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SYNTHESIS AND PHARMACOLOGICAL ACTIVITIES OF A NOVEL TRIPEPTIDE MIMETIC DOPAMINE PRODRUG

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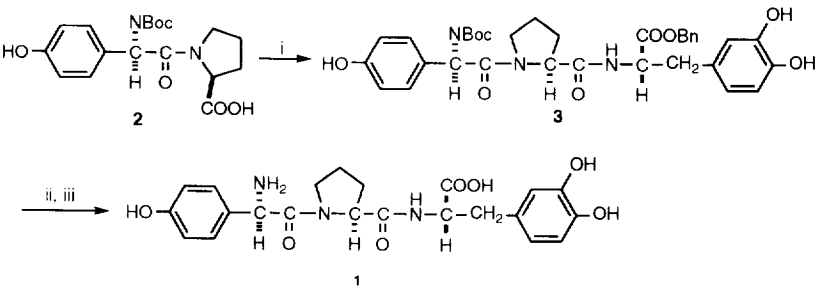
Abstract. D-*p*-hydroxyphenylglycine-L-proline was attached to L-dopa as delivery tool for improving the oral absorption. This tripeptide prodrug **1** of dopamine was well absorbed in a perfusion study in rat intestine, devoid of dopamine-like side effects on isolated smooth muscles and significantly inhibited the (+)-methamphetamine-induced rotation in nigrostriatal-lesioned rats, suggesting of anti-Parkinsonism effect.

L-Dopa is the drug of choice in management of Parkinson's disease. However, as a prodrug of dopamine most of the circulating L-dopa does not penetrate blood brain barrier (AUC_{CSF} / AUC_{plasma} = 12 / 100).[1] As a consequence, extensive metabolic decarboxylation of L-dopa generated peripheral dopamine-related side effects.[2] Moreover, the oral bioavailability of L-dopa is only 33% at up to 1000 mg/day[3] and a wide range of inter- and intra-patient variations in the rate and extent of absorption was reported.[4] Lipophilicity has been a major concern in intestinal drug absorption. The absorption of hydrophilic especially amphoteric compounds such as L-dopa, on the other hand, remains a challenge.

Studies have revealed that certain dipeptide-mediated carrier transport systems are responsible for the intestinal absorption of orally absorbable amino-β-lactams.[5-8] Most of these amino-β-lactams are actually tripeptide mimetics containing D-phenylglycine or D-*p*-hydroxyphenylglycine. Since the carrier systems showed broad specificity with less structural requirement for the substrates,[9,10] it is worthwhile to investigate if the carrier systems are accessible for the absorption of di- or tripeptide mimetics other than amino-β-lactams. The purpose of this study is to prepare di- and tripeptide prodrugs of dopamine in which D-phenylglycine or D-*p*-hydroxyphenylglycine is attached onto L-dopa as tools for intestinal delivery. The peptide derivatives may also prevent L-dopa from fast decarboxylation in the circulation. This report describes the preliminary results of the intestinal absorption and anti-Parkinsonism effect of a tripeptide mimetic prodrug, D-*p*-hydroxyphenylglycine-L-proline-L-dopa (**1**). L-proline was introduced in the molecule as a linkage between D-*p*-hydroxyphenylglycine and L-dopa with the thought that the tripeptide will be metabolized by prolinase rich in the body[11] and release L-dopa.

Synthesis of compound **1** is summarized in Scheme **1**. Coupling of D-N(Boc)-*p*-hydroxyphenylglycine-L-proline (**2**) with L-dopa benzyl ester in the presence of dicyclohexyl carbodiimide (DCC)[12] and N-hydroxybenzotriazole (HOBt)[13] gave **3**. Subsequent deprotection of **3** by treatment with trifluoroacetic acid followed by catalytic hydrogenation gave compound **1**.[14] As tripeptide comprising nonessential amino acids, compound **1** demonstrated sufficient stability toward enzymatic degradation prior to absorption. Only 19% of L-glycine-L-phenylalanine was recovered after 2 min of incubation in a rat intestinal mucosa suspension[15] at 37°C. However, 94 % (n=2) of compound **1** was recovered after incubation in the same preparation for 90 min.



Scheme 1. i. L-dopa benzyl ester/DCC/HOBt/Na_2CO_3/dioxane ii. TFA/CH_2Cl_2 iii. H_2/Pd(OH)$_2$-C/MeOH

According to Johnson and Amidon,[16, 17] the absorption of oral drugs can be evaluated as dimensionless membrane permeability (Pm*) from *in situ* single-pass rat jejunal perfusion experiments when the perfusion reaches steady-state. Assuming that chemical stability, first-pass metabolism and solubility/dissolution are not the rate controlling factors, then Pm* is the fundamental parameter for measuring human bioavailability of oral drugs. This correlation is independent of transport mechanism and structural class of compounds. In such correlation, Pm* less than 1.0 represents incomplete absorption whereas Pm* of 1.0 or greater correlates with 100% absorption from oral drug solutions. The Pm* for compound **1** (0.1 mM) and L-dopa (0.1 mM) in this study[18] (Table 1) indicated that both compounds were well absorbed from the intestine. The phenomenum of net water uptake[19] accompanied with compound **1** implied that a carrier-mediated transport mechanism might be involved in the absorption of this compound.

Table 1. Summary of the *in situ* rat jejunal perfusion study.

compound	no. of experiment.	Pm* (mean ± s.e.m)	water uptake %/cm (mean±s.e.m.)
L-dopa	4	5.38 ± 1.07	0.03 ± 0.07
1	4	1.53 ± 0.64	1.64 ± 0.32

6-Hydroxydopamine (6-OHDA), a neurotoxin, has been used in unilaterally lesioning the nigrostriatal dopaminergic pathways.[20] The unilateral lesion can be demonstrated by either contralateral turning induced by apomorphine or ipsilateral rotation induced by (+)-amphetamine.[21] This model system has been extensively used in exploring mechanisms that may underlie Parkinson's disease and in screening therapeutic agents for the treatment of this movement disorder.[22,23] Hudson *et al* reported that animals with unilateral nigrostriatum lesion elicited by 6-OHDA rotating more than 300 turns/h in rotometers in response to 5.0 mg/kg s.c. treatment of (+)-amphetamine had more than 90% probability of possessing a greater than 90% depletion of dopamine in lesioned striatum.[24] This rotational behavior was also observed on 6-OHDA-treated rats upon treatment with (+)-methamphetamine (MA) in our laboratory. The rotation was persistent and reproducible for at least 6 months . Thus, in the pesent study, only animals with unilateral 6-OHDA-elicited lesion showing greater than 300 turns/h of rotation upon MA treatment were chosen for the experiment. In brief, 6-OHDA (9 ug/4 ul/4min) in ascorbate (0.2 ug/ul) was slowly infused by an infusion pump (0.2 ul/min) through a 30 gauge stainless steel needle into unilateral substantia nigra compacta (SNc) of male Wistar rats using the Paxinos and Watson

coordinates: AP 5.3, L 2.0, H 7.8 mm.[25] The animals were then placed in automated rotometer bowls and secured to the counting head by a thoracic harness. After acclimation for at least 10 min, MA (4.0 mg/kg) was administered s. c. and the circling behavior were continuously recorded for 2 hours. The circling behavior of rats were monitored for 4 weeks after intranigral injection.

Both MA and L-dopa increased the dopamine content in the brain. However, with yet unclear mechanism, we found that i. p. injection of L-dopa one hour prior to MA treatment significantly decreased the rotation in 6-OHDA-treated rats (Table 2). It may be a consequence that (1) the effect of MA on the release and uptake of dopamine in the intact side of the brain was saturable and (2) L-dopa may act on the undamaged neuon on the lesioned side of the brain. Further study is needed to clarify the suggestions. Compound **1** was administered i.p. one hour prior to MA treatment. In consideration of drug latentiation, this compound was also administered two hours prior to MA treatment in another experiments. As a prodrug of L-dopa, this compound exhibited an effect similar to that of L-dopa. In both cases of one and two hour pretreatment with compound **1**, the rotation of the rats induced by MA was significantly inhibited. The effect was slightly higher than that of L-dopa.

Table 2. Reduction of (+)-methamphetamine-induced rotational counts by L-dopa and compound **1** on unilateral-lesion rats.

compound	dose (mg/kg)	time after drug treatment (h)[a]	no. of experiment	counts of rotation (turns/h)
control saline	--	--	19	552 ± 47
L-dopa	4.20	1	6	287 ± 86
1	9.60	1	6	223 ± 95
1	9.60	2	6	247 ± 67

[a] Time of (+)-methamphetamine injection after treatment with L-dopa or compound **1**.

Peripheral dopamine-like side effects of compound **1** were evaluated by measuring the resting tension on pulmonary artery and vas deferences, and the spontaneous mechanical contraction on the circular muscle of stomach in male guinea pigs (250-350 g) according to established procedures.[26] The mean responses before and after drug treatment were compared by means of Student's t test with $p < 0.05$ (n=3) indicating significance. The contractile force of both pulmonary artery and vas deferences was elicited after continuous perfusion of dopamine (1 mg/mL) for one min. However, no significant contraction was obseved after continuous perfusion of compound **1** (1 mg/mL) for 1 min. The spontaneous contraction of the circular muscle of stomach was significantly increased, after a transient decrease, upon continuous perfusion of dopamine (1 mg/mL) for one min. On the other hand, the frequency of spontaneous contration was not altered when compound **1** (1 mg/mL) was perfused for the same period. This compound apparently had little effect on the smooth muscles tested.

In conclusion, we designed and synthesized D-*p*-hydroxyphenylglycine-L-proline-L-dopa (**1**) as dopamine prodrug in which D-*p*-hydroxyphenylglycine was attached as a tool for oral drug delivery. Perfusion studies indicated that this compound was well absorbed in the rat intestine. The oral bioavailability is currently being conducted. Whether the absorption is via the dipeptide-mediated transport systems is being investigated in an uptake study in the brush-border membrane vesicle of rat intestine.[27] This prodrug significantly decreased the MA-induced rotational behavior in nigrostriatal-lesioned rats, suggesting that it has CNS activity related to

dopamine and might be an effective agent for Parkinson's disease. Whether the metabolic transformation from compound **1** to L-dopa and then to dopamine took place before or after the prodrug penetrates to CNS created interesting points for further study. Such studies on this compound and other di-and tri-peptide mimetic prodrugs are currently being conducted by virtue of CNS microdialysis experiments.

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