Suppression of Noradrenaline Spillover by the Dopamine Prodrug \( \gamma \)-L-Glutamyl-L-Dopa: A Central Effect?

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The DA prodrug \( \gamma \)-L-glutamyl-L-dopa (gludopa) has a high degree of renal selectivity with 2-step conversion to DA in the kidney. The effects of gludopa, with and without DA-2 receptor blockade, on renal and total noradrenaline (NA) spillover, were studied in two groups of rabbits. Eight rabbits received gludopa infusion (25 and 100 \( \mu \)g/kg/min and 8 received an infusion of gludopa and DA-2 receptor antagonist, YM-09151 (50 \( \mu \)g/kg i.v.). Renal and total NA spillover rates were measured by \(^3\)H-NA tracer method before and after gludopa infusion. Brain NA, DA, gludopa and L-dopa content were measured after gludopa infusion in 5 rabbits; control values for tissue catecholamine and drug levels were obtained in 5 untreated rabbits. Gludopa infusion markedly increased kidney DA content (300 - fold) and DA excretion (6000 - fold) but had little effect on plasma DA. It produced a dose-related fall in mean (± SEM) renal NA spillover (21.6 ± 3.7 to 10.6 ± 2.7, 7.2 ± 2.7 ng/min, \( p < 0.01 \)). Even greater falls were observed in total NA spillover after gludopa (43.1 ± 10.2 to 19.7 ± 3.4, 9.4 ± 1.8 ng/min, \( p < 0.01 \)). DA-2 receptor antagonism had no influence on the effects of gludopa on either renal or total NA spillover. Significant amounts of gludopa were detected in the brain after drug infusion (0.28 ± 13 nmol/g brain tissue). Gludopa, a putative renal selective dopamine prodrug with effects mediated via DA-1 receptors also significantly inhibits both renal and extra-renal NA spillover. This effect is not a DA-2 effect but may be mediated centrally. (Hypertens Res 1995; 18 Suppl. I: S113-S118)

Key Words: DA-2 receptor, gludopa, levodopa, noradrenaline, sympathetic nerve system, YM-09151

The sympathetic nervous system plays a central role in the regulation of the renal circulation, sodium and water homeostasis and renin secretions as well as intrarenal release of other vasoactive substances (1). Sympathetic activity is regulated not only by the number of centrally-induced efferent impulses but also locally by multiple inhibitory and facilitatory presynaptic auto- or heteroreceptors. Of particular interest is the inhibitory presynaptic receptors, expecially \( \alpha \)-2 adrenoceptors, but also adenosine, muscarine, opiate, prostaglandin and dopamine-2 (DA-2) receptors, which, upon stimulation, diminish neuronal noradrenaline (NA) release from varicosities and inhibit adrenergic neurotransmission (2). Previous studies have shown that dopamine (DA) agonists, such as bromocriptine, co-dergocrine, ibopamine and carmoxirole, exert their therapeutic effects in hypertension and/or congestive heart failure at least partly through inhibition of sympathetic nerve activity mediated via DA-2 receptors (3, 4).

Sympathetic nervous activation often occurs in a highly differentiated way. Renal sympathetic nerve endings have been shown to possess presynaptic DA-2 and \( \alpha \)-2 receptors (5-9), which inhibit the amount of NA released per nerve impulse, although renal DA-2 receptors are thought not to play a physiologically significant role (7). As a DA prodrug with renal selectivity, \( \gamma \)-L-glutamyl-L-dopa (gludopa) leads to intrarenal DA synthesis, accumulation of DA relatively confined to the renal parenchyma, and produces renal vasoconstriction and natriuresis via DA-1 receptors (renal tubular and vascular) without major systemic haemodynamic effects in animals and man (9–11). Gludopa infusion was shown to increase renal blood flow and urine sodium excretion in control rabbits and rabbits with doxorubicin-induced congestive heart failure (11, 12). These effects were blocked by the DA-1 antagonist, SCH23390 (12). On the other hand, gludopa-induced tissue accumulation of DA may suppress NA release via activation of presynaptic DA-2 receptors. This has been postulated as the reason for gludopa-induced suppression of renin in normal and hypertensive subjects (13). The aims of this study were to determine the effects of gludopa on renal and overall sympathetic nervous activity, using the \(^{[3]}\)H-NA radiotracer kinetic technique, and to define the role of DA-2 receptors in mediating these responses in conscious rabbits.

Methods

Twenty six male rabbits (New Zealand White and mixed strains, 2.5–4.0 kg in body weight), obtained from the Central Animal House of Monash Uni-
Urine was collected for 20 min during the whole period. After an hour of equilibration, saline (0.1 ml/min) for 20 min as the control period and gludopa was given i.v. at 1 mg/min and [3H]-NA at 0.04 pCi/kg/min (Harvard Apparatus) throughout the experimental period. The marginal ear vein and central ear artery were cannulated under local anaesthesia with 0.5% lignocaine. A bladder catheter (8 Fr Foley catheter with 3-ml balloon) was inserted under brief anaesthesia with a i.v. dose of 20 mg methohexitone sodium. Mean arterial pressure (MAP) was measured using a Hewlett-Packard transducer and the phasic signal was used to trigger a heart rate meter (Model 173, Baker Medical Research Institute, Melbourne, Australia). MAP and HR were continuously recorded on a Macintosh SE computer (Apple Computer Inc., Cupertino, California, USA) via a MacLab A/D converter (Analog-Digital Instruments, Dunedin, New Zealand). The rabbit was allowed to recover in a study box for 1 h prior to the experiment.

After control samples of blood and urine were taken for blank measurements, a priming dose of 10 mg/kg p-aminophippurate (PAH) and 0.8 μCi/kg radiolabeled norepinephrine ([3H]-NA, ring-2,5,6-trititated NA, specific activity 40.8-43.4 Ci/mmol, New England Nuclear, Mass, USA) was given i.v. as a bolus, followed by a constant infusion of PAH at 1 mg/min and [3H]-NA at 0.04 μCi/kg/min (Harvard Apparatus) throughout the experimental period. After an hour of equilibration, saline (0.1 ml/min) for 20 min as the control period and gludopa (UCB Bioproducts, Brussels, Belgium) at 25 and 100 μg/kg/min, each for 50 min, were infused i.v. Urine was collected for 20 min during the whole control period and 30 min after commencing gludopa administration at different doses. In the YM-09151 + gludopa group, YM-09151 (Yamanouchi Pharmaceutical Co. Ltd., Japan) was injected at 50 μg/kg i.v. as a bolus just before the commencement of gludopa infusion. Blood samples (2.5 ml each) were taken simultaneously from the ear artery and renal vein at the middle of each urine collection period. The same amount of blood was replaced after each blood sampling from a donor rabbit.

A group of five rabbits were infused i.v. sequentially with saline vehicle and gludopa at 25 and 100 μg/kg/min as described above. Arterial blood was collected after each infusion period for gludopa and L-dopa measurement. At the end of the experiment, the rabbit was sacrificed by i.v. injection of pentobarbitone sodium. The kidneys and brains were collected for the measurement of tissue NA and DA in the kidney, and tissue gludopa, L-dopa, NA and DA content in the brain. Another group of five control rabbits which did not receive gludopa infusion was killed for basal measurements. The kidneys and brains were quickly removed, weighed and snap frozen in liquid nitrogen and then stored at -70°C until subsequently assayed.

**Biochemical Analysis**

PAH was determined by a photometric method (17).Renal plasma flow was estimated from the steady-state clearance of infused PAH corrected for the renal extraction. Plasma [3H]-NA was extracted by alumina and the radioactivity directly counted on a liquid scintillation counter (LKB Wallac 1409) (13 -15). NA and DA in plasma and urine were measured by the radioenzymatic assay (18). Plasma L-dopa and gludopa were determined using high performance liquid chromatography with electrochemical detection (HPLC-ECD) as described previously (19). The kidneys and brains were homogenised with 0.1 M perchloric acid, and 2.5 ml of the supernatants extracted and then quantitated by HPLC-ECD for tissue gludopa, L-dopa, NA and DA content (19-21). Renal NA spillover rate was calculated according to the Fick principle corrected for the fractional extraction of [3H]-NA across the kidney while total body NA spillover rate and total NA clearance were calculated based on arterial sampling using the following equations (22):

Renal NA spillover rate

\[ \frac{[(\text{NA}_R - \text{NA}_A) + \text{NA}_A \times \text{EX}_{\text{[3H]-NA}}] \times \text{RPF}}{} \]

Total NA spillover rate

\[ \frac{[\text{[3H]-NA}] \times \text{arterial plasma [3H]-NA specific activity}}{} \]

Total NA clearance rate

\[ [\text{[3H]-NA}] \times \text{arterial plasma [3H]-NA concentration} \]

where NA_R is renal venous NA concentration, NA_A is arterial NA concentration, EX_{[3H]-NA} is fractional extraction of [3H]-NA across the kidney and RPF is renal plasma flow.

**Data Analysis**

Statistical analysis was performed using the Macintosh StatView SE program (Abacus Concepts, Inc. Berkeley, CA, USA). The statistical significance of
differences between variables was assessed by either Student’s t test or analysis of variance (ANOVA) followed by Fisher’s protected least significance difference (PLSD) when appropriate. A p-value less than 0.05 was considered statistically significant. All data were presented as mean ± SEM.

Results

Mean arterial pressure and heart rate remained constant during gludopa infusion. Renal plasma flow was significantly increased, the changes being similar with gludopa and gludopa + YM-09151 infusions (Table 1).

During gludopa infusion at 25 and 100 μg/kg/min, urinary DA excretion increased from 0.05 ± 0.01 to 39.95 ± 2.93, 309.68 ± 35.64 nmol/min (n = 5, p < 0.001). Renal venous DA rose after gludopa infusion (0.21 ± 0.14, 0.94 ± 0.28 and 1.67 ± 0.55 pmol/ml, n = 8, p < 0.01); arterial plasma DA concentrations were not significantly elevated (0.14 ± 0.06, 0.35 ± 0.14 and 0.62 ± 0.24 pmol/ml, n = 8, p > 0.05). Mean arterial plasma gludopa concentrations were 890, 3,190 ng/ml, and L-dopa concentrations were 3, 10 ng/ml at low and high doses respectively. Renal DA content was also markedly increased at the end of the experiment, after the high dose gludopa infusion, when compared to untreated controls (40.41 ± 11.94 vs. 0.34 ± 0.29 nmol/g, n = 5, p < 0.01). Renal NA content in gludopa-infused rabbits was higher than that in the control group (1.899.6 ± 437.0 vs. 817.2 ± 205.5 nmol/g, n = 5, p < 0.05).

**Effects of Gludopa Treatment on NA Spillover**

Gludopa infusion at 25 and 100 μg/kg/min resulted in pronounced dose-related falls in renal NA spillover (51 ± 6%, 75 ± 4%) and total NA spillover (60 ± 17%, 72 ± 6%) (Table 1). Total and renal NA spillover rates during gludopa infusion were not significantly different in the presence and absence of DA-2 receptor blockade by YM-09151 (Figs. 1, 2). The mean ratio of renal:total NA spillover was 0.44 during saline infusion, 0.51 at 25 μg/kg/min gludopa and 0.51 at 100 μg/kg/min gludopa infusion.

Total clearance of NA from plasma and renal extraction of [3H]-NA were unchanged during gludopa or gludopa + YM-09151 infusions.

**Effect of Gludopa on Catecholamine Content of the Brain**

As shown in Table 2, the brain tissue L-dopa and gludopa contents were higher after gludopa infusion compared with control levels (L-dopa: 0.55 ± 0.03 vs. 0.48 ± 0.08 nmol/g, p < 0.05; gludopa: 0.28 ± 0.13 nmol/g vs. not detectable, p < 0.01). The tissue NA and DA contents did not show differences after gludopa infusion compared to control (NA: 1.30 ± 0.06 vs. 1.36 ± 0.12; DA: 3.26 ± 0.26 vs. 3.66 ± 0.98 nmol/g). There were no significant differences between the groups with and without DA-2 blockade.

**Discussion**

The effects of gludopa on mean arterial pressure, heart rate and renal plasma flow were similar to those previously reported in animals and man (9-11). This study also confirmed the relative renal selectivity of gludopa and has provided evidence that gludopa produced a significant reduction in renal NA spillover to plasma, suggesting that the increase in intra-renal DA afforded by gludopa treatment may inhibit NA release from nerve endings and/or reduce renal sympathetic nerve activity. The most surprising finding of this study was that gludopa treatment had a suppressive effect on extrarenal NA spillover.

Gludopa undergoes sequential conversion by the brush border enzyme γ-glutamyl transpeptidase (γ-GT) to L-dopa and then via the intracellular enzyme amino acid decarboxylase (AADC) to DA predomi-
nantly in the proximal tubular cells of the kidney, where both enzymes involved in the process are present in abundance (23, 24). When added to the perfusate of the isolated perfused rat kidney, it led to renal vasodilation and release of DA both into urine and renal venous perfusate. Inhibition of γ-GT and AADC with AT-125 and carbidopa significantly decreased DA synthesis and renal vasodilation (9). A study of the tissue distribution of gludopa in rats also demonstrated its high renal selectivity, with renal DA content at least six orders of magnitude higher than endogenous values (25). The results of the present study confirms the renal specificity of gludopa as shown by the marked increase in urinary DA excretion and renal DA content during gludopa infusion, without parallel change in plasma DA concentration. There was some overflow of DA into the renal vein after gludopa but arterial DA content was not significantly elevated, which is in accord with the results obtained in the isolated rat kidney (9). Increased circulating L-dopa in the present study is consistent with the previously reported disposition of gludopa in rats and man (25-27). Our results that tissue gludopa in the brain was increased suggests that gludopa can pass across the blood-brain barrier and may therefore exert additional central effects. The lack of change in brain catecholamines (DA, NA) indicates that no apparent metabolism of gludopa occurred in the brain.

The present study demonstrated that gludopa produced a marked reduction in renal NA spillover in conscious rabbits, but this reduction was not altered by the specific DA-2 antagonist YM-09151, a potent benzamide neuroleptic with a high affinity for central D-2 and peripheral DA-2 receptors (28-30). The decline in renal NA spillover to plasma could not be accounted for by changes in flow-dependent NA washout or altered neuronal re-uptake of NA, because renal plasma flow was actually increased with gludopa administration. Nor was it thought to be due to [3H]-NA extraction, an index of neuronal NA re-uptake, remained unchanged. Rather, it reflects either reduced renal sympathetic nerve firing and/or reduced adrenergic neurotransmission.

The rate of NA escape into the renal vein is largely proportional to the rate of renal sympathetic nerve firing that triggers exocytosis (16, 31, 32). It is also regulated by other factors including neuronal reuptake, displacement of vesicular stores and presynaptic modulation of NA release. In the present study, the increase in renal NA content after gludopa infusions is consistent with inhibition of NA release but may also be due to accelerated renal NA synthesis as a result of increased availability of catecholamine precursors (i.e. L-dopa and DA). Presynaptical DA-2 receptors have been identified in the adventitia of renal vessels (33). Stimulation of these receptors diminishes the amount of NA released per action potential. However the decline of NA spillover after gludopa infusion was not significantly altered by DA-2 blockade, although there was tendency to be lower. It suggests this gludopa effects on NA spillover was not mediated by DA-2 receptors. What is the possible mechanisms of gludopa effects on spillover? Firstly when a very high concentration of DA accumulates in the kidney, DA can also activate presynaptic α-2 adrenoceptors.

Table 2. BRAIN Catecholamine Content (ng/g) after GLUDOPA Infusion

<table>
<thead>
<tr>
<th>Catecholamine Content (ng/g)</th>
<th>Control</th>
<th>Gludopa</th>
<th>Gludopa + YM09151</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>0.23±0.02</td>
<td>0.22±0.01</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td>DA</td>
<td>0.56±0.15</td>
<td>0.50±0.06</td>
<td>0.37±0.07</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>0.10±0.02</td>
<td>0.12±0.01</td>
<td>0.13±0.01*</td>
</tr>
<tr>
<td>Gludopa</td>
<td>0</td>
<td>0.09±0.04*</td>
<td>0.09±0.08*</td>
</tr>
</tbody>
</table>

Fig. 1. Changes in total noradrenaline (NA) spillover in response to gludopa at 25 μg/kg/min (low dose) and 100 μg/kg/min (high dose) compared to gludopa plus DA-2 antagonist YM-09151 50 μg/kg (gludopa + DA-2 blocker). *p<0.05 vs. saline control, **p<0.01 vs. saline control.

Fig. 2. Changes in renal noradrenaline spillover in response to gludopa at 25 μg/kg/min (low dose) and 100 μg/kg/min (high dose) compared to gludopa plus DA-2 antagonist YM-09151 50 μg/kg (gludopa + DA-2 blocker). *p<0.05 vs. saline control, **p<0.01 vs. saline control.
Mean arterial pressure and heart rate were not significantly affected by YM-09151. DA-2 receptors exist not only presynaptically on sympathetic nerve terminals but also postsynaptically in the intimal layer of these vessels and in the glomerulus as well (33). Blockade of these postsynaptic renal DA-2 receptors with YM-09151 has been shown to produce renal vasodilatation and increase in GFR (28). Renal effects of attenuation of gludopa induced-reduction in the sympathetic activity by YM-09151 could have been compromised by the concurrent antagonism of renal postsynaptic DA-2 receptors.

Mean arterial pressure and heart rate were not altered significantly in spite of gludopa-induced sympathoinhibition in conscious rabbits. Our previous study showed PRA tended to increase after gludopa in rabbits (12). One week treatment with gludopa in conscious diabetic rats increased urinary DA excretion by 180 times and suppressed renal sympathetic nerve activity. Our results showed a significant increase in arterial plasma L-dopa without any significant rise of arterial DA concentration. Direct treatment with L-dopa failed to affect endogenous NA overflow in the isolated canine saphenous vein (34). However, L-dopa was shown to produce an increase in brain NA and DA content (35, 36) and reduce directly-recorded efferent sympathetic nervous activity (36, 37). In addition, L-dopa-induced hypotensive effect in hypertensive patients has been ascribed, at least partly, to suppression of efferent sympathetic activity (38-40). Therefore, increased circulating L-dopa may also directly suppress efferent sympathetic nervous activity via a central mechanism.

Gludopa-induced increase in renal plasma flow was not significantly affected by DA-2 blockade with YM-09151. DA-2 receptors exist not only presynaptically on sympathetic nerve terminals but also postsynaptically in the intimal layer of these vessels and in the glomerulus as well (33). Blockade of these postsynaptic renal DA-2 receptors with YM-09151 has been shown to produce renal vasodilatation and increase in GFR (28). Renal effects of attenuation of gludopa induced-reduction in the sympathetic activity by YM-09151 could have been compromised by the concurrent antagonism of renal postsynaptic DA-2 receptors.

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