ORIGINAL

L-DOPA inhibits nitric oxide-dependent vasorelaxation via production of reactive oxygen species in rat aorta

Yinhua[1], Nagakatsu Harada[2], Kazuaki Mawatari[2], Sonoko Yasui[2], Hiroko Segawa[3], Akira Takahashi[2], Shuzo Oshita[1], and Yutaka Nakaya[2]

[1]*Department of Anesthesiology,* [2]*Department of Nutrition and Metabolism, and* [3]*Department of Molecular Nutrition, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan*

Abstract : *Objectives* : To clarify the underlying mechanisms of L-DOPA induced vasoconstriction in rat aorta. *Methods* : The effect of L-DOPA on phenylephrine-induced contractile force of blood vessels was examined *in vitro* using rat aortic ring preparations by isometric tension experiment. Involvement of nitric oxide (NO) in the effect of L-DOPA on vascular smooth muscle was studied by using N_{ω}-Nitro-L-arginine (L-NNA), Sodium nitroprusside (SNP) in endothelium-intact and endothelium-denuded aortic rings. *Results* : L-DOPA potentiated α-adrenergic receptor- and depolarization-induced vascular contraction and inhibited acetylcholine-induced vasorelaxation. This effect was diminished by pretreatment of the aortic rings with L-NNA, an inhibitor of NO synthesis, or by removing the endothelium from the ring preparations. In endothelium-denuded rings, L-DOPA inhibited exogenous NO-dependent but not cGMP-mediated vasorelaxation. Increases in cGMP levels in response to an NO donor were attenuated by L-DOPA in cultured rat aortic smooth muscle cells. L-DOPA could not contract rings (without endothelium) pretreated with 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1), an activator of guanylyl cyclase, but SOD (150 U/ml) pretreatment of rings with endothelium inhibited contraction by L-DOPA. *Conclusions* : These results suggest that L-DOPA inhibits nitric-dependent vasorelaxation on vascular smooth muscle cells via production of reactive oxygen species. J. Med. Invest. 56 : 120-129, August, 2009

Keywords **:** *L-DOPA, aortic rings, nitric oxide, vasorelaxation, reactive oxygen species*

INTRODUCTION

L-DOPA (L-3,4-dihydroxyphenylalanine) is a widely used drug for the treatment of Parkinson's disease (PD) (1, 2). Many studies showed that some doses of L-DOPA caused hypertensive response in human and laboratory animals. Early study reported that injection of L-DOPA could cause the cat renal hypertension (3), and that conscious rats orally administered L-DOPA following parenteral administration (4) and intraperitoneal administration of L-DOPA showed hypertensive responses (5). Intravenous L-DOPA evoked some tachycardia (2) and larger rises in blood pressure in human (6). However, these studies did not show precise mechanism of L-DOPA-induced hypertensive response. It has been known that tension in aortic vascular smooth muscle induced by either α-adrenergic agonists or

Received for publication February 9, 2009 ; accepted March 4, 2009.

Address correspondence and reprint requests to Yinhua, Department of Anesthesiology, the University of Tokushima Graduate School, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-088-633-7182.

passive external stretch is related to NO release from the endothelium (7-9), On the other hand, phenylephrine induces NO release by α_2 receptor in endothelium cells (10), in addition to direct contraction effect on smooth muscle. These NO-releasing systems of the vasculature is considered to function as a kind of self-protecting mechanism against excessive force generation by vascular smooth muscle cells (7). However, whether hypertensive effect of L-DOPA is related with NO signaling pathway is unknown. In this study, we investigated the mechanism of effect of L-DOPA on phenylephrine-induced contractile force of blood vessels *in vitro* by using several pharmacological blockers The results indicate that the effect of L-DOPA on contractile force of blood vessels was mediated by NO signaling pathway via production of reactive oxygen species.

METHODS

Agents

Phenylephrine(phe), acetylcholine(Ach), sodium nitroprusside (SNP), S-nitroso-N-acetyl-penicillamine (SNAP), L-3,4-dihydroxyphenylalanine(L-DOPA), N_{ω}-Nitro-L-arginine (L-NNA), 8-(4-chlorophenylthio) guanosine 3' 5'-cyclic monophosphate (cpt-cGMP) , 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1), superoxide dismutase (SOD) and indomethacin were purchased from Sigma (St. Louis, MO, USA).

Preparation of aortic rings and tension measurement

All animal procedures were in accordance with the institutional guidelines for the care and use of laboratory animals of Tokushima University. Male Wistar rats (Japan SLC, Shizuoka, Japan) weighing 150-250 g were anesthetized by an intraperitoneal injection of pentobarbital. The thoracic aortas were dissected free of connective tissue and cut into ring segments, 3- to 4- mm in length. In some experiments, the aortic endothelium was removed by inserting a cotton thread into the lumen followed by gentle rubbing. Each ring was then placed in a 3 ml organ bath (Micro Easy Magnus, Kishimoto Medical ; Kyoto, Japan) and mounted on two stainless steel wires, one of which was fastened to the bath and the other connected to a force transducer for the measurement of isometric tension. The bath was filled with Krebs-Ringer bicarbonate buffer (KRB) solution at 37°C and bubbled with a mixture of 95% O_2-5% CO_2. The KRB contained (in mmol/L) 118 NaCl, 4.6 KCl, 2.5 $CaCl_2$, 24.8 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, and 5.6 glucose. The ring was equilibrated for 60 mins under a resting tension of 1.5 g and the solution was changed at 30 min intervals. The presence or absence of endothelium was confirmed by the addition of the endothelium-dependent vasodilator, acetylcholine (10^{-6} M), after contractions had been induced by phenylephrine (α_1-adrenergic receptor agonist) (10^{-6} M). Following washout of phenylephrine and acetylcholine with KRB, the aortic rings were contracted by adding a solution to the bath with a high K^+ concentration (50 mM KCl). After recording the contractile force of each ring in response to high K^+, the KCl was washed out with KRB. Each ring was then allowed to equilibrate at a resting tension of 1.5 g for 20 min. The contractile response in the test experiments described below was expressed relative to that measured for the KCl-induced contraction (1^{st} KCl) (11, 12).

Preparation of rat smooth muscle cells

Isolation of vascular smooth muscle cells from rats was conducted as described previously (13). Briefly, male Wistar rats aged 8 to 10 weeks were anesthetized with ether, and 1.0 U/g heparin was injected into the peritoneal cavity 30 min before surgery. Aortas were dissected and longitudinally opened in the longitudinal direction, and endothelium and adventitia were removed. The tissue was then minced into small pieces in normal Tyrode's solution. The pieces were then explanted on glass coverslips in tissue culture dishes filled with medium 199 (Nissui Chemicals, Tokyo, Japan), supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY), 100 μg/ml streptomycin, and 100 μg/ml penicillin and stored in a carbon dioxide incubator (5% CO_2, 37°C). Single smooth muscle cells migrated out of the tissues and adhered to the coverslips within a few days. After culturing for 6-10 days, they were used in the experiments described below.

Effect of L-DOPA on aortic ring contraction

Test experiments involving L-DOPA were conducted on aortic rings in which contraction was induced by either phenylephrine (10^{-9}-$10^{-4.5}$ M) or KCl (30, 50, or 80 mM). L-DOPA (10^{-7} to $10^{-4.5}$ M) was added to the incubation system either 20 min prior to or during the contraction induced by each constrictor. Vehicle (water)-treated rings were used as controls.

NO synthesis Inhibitor treatment of aortic rings

In some experiments, endothelium-derived nitric oxide (NO) or prostaglandins were blocked by incubating aortic rings with L-NNA (3×10^{-5} M) or indomethacin (10^{-5} M) to inhibit NO or prostaglandin synthesis, respectively. The aortic rings were pretreated with each inhibitor for 20 min and then contraction was induced by phenylephrine or KCl. Each inhibitor was present in the incubation system during contraction.

Determination of L-DOPA-inhibited endothelium-dependent aortic relaxation

In the experiment of effect of SNP, cpt-cGMP or acetylcholine, SNP (NO donor ; 10^{-11}-10^{-6} M) or cpt-cGMP (10^{-9}-10^{-4} M) were added to the bath during the contraction induced by phenylephrine (10^{-5} M) or KCl (50 mM). In other experiments, acetylcholine (10^{-6} M)-induced vasorelaxation was assessed in aortic rings that had been contracted by phenylephrine (10^{-5} M).

Effect of YC-1 on L-DOPA-inhibited relaxation of aortic rings

Aortic rings without endothelium pretreated with L-DOPA (10^{-5} M) for 20 mins and then induced contraction by phenylephrine (10^{-5} M). YC-1(0.1-100 uM) per 10 mins added into the bath with the subsequent contraction by phenylephrine. Vehicle (water)-treated rings were used as controls.

Effect of SOD on L-DOPA-inhibited endothelium-dependent relaxation of aortic rings

Aortic rings with endothelium were pretreated with SOD (150 U/ml) for 20 mins and then induced contraction by phenylephrine (10^{-5} M). L-DOPA (10^{-7}-$10^{-4.5}$ M) were added to the bath after induced contraction. Vehicle (water)-treated rings were used as controls.

Measurement of intracellular cGMP

The rat smooth muscle cells cultured in a 35 mm dish were treated with or without L-DOPA (10^{-5} M) in the Krebs-Ringer Hepes buffer solution (containing 125 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 2.6 mM $MgSO_4$, and 5 mM Hepes, pH 7.4) for 5 min at 37°C. Cells were then stimulated with SNAP (1, 10, or 100 M) for 5 mins. Following washing two times with ice-cold phosphate-buffered saline, intracellular cGMP was extracted with 1 ml of HCl (0.1 M) under shaking for 20 minutes at room temperature. The extracted solution was centrifuged at 80× g for 5 minutes. The concentration of cGMP in the supernatant was measured with a cGMP EIA kit (Cayman Chemical, Michigan, USA) according to the manufacturer's protocol. The concentration of intracellular cGMP was expressed as pmol per mg protein.

Statistical analysis

Data are expressed as the mean±S.D. and were analyzed by ANOVA plus Bonferroni multiple comparison tests. A P-value<0.05 was regarded as statistically significant.

RESULTS

L-DOPA potentiated phenylephrine-induced contraction through endothelium- and NO-dependent pathways

Firstly, we examined the effect of L-DOPA on vascular contraction using rat aortic rings precontracted by phenylephrine (Fig. 1-a). Fig. 1-b showed a trace demonstrating the effect of L-DOPA on rat aortic rings pretreated with phenylephrine. As shown in Fig. 1-c, administration of L-DOPA alone to the rings without phenylephrine (vehicle group) did not induce contraction. However, when the rings were precontracted by phenylephrine, subsequent administration of L-DOPA potentiated contraction in a dose-dependent manner. After washed with KRB, the contractile effect of the rings caused by L-DOPA disappeared and L-DOPA retreatment restored rat aortic ring contraction to phenylephrine precontraction state. This effect of L-DOPA was not affected by pretreatment of the rings with indomethacin (10^{-5} M), an inhibitor of prostaglandin synthesis (Fig. 1-c). In contrast, pretreatment of the rings with L-NNA (3×10^{-5} M), an inhibitor of NO synthesis, or the removal of endothelium from the rings (Fig. 1-d) significantly inhibited the L-DOPA-mediated increase in contractile force. Dopamine and its metabolite, DOPAC (L-3,4-dihydroxyphenylacetic-acid), failed to enhance the phenylephrine-induced aortic contraction when these substances were used at concentrations up to 10^{-5} M (data not shown).

To examine the effect of L-DOPA pretreatment on subsequent phenylephrine-induced contraction, L-DOPA (10^{-5} M) was administrated 20 min prior to phenylephrine (10^{-9}-$10^{-4.5}$ M) (Fig. 2-a). As shown in Fig. 2-b, L-DOPA pretreatment potentiated the subsequent phenylephrine-induced contraction in rings

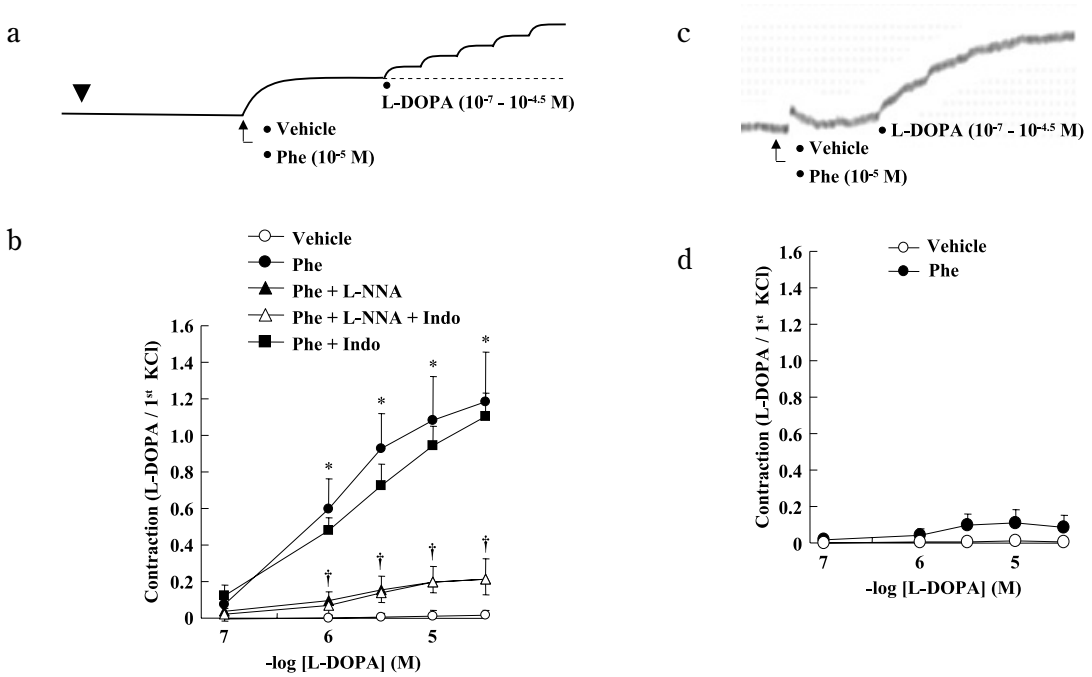

Fig. 1 L-DOPA potentiated phenylephrine-induced contraction of rat aortic rings through endothelium- and NO- dependent pathways (I). (a) Experimental design. L-DOPA (10^{-7} -$10^{-4.5}$ M) was administrated cumulatively to enthothelium-intact/-denuded aortic rings which had been pretreated with or without (vehicle) phenylephrine (10^{-5} M). In some groups, L-NNA (3×10^{-5} M) and/or indomethacin (Indo ; 10^{-5} M) were administrated to the aortic rings 20 min prior to phenylephrine (or vehicle) administration (inverted triangle). (b) Trace demonstrating the effect of L-DOPA on enthothelium-intact rat aortic rings pretreated with phenylephrine. (c) Concentration-response curves for L-DOPA-induced change in the contractile force of endothelium-intact aortic rings. (d) Concentration-response curves for L-DOPA-induced change in the contractile force of endothelium-denuded aortic rings. Data are expressed as means ± S.D. for 4 aortic rings in each group. * $p < 0.05$ compared with vehicle group (open circles). † $p < 0.05$ compared with Phenylephrine group (closed circles).

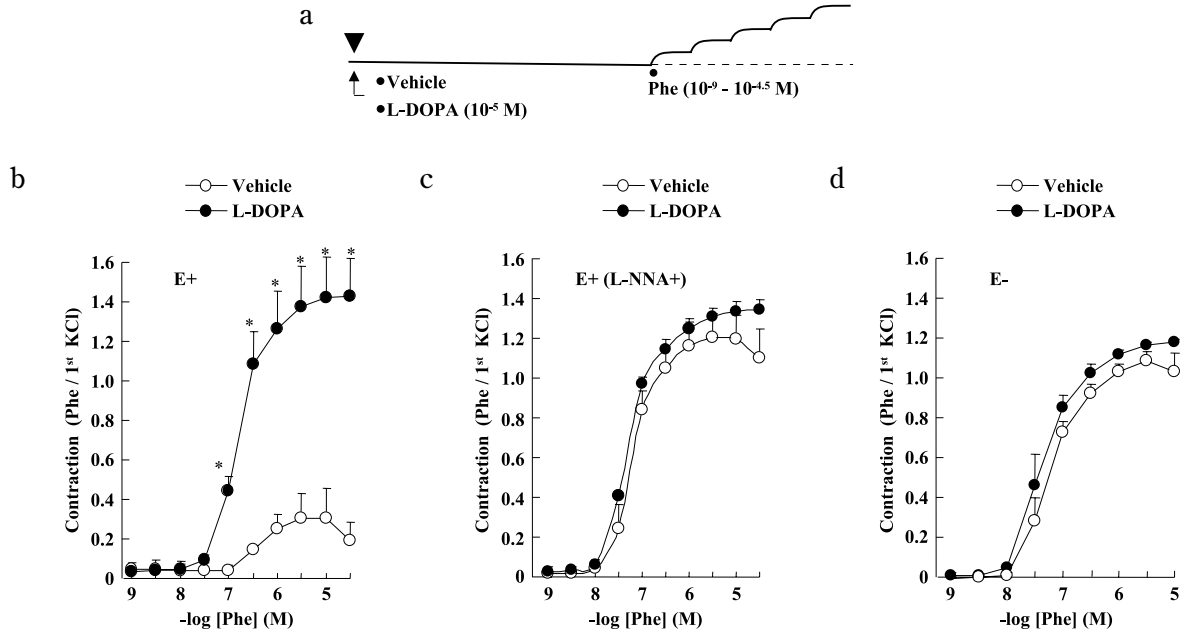

Fig. 2 L-DOPA potentiated phenylephrine-induced contraction of rat aortic rings through endothelium- and NO- dependent pathways (II). (a) Experimental design. Phenylephrine (Phe : 10^{-9} -$10^{-4.5}$ M) was administrated cumulatively to endothelium-intact/denuded rat aortic rings which had been pretreated with or without (vehicle) L-DOPA (10^{-5} M). In some groups (panel c), L-NNA (3×10^{-5} M) was administrated to the aortic rings together with L-DOPA (or vehicle) (inverted triangle). (b) Concentration-response curves for phenylephrine-induced changes in contractile force of endothelium-intact (E+) aortic rings. (c) Concentration-response curves for phenylephrine-induced change in contractile force of endothelium-intact (E+) aortic rings pretreated with L-NNA. (d) Concentration-response curves for phenylephrine-induced changes in contractile force of endothelium-denuded (E-) aortic rings. There are no difference between experimental group and control in (c) and (d). Data are expressed as means ± S.D. for 4 aortic rings in each group. * $p < 0.05$ compared with vehicle group (open circles).

with intact endothelium. It is well known that the blockade of endothelium-derived NO or removal of endothelium itself induces hypercontractility of blood vessels (11). L-DOPA pretreatment did not further increase contractile force in these NO-blocked and endothelium-denuded aortic rings (Fig. 2c and d). These results suggest that L-DOPA potentiated phenylephrine-induced aortic contraction through endothelium- and NO- dependent pathways.

We also examined the effect of L-DOPA on depolarization-induced contraction of rat aortic rings precontracted by KCl (50 mM). Administration of L-DOPA during KCl-induced contraction increased contractile force in a dose-dependent manner. This effect of L-DOPA was almost completely inhibited by pretreatment of the rings with L-NNA (3×10^{-5} M) as well as in the rings without endothelium (data not shown).

L-DOPA inhibited endothelium-dependent relaxation of the aorta

Next, we tested whether potentiation of L-DOPA is due to inhibition of endothelium-dependent relaxation by NO signal. The effect of L-DOPA on endothelium-dependent vasorelaxation was examined using aortic rings which had been precontracted by phenylephrine (Fig. 3-a). As shown in Fig. 3-b, acetylcholine (10^{-6} M) almost completely relaxed the tension in aortic ring with intact endothelium.

Since acetylcholine-induced vasorelaxation depends largely on endothelium-derived NO in conduit arteries (14), this relaxation was inhibited by pretreatment with L-NNA (Fig. 3-c). L-DOPA pretreatment also inhibited acetylcholine-induced vasorelaxation to the same extent as that observed in the L-NNA-treated rings (Fig. 3-c).

L-DOPA inhibited exogenous NO-dependent but not cGMP-mediated vasorelaxation

NO activates guanylate cyclase which in turn increases the concentration of intracellular cGMP in the smooth muscle cells (15). The rise of cGMP levels triggers subsequent vasorelaxation (15). To further study the mechanism of L-DOPA, we tested the effect of L-DOPA on cGMP induced vasorelaxation using the membrane-permeable cGMP analog, cpt-cGMP. Endothelium-denuded aortic rings were first precontracted by phenylephrine or KCl (Fig.

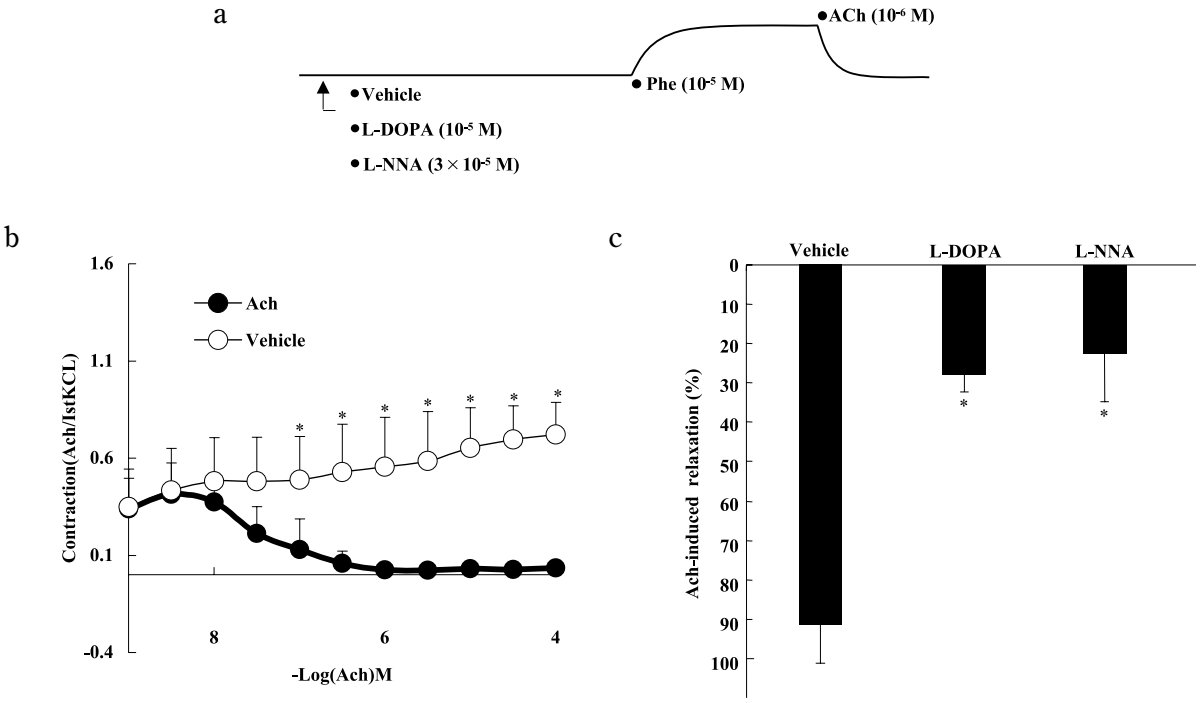

Fig. 3 L-DOPA inhibited acetylcholine-induced vasorelaxation in rat aortic rings. (a) Experimental design. Acetylcholine (ACh) (10^{-6} M) was administrated to endothelium-intact aortic rings that had been precontracted by phenylephrine (Phe ; 10^{-5} M). L-DOPA (10^{-5} M), L-NNA (3×10^{-5} M) or vehicle was administrated to the endothelium-intact aortic rings 20 min prior to phenylephrine administration. (b) Acetylcholine concentration induced maximum relaxation in endothelium-intact ring. Data are expressed as means ± S. D. for 4 aortic rings in each group. * $p<0.05$ compared with vehicle group. (c) Acetylcholine-induced maximum relaxation in each endothelium-intact ring.

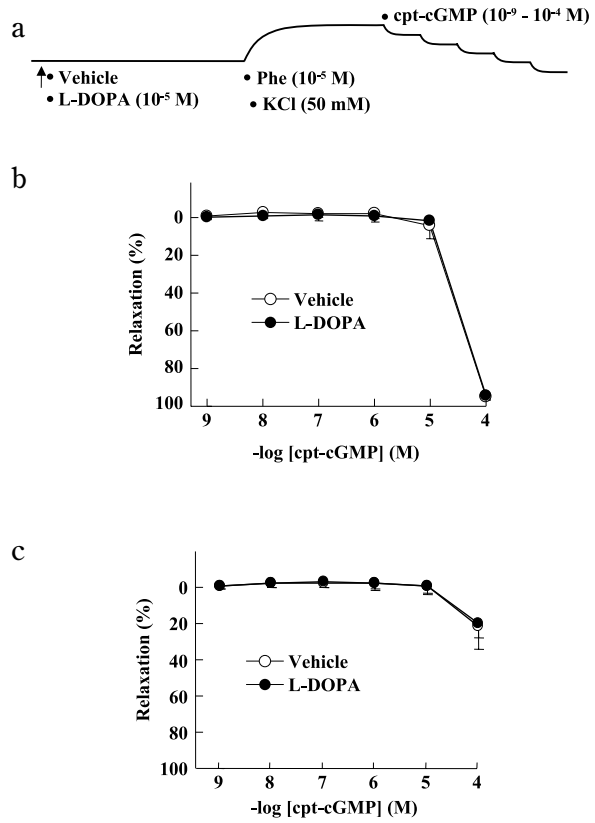

Fig. 4 L-DOPA did not attenuated exogenous cGMP-mediated vasorelaxation. (a) Experimental design. cpt-cGMP (10^{-9}-10^{-4} M) was administrated cumulatively to endothelium-denuded aortic rings that had been precontracted by phenylephrine (Phe ; 10^{-5} M, panel b) or KCl (50 mM, panel c). L-DOPA (10^{-5} M) or vehicle was administrated to the aortic rings 20 min prior to phenylephrine or KCl administration. (b) Concentration-response curves for phenylephrine or (c) KCl-induced change in contractile force of endothelium-denuded aortic rings. Data are expressed as means ± S.D. for 4 aortic rings in each group. $p > 0.05$ compared with vehicle group (open circles).

4-a), and after the contraction reached a steady level, subsequent relaxing responses induced by cpt-cGMP (10^{-9}-10^{-4} M) were assessed in the presence or absence of L-DOPA (10^{-5} M). L-DOPA pretreatment failed to affect cpt-cGMP-mediated vasorelaxation in the rings contracted with phenylephrine (Fig. 4-b) or KCl- (Fig. 4-c).

L-DOPA-inhibited vasorelaxation was not soluble guanylyl cyclase-dependent

Soluble guanylyl cyclase (sGC) expressed by endothelium of blood vessel is considered the key enzyme mediating vascular relaxation induced by NO. By the formation of cyclic GMP, this enzyme mediates NO-mediated vascular smooth muscle relaxation (16, 17). In order to probe whether L-DOPA-inhibited vasorelaxation was caused by blocking the action of soluble guanylyl cyclase, effect of YC-1 (an activator of guanylyl cyclase) (18) on the aortic ring contraction was examined. In aortic rings without endothelium pretreatmented with L-DOPA (10^{-5} M), YC-1was added into the bath and subsequent contraction effect of rings by phenylephrine was studied (Fig. 5-a). YC-1 (0.1 μM-100 μM) could relax the rings precontracted by phenylephrine in dose-dependent manner. There was no significant difference in relaxation between the ring with and without pretreatment by L-DOPA (Fig. 5-b), suggesting that solube guanylyl cyclase is not a target of L-DOPA-inhibited vasorelaxation. Similar effect was observed in the experiment using atrial natriuretic peptide, an activator of membrane guanylyl cyclases (data not shown).

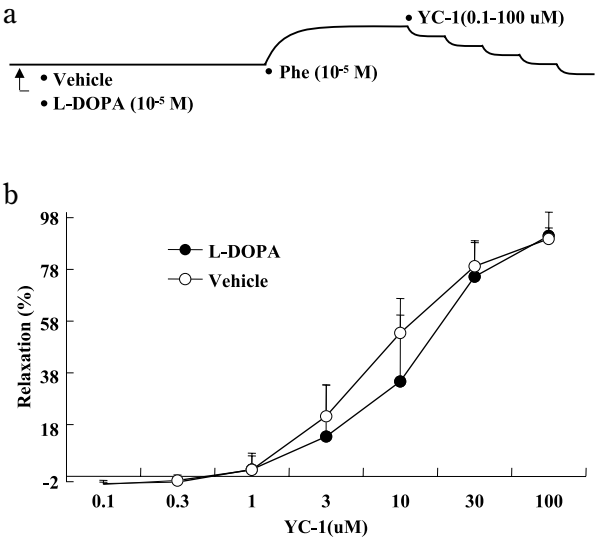

Fig. 5 Effects of YC-1 on tension of rat aortic rings (a) Experimental design. Aortic rings without endothelium pretreatmented with L-DOPA (10^{-5} M) for 20 mins YC-1 (0.1-100 uM) per 10 mins were added into the bath with the subsequent precontraction of rings by pheylephrine (10^{-5} M). Vehicle(water)-treated rings were used as control. (b) Concentration-response curves for YC-1-induced L-DOPA-mediated relaxation of rings without endothelium of YC-1 treatment did not show significant difference compared with that of rings of L-DOPA no-pretreatment. Data are expressed as means ± S.D. for 4 aortic rings in each group. $P > 0.1$

L-DOPA attenuates NO-donor-induced cGMP production in cultured rat aortic smooth muscle cells and inhibits acetylcholine-induced relaxation

To examine whether L-DOPA affects cGMP production by NO, cultured rat aortic smooth muscle cells were stimulated with a donor of NO, SNAP (1-100 μM), and intracellular cGMP levels were measured in the presence or absence of L-DOPA (10^{-5}

M). As shown in Fig. 6, L-DOPA treatment inhibited cGMP production at higher SNAP concentrations. We also tested effect of L-DOPA of exogenous

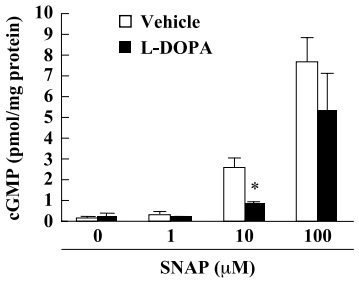

Fig. 6 L-DOPA attenuated NO-donor-induced cGMP production in cultured rat aortic smooth muscle cells. Cells were treated with or without (vehicle) L-DOPA (10^{-5} M) for 5 min and then stimulated with SNAP (1, 10, or 100 μM) for 5 min. After washing two times with ice-cold phosphate-buffered saline, intracellular cGMP was extracted and quantified as described in "Materials and Methods" section. Data are expressed as means ± S.D. for 4 culture dishes (35 mm dishes) in each group. * $p < 0.05$ compared with vehicle group.

NO on SNP-induced relaxation. The experimental design was showed by Fig. 7-a Namely the L-DOPA-Pretreatmented endothelium-denuded aortic rings were precontreated by phenylephrine or KCl and then added SNP (NO doner) to observe L-DOPA attenuated effect on exogenous NO-dependent Vasorelaxtion the results showed that L-DOPA pretreatment significantly attenuated vasorelaxation responses induced by SNP (10^{-11}-10^{-6} M) (Figs. 7-b and 7-c).

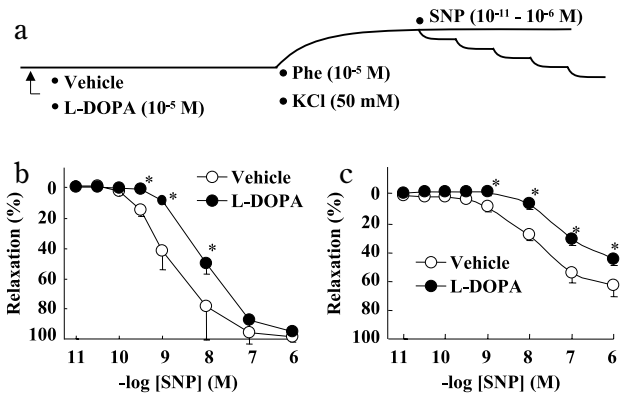

Fig. 7 L-DOPA attenuated exogenous NO-dependent vasorelaxation. (a) Experimental design. SNP (10^{-11}-10^{-6} M) was administrated cumulatively to endothelium-denuded aortic rings that had been precontracted by phenylephrine (10^{-5} M) or KCl (50 mM). L-DOPA (10^{-5} M) or vehicle was administrated to the aortic rings 20 min prior to phenylephrine or KCl administration. (b) Concentration-response curves for phenylephrine or (c) KCl-induced change in contractile force of endothelium-denuded aortic rings. Data are expressed as means ± S.D. for 4 aortic rings in each group. * $p < 0.05$ compared with vehicle group (open circles).

L-DOPA mediated inhibition of relaxation is due to inactivation of NO by oxygen free radicals

Increased production of reactive oxygen species (ROS) reduces the effect and/or bioavailability of NO, leading to an impaired endothelial function. Through auto-oxidation, L-DOPA forms reactive free radicals such as hydroxyl radicals, oxygen free radicals. Oxygen free radicals (O_2^-) can decrease ring relaxation by inactivation of NO (19, 20). In this experiment we used SOD to decrease O_2^- generation in order to further confirm NO effect in L-DOPA-mediated contraction. Aortic rings with endothelium were pretreated with SOD (150 u/ml) for 20 mins. L-DOPA (10^{-7}-$10^{-4.5}$ M) were added to the bath with the subsequent contraction by phenylephrine (10^{-5} M) (Fig. 8-a). Fig. 8-b showed that SOD (150 U/ml) pretreatment inhibited contraction by L-DOPA of rings with endothelium.

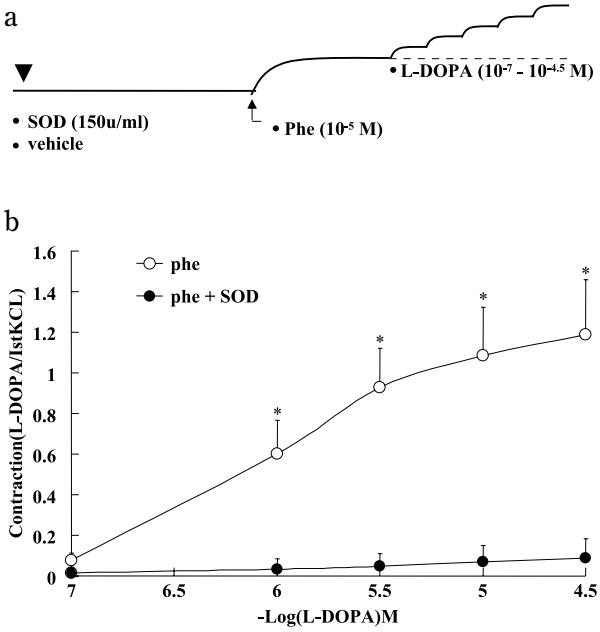

Fig. 8 Effect of SOD on L-DOPA-mediated contraction of aortic rings. (a) Experimental design. Aortic rings with endothelium were pretreated with SOD (150 u/ml) for 20 mins. L-DOPA (10^{-7}-$10^{-4.5}$ M) were added to the bath with the subsequent contraction by phenylephrine (10^{-5} M). Vehicle (water)-treated rings were used as controls. (b) Contractile response of endothelium- intact aortic rings pretreated by SOD in the condition of various L-DOPA concentration. Data are expressed as means ± S.D. for 4 aortic rings in each group, * $p < 0.01$.

DISCUSSION

Our present studies indicated that L-DOPA

increased the α-adrenergic receptor-mediated and depolarization-induced vascular contraction and decreased ACh-induced vascular relaxation in endothelium-intact rat aorta, which was inhibited by L-NNA pretreatment. L-DOPA attenuated the rise in the intracellular cGMP and inhibited vasorelaxation with an NO donor, but not those with cpt-cGMP, a membrane-permeable cGMP analog, or YC-1, an activator of guanylyl cyclase, suggesting that action of L-DOPA might be upstream of guanylyl cyclase. SOD (150 U/ml) pretreatment inhibited contraction by L-DOPA of rings with endothelium, suggesting that production of reactive oxygen species by L-DOPA decreases the NO action. All these data demonstrated that the underlying mechanism of vasocontractive effect of L-DOPA was inhibition of NO-dependent vasorelaxation via production of reactive oxygen species from L-DOPA.

It is well know that nitric oxide (NO) is the major mediator of endothelium-dependent relaxation in aorta (15, 21, 22), which is formed from the conversion of L-arginine by nitric oxide synthase (NOS) in endothelium cell and released. This endothelium-derived NO stimulates the activity of soluble guanylate cyclase (sGC) in smooth muscle cells, leading to an increase in cyclic guanosine-3',5'- monophosphate (cGMP) and finally to calcium depletion from the cytosolic space and vascular smooth muscle relaxation (15, 21-23). Various agonists such as NE (noradrenaline), phenylephrine, 5HT can stimulate the release of NO from the endothelium during vasocontractile response to them (9). In our present study, we found that L-DOPA increased the phenylephrine (α_1-adrenergic receptor)-mediated and depolarization-induced vascular contraction in endothelium-intact rat aorta, which was inhibited by L-NNA pretreatment, suggesting this reaction is mediated by NO pathway. Meanwhile, we also found that L-DOPA pretreatment inhibited acetylcholine-induced vasorelaxation. The acetylcholine-induced vasorelaxation is largely mediated by endothelium-derived NO in conduit arteris (9). Effect of NO is mainly mediated by production of cGMP. Thus, we studied the effect of L-DOPA on cGMP. L-DOPA could not reduced relaxation by cGMP, such as membrane permeable cGMP (cpt-cGMP), or soluble guanylate cyclase activator (YC-1), suggesting that the intracellular signaling pathway downstream from cGMP would not contribute this mechanism. Nakatsubo *et al.* also found that L-DOPA decreased fluorescence levels of diaminofluorescein-2 (DAF-2), a widely used indicator of NO (24). In our preliminary study, L-DOPA decreased nitroprusside-induced fluorescence levels of DAF-2 in the presence of an NO donor in cell-free system (data not shown). This hypothesis is also supported by our data that SOD pretreatment of rings with endothelium induced the subsequent ring relaxation.

L-DOPA is molecularly unstable and can rapidly auto-oxidize in the presence of oxygen or light and oxidize enzymatically to yield free radicals : semiquinones, O-quinone derivatives and several free radicals such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-). Rocchitta *et al.* (25) reported that auto-oxidation of exogenous L-DOPA occurs *in vivo* in the extracellular compartment of the freely moving rat with a consequent formation of L-DOPA semiquinone (L-DOPA-SQ) in dialysates. NO reacting with superoxide generates peroxynitrite ($O=NOO^-$) to block NO signaling pathway by inactivating NO (20, 26, 27), leading to decrease of ring relaxation (12, 13). L-DOPA is transported into mammalian cells through the L-type amino acid transporter 1 (LAT1) (13), which is also expressed in vascular endothelium (28). *In vitro* experiments showed that L-DOPA can lead to accumulation of O_2^-, and the addition of SOD resulted in higher levels of NO_2^- (27). *In vivo* experiment also revealed that SOD can completely inhibit L-DOPA auto-oxidation (20) and decrease spontaneous tone in endothelium-intact rings by scavenging O_2^- and thereby freeing endothelium-derived NO to relax the blood vessel (29). Therefore, we concluded that L-DOPA mediated inhibition of relaxation is due to inactivation of NO by oxygen free radicals. We do not know whether L-DOPA could produce vasoconstriction via production of reactive oxygen species *in vivo*, because *in vivo* endogenous antioxidants, such as ascorbic acid or Vitamin E. Further investigation is needed to clarify *in vivo* effect of L-DOPA.

MAO-A/B mixed inhibitors may be efficacious in the inhibition of dopamine metabolism and the treatment of PD (30, 32), but there are some side effects of increasing the blood pressure in the presence of a non-selective monoamine oxidase (MAO)-A/B inhibitor or a selective MAO-A inhibitor, L-DOPA increased blood pressure in both PD patients and rats (4, 33). These results indicate that L-DOPA exerts directly on its vasoconstrictive effect without conversion to dopamine.

Minimizing the side effects of hypertension by L-DOPA treatment would be necessary for the maintenance of physical as well as mental well-being of PD patients. The NO-inhibiting effect of L-DOPA on

the vasculature could be applicable to the establishment of a novel therapeutic strategy for the treatment of cardiovascular disorders in PD patients. The concentration of L-DOPA used in the present study (mainly 10^{-5} M) is near the plasma concentration in L-DOPA-treated PD patients (approximately 0.5-1.0×10^{-5} M) (34). Thus, L-DOPA at this concentration is considered to be enough to inhibit the NO pathway in these patients. From our study, however, in the absence of vasoconstrictors, L-DOPA did not induce contraction of rat aortic rings and the effect of L-DOPA on blood pressure in PD patients was also complex. L-DOPA-mediated inhibition of the NO pathway would result in vasoconstriction when endothelial production of NO was high. However, vasoconstriction due to NO inhibition might be offset by an L-DOPA-mediated sympathetic inhibition. Thus, further studies are needed to clarify the interaction of these different mechanisms on the regulation of the cardiovascular system in PD patients treated with L-DOPA. All together, our experimental results revealed the mechanism about hypertensive effect of L-DOPA and provided a new possibility to the establishment of a novel therapeutic strategy for the treatment of PD patients to avoid side effects (e.g. hypertension) caused by therapy.

REFERENCES

1. Mercuri NB, Bernardi G : The 'magic' of L-dopa : why is it the gold standard Parkinson's disease therapy? Trends Pharmacol Sci 26 : 341-344, 2005
2. Hornykiewicz O : L-DOPA : from a biologically inactive amino acid to a successful therapeutic agent. Amino Acids 23 : 65-70, 2002
3. Bing RJ : The formation of hydroxytyramine by extracts of renal cortex and by perfused kidneys. Am J Physiol 132 : 497-503, 1941
4. Finberg JP, Gross A, Bar-Am O, Friedman R, Loboda Y, Youdim MB : Cardiovascular responses to combined treatment with selective monoamine oxidase type B inhibitors and L-DOPA in the rat. Br J Pharmacol 149 : 647-656, 2006
5. Henning M, Rubenson A : Effects of L-DOPA and structurally related compounds on blood pressure. Acta Pharmacol Toxicol (Copenh) 28 : 50, 1970
6. Oster KA, Sorkin SZ : Effects of intravenous injections of L-dopa upon blood pressure. Proc Soc Exp Biol Med 51 : 67-70, 1942
7. Yoshimura M, Kambara S, Takahashi H, Okabayashi H, Ijichi H : Involvement of dopamine in development of hypertension in spontaneously hypertensive rat : effect of carbidopa, inhibitor of peripheral dopa decarboxylase. Clin Exp Hypertens A 9 : 1585-1599, 1987
8. Nakaki T, Otsuka YY, Kato R : Tension-induced release of endothelium-derived relaxing factor ; possible role in establishment of desensitization of norepinephrine-induced contraction in rat aorta. Jpn J Pharmacol 54 : 491-494, 1990
9. Gürdal H, Can A, Uğur M : The role of nitric oxide synthase in reduced vasocontractile responsiveness induced by prolonged alpha1-adrenergic receptor stimulation in rat thoracic aorta. Br J Pharmacol 145 : 203-210, 2005
10. Vanhoutte PM : Endothelial adrenoceptors. J Cardiovasc Pharmacol 38 : 796-808, 2001
11. Nakamura A, Harada N, Takahashi A, Mawatari K, Nakano M, Tsutsumi K, Nakaya Y : NO-1886, a lipoprotein lipase activator, attenuates vascular smooth muscle contraction in rat aorta. Eur J Pharmacol 554 : 183-190, 2007
12. Harada N, Sakamoto S, Niwa Y, Nakaya Y : Involvement of adenosine in vascular contractile preconditioning. Am J Physiol Heart Circ Physiol 280 : H2911-9, 2001
13. Tanaka K, Kawano T, Nakamura A, Nazari H, Kawahito S, Oshita S, Takahashi A, Nakaya Y : Isoflurane activates sarcolemmal adenosine triphosphate-sensitive potassium channels in vascular smooth muscle cells : a role for protein kinase A. Anesthesiology 106 : 984-991, 2007
14. Woodman OL, Wongsawatkul O, Sobey CG : Contribution of nitric oxide, cyclic GMP and K^+ channels to acetylcholine-induced dilatation of rat conduit and resistance arteries. Clin Exp Pharmacol Physiol 27 : 34-40, 2000
15. Mitchell JA, Ali F, Bailey L, Moreno L, Harrington LS : Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. Exp Physiol 93 : 141-147, 2008
16. Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ : Relationship between cyclic guanosine 3' : 5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide : effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 219 : 181-186, 1981

17. Lin PL, Huang HH, Fan SZ, Tsai MC, Lin CH, Huang CH : Effect of ropivacaine on endothelium-dependent phenylephrine-induced contraction in guinea pig aorta. Acta Anaesthesiol Scand 51 : 1388-1393, 2007
18. Wegener JW, Gath I, Förstermann U, Nawrath H : Activation of soluble guanylyl cyclase by YC-1 in aortic smooth muscle but not in ventricular myocardium from rat. Br J Pharmacol 122 : 1523-1529, 1997
19. Pattison DI, Dean RT, Davies MJ : Oxidation of DNA, proteins and lipids by DOPA, protein-bound DOPA, and related catechol(amine)s. Toxicology 177 : 23-37, 2002
20. Soliman MK, Mazzio E, Soliman KF : Levodopa modulating effects of inducible nitric oxide synthase and reactive oxygen species in glioma cells. Life Sci 72 : 185-198, 2002
21. Ignarro LJ : Nitric Oxide : A Unique Endogenous Signaling Molecule in Vascular Biology. Bioscience Reports 19 : 51-70, 1999
22. Moncada S, Palmer RMJ, Higgs EA : Nitric oxide : physiology, pathophysiology and pharmacology. Pharmacological reviews 43 : 109-142, 1991
23. Ghalayini IF : Nitric oxide-cyclic GMP pathway with some emphasis on cavernosal contractility. Int J Import Res 16, 459-469, 2004
24. Nakatsubo N, Kojima H, Kikuchi K, Nagoshi H, Hirata Y, Maeda D, Imai Y, Irimura T, Nagano T : Direct evidence of nitric oxide production from bovine aortic endothelial cells using new fluorescence indicators : diaminofluoresceins. FEBS Lett 427 : 263-266, 1998
25. Rocchitta G, Migheli R, Esposito G, Marchetti B, Desole MS, Miele E, Serra PA : Endogenous melatonin protects L-DOPA from autoxidation in the striatal extracellular compartment of the freely moving rat : potential implication for long-term L-DOPA therapy in Parkinson's disease. J Pineal Res 40(3) : 204-13, 2006
26. Pryor WA, Houk KN, Foote CS, Fukuto JM, Ignarro LJ, Squadrito GL, Davies KJ : Free radical biology and medicine : it's a gas, man! Am J Physiol Regul Integr Comp Physiol 291 : R 491-511, 2006
27. Uchino H, Kanai Y, Kim DK, Wempe MF, Chairoungdua A, Morimoto E, Anders MW, Endou H : Transport of amino acid-related compounds mediated by L-type amino acid transporter 1 (LAT1) : insights into the mechanisms of substrate recognition. Mol Pharmacol 61 : 729-737, 2002
28. Takabe W, Kanai Y, Chairoungdua A, Shibata N, Toi S, Kobayashi M, Kodama T, Noguchi N : Lysophosphatidylcholine enhances cytokine production of endothelial cells via induction of L-type amino acid transporter 1 and cell surface antigen 4F2. Arterioscler Thromb Vasc Biol 24 : 1640-1645, 2004
29. Ghosh M, Wang HD, McNeill JR : Role of oxidative stress and nitric oxide in regulation of spontaneous tone in aorta of DOCA-salt hypertensive rats. Br J Pharmacol 141 : 562-73, 2004
30. Petzer JP, Castagnoli N Jr, Schwarzschild MA, Chen JF, Van der Schyf CJ : Dual-target-directed drugs that block monoamine oxidase B and adenosine A(2A) receptors for Parkinson's disease. Neurotherapeutics 6 : 141-51, 2009
31. Di Monte DA, DeLanney LE, Irwin I : Monoamine oxidasedependent metabolism of dopamine in the striatum and substantia nigra of L-DOPA-treated monkeys. Brain Res 738 : 53-59, 1996
32. Youdim MB, Bakhle YS : Monoamine oxidase : isoforms and inhibitors in Parkinson's disease and depressive illness. Br J Pharmacol 147 : S287-S296, 2006
33. Hunter KR, Boakes AJ, Laurence DR, Stern GM : Monoamine oxidase inhibitors and L-dopa. Br Med J 3 : 388, 1970
34. Blandini F, Nappi G, Fancellu R, Mangiagalli A, Samuele A, Riboldazzi G, Calandrella D, Pacchetti C, Bono G, Martignoni E : Modifications of plasma and platelet levels of L-DOPA and its direct metabolites during treatment with tolcapone or entacapone in patients with Parkinson's disease. J Neural Transm 110 : 911-922, 2003