BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 193—197

Sympatho-inhibitory effects of γ-*l*-glutamyl-*l*-dopa are not mediated by activation of dopamine-2 receptors in conscious rabbits[1]

Zhi-Qin WANG[2], Kazumasa SHIMIZU, Diana WAY, John SECOMBE[3], Barry P McGRATH
(*[1]University Department of Medicine and [2]Departemnt of Clinical Biochemistry, Monash Medical Center, Melbourne, Victoria 3168, Australia*)

AIM: To define the role of dopamine-2 receptors in the sympatho-inhibitory effects of γ-*l*-glutamyl-*l*-dopa in conscious rabbits. **METHOD:** γ-*l*-glutamyl-*l*-dopa (gludopa) was infused iv at 25 and 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$ with and without prior dopamine-2 receptor blockade by YM-09151-2 (50 $\mu g \cdot kg^{-1}$ iv) in conscious rabbits. **RESULTS:** Mean arterial pressure and heart rate remained unchanged while renal plasma flow increased. Arterial norepinephrine (NE) concentration, total and renal NE spillover rate were markedly decreased in a dose-related manner, which were not affected by prior dopamine-2 receptor blockade. Gludopa was detected in the whole brain (92 ±112 ng/g wet brain tissue) at the end of experiment although brain tissue levodopa, NE, and dopamine contents were not much different from those in the control group. **CONCLUSION:** Gludopa decreased dose-dependently plasma NE concentration, and total and renal NE overflow to plasma, which were not mediated by activation of dopamine D_2 receptors.

KEY WORDS dopamine D_2 receptors; dopamine; gludopa; levodopa; norepinephrine; sympathetic nervous system; YM-09151-2

As a putative dopamine (DA) prodrug with relative renal selectivity, γ-*l*-glutamyl-*l*-dopa (gludopa) leads to synthesis and accumulation of DA predominantly confined to the renal parenchyma[1,2]. Gludopa produced similar renal vasodilation and natriuresis in conscious control rabbits and rabbits with congestive heart failure via renal dopamine D_1 receptor stimulation without major systemic hemodynamic effects[3]. It also suppressed norepinephrine (NE) release possibly via peripheral presynaptic and/or some central mechanisms[4]. The present study was performed to define the role of dopamine D_2 receptors in mediating sympatho-inhibitory effects of gludopa in conscious rabbits.

METHODS

Rabbit preparation Twenty-one male rabbits (New Zealand White and mixed strains, 3.2 ± *s* 0.3 kg) were anesthetized with halothane after induction with methohexital sodium (20 mg iv) and endotracheal intubation. Through a mid-abdominal incision a catheter (Clear Vinyl tube SV .55, ID 0.8 mm, OD 1.2 mm, Dural Plastics & Engineering, NSW, Australia) was introduced into the left renal vein against the blood flow via the left adrenolumbar ($n = 12$) or spermatic ($n = 4$) vein in 16 rabbits[4]. The adrenolumbar vein was tied off and severed in proximity to the adrenal gland, and the spermatic vein was ligated. The catheter was fixed in a position to keep its tip close to the renal hilus. The free end of the catheter was tunneled subcutaneously to the nucha of the rabbit. The patency of the catheter was maintained

Parts of this study were presented at the 5th International Conference on Peripheral Dopamine (Kyoto, Japan, 1994 Oct 20—22), abstract № L-21.

Correspondence to Dr Zhi-Qin WANG, *Rm 2035, MR-4 Building, Division of Endocrinology & Metabolism, Department of Internal Medicine, Health Sciences Center, University of Virginia, Charlottesville VA 22908, USA*

Received 1994-11-21 Accepted 1995-01-03

by flushing with heparinized saline (1 IU · L^{-1}) 3 times /wk. The position of the catheter tip at the renal vein was confirmed at autopsy.

Study protocol The effects of gludopa on renal and wholebody NE spillover rates with and without prior dopamine D_2 receptor blockade by YM-09151-2 ($n=8$ in each group) were examined 1 wk after the surgery[4,5]. The marginal ear vein and central ear artery were cannulated under local anesthesia with 0.5 % lidocaine. A bladder catheter (8 Fr Foley catheter with 3-mL balloon) was inserted under brief anesthesia with 20 mg iv methohexital sodium. Mean arterial pressure (MAP) was measured using a Hewlett-Packard transducer and the phasic signal was used to trigger a heart rate (HR) meter (Model 173, Baker Medical Research Institute, Melbourne, Australia). 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 experiment .

After control samples of blood and urine were taken for blank measurements, *p*-aminohippurate (PAH) 10 mg · kg^{-1} and [^{3}H]NE (ring-2,5,6-tritiated NE, New England Nuclear, Boston MA, USA) 29.6 kBq · kg^{-1} were given iv as a bolus, followed by a constant infusion of PAH 1 mg · min^{-1} and [^{3}H]NE 1.48 kBq · kg^{-1} · min^{-1} throughout the experiment. After 1 h of equilibration, saline (0.1 mL · min^{-1}) for 20 min in the control period and gludopa (UCB Bioproducts, Brussels, Belgium) 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 administration. In YM-09151-2 + Gludopa group, YM-09151-2 (Yamanuchi Pharmaceutical Co Ltd, Japan) was injected at 50 μg · kg^{-1} iv as a bolus 5 min before gludopa iv infusion started. The dose of YM-09151-2 used in the present study was 10- to 50-fold greater than what has been shown to produce dopamine D_2 receptor blockade[6,7]. Blood samples (2.5 mL) 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.

At the end of the experiment the rabbit was killed by iv pentobarbital sodium. The whole brain ($n=5$ in each group) was collected, frozen in liquid nitrogen and stored at −70℃ until subsequently assayed for gludopa, levodopa, NE, and DA contents. Another 5 rabbits which received neither gludopa nor YM-09151-2 were killed for control measurements.

Analysis PAH was determined photometrically[4,5]. Plasma [^{3}H]NE was extracted by alumina and the radioactivity was counted in a liquid scintillation counter (Wallac 1409, LKB)[4,5]. Plasma NE was measured by the radioenzymatic assay[3-5]. Renal plasma flow was estimated from the steady-state clearance of infused PAH corrected for the renal extraction. The brains were homogenized with perchloric acid 0.1 mol · L^{-1} and 2.5 mL of the supernant was quantitated by HPLC with electrochemical detection for gludopa, levodopa, NE, and DA contents in whole brain[3,4,8].

Renal NE spillover rate was calculated according to the Fick Principle corrected for the fractional extraction of [^{3}H]NE across the kidney while total body NE spillover rate and total NE clearance were calculated based on arterial sampling using the following equations[4,5]:

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

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

Total NE clearance rate = [^{3}H]NE infusion rate/arterial 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.

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 ANOVA followed by Fisher's protected least significance difference (PLSD) when appropriate. All data were presented as $\bar{x} \pm s$.

RESULTS

In both groups gludopa was detected in the whole brain collected at the end of graded gludopa infusions, but levodopa, NE, and DA contents were not significantly different from those in the control group (Tab 1).

Tab 1. **Gludopa and its metabolites (ng/g wet brain tissue) at the end of iv infusion of gludopa 25 and 100 μg · kg^{-1} · min^{-1} with/without prior dopamine-2 receptor blockade by YM-09151-2 (50 μg · kg^{-1} iv) in rabbits. $n=5$, $\bar{x}\pm s$. $^{a}P>0.05$ vs control group.**

Group	Control	Gludopa	YM+Gludopa
Gludopa	—	92±112	89±123
Levodopa	95±45	108±36[a]	116±25[a]
Dopamine	567±314	500±244[a]	492±258[a]
Norepinephrine	230±94	220±84[a]	210±56[a]

MAP and HR remained unaltered during gludopa infusions in both groups of rabbits. Gludopa infusion at 25 and 100 μg · kg^{-1} · min^{-1} resulted in pronounced dose-related falls in arterial NE concentration, renal and total NE spillover. Total clearance of NE from plasma and renal extraction of [^{3}H]NE was unchanged. Renal plasma flow increased with gludopa infusion. With prior dopamie D_2 receptor blockade by YM-09151-2, gludopa-induced renal and total NE spillover was not much affected. Renal plasma flow was similarly increased (Tab 2).

Tab 2. **Hemodynamic variables and [^{3}H]NE kinetics during iv infusion of gludopa 25 and 100 μg·kg^{-1}·min^{-1} with/without prior dopamine 2 receptor blockade by YM-09151-2 (50 μg·kg^{-1} iv) in conscious rabbits. $n=8$, $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ vs saline, $^{d}P>0.05$ vs gludopa.**

		Saline 0.1 mL·min^{-1}	Gludopa 25 μg·kg^{-1}·min^{-1}	Gludopa 100 μg·kg^{-1}·min^{-1}
Mean arterial pressure	/kPa			
Gludopa		11.1±0.7	10.2±0.7[a]	10.0±0.6[a]
YM+Gludopa		10.4±0.6[d]	10.8±0.4[ad]	9.9±0.7[ad]
Heart rate	/bpm			
Gludopa		260±25	255±21[a]	262±24[a]
YM+Gludopa		252±28[d]	249±26[ad]	257±25[ad]
Renal plasma flow	/mL·min^{-1}			
Gludopa		71±13	83±16[b]	97±19[c]
YM+Gludopa		68±14[d]	78±15[cd]	103±20[cd]
Arterial norepinephrine	/ng·mL^{-1}			
Gludopa		254±116	108±31[c]	47±29[c]
YM+Gludopa		263±104[d]	114±39[cd]	56±22[cd]
Renal venous norepinephrine	/ng·mL^{-1}			
Gludopa		397±188	155±76[c]	98±51[c]
YM+Gludopa		386±161[d]	164±68[cd]	105±49[cd]
Renal [^{3}H]NE extraction				
Gludopa		0.51±0.04	0.47±0.07[a]	0.45±0.05[a]
YM+Gludopa		0.49±0.03[d]	0.41±0.05[ad]	0.47±0.06[ad]
Renal norepinephrine spillover	/ng·min^{-1}			
Gludopa		22±10	11±8[c]	7±8[c]
YM+Gludopa		25±12[d]	13±7[cd]	5±4[cd]
Total norepinephrine spillover	/ng·min^{-1}			
Gludopa		43±29	20±10[c]	9±5[c]
YM+Gludopa		47±27[d]	26±11[cd]	8±5[cd]
Total norepinephrine clearance	/mL·min^{-1}			
Gludopa		231±56	224±45[a]	219±48[a]
YM+Gludopa		220±47[d]	213±39[ad]	233±51[ad]

DISCUSSION

Changes in mean arterial pressure, heart rate and renal plasma flow after gludopa iv infusion were similar to those previously reported in animals and man[1-4,9]. A study of the tissue distribution of gludopa in rats demonstrated its high renal selectivity, with renal DA content at least six orders of magnitude higher than endogenous values[1]. But there have been no previous reports on whether this agent can permeate through the blood-brain barrier. Our results, that tissue gludopa in the whole brain was significantly increased following gludopa infusion, suggest for the first time that gludopa itself can pass across the blood-brain barrier and possibly exert some central effects.

Reduction in arterial NE concentration, total and renal NE spillover rates after gludopa infusion confirmed sympatho-inhibitory property of gludopa in conscious rabbits[4], which is also in keeping with gludopa-induced decrease in plasma renin activity in man[9]. No significant change in gludopa-induced fall in total and renal NE spillover after dopamine-2 blockade by YM-09151-2 (a potent benzamide neuroleptic with a high affinity for central D-2 and peripheral DA-2 receptors[10,11]) in the present study suggested that dopamine D_2 receptors played little role in the inhibition of NE release after gludopa. The present study is consistent with previous result of Lokhandwala *et al.*[12], who showed that renal presynptic dopamine D_2 receptors were not physiologically functional in the rat.

Our previous results showed significant increase in circulating levodopa following gludopa administration without parallel rise of DA concentration[4]. Direct treatment with levodopa failed to affect endogenous NE overflow in the isolated canine saphenous vein[13]. When given peripherally, levodopa was sho to produce an increase in regional brain and DA content[13,14] and reduce direct recorded efferent sympathetic nervous o put[15]. Significant amount of gludopa detec in the brain might serve as a precursor levodopa, DA and NE in the central nervo system and suppress sympathetic nervo activity centrally. In tne present study, m surement of tissue levodopa, NE and DA c tent in whole brain represented only an aver ing of all regions, with the greater contri tion from brainstem and hypothalamus[There may be some differences in regional take of the precursor and activities of synth ic/degradative enzymes in the brain. The fore, further studies employing regional ana sis of transmitter turnover and microdialy technique are warranted to clarify mechanis of sympatho-inhibitory effects of gludopa.

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BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; **16** (3): 197—200

Pharmacokinetics of 4-[4″-(2″,2″,6″,6″-tetramethyl-1″-piperidinyloxy)amino]-4′-demethylepipodophyllotoxin in mice bearing sarcoma 180

JIA Zheng-Ping, XIE Jing-Wen, XIE Ting-Quan (*Department of Pharmacy, Lanzhou General Hospital, Lanzhou Command, PLA, Lanzhou 730050, China*)

AIM: To study the pharmacokinetics of 4-[4″-(2″,2″,6″,6″-tetramethyl-1″-piperindyloxy) amino]-4′-demethylepipodophyllotoxin (GP-7) in mice bearing sarcoma 180. **METHOD**: Using HPLC with a uv detector at 285 nm. **RESULTS**: The plasma concentration-time course of GP-7 in mice was best fitted to a 2-compartment open modle after iv 20, 60 mg·kg^{-1}. At both doses the plasma $T_{\frac{1}{2}\beta}$ was around 40 min. The highest concentration was found in liver and lung. The level of GP-7 was higher in tumor than in kidney, spleen, and bone marrow after ip 20 mg·kg^{-1} for 10 d. Urinary excretion of GP-7 as unchanged drug accounted for about 20 % of the administered doses 72 h after injection. **CONCLUSION**: GP-7 disappeared more slowly from the plasma of mice bearing sarcoma 180, distributed extensively over the tissues and was partially excreted from urine. The concentrition of GP-7 in tumor was higher.

KEY WORDS podophyllotoxin; pharmacokinetics; sarcoma 180

4-[4″-(2″, 2″, 6″, 6″-Tetramethyl-1″-piperidinyloxy) amino]-4′-demethylepipodophyllotoxin (GP-7) is a new spin-labeled podophyllotoxin derivative synthesized by Lanzhou University, China[1]. In our previous

Received 1993-06-07 Accepted 1994-08-31