)-sulpiride treatment (▨).

Figure 5 Serum prolactin concentrations with treatment with placebo (○) or (+)-sulpiride (Δ).

Figure 6 Mean serum (+)-sulpiride concentration with time.

(Massara & Camanni, 1979). Whether this effect is due to DA_2 -receptor antagonism in the pituitary or higher centres (MacLeod & Robyn, 1977) or a direct non specific effect on the pituitary (Debeljuk *et al.*, 1974) is unclear. It is likely that in the dose we used there was some residual D_2 and DA_2 -receptor antagonist activity present although this is difficult to measure accurately.

Metoclopramide, another benzamide possessing DA_1 and DA_2 -receptor antagonist properties has been shown to cause significant decreases in renal plasma flow when given in high dose to patients prior to cytotoxic therapy (Israel *et al.*, 1986). However in this study, there was no suggestion of a fall in ERPF following (+)-sulpiride in the dose used.

This study provides clear evidence that (+)-sulpiride antagonises the renal and hormonal effects of gludopa and provides strong support that the dipeptide acts by being converted to dopamine stimulating renal dopamine receptors.

It is likely that the receptors responsible for the natriuretic effect of gludopa are of the DA₁ subtype as Worth *et al.* (1986) have previously shown no attenuation of this by the relatively specific DA₂-receptor antagonist domperidone. As the dose of gludopa used by Worth and colleagues (1986) failed to increase ERPF or GFR significantly, it is not possible to rule out a DA₂-receptor modulation of renal haemodynamics with certainty.

In this study the effects of gludopa increased with the duration of infusion. This is probably due to increasing intrarenal conversion of gludopa to dopamine with time as is shown by the urine dopamine excretion. In the presence of (+)-sulpiride, gludopa still produced a significant increase in sodium excretion despite a fall in filtration fraction. This may be due to high intrarenal concentrations of dopamine overcoming the DA₁-receptor antagonism in a competitive manner with time. This may occur preferentially at the proximal tubular receptors (Felder *et al.*, 1984) close to the site at which dopamine is thought to be generated (Hagege & Richet, 1985) and secreted (Baines & Chan, 1980). Serum sulpiride concentrations were falling throughout the gludopa infusion and may have contributed to an increasing effect of gludopa.

The acute fall in PRA seen with gludopa treated volunteers was abolished by (+)-sulpiride. This is of interest as Worth *et al.* (1986) found that domperidone clearly antagonised this effect of gludopa. It is possible that renin release is under both DA₁ and DA₂ control, or that the DA₂-receptor antagonist effect of (+)-sulpiride was sufficient to antagonise the fall in PRA. PRA fell in both groups towards the end of the infusion. Diurnal variation makes these results difficult to

interpret in the absence of a placebo control for the gludopa infusion.

It is unlikely that (+)-sulpiride affected the conversion of gludopa to dopamine as urine dopamine excretion rates remained unchanged. The definitive elucidation of these mechanisms in man must await the development of more specific DA₁-receptor antagonists.

We have recently shown that effects of gludopa are abolished by prior treatment with carbidopa (Jeffrey *et al.*, 1988) which prevents conversion of gludopa to dopamine. Glutyrasine, which is structurally similar to gludopa does not increase urine dopamine and is not natriuretic (Jeffrey *et al.*, 1987) providing evidence against a non specific effect of gludopa. In conclusion, we have shown that effects of gludopa are antagonised by a relatively specific DA₁-receptor antagonist (+)-sulpiride. We believe that gludopa is a useful investigational agent of potential therapeutic value which has little cardiovascular effect. It acts by being converted to dopamine intrarenally stimulating specific renal dopamine receptors which are principally of the DA₁ subtype.

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