Regional haemodynamic effects of dopamine and its prodrugs L-dopa and gludopa in the rat and in the glycerol-treated rat as a model for acute renal failure

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1 In this study the renal selectivity of dopamine and its prodrugs L-dopa and gludopa, with respect to their effects on regional blood flow, vascular resistance and central haemodynamics was investigated in normal rats and in rats with glycerol-induced acute renal failure (ARF).

2 In normal, anaesthetized rats, dopamine as well as its prodrugs caused a dose-dependent reduction of vascular resistance in the kidney (RR), mesentry (MR) and hindquarters (HQR) (dose range: dopamine: 0.1–5 μmol kg\(^{-1}\) h\(^{-1}\); L-dopa and gludopa: 1–200 μmol kg\(^{-1}\) h\(^{-1}\)). Blood pressure and heart rate were affected at the highest dose only.

3 Administration of dopamine induced a preferential renal vasoconstriction; renal blood flow (−60%) and vascular resistance (+190%) were significantly more affected than MR (+40%) and HQR (+60%). This was only ameliorated by a low rate (10 μmol kg\(^{-1}\) h\(^{-1}\)) infusion of gludopa: the glycerol-induced reduction of renal flow and increase in RR were significantly attenuated. A high dose of gludopa (100 μmol kg\(^{-1}\) h\(^{-1}\)) or any dose of L-dopa or dopamine did not induce this beneficial effect. The glycerol-induced increase in MR and HQR was not attenuated by any of the treatments used.

4 The results indicate that gludopa is not renally selective at a pharmacodynamic level in normal, anaesthetized rats. Contrary to this, a low dose of gludopa does cause a renal selective vasodilatation and reduction of RR in rats with glycerol-induced ARF. This difference could be explained by a difference in renal vascular tone between normal rats and glycerol-induced ARF rats. A high dose of gludopa does not cause these renal-selective effects: renal resistance and renal flow are at the same level as following glycerol and saline. This is probably due to the systemic effects of the released dopamine.

Keywords: Dopamine; gludopa; glycerol; L-dopa; haemodynamics; renal failure; prodrug

Introduction

Gludopa is a prodrug of dopamine (Wilk et al., 1978). It is converted by γ-glutamyl transpeptidase to L-dopa and subsequently by aromatic aminoacid decarboxylase to dopamine. This metabolism occurs predominantly in the kidney (Barthelmebs et al., 1990; Boateng et al., 1990; Cummings et al., 1990). The effects of gludopa on the kidney have been described by several investigators in normal rats (Wilk et al., 1978; 1979), rats with decreased renal function (Casson et al., 1982; 1983; Boateng et al., 1990; Barthelmebs et al., 1991), rabbit (Wang, Z-Q. et al., 1992) and man (Jeffrey et al., 1988a; Casagrande et al., 1989; MacDonald et al., 1989).

Based on these results it was concluded that gludopa is a kidney-specific dopamine precursor and has kidney-selective actions. This selectivity was defined by comparison of renal actions with effects on heart and blood pressure, but no comparison has been made with other vascular regions, except for hindlimb flow in the conscious rabbit (Wang, Z-Q. et al., 1992); it was found that gludopa has renal effects at doses that do not change hindlimb flow.

Other γ-glutamyl derived renal selective prodrugs are for instance N-acetyl-γ-glutamyl sulphamethoxazole (Orłowski et al., 1980; Drieman et al., 1990a), the renal vasodilator CGP 22979 (Smits & Struijker Boudier, 1985) and the dopamine-β-hydroxylase inhibitor prodrug, SC-47792 (Koecke et al., 1991). The selectivity of these prodrugs is determined in part by the presence of a transport system for the prodrug in the kidney and of intracellular converting enzymes (Drieman et al., 1990a,b; Hwang & Elfarra, 1991). N-acetyl-γ-glutamyl prodrugs are handled this way by the kidney, unlike γ-glutamyl prodrugs, which are converted extracellularly and cause relatively high plasma concentrations of active drug, released through the action of the extracellular enzyme γ-glutamyl transpeptidase (Drieman et al., 1990a; 1993).

As gludopa is not an acetylated prodrug, it is likely that the dopamine released from gludopa is also systemically available. This led us to investigate the selectivity of gludopa with respect to different vascular regions in the rat.

The second question investigated in this study is whether the protective effects of gludopa on the kidney in the glycerol-treated rat model of acute renal failure (ARF), as has been described by Casson et al. (1982, 1983), is due exclusively to local renal effects of gludopa or also to systemic effects. The decreased glomerular filtration rate in this model of ARF is associated with pregglomerular vasoconstriction (Hsu et al., 1979; Wolfert & Oken, 1989). Dopamine increases renal plasma flow and glomerular filtration rate through DA1 receptor stimulation in efferent and afferent arteries (see for review e.g. Lee, 1982; Felder et al., 1989). Furthermore, DA1 receptor stimulation results in normalization of the glomerular filtration rate (GFR) in a state of hyperfiltration caused by glycine (Wang Y.W. et al., 1992) or streptozotocin-induced diabetes (Barthelmebs et al., 1991). Because we found gludopa to have a vasodilator action not only in the kidney, but also in the mesentery and hindlimb, we investigated whether gludopa has normalizing effects only on renal blood flow or also on other flows in the glycerol-treated rat model for acute renal failure.

Methods

Animals

Male wistar rats weighing 220–300 g (Winkelman, Borchen, Germany) were used throughout the study.

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Surgery

In all experiments, miniaturized Doppler flowprobes were implanted on the left renal artery, superior mesenteric artery and abdominal aorta of the rats under pentobarbitone anaesthesia (50 mg kg⁻¹, i.p.), as described by Smits & Struyker Boudier (1985). Doppler flow probes record changes in blood flows, which are only relative and not absolute blood flows are recorded. The animals were provided with polyethylene catheters for infusion of pentobarbitone and drugs in both femoral veins, and in the abdominal aorta via the left femoral artery for blood pressure measurements. The body temperature of the rats was kept constant at 37°C.

Anaesthesia was maintained by a constant rate infusion of pentobarbitone (24 mg kg⁻¹ h⁻¹). Following stabilization, mean arterial pressure (MAP), systolic pressure (SP), heart rate (HR) and the relative blood flows through the left renal artery (renal flow: RF), superior mesenteric artery (mesenteric flow: MF) and abdominal aorta (hindquarter flow: HQF) were recorded.

Experiments

Dose-response curves of dopamine (0.1–5 μmol kg⁻¹ h⁻¹) and dopamine prodrugs (1–20 μmol kg⁻¹ h⁻¹) on MAP, SP, HR (flow rate: 5 ml kg⁻¹ h⁻¹) and the changes in regional blood flows were recorded during cumulative infusions of the appropriate drugs. When all recorded parameters were stable, a 20 min control period was followed by the infusions. Infusion of each dose lasted 20 min, as it was found that all responses on a dose of a dopamine prodrug were maximal at approximately 15 min. Vascular resistances of kidney (RR), mesenteric (MR) and hindquarter (HQR) were derived from the MAP values and flow changes in each rat.

Acute renal failure (ARF) was induced by subcutaneous injection of 7 ml kg⁻¹ of glycerol (50% v/v in water) to the anaesthetized rats. This dose was chosen because it causes considerable renal failure with a minimal of losses (Finckh, 1957; Carrol et al., 1965; Casson et al., 1983).

The effect of glycerol-induced ARF on haemodynamic parameters and regional blood flows was studied as follows. Rats, instrumented as described above, were infused with pentobarbitone (24 mg kg⁻¹ h⁻¹; 2.5 ml kg⁻¹ h⁻¹) and saline (2.5 ml kg⁻¹ h⁻¹). A pilot experiment showed that the rate of total saline infusion was important: when the rate was reduced by 50% most of the animals did not survive for 4 h; a higher (200%) rate of infusion had a too large protective effect on the kidneys, as has been described for conscious rats (Casson et al., 1982; Cusner et al., 1986). For the same reason, the rats were not dehydrated 24 h prior to induction of ARF. After the rats were stable for about 30 min, glycerol was injected s.c. (t = 0) and all parameters were recorded for 4 h. Changes in blood flow and vascular resistance are expressed as percentage change at t = 4 h (average of the last 15 min of these 4 h) compared to control period at t = −15 min to t = 0, changes in the other haemodynamic parameters in absolute differences (beats per min (b.p.m.) and mmHg).

Effects of dopamine and dopamine prodrugs on ARF Experiments were conducted as described above, except that at t = −30 min (i.e. 30 min before glycerol injection, which is t = 0), a continuous infusion of dopamine (0.5 and 5 μmol kg⁻¹ h⁻¹), L-dopa (10 and 100 μmol kg⁻¹ h⁻¹) or gludopa (10 and 100 μmol kg⁻¹ h⁻¹) in saline was started instead of the saline infusion; total infusion rate (5 ml kg⁻¹ h⁻¹) was the same in all experiments. Changes in parameters measured at t = 4 h (average of the last 15 min of these 4 h) are compared to the drug infusion control period (t = −15 min to t = 0).

Figure 1 Dose-response curve of dopamine on haemodynamics (a), vascular resistance; (b) and regional blood flows (c). In (a), hatched columns, mean arterial pressure; solid columns, systolic pressure; cross-hatched columns, heart rate; In (b), hatched columns, renal resistance; solid columns, mesenteric resistance; cross-hatched columns, hindquarter resistance. In (c), hatched columns, renal blood flow (%); solid columns, mesenteric blood flow (%); cross-hatched columns, hindquarter blood flow (%).
Figure 2 Dose-response curve of L-dopa on haemodynamics (a), vascular resistance (b) and regional blood flows (c). Columns as in Figure 1.

Figure 3 Dose-response curve of gludopa on haemodynamics (a), vascular resistance; (b) and regional blood flow (c). Columns as in Figure 1.
Drugs

Dopamine and glycerol were obtained from Sigma Chemical company (St Louis, U.S.A.), L-Dopa from Janssen Chimica (Beirse, Belgium); gludopa was a kind gift from Prof. M.R. Lee (University of Edinburgh, Scotland) and from Prof. C. Casagrande (Simes Cardiovascular Research Centre, Milan, Italy).

Statistics

Data are presented as mean ± s.e. Groups were compared by one-way analysis of variance, followed by Duncan’s range test. Differences were considered to be statistically significant when P < 0.05.

Results

Effects of dopamine, L-dopa and gludopa on regional blood flows and haemodynamic parameters

The results of these experiments are summarized in Figures 1–3. Baseline values (in the absence of drug) of MAP (mmHg) and HR (b.p.m.) were: dopamine, MAP: 108 ± 5, HR: 370 ± 15; L-dopa, MAP: 112 ± 4, HR: 378 ± 12; gludopa, MAP: 107 ± 5, HR: 385 ± 11. Dopamine decreased vascular resistance and increased blood flow in all regions studied (Figure 1b,c). At the highest dose the changes in MR and HQR, but not RR were diminished. This is probably due to stimulation of cardiac adrenoceptors by dopamine. No significant differences between changes in RR, MR and HQR were found at 0.1–1.0 μmol kg⁻¹ h⁻¹, indicating that dopamine is not renal selective in the rat at low dose. L-Dopa and gludopa, both in a 10 fold higher dose, increased blood flows and decreased vascular resistance dose-dependently in all regions studied (Figures 2b,c and 3b,c), suggesting no renal selectivity for gludopa at the level of renal resistance under the circumstances studied. At a dose of 200 μmol kg⁻¹ L-dopa had similar effects as dopamine at 5 μmol kg⁻¹.

The effects of dopamine and L-dopa on MAP, SP and HR were similar: no effects in low doses and a significant rise of all parameters at high doses (Figures 1a and 2a). At the high dose of 200 μmol kg⁻¹ h⁻¹ gludopa caused an increase in systolic pressure, but not in HR or MAP (Figure 3a).

These results indicate that dopamine and its prodrugs L-dopa and gludopa are not renal-selective at low doses in the rat, but that all resistances measured decrease at a dose which does not affect heart rate or blood pressure. Based on the results of these experiments, we chose to study doses of gludopa, L-dopa and dopamine in the ARF studies that decreased RR, MR and HQR without affecting blood pressure and heart rate, and higher doses which do affect these parameters.

Glycerol-induced ARF

The administration of glycerol had the following effects. Immediately following injection a large, transient reduction (typically about 30–40%) of all blood flows was observed which lasted for about 30 min. After this period, all flows

Figure 4 Effects of dopamine (0.5 μmol kg⁻¹ h⁻¹), L-dopa (10 and 100 μmol kg⁻¹ h⁻¹) and gludopa (10 and 100 μmol kg⁻¹ h⁻¹) on haemodynamics (a), vascular resistance; (b) and regional blood flows (c) in glycerol-treated rats at t = 4 h. In (a), hatched columns, mean arterial pressure; solid columns, systolic pressure; cross-hatched columns, heart rate. In (b), hatched columns, renal resistance; solid columns, mesenteric resistance; cross-hatched columns, hindquarters resistance. In (c), hatched columns, renal blood flow (%); solid columns, mesenteric blood flow (%), cross-hatched columns, hindquarters blood flow (%). *Significantly (P < 0.05) different from kidney; **Significantly (P < 0.05) different from saline control.
decreased slowly. At \( t = 4 \) h, MAP, SP and HR were not changed significantly (Figure 4a). RR showed a significantly larger increase than HQR and MR (Figure 4b). The latter two did not differ significantly.

Effects of dopamine and its prodrugs on glycerol-induced ARF

Baseline values of MAP (mmHg) and HR (b.p.m.) in these experiments (before start of drug infusion) were: saline, MAP: 110 ± 6, HR: 383 ± 15; dopamine, MAP: 106 ± 5, HR: 373 ± 11; L-dopa low dose, MAP: 110 ± 3, HR: 385 ± 10; L-dopa high dose, MAP: 111 ± 4, HR: 391 ± 10; gludopa low dose, MAP: 101 ± 4, HR: 384 ± 5; gludopa high dose, MAP: 110 ± 4, HR: 378 ± 12.

Dopamine at a low dose (0.5 \( \mu \text{mol kg}^{-1} \text{h}^{-1} \)) did not significantly affect the changes in vascular resistance or flow compared to saline control (Figure 4b and 4c), nor were differences between saline control (Figure 4a). Unlike the saline control it was found that not only RF, but also HQR was significantly lower than MF. Most of the glycerol-treated animals did not survive the high dose (5 \( \mu \text{mol kg}^{-1} \text{h}^{-1} \) dopamine) for 4 h.

L-dopa at a low dose (10 \( \mu \text{mol kg}^{-1} \text{h}^{-1} \)) caused effects comparable to dopamine (0.5 \( \mu \text{mol kg}^{-1} \text{h}^{-1} \)). The high dose (100 \( \mu \text{mol kg}^{-1} \text{h}^{-1} \)) caused a large increase in HR (significantly different from saline control), but no change of MAP or SP. No amelioration of the glycerol-effects on RF, MF and HQR were found, compared to saline control.

Gludopa at a low dose (10 \( \mu \text{mol kg}^{-1} \text{h}^{-1} \)) prevented the sharp rise in renal resistance: RR was significantly less increased in this case than in the case of saline control or any of the other treatments and was now of the same magnitude as MR and HQR (no significant differences between ΔRR, ΔMR and ΔHQR). Gludopa at a high dose (100 \( \mu \text{mol kg}^{-1} \text{h}^{-1} \)) did not produce this amelioration of RR and RF, but had similar effects on regional blood flow and resistance to dopamine or L-dopa at a low dose. HQR was significantly increased. The effects of gludopa at a high dose on the haemodynamics were comparable to the haemodynamic effects of L-dopa at a high dose.

Discussion

The kidney is rich in dopamine receptors. \( D_1 \) and \( D_2 \) receptors are found in renal arteries, proximal tubule and at juxtaglomerular cells (see for review Felder et al., 1989), where they may cause vasodilatation, decrease renal plasma flow and influence renin secretion (\( D_1 \); increase, \( D_2 \); decrease). Selective stimulation of \( D_1 \) receptors in the kidney may have beneficial effects in several renal disease states (Casson et al., 1983; Barthelmebs et al., 1991; Brooks et al., 1991; Wang, Y.-W. et al., 1992). Hence, a renal selective dopamine prodrug, without systemic side-effects, would be of great therapeutic value.

Gludopa has been described as renal selective in mice, rat and man (e.g. Wilk et al., 1978; 1979; Lee, 1987; Jeffrey et al., 1988a,b; MacDonald et al., 1989; Cummings et al., 1990). Dopamine concentrations following gludopa administration were found to be much higher in the kidney than in mouse heart (Wilk et al., 1978) or rat liver (Cummings et al., 1990). Gludopa may cause vasodilatation, decrease renal plasma flow and influence renin secretion (\( D_1 \); increase, \( D_2 \); decrease). Selective stimulation of \( D_1 \) receptors in the kidney may have beneficial effects in several renal disease states (Casson et al., 1983; Barthelmebs et al., 1991; Brooks et al., 1991; Wang, Y.-W. et al., 1992). Hence, a renal selective dopamine prodrug, without systemic side-effects, would be of great therapeutic value.

The lack of renal selectivity of gludopa implies that the dopamine, released from gludopa is systemically available. Pharmacokinetic studies with gludopa in the rat support our pharmacodynamic findings. After a bolus injection of gludopa, considerable amounts of L-dopa and dopamine were found in plasma (Boateng et al., 1990; Cummings et al., 1990). Due to the high doses of dopamine, renal is difficult to estimate the amount of dopamine that is available systemically in vivo. In vitro in rat isolated kidney it was found that the rate of dopamine release from gludopa to the perfusate was about 3 times higher than to the urine (Barthelmebs et al., 1990). This high leakage from the kidney is probably due to the nature of the prodrug. Gludopa is the \( \gamma \)-glutamyl prodrug of L-dopa. We have found with the model prodrugs \( \gamma \)-glutamyl sulphamethoxazole and \( \gamma \)-glutamyl sulphamethoxazole that the N-acetyl group is essential for high kidney-selectivity: the site of conversion is intracellular in the latter case, unlike that of the \( \gamma \)-glutamyl prodrug (Drieman et al., 1990a). Sulphamethoxazole, released intracellularly, is retained by the kidney, whereas extracellularly released drug causes relatively high plasma concentrations. It would therefore be very interesting to investigate the N-acetyl derivative of gludopa with respect to its renal selectivity. Casson et al. (1983) did, however, report that gludopa has a protective effect on the glycerol-treated rat model of ARF. In the present study we investigated whether this protective effect is due to local or systemic dopamine effects. We chose to do this in the early phases of the induction of ARF, because it has been suggested that maintenance renal blood flow during the first hours after glycerol administration protects the kidney from ARF (Cushner et al., 1986).

Our results show that glycerol decreases renal blood flow significantly more than mesenteric flow or hindlimb flow (Figure 4c). This decrease was ameliorated only by a continuous infusion of gludopa at a low rate. Gludopa at a high rate reversed this beneficial effect, which indicates that the systemic effects of dopamine (sulphamethoxazole and N-acetyl \( \gamma \)-glutamyl sulphamethoxazole) are involved in the local beneficial effects of dopamine. The effects of the low dose gludopa on renal blood flow, in combination with the finding of Casson et al. (1983) that gludopa protects from ARF, confirm the suggestion of Cushner et al. (1986) that preservation of the renal blood flow may cause a reduction or prevention of ARF. The dose of gludopa was not adjusted to the decreased kidney function, since the pharmacokinetics of gludopa are not changed in rats with glycerol-induced ARF (Boateng et al., 1990). The renal effects of gludopa in glycerol-induced ARF in rats are different from those in normal rats. This could be caused by the difference in vascular diameter in these situations. When the diameter is near maximal, a dopamine-induced arteriolar dilatation will not result in a significant decrease of renal resistance, whereas when the renal vessels are contracted, a dilatation will have large effects on the resistance.

The pharmacokinetic studies combined with the pharmacodynamic studies lead to the conclusion that although gludopa may cause relatively high renal concentrations of dopamine, it does not seem to be kidney-selective under physiological circumstances. In pathological circumstances, on the other hand, gludopa has beneficial effects on the kidney (Casson et al., 1983; Barthelmebs et al., 1991). This does not seem to be due to systemic dopamine effects, as dopamine and L-dopa did not increase kidney function. Local stimulation of \( D_1 \) receptors in the kidney is supposed to be responsible for this effect in fenoldopam acetate, whereas no increase in kidney function in streptozotocin-induced diabetes
The dose of gludopa should not be too high in order to avoid systemic effects that counteract the beneficial renal effects. A more renal selective prodrug of dopamine would have a higher therapeutic index. N-acetyl gludopa is in our opinion an interesting candidate, considering the effects of gludopa itself and the beneficial effects of an N-acetyl group on the renal selectivity of prodrugs (Drieman et al., 1990a; 1993). An alternative would be a renal selective D1 agonist. Such a compound might become a useful therapeutic agent, for instance in protecting patients against the nephrotoxic side effects of cytostatics.

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References


