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Sympatho-inhibitory effects of *r-l*-glutamyl-*l*-dopa in conscious rabbits

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ABSTRACT Renal and total norepinephrine (NE) spillover rates were studied with [^{3}H] NE kinetic method during graded *r-l*-glutamyl-*l*-dopa (gludopa) iv infusion in conscious rabbits. Mean arterial pressure (MAP) and heart rate (HR) remained constant during the experiment. Gludopa iv infusion at 25 and 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$ produced marked increases in urinary dopamine (DA) excretion and renal DA content. Although renal venous DA rose after gludopa infusion, the arterial DA was not significantly altered. Arterial plasma gludopa and levodopa levels reached 0.9 ± 0.5, 3.2 ± 0.8 $\mu g \cdot ml^{-1}$ and 3.0 ± 1.8, 10.1 ± 5.1 $ng \cdot ml^{-1}$ at the lower and higher gludopa doses, respectively. Gludopa elicited a pronounced dose-related fall in renal NE spillover, which only accounted for about one half of the reduction in overall NE spillover rate. Renal NE content was doubled. These results indicated that gludopa decreased the renal and extrarenal NE spillover to plasma. This reduction may be mediated by intrarenally synthesized DA via presynaptic DA-2 and α-2 receptors, but could also be explained by some central sympatbo-inhibitory mechanism.

KEY WORDS catecholamines; dopamine; dopa; kidney; levodopa; norepinephrine

Sympathetic nerve activity is regulated not only by the number of centrally-induced efferent impulses but also by peripherally inhibitory and facilitatory presynaptic mechanisms. Activation of presynaptic DA-2 receptors and α-2 adrenoceptors inhibits neuronal NE release from renal sympathetic nerve endings[1,2]. As a DA prodrug with relative renal selectivity, *r-l*-glutamyl-levodopa (gludopa) induces renal vasodilation and natriuresis via renal tubular and vascular DA-1 receptors without major systemic hemodynamic effects[3]. The present study was conducted to determine the effect of gludopa on renal and overall sympathetic activity, using [^{3}H] NE technique in conscious rabbits.

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METHODS

Experiments were carried out in 3 groups of 18 ♀ rabbits (New Zealand white and mixed strains, weighing $3.1 \pm s$ 0.2 kg).

Effect of gludopa on NE spillover General anesthesia was maintained with halothane-air mixture after induction with 20 mg methohexitone sodium and endotracheal intubation. Through a midabdominal incision, a clear vinyl tube (SV 55, ID 0.8 mm, OD 1.2 mm, Dural Plastics & Engineering, Auburn, NSW, Australia) was introduced into the left renal vein against the blood stream via the adrenolumbar or spermatic vein in 8 rabbits. The adrenolumbar vein was ligated in proximity to the adrenal gland, and the catheter was fixed to keep its tip close to the renal hilus. The free end of the catheter was tunneled subcutaneously to the back of the rabbit. The patency was maintained by flushing with heparinized saline (1 $IU \cdot L^{-1}$) 3 times per week. The position of the catheter tip in the renal vein was confirmed at autopsy.

One week after surgery the effect of gludopa on renal and whole-body NE spillover rates was examined as described previously[4]. On the day of experiment, the marginal vein and central artery of the ear were cannulated under local anesthesia with 0.5 % lignocaine. MAP was measured using a Hewlett-Packard transducer and phasic signal was used to trigger a heart rate meter (Model 173, Baker Medical Research Institute, Melbourne, Australia). A bladder catheter (8 Fr Foley catheter with 3-ml ballon) was inserted under brief anesthesia with iv 20 mg methohexitone sodium.

MAP and HR were continuously recorded on a Macintosh SE computer (Apple Computer Inc Cupertino CA, USA) via a MacLab A/D converter (Analog-Digital Instruments, Dunedin, New Zealand). The rabbit was allowed to recover for 1 h before the experiment.

After blood and urine were taken for blank measurements, *p*-aminohippurate (PAH) 10 mg · kg^{-1} and [^{3}H]NE 29.6 kBq · kg^{-1} (*ring*-2,5,6-^{3}H NE, New England Nuclear, Bosron MA, USA) were given iv as a bolus, followed by a constant iv infusion of PAH 1 mg · min^{-1} and [^{3}H]NE 1.48 kBq · kg^{-1} · min^{-1} throughout the experiment. After 1-h equilibration, saline vehicle at 0.1 ml · min^{-1} for 20 min in the control period and gludopa (UCB Bioproducts, Brussels, Belgium) at 25 and 100 μg · kg^{-1} · min^{-1}, each for 50 min, were infused iv sequentially. Urine was collected for 20 min during the whole control period and 30 min after the commencement of gludopa infusion. Blood samples (2.5 ml each) were taken simultaneously from the ear artery and renal vein in the middle of each urine collection period. The same amount of blood was replaced after each blood sampling from a donor rabbit.

Effect of gludopa on renal DA and NE contents

A group of 5 rabbits were infused iv with saline and then gludopa at 25 and 100 μg · kg^{-1} · min^{-1} as described above. Arterial blood was collected after each infusion period for gludopa and *l*-dopa measurements. At the end of the infusion the rabbit was killed (by overdose of iv pentobarbitone sodium) for the measurement of renal DA and NE contents. Control group of 5 rabbits that did not receive saline and gludopa infusions was killed only for basal DA and NE contents in the kidneys. The kidneys were quickly excised, cleaned of fat and connective tissue, frozen in liquid nitrogen, and stored at −70 ℃ until assay for renal DA and NE.

Analysis PAH was determined photometrically[12]. Plasma [^{3}H]NE was extracted by alumina and measured in a liquid scintillation counter (Wallac 1409, LKB)[14]. The DA and NE in plasma and urine were measured by radioenzymatic assay[13]. Renal plasma flow was estimated from steady-state clearance of infused PAH corrected for renal extraction. Plasma gludopa and *l*-dopa were determined by HPLC-ECD[15]. The kidneys were homogenized with perchloric acid 0.1 mol · L^{-1}, and 2.5 ml of supernatant were quantitated by HPLC-ECD for renal DA and NE contents[15]. Renal and total NE spillover rates and total NE clearance were calculated[14]:

Renal NE spillover rate = $[(NE_R - NE_A) + NE_A \times Ex_{[^3H]NE}] \cdot RPF$

Total NE spillover rate = [^{3}H]NE infusion rate/plasma NE specific activity

Total NE clearance rate = [^{3}H]NE infusion rate/plasma [^{3}H]NE concentration

Where NE_R is renal venous NE concentration; NE_A is arterial NE concentration; $Ex_{[^3H]NE}$ is fractional extraction of [^{3}H]NE across the kidney; and RPF is renal plasma flow.

All data were presented as $\bar{x} \pm s$. The statistical significance of differences between variables was assessed using the Macintosh StatView SE program (Abacus Concepts Inc, Berkeley CA, USA) by either *t* test or prorected least significance difference (PLSD) when appropriate.

RESULTS

After gludopa infusion urinary DA excretion increased markedly from 0.05 ± 0.02 to 310 ± 78 nmol · min^{-1} ($n = 5$, $P < 0.1$), and the renal DA content also rose (0.3 ± 0.6 in controls and 40 ± 26 nmol · g^{-1} at the end of the experiment, $n = 5$, $P < 0.01$). Although renal venous DA was elevated during gludopa infusion (0.2 ± 0.4, 0.9 ± 0.8, and 1.7 ± 1.5 pmol · ml^{-1}, $n = 8$, $P < 0.01$), changes in arterial DA concentration were not statistically significant (0.1 ± 0.2, 0.4 ± 0.4, and 0.6 ± 0.7 pmol · ml^{-1}, $n = 8$, $P > 0.05$).

The MAP and HR remained unchanged during the experiment period. Arterial plasma NE concentration was greatly lowered. Gludopa iv infusion at 25 and 100 μg · kg^{-1} · min^{-1} reduced both renal and total NE spillover. The fall in renal NE spillover accounted only for 48 ± 20 % of the total reduction in whole-body NE spillover. Total clearance of NE from plasma and renal fractional extraction of [^{3}H]NE were unchanged while

Tab 1. Hemodynamic variables and [^{3}H]NE kinetics during iv infusion of gludopa 25 and 100 μg · kg^{-1} · min^{-1} in conscious rabbits. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline. RPF = renal plasma flow. Renal [^{3}H]NE Ex = renal [^{3}H]NE fractional extraction.

	Saline vehicle 0.1 ml·min^{-1}	Gludopa/μg·kg^{-1}·min^{-1} 25	100
MAP/kPa	10.2±0.8	10.1±0.6^a	9.9±0.8^a
HR/bpm	251±23	244±28^a	247±25^a
RPF·ml^{-1}·min^{-1}	75±14	88±15^b	101±18^c
Plasma NE/pg·ml^{-1}			
Arterial	268±133	104±29^c	50±35^c
Renal venous	406±230	178±100^c	115±68^c
NE spillover/ng·min^{-1}			
Renal	22±12	11±9^c	5±6^c
Total	55±31	21±9^c	11±7^c
Renal [^{3}H]NEEx	0.48±0.06	0.41±0.08^a	0.45±0.08^a
Total NE clearance/ml·min^{-1}	218±41	211±33^a	225±72^a

total renal plasma flow increased with gludopa infusion (Tab 1).

The renal NE content in the gludopa-infused rabbits was higher than that in the control group (1.9 ± 0.9 *vs* 0.8 ± 0.5 nmol·g^{-1}, $n=5$, $P<0.05$). Arterial plasma gludopa and *l*-dopa levels reached 0.9 ± 0.5, 3.2 ± 0.8 μg·ml^{-1} and 3.0±1.8, 10.1±5.1 ng·ml^{-1} at the lower and higher doses, respectively.

DISCUSSION

Gludopa is converted by *r*-glutamyl transpeptidase to levodopa and then by amino acid decarboxylase to DA predominantly in the renal proximal tubular cells, where both enzymes involved in the process are present in abundance by comparison with those in liver, pancreas and brain[3]. In the present study, the marked increases in urinary DA excretion and renal DA content during gludopa infusion are consistent with our previous work[3], confirming the renal selectivity of gludopa. There was some overflow of DA into the renal vein, but arterial DA concentration was only slightly elevated after gludopa administration. Increased circulating *l*-dopa in the present study is in agreement with previously reported disposition of gludopa in rats and humans[7,8].

The decline in renal NE spillover provided an evidence of inhibition of renal NE overflow by gludopa, the lower NE spillover was not accountable in terms of other determinants, such as flow-dependent NE washout or altered neuronal re-uptake of NE, because the renal plasma flow was actually increased with gludopa infusion, and the [^{3}H]NE extractional fraction remained unchanged. The inhibition of NE release from the postganglionic sympathetic nerve endings was further supported by the increased renal NE content even though renal NE synthesis may have been accelerated as a result of increased availability of catecholamine precursors (ie, *l*-dopa and DA). Gludopa-induced renal DA loading could suppress renal adrenergic neurotransmission by activation of presynaptic DA-2 and α-2 receptors.

The fall in extrarenal NE overflow also accounted for about 50 % of the reduction in total NE spillover although arterial DA level did not change significantly. There have been no previous reports on whether gludopa itself can pass through the blood-brain barrier and

exert a central sympatho-inhibitory effect. On the other hand, direct treatment with *l*-dopa reportedly failed to affect the endogenous NE overflow in the isolated saphenous vein[9]. When given peripherally, *l*-dopa was shown to increase regional brain DA and NE contents and reduced directly-recorded efferent sympathetic traffic[10,11]. Therefore, an increased circulating *l*-dopa after gludopa infusion may inhibit efferent sympathetic outflow via some central mechanism.

In spite of gludopa-induced sympatho-inhibition in conscious rabbits, MAP and HR did not change significantly. This is in agreement with previous observations in rats and humans[12,13], but prolonged iv infusion (10 h) of gludopa did reduce blood pressure in healthy subjects[14]. Our previous study showed that plasma renin activity tended to increase after gludopa infusion[15]. Different responses of blood pressure to *l*-dopa under normotensive and hypertensive states have been observed[11,15]. Therefore, relative short infusion period, activation of other vasoactive systems and different basal sympathetic tone might explain the absence of MAP and HR changes in the present study on conscious normal rabbits.

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谷酰多巴在清醒兔的交感神经抑制作用

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摘要 给清醒兔 iv 输注谷酰多巴（GD）25 和 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$，MAP、HR 和动脉血浆多巴胺(DA)浓度无显著变化. 尿 DA 排泌率和肾 DA 增加. 动脉血浆 GD 和 *L*-多巴分别达到3.2±0.8 $\mu g \cdot ml^{-1}$和16.1±5.1 $ng \cdot ml^{-1}$. 肾脏和肾外去甲肾上腺素(NE)溢出率均降低，肾 NE 增加. 作为具有相对肾脏选择性的 DA 前体，GD 能抑制清醒家兔肾脏及肾外交感神经活性.

关键词 儿茶酚胺；多巴胺；多巴；肾；左旋多巴；去甲肾上腺素

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Presence of endothelium masks direct vasodilator effects of pyrogallol and methylthioninium chloride in perfused rat mesenteric artery[1]

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ABSTRACT In the perfused rat mesenteric artery vasoconstrictor responses to transmural nerve stimulation (TNS) were enhanced by pyrogallol (Pyr) 0.1 $mmol \cdot L^{-1}$ or methylthioninium chloride (Met) 0.01 $mmol \cdot L^{-1}$. But the duration of the effect of Pyr was brief, while the effect of Met remained stable. Met, but not Pyr, slightly increased the basal level of perfusion pressure. Contractile responses to the alpha adrenergic agonist methoxamine were also potentiated by both Pyr and Met, and in both cases their effects persisted as long as Pyr or Met was present. Superoxide dismutase (SOD) abolished or inhibited the potentiation produced by Pyr or Met. Both Pyr and Met inhibited the vasodilation produced by acetylcholine (ACh). However, after blockade of endothelial function both Pyr and Met inhibited vasoconstrictor responses to TNS in the presence of N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME) 0.1 $mmol \cdot L^{-1}$, an inhibitor of nitric oxide synthesis, or removal of endothelium. After removal of endothelium both Pyr and Met produced vasodilator responses in a concentration-dependent manner. These results suggest that the ability of both Pyr and Met to potentiate contractile responses and inhibit vasodilator responses to ACh is due to generation of superoxide anion, and that the actions of Met may also involve direct inactivation of guanylate cyclase. The present study also suggests that both Pyr and Met have direct relaxing effects on vascular smooth muscle, effects which are masked by enhancing actions in the presence of endothelium.

KEY WORDS mesenteric arteries; pyrogallol; methylthioninium chloride; N^{ω}-nitro-*L*-arginine methyl ester; superoxide dismutase

Methylthioninium chloride (methylene blue, Met), an inhibitor of guanylate cyclase, is widely used as a tool to evaluate the mechanism of action of vasodilators[1,2]. Besides inhibiting guanylate cyclase, Met has been shown to produce superoxide anion[3].

Pyrogallol (Pyr), a generator of superoxide anion, produces pharmacological responses similar to those of Met, including inhibition of relaxation to endothelium-dependent vasodilator and potentiation of contractile responses to vasoconstrictors, as well as inactivation of

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