Antihypertensive Activity of Redox Derivatives of Tryptophan†

Emil Pop,‡ Wesley Anderson,‡ Katalin Prókai-Tâtrai,‡ Marcus E. Brewster,‡ Melvin Fregly,§ and Nicholas Bodor*,‡

Center for Drug Design and Delivery, College of Pharmacy, University of Florida, P.O. Box J-497, J. Hillis Miller Health Center, Gainesville, Florida 32610, Pharmatec, Inc., P.O. Box 730, Alachua, Florida 32615, and the Department of Physiology, University of Florida, P.O. Box J-274, Gainesville, Florida 32610. *Received January 12, 1990*

The essential amino acid, tryptophan, has been shown to lower blood pressure in rats when administered orally or intravenously. In order to potentially enhance this action, a brain-targeting chemical delivery system (CDS) approach was applied to this compound. The CDS is based on a dihydropyridine ↔ pyridinium ion redox system, chemically analogous to the naturally occurring NADH ↔ NAD^+ system. The dihydropyridine moiety containing carrier is chemically attached to the amino group by an amide-type bonding while the carboxylic acid functionality is esterified to various alcohols. Physicochemical studies of the new derivatives were performed. The determined chromatographic R_m values indicate an increased lipophilicity for the CDSs compared to the parent compound. Oxidation stability studies performed on selected compounds using a ferricyanide-mediated method showed that the CDSs are oxidized to the respective quaternary salt forms. Activity studies performed in deoxycorticosterone acetate induced hypertensive rats, demonstrated that the delivery system for tryptophan reduced blood pressure more efficiently for a longer time than did the parent compound.

Introduction

Hypertension, or high blood pressure, is a condition that affects a large portion of the population. Although it is often symptomless, sustained hypertension ultimately produces disability and death by stroke or coronary disease. In most cases the pathogenesis of hypertension is obscure. Nevertheless, it was proved that lowering blood pressure (BP) prevents blood vessel damage and reduces morbidity and mortality. It is recommended[1] that all patients with moderate (diastolic BP 105–114 mmHg) and severe (diastolic BP > 115 mmHg) hypertension should be treated with antihypertensive drugs with strong consideration being given to people with mild hypertension (diastolic BP 90–104 mmHg). Numerous drugs have been developed for reducing BP, and these act through a variety of mechanisms. Antidiuretic, adrenergic-blocking agents and vasodilators are some of the most prescribed classes of drugs in the treatment of hypertension. However, the search for new antihypertension agents, which are more effective or have fewer side effects, continues and clearly is warranted, given the social and medical ramifications of this malady.

One endogenous compound which has been recently investigated as a potential antihypertensive agent is L-tryptophan (L-Trp). L-Trp is an essential amino acid, commonly used as a nutrient and more recently proposed as an antidepressant agent. A number of reports[2-7] have shown that acute administration of L-Trp to spontaneously hypertensive rats lowers BP. The maximal reduction in BP (30 mmHg) was shown to be manifested within 2 h of treatment after administration of 50 mg L-Trp/kg with basal BP levels being reestablished by 4 h posttreatment. Administration of 125 mg L-Trp/kg caused a 42 mmHg decrease in blood pressure in rats within 3 h.[8] A series of studies with L-Trp examined the effect of the chronic treatment in the established deoxycorticosterone acetate (DOCA) salt induced hypertension[9-10] model and hypertension induced by bilateral encapsulation of the kidneys.[11] These studies suggested that L-Trp prevented the development of hypertension, polydipsia, and cardiac hypertrophy. A modest reduction in the blood pressure was noted in humans with essential hypertension when 50 mg Trp/kg was orally administered.[12]

Many potential mechanisms could explain the antihypertensive effect of L-Trp including: (1) alterations in ingestion of NaCl; (2) an increase in the turnover of serotonin and;[2,4,5,8] (3) a reduction of specific angiotensin II binding sites in brain. However there is a spirited debate in the literature in regards to the actual mechanism.[3,6,7,13-16] It is clear from the literature that controversy even surrounds the probable metabolic pathways of L-Trp (i.e., serotoninergic, kynurenine and/or tryptamine), and it is possible that L-Trp exerts some activity directly.[7] While there are still many questions to be answered relative to the mechanism of action, the majority of evidence seems to indicate that L-Trp exerts its primary action in the central nervous system (CNS). Amino acids are transported into the CNS through the blood–brain barrier (BBB) by the large neutral amino acid carrier (LNAA)[17]

* To whom correspondence should be addressed at Center for Drug Design and Delivery.

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‡ Center for Drug Design and Delivery, College of Pharmacy, University of Florida, P.O. Box J-497, J. Hillis Miller Health Center, Gainesville, FL 32610 and Pharmatec, Inc., P.O. Box 730, Alachua, FL 32615.

§ Department of Physiology, University of Florida, P.O. Box J-274, Gainesville, FL 32610.

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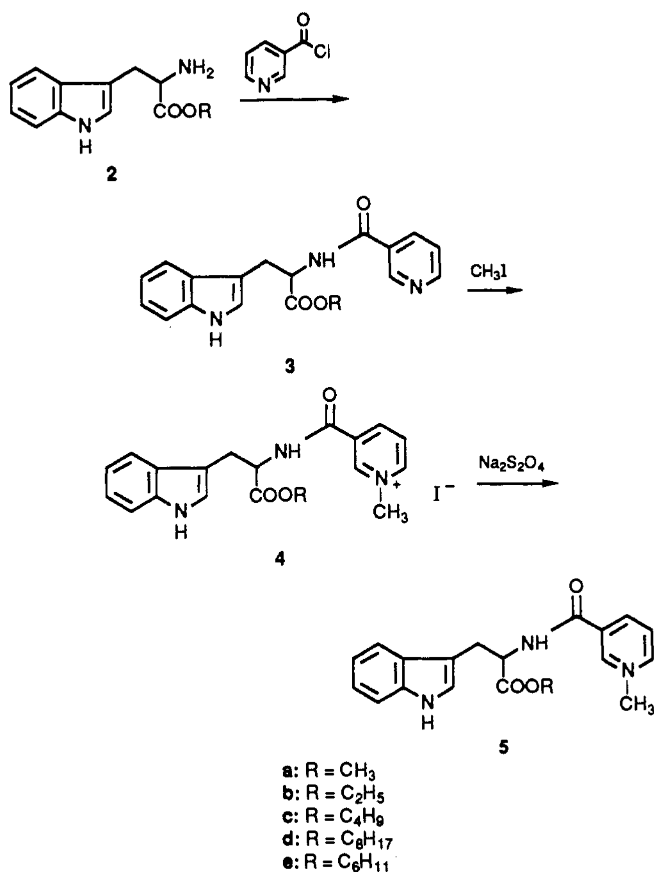
Scheme I



in spite of the unfavorable physicochemical properties of the amino acid (low lipophilicity, polar molecule). However, L-Trp is rapidly eliminated from the CNS by active transport which makes the transit through the BBB bidirectional and results in an equilibrium between blood and brain. In addition, L-Trp is the only amino acid which significantly binds to serum albumin. Due to these factors, the CNS concentration of L-Trp is not significantly elevated after administration of low doses of the amino acid; on the other hand, there is evidence that high doses of Trp may cause bladder cancer[18] or blood disorders. A method for enhanced Trp concentrations in the CNS which may circumvent the natural amino acid carrier may be useful.

Recently, a chemical delivery system (CDS) approach has been developed in order to deliver centrally acting drugs to the brain in a site-selective and sustained manner.[19-21] This system is based on a dihydropyridine ↔ pyridinium salt type molecular carrier and is similar to the endogenous NADH ↔ NAD^+ coenzyme system. The desired centrally mediated effects of the drug can be achieved without the exposure of the body to high drug levels which may be responsible for the toxic side effects of this agent. The CDS approach has been reviewed elsewhere and applied to a number of drugs.[22-30]

Table I. Second-Order Rates of Ferricyanide-Mediated Oxidation of Tryptophan CDSs

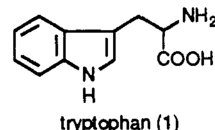
compd	k_o, s^{-1} M^{-1}	correlation coefficient: r
5a	13.8	0.999
5b	13.5	0.997
5d	7.61	0.984
5e	12.20	0.992

Table II. R_m Values[a] for Tryptophan CDSs in Different Aqueous Mobile Phases (Acetone/Water)

	acetone, %				
compd	30	50	60	70	80
5a	0	0.3952	−0.0209	−0.3475	−0.6917
5b	1.4400	0.5702	0.1082	−0.2518	−0.5910
5c	0	0.9447	0.3909	−0.0469	−0.4656
5d	0	0	1.0490	0.4210	−0.1170
5e	0	1.1102	0.6297	0.0590	−0.3680

[a] $R_m = \log(1/R_f - 1)$.

The derivatization of Trp in order to generate brain-targeting redox CDSs and the study of the novel delivery systems was the aim of this report. The tryptophan chemical delivery system (Trp-CDS) should enhance both the CNS penetration, and the retention and the duration of action of Trp, and therefore may improve the pharmacodynamics and pharmacokinetics of the hypotensive agent. Racemic, DL-tryptophan (Trp) (1) has been used for these preliminary experiments.



tryptophan (1)

Results and Discussion

Several CDS derivatives of Trp (1) were synthesized as shown in Scheme I. Various esters of **1** (**2a–d**) are commercially available. The cyclohexyl ester **2e** was synthesized by reacting cyclohexanol with Trp in the presence of thionyl chloride. The amino group of **2a–e** was acylated with nicotinoyl chloride in methylene chloride in the presence of triethylamine to give the nicotinamide-type derivatives **3a–e**. N-Alkylations, were subsequently carried out with methyl iodide in acetone to generate the pyridinium salts **4a–e**, and the reduction of these materials to the 1,4-dihydro derivatives **5a–e** (CDSs) was carried out by using sodium dithionite in a biphasic system containing ethyl acetate and aqueous sodium bicarbonate.

All prepared compounds were fully characterized by their microcombustion analyses and ultraviolet (UV) and proton nuclear magnetic resonance (^{1}H NMR) spectra. The quaternary salts **4a–e** were also analyzed by mass spectrometry (MS). Chromatographic techniques (TLC, HPLC) indicated the presence of only one component of

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Table III. Lipophilic Indexes (Extrapolated R_m Values) for Tryptophan CDSs

compd	R_m[a]	r[b]
5a	2.16	0.9985
5b	2.63	0.9982
5c	3.24	0.9977
5d	4.53	0.9990
5e	3.61	0.9986

[a] Mobile phases were 30–80% acetone in water. R_m values at 0% acetone were calculated by extrapolating from the linear part of the R_m value curve obtained from various mobile phases. [b] r = correlation coefficient.

Table IV. Incorporation of Tryptophan CDSs into 2-(Hydroxypropyl)-β-cyclodextrin (HPCD)

compd	incorporation, mg/g
5a	43.98
5b	37.55
5c	18.28

each derivative. The dihydropyridine derivatives were shown to be the 1,4 isomers by their UV characteristic maxima and appropriate ^1H NMR absorbances.[31]

All dihydropyridine derivatives could be oxidized to the corresponding quaternary salts by methanolic silver nitrate or H_2O_2 in the presence of cupric ions. A ferricyanide-mediated oxidation study was performed for some of the CDSs by using a spectrophotometric method.[32] The data are summarized in Table I. The second-order oxidation rates were found to fall within a range of 7.61–13.8 s^{-1} M^{-1} indicating that the CDSs have adequate stability towards oxidation. These values correlate empirically with successful CDS examples.

Lipophilicity is an important factor controlling the interaction of drugs with biological systems. Lipophilic character is essential for a CDS to penetrate the BBB. It has been shown that the chromatographic R_m value is related to the partition coefficient between the mobile and stationary phase of a chromatographic system.[33-36] The R_m values were determined as a measure of lipophilicity in this study.[37] The R_m values were measured by means of a reversed-phase TLC method with various concentrations of aqueous acetone as the mobile phase. The determined R_m values are indicated in Table II. The lipophilic indices presented in Table III were calculated by extrapolation of the linear part of R_m value curves to a totally aqueous mobile phase. All CDSs were more lipophilic than Trp (R_m = 0). The octyl ester **5d** was the most lipophilic as expected and the R_m values decreased in the order of the lengths of the ester substituent: octyl > cyclohexyl > butyl > ethyl > methyl or **5a** > **5e** > **5c** > **5b** > **5a**, respectively. These data suggest a good penetration of the BBB for all CDSs.

Solubility Studies. The use of hydroxyalkylated cyclodextrins such as of 2-(hydroxypropyl)-β-cyclodextrin (HPCD) to enhance the aqueous solubility of lipophilic compounds has been recently described.[38,39] Cyclodextrins

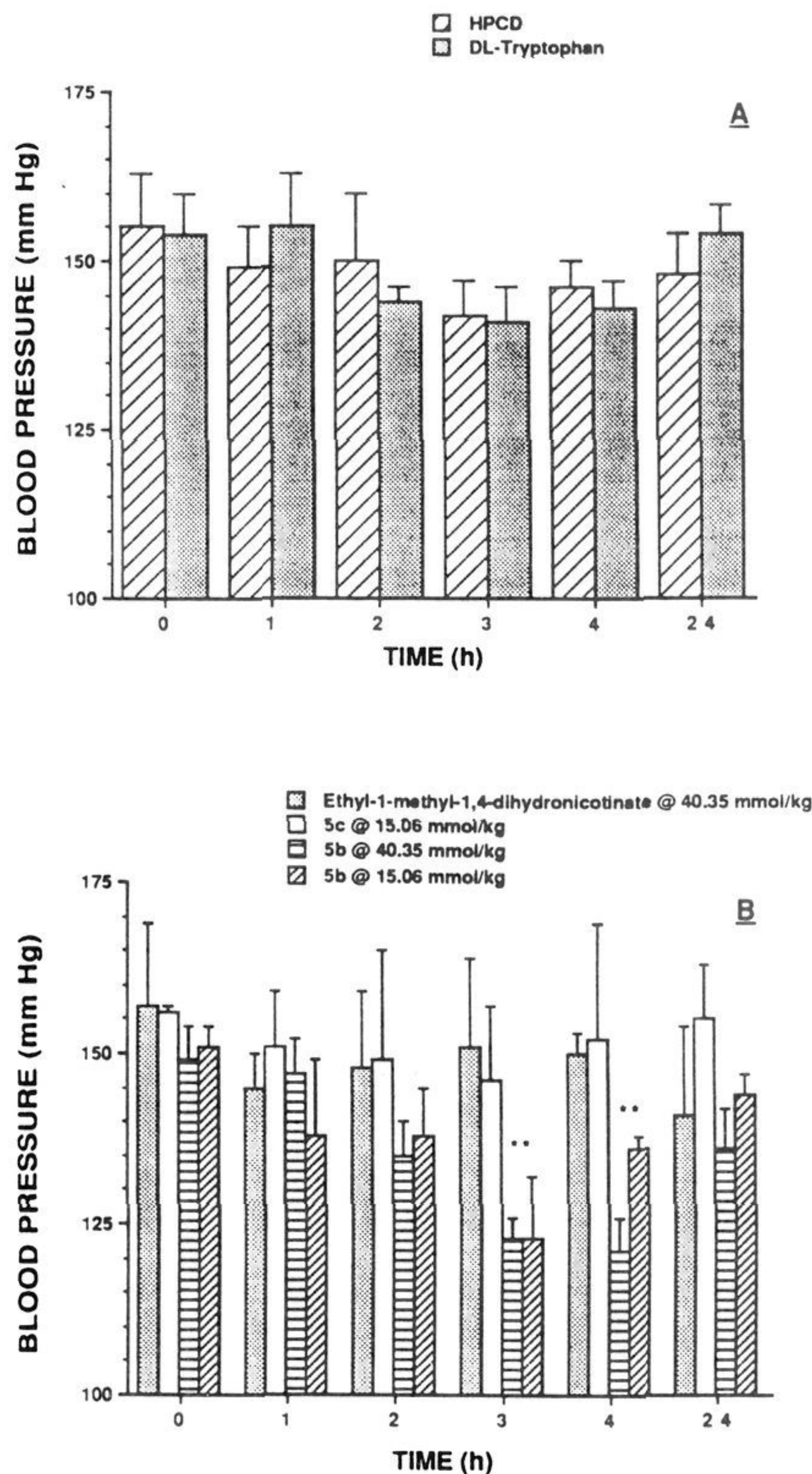

Figure 1. Effect of chemical delivery systems for tryptophan on systolic blood pressures on DOCA-induced hypertension in rats. Panel A compares DL-tryptophan with the drug vehicle, HPCD and panel B compares blood pressure effects of CDSs **5b** and **5c** and the CDS control, ethyl 1-methyl-1,4-dihydronicotinate. The asterisk indicates significant differences of a compound compared to the vehicle control, HPCD, at 0 h.

form reversible, water-soluble inclusion complexes with many lipophilic compounds. These complexes represent potentially useful formulations for drugs intended for parenteral use since these excipients are nontoxic. Cyclodextrins are particularly applicable in the case of CDSs as they stabilize as well as solubilize these highly lipophilic compounds.[40] The results of the solubilization study are presented in Table IV. The degree of incorporation decreased in the series **5a** > **5b** > **5c**, proportionally with the length of the alkyl group R present in the molecules. Aqueous solution of these 2-(hydroxypropyl)-β-cyclodextrin complexes were used in the pharmacological studies.

Activity Study. To assess the effects of Trp-CDS derivatives on the reduction of hypertension, tail blood

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pressures were recorded at various timepoints after administration of the compounds in deoxycorticosterone acetate model of hypertension. Figure 1A shows that blood pressures were not significantly reduced by administration of either the drug vehicle, 2-(hydroxypropyl)-β-cyclodextrin, or the parent compound, Trp at a dose of 40 mmol/kg. Effects of the Trp-CDS derivatives on blood pressure are indicated in Figure 1B. The most potent compound tested was **5b**, which was an ethyl ester. Blood pressure (BP) was lowered by 14% at 3 h and by 25% 4 h after injection. When the dose of **5b** was decreased by 63%, an equivalent suppression of BP was observed at 3 h. The hypotensive response to the lower dose of **5b** at 4 h was not as dramatic as previously observed but BP was significantly lowered by 7%. Because of limited solubility of **5c** in HPCD, this CDS was administered to animals at a dose of 15.06 mmol/kg instead of 40.35 mmol/kg. This dose of **5c** was not effective in eliciting a hypotensive response.

It is premature at this point to speculate as to the mechanism by which the improved hypotensive effect exerted by the Trp-CDS is manifested. It is possible that the tryptophan derivatives, i.e. either the dihydronicotinamide **5b** or the nicotinamide salt adducts **4b** are themselves active and are thus analogues acting as amino acid. Another possibility however, is that these derivatives act as delivery systems for the parent amino acid. The high lipophilicity of the dihydronicotinamides ensures brain uptake and the subsequent conversion to the pyridinium salt can result in brain sequestration. Hydrolysis of the ester group and further amide cleavage would release the active substance. Extensive studies on a similar CDS for dopamine have been completed and have shown that this series of events can, in fact, occur and that the result of this manipulation is profound and prolonged central dopaminergic action.[23,41-42] Specifically, a 1-methyl-1,4-dihydronicotinamide derivative of dopamine which was also esterified at the catechol hydroxy groups with pivaloyl moieties was found to readily enter the CNS and undergo a series of oxidative and hydrolytic conversions to result in formation of the catechol–nicotinamide salt. Dopamine levels in rats treated with the CDS were significantly increased relative to animals dosed with dopamine hydrochloride. In addition, various pharmacological parameters such as suppression of prolactin release were more responsive to the CDS relative to the parent catecholamine.

In the experiments herein described, it is possible that the enhanced antihypertensive effect may be associated with direct release of tryptophan in the CNS. In any case, the profound and prolonged effect of the described compounds on BP are significant and should be pursued.

Conclusion

The CDSs synthesized for Trp possess the required properties to deliver the drug through the BBB into the CNS. The study of some physicochemical properties support this statement. The remarkable improvement of the hypotensive activity of Trp achieved by using CDSs justifies further studies which include other types of CDSs and the in vivo distribution and similar studies of the CDSs of L-Trp.

Experimental Section

Uncorrected melting points were determined on an Electrothermal melting point apparatus (Fischer Scientific). Elemental microcombustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. Ultraviolet spectra (UV) were obtained on a Hewlett-Packard 8451A diode array spectrophotometer. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian XL 200 (200-MH2; FT mode) spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported as parts per million (δ) relative to tetramethylsilane which was used as an internal standard. Coupling constants (J) are reported in hertz (Hz). Mass spectra were recorded on a Kratos, MS 80-RFA double focusing instrument. Fast-atom bombardment (FAB) ionization was performed with use of a xenon beam (6 keV) and dissolving the samples in a glycerol matrix, as described in literature.[43] Thin layer chromatography (TLC) was performed on EM Reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel 60. A mixture of 2-propanol/chloroform (1:8) was used as solvent for development to determine R_f. All chemicals were reagent grade. Tryptophan esters **2a–d** were obtained from SIGMA. Compound **2e** was synthesized as follows.

DL-Tryptophan, Cyclohexyl Ester Hydrochloride (2e). To 100 g (1 mol) of cyclohexanol cooled to 0–5 °C was added dropwise 20 mL (0.25 mol) of thionyl chloride over a 15-min period. To the resulting solution was added 10.21 g (50 mmol) of DL-tryptophan, and the solution was stirred for 7 days at 80 °C. The reaction mixture was cooled to room temperature and 200 mL of ethyl ether was added. That mixture was stirred for 2 h, then the solid was removed by filtration and rinsed with ether, affording 3.25 g of product, mp 191–193 °C. Calcd for $C_{17}H_{23}ClN_2O_2$: C, H, Cl, N.

***N*-Nicotinoyl-DL-tryptophan, Methyl Ester (3a).** To a suspension of 3.82 g (15 mmol) of DL-tryptophan, methyl ester hydrochloride and 4.05 g (22.5 mmol) of nicotinoyl chloride hydrochloride in 100 mL of methylene chloride was added 5.31 g (52.5 mmol) of triethylamine in 20 mL of methylene chloride at 5–10 °C. The resulting mixture was stirred for 1 h. The mixture was washed successively with water, 0.1 N aqueous hydrochloric acid, water, 5% aqueous sodium bicarbonate solution, and water, dried over sodium sulfate, and evaporated. Then, 100 mL of ether was added and the solution was evaporated again. The product was obtained as yellow, hygroscopic crystals (3.5 g, 72.1% yield), melting at 55–57 °C, R_f 0.69. ^{1}H NMR ($CDCl_3$): δ 3.41–3.46 (m, 2 H), 3.71 (s, 3 H), 5.09–5.13 (m, 1 H), 6.95–7.29 (m, 7 H), 7.48 (d, 1 H, J = 7.64), 7.94 (d, 1 H, J = 8.10), 8.68 (b s, 1 H), 8.87 (s, 1 H). Anal. Calcd for $C_{18}H_{17}N_3O_3 \cdot 0.5H_2O$: C, H, N.

***N*-Nicotinoyl-DL-tryptophan, Ethyl Ester (3b).** With use of a similar procedure to that described above, 10 g (37.2 mmol) of DL-tryptophan, ethyl ester hydrochloride, 9.93 g (55.8 mmol) of nicotinoyl chloride hydrochloride, 360 mL of methylene chloride, and 13.17 g (130 mmol) of triethylamine were combined and gave a yield of 10.5 g (83.7%). The product was obtained as a white substance, melting at 58–61 °C, R_f 0.48. ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3 H, J = 7.21), 3.39–3.43 (m, 2 H), 4.15 (q, 2 H, J = 7.14), 5.06–5.10 (m, 1 H), 6.94–7.27 (m, 7 H), 7.51 (d, 1 H, J = 7.42), 7.91 (d, 1 H, J = 6.51), 8.51 (b s, 1 H), 8.87 (s, 1 H). Anal. Calcd for $C_{19}H_{19}N_3O_3 \cdot 0.25H_2O$: C, H, N.

***N*-Nicotinoyl-DL-tryptophan, Butyl Ester (3c).** Following the same procedure, 2.97 g (10 mmol) of DL-tryptophan, butyl ester hydrochloride, 2.67 g (15 mmol) of nicotinoyl chloride hydrochloride, 80 mL of methylene chloride, and 3.54 g (35 mmol) of triethylamine yielded 3.28 g product (90% yield), melting at 65–68 °C, R_f 0.60. ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3 H, J = 7.12), 1.30–1.34 (m, 2 H), 1.55–1.59 (m, 2 H), 3.41–3.45 (m, 2 H), 4.09–4.15 (m, 2 H), 5.09–5.13 (m, 1 H), 6.95–7.26 (m, 7 H), 7.52 (d, 1 H, J = 7.42), 7.95 (d, 1 H, J = 6.19), 8.59 (b s, 1 H), 8.71 (s, 1 H). Anal. Calcd for $C_{21}H_{23}N_3O_3$: C, H, N.

***N*-Nicotinoyl-DL-tryptophan, Octyl Ester (3d).** The described procedure was repeated, using 3.53 g (10 mmol) of DL-tryptophan, octyl ester hydrochloride, 2.67 g (15 mmol) of nicotinoyl chloride hydrochloride, 100 mL of methylene chloride, and 3.54 g (35 mmol) of triethylamine. The reaction time was 1.5 h. The product (3.96 g, 94% yield) was obtained as a white solid, melting at 103–105 °C, R_f 0.65. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3

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H, J = 6.74), 1.20–1.25 (m, 10 H), 1.56–1.59 (m, 2 H), 3.41–3.44 (m, 2 H), 4.06–4.12 (m, 2 H), 5.09–5.13 (m, 1 H), 6.48–7.29 (m, 7 H), 7.52 (d, 1 H, J = 7.58), 7.93 (d, 1 H, J = 3.34), 8.58 (bs, 1 H), 8.81 (s, 1 H). Anal. Calcd for $C_{25}H_{31}N_3O_3$: C, H, N.

***N*-Nicotinoyl-DL-tryptophan, Cyclohexyl Ester (3e).** When 0.8 g (2.5 mmol) of DL-tryptophan, cyclohexyl ester hydrochloride, 0.67 g (3.75 mmol) of nicotinoyl chloride hydrochloride, 15 mL of methylene chloride, and 0.88 g (8.75 mmol) of triethylamine were used, 0.92 g (95.2% yield) of product was obtained as a white solid, melting at 70–72 °C, R_f 0.58. ^{1}H NMR (DMSO-d_6): 1.25–1.61 (m, 10 H), 3.24–3.25 (m, 2 H), 4.68–4.71 (m, 2 H), 7.00–7.51 (m, 6 H), 7.58 (d, 1 H, J = 7.93), 8.17 (d, 1 H, J = 7.92), 8.71 (b s, 1 H), 8.98 (s, 1 H), 10.87 (b s, 1 H). Anal. Calcd for $C_{23}H_{25}N_3O_3$: C, H, N.

3-[[(*R*,*S*)-1-Carboxy-2-indol-3-ylethyl]carbamoyl]-1-methylpyridinium Iodide, Methyl Ester (4a). A solution of 3.5 g (10.8 mmol) of *N*-nicotinoyl-DL-tryptophan, methyl ester and 11.4 g (5 mL, 80 mmol) of methyl iodide in 70 mL of acetone was stirred at room temperature for 48 h. The solvent was removed in vacuo, ether was added, and the mixture was evaporated to give 4.4 g (87.4% yield) of the product as a yellow solid, melting at 156–160 °C. UV (methanol): 217, 272 nm. ^{1}H NMR (DMSO-d_6): δ 3.27–3.38 (m, 2 H), 3.68 (s, 3 H), 4.39 (s, 3 H), 4.80–4.82 (m, 1 H), 6.99–7.52 (m, 5 H), 8.25–8.29 (m, 1 H); 8.87 (d, 1 H, J = 8.25), 9.12 (d, 1 H, J = 6.07), 9.34 (s, 1 H), 9.52 (d, 1 H, J = 7.54), 10.87 (b s, 1 H). MS (FAB): C^+ m/z 337. Anal. Calcd for $C_{19}H_{20}IN_3O_3$: C, H, I, N.

3-[[(*R*,*S*)-1-Carboxy-2-indol-3-ylethyl]carbamoyl]-1-methylpyridinium Iodide, Ethyl Ester (4b). Similarly, 14.91 g (44 mmol) of *N*-nicotinoyl-DL-tryptophan, ethyl ester and 10 mL (160 mmol) of methyl iodide in 150 mL of acetone produced 21.26 g (97% yield) of the product which was obtained as a yellow solid, melting at 99–101 °C. UV (methanol): 217, 271 nm. ^{1}H NMR (DMSO-d_6): δ 1.21 (t, 3 H, J = 7.15), 3.33–3.42 (m, 2 H), 4.10 (q, 2 H, J = 7.13), 4.46 (s, 3 H), 4.77–4.80 (m, 1 H), 7.00–7.58 (m, 5 H), 8.27–8.30 (m, 1 H), 8.89 (d, 1 H, J = 8.0), 9.14 (d, 1 H, J = 7.94), 9.36 (s, 1 H), 9.80 (d, 1 H, J = 7.40), 10.88 (b s, 1 H). MS (FAB): C^+ m/z 351. Anal. Calcd for $C_{20}H_{22}IN_3O_3 \cdot 0.25H_2O$: C, H, I, N.

[[(*R*,*S*)-1-Carboxy-2-indol-3-ylethyl]carbamoyl]-1-methylpyridinium Iodide, Butyl Ester (4c). By reacting in the same way 3.28 g (0.9 mmol) of *N*-nicotinoyl-DL-tryptophan, butyl ester and 10 mL (160 mmol) of methyl iodide in 60 mL of acetone, 4.8 g (95% yield) of product was obtained as a yellow hygroscopic solid, melting at 55–62 °C. UV (methanol): 240, 289 nm. ^{1}H NMR (DMSO-d_6): δ 0.82 (t, 3 H, J = 7.17), 1.15–1.18 (m, 2 H), 1.46–1.50 (m, 2 H), 3.31–3.35 (m, 2 H), 4.04–4.07 (m, 2 H), 4.41 (s, 3 H), 4.78–4.82 (m, 1 H), 7.00–7.58 (m, 5 H), 8.27–8.31 (m, 1 H), 8.90 (d, 1 H, J = 8.06), 9.15 (d, 1 H, J = 5.91), 9.36 (s, 1 H), 9.80 (d, 1 H, J = 6.94), 10.87 (b s, 1 H). MS (FAB): C^+ m/z 380. Anal. Calcd for $C_{22}H_{26}IN_3O_3 \cdot 0.5H_2O$: C, H, I, N.

3-[[(*R*,*S*)-1-Carboxy-2-indol-3-ylethyl]carbamoyl]-1-methylpyridinium Iodide, Octyl Ester (4d). From 3.5 g (8.3 mmol) of *N*-nicotinoyl-DL-tryptophan, octyl ester and 10 mL (160 mmol) of methyl iodide in 60 mL of acetone, 4.21 g (90% yield) of the product was obtained as a yellow solid melting at 55–62 °C. UV (methanol): 217, 274 nm. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3 H, J = 6.77), 1.16–1.20 (m, 10 H), 1.45–1.48 (m, 2 H), 3.31–3.38 (m, 2 H), 4.01–4.07 (m, 2 H), 4.42 (s, 3 H), 4.71–4.81 (m, 1 H), 6.99–7.51 (m, 5 H), 8.17–8.31 (m, 1 H), 8.85 (d, 1 H, J = 8.25), 9.17 (d, 1 H, J = 5.87), 9.38 (s, 1 H), 9.49 (d, 1 H, J = 8.0), 10.87 (bs, 1 H). MS (FAB): C^+ m/z 436. Anal. Calcd for $C_{26}H_{34}IN_3O_4 \cdot 0.5H_2O$: C, H, I, N.

3-[[(*R*,*S*)-1-Carboxy-2-indol-3-ylethyl]carbamoyl]-1-methylpyridinium Iodide, Cyclohexyl Ester (4e). Similarly from 0.7 g (1.78 mmol) of *N*-nicotinoyl-DL-tryptophan, cyclohexyl ester and 5 mL (80 mmol) of methyl iodide in 20 mL of acetone, 0.9 g (94.8% yield) of product was obtained as a yellow solid, melting at 94–96 °C. UV (methanol): 217, 271 nm. ^{1}H NMR (DMSO-d_6): δ 1.14–1.58 (m, 10 H), 3.24–3.30 (m, 2 H), 4.39 (s, 3 H), 4.61–4.78 (m, 2 H), 7.00–7.57 (m, 5 H), 8.26–8.28 (m, 1 H), 8.89 (d, 1 H, J = 7.81), 9.13 (d, 1 H, J = 5.67), 9.36 (s, 1 H), 9.51 (d, 1 H, J = 7.52), 10.88 (b s, 1 H) MS (FAB): C^+ m/z 406. Anal. Calcd for $C_{24}H_{28}IN_3O_3 \cdot 0.5H_2O$: C, H, I, N.

***N*-(1,4-Dihydro-1-methylnicotinoyl)-DL-tryptophan, Methyl Ester (5a).** To a deaerated solution of 4 g (8.6 mmol) of **4a** in 350 mL of water and 250 mL of ethyl acetate were added 4.34 g (51.6 mmol) of sodium bicarbonate and 6 g (34.4 mmol) of sodium dithionite at 5 °C. The reaction mixture was stirred for 5 h at which time the organic layer was removed, washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give 2.1 g (72% yield) of the product as a yellow solid, melting at 110–112 °C, R_f 0.60. UV (methanol): 216, 356 nm. ^{1}H NMR ($CDCl_3$): δ 2.92 (s, 3 H), 3.28–3.31 (m, 4 H), 3.70 (s, 3 H), 4.82–4.97 (m, 2 H), 5.71 (d, 1 H, J = 8.16), 6.92–7.52 (m, 5 H), 9.01 (b s, 1 H). Anal. Calcd for $C_{19}H_{21}N_3O_3 \cdot 0.5H_2O$: C, H, N.

***N*-(1,4-Dihydro-1-methylnicotinoyl)-DL-tryptophan, Ethyl Ester (5b).** The procedure of the previous experiment was repeated, substituting 4.79 g (10 mmol) of **4b** in a mixture of 500 mL of water and 350 mL of ethyl acetate, 5 g (60 mmol) of sodium bicarbonate, and 7 g (40 mmol) of sodium dithionite. The product (2.46 g, 69.7% yield) which was obtained melted at 53–59 °C, R_f 0.73. UV (methanol): 218, 356 nm. ^{1}H NMR (DMSO-d_6): 1.09 (t, 3 H, J = 7.01), 2.85 (s, 3 H), 3.16–3.43 (m, 6 H), 4.02 (q, 2 H, J = 7.14), 4.54–4.61 (m, 2 H), 5.78 (d, 1 H, J = 7.93), 6.67–7.55 (m, 6 H). Anal. Calcd. for $C_{20}H_{23}N_3O_3$: C, H, N.

***N*-(1,4-Dihydro-1-methylnicotinoyl)-DL-tryptophan, Butyl Ester (5c).** In a similar manner, 4 g (9.5 mmol) of **4c** was added to a mixture of 350 mL of water and 250 mL of ethyl acetate, 4.78 g (57 mmol) of sodium bicarbonate, and 6.61 g (38 mmol) of sodium dithionite. Title product (2.56 g, 70.7% yield) was obtained as a yellow solid, melting at 74–78 °C, R_f 0.68. UV (methanol): 238, 376 nm. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3 H, J = 7.01), 1.20–1.29 (m, 2 H), 1.56–1.80 (m, 2 H), 2.83 (s, 3 H), 3.22–3.49 (m, 4 H), 4.11–4,12 (m, 2 H), 4.61–4.79 (m, 3 H), 5.81 (d, 1 H, J = 7.13), 6.94–7.52 (m, 7 H). Anal. Calcd for $C_{22}H_{27}N_3O_3$: C, H, N.

***N*-(1,4-Dihydro-1-methylnicotinoyl)-DL-tryptophan, Octyl Ester (5d).** Similarly, 4.5 g (8 mmol) of **4d** in a mixture of 500 mL of water and 480 mL of ethyl acetate, 4.03 g (48 mmol) of sodium bicarbonate, and 5.57 g (32 mmol) of sodium dithionite. Title product (2.26 g, 64.6% yield) was obtained as a yellow solid, melting at 84–87 °C, R_f 0.73. UV (methanol): 219, 357 nm. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, J = 6.11), 1.22–1.52 (m, 10 H), 1.48–1.54 (m, 2 H), 2.83 (s, 3 H), 2.90–2.92 (m, 2 H), 3.30–3.37 (m, 2 H), 4.01–4.03 (m, 2 H), 4.58–4.60 (m, 1 H), 4.99–5.03 (m, 1 H), 5.74 (d, 1 H, J = 7.42), 6.93–7.54 (m, 7 H), 8.81 (b s, 1 H). Anal. Calcd for $C_{26}H_{35}N_3O_3 \cdot 1.5H_2O$: C, H, N. Found: C, H, N.

***N*-(1,4-Dihydro-1-methylnicotinoyl)-DL-tryptophan, Cyclohexyl Ester (5e).** From 0.200 g (3.7 mmol) of **4e** in a mixture of 20 mL of water and 25 mL of ethyl acetate, 0.186 g of sodium bicarbonate, and 0.257 g of sodium dithionite, 0.130 g (86.7% yield) product melting at 68–70 °C was obtained. UV (methanol): 217, 356 nm. ^{1}H NMR (DMSO-d_6): δ 1.17–1.57 (m, 10 H), 2.86 (s, 3 H), 3.11–3.16 (m, 4 H), 4.05–4.62 (m, 3 H), 5.79 (d, 1 H, J = 7.11); 6.98–7.53 (m, 8 H). Anal. Calcd. for $C_{24}H_{29}N_3O_3$: C, H, N.

Chemical Oxidation Studies. Ferricyanide oxidation kinetic study was performed according to a modified literature method: oxidations were followed by UV spectroscopy (350 nm) in solutions containing both $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ in 20% acetonitrile/water under an oxygen-free atmosphere at 37 °C; pseudo-first-order conditions were used, meaning that the concentration of $Fe(CN)_6^{3-}$ was much higher than the concentrations of CDSs in these studies. pH was held constant. The second-order rate constants of oxidation (k_0) were determined from the pseudo-first-order rates at different concentrations of $Fe(CN)_6^{3-}$.

Lipophilicity Measurements. R_m determinations were carried out with use of TLC plates [Baker, Si-C18F 19C, 20 × 20 glass plates precoated with octadecylsilane (C_{18}) reversed-phase bonded to silica gel, approximately 20-μm particle size, 200-μm hard surface layer with 254-nm fluorescent indicator and 19 channels each of 8-mm width]. The compounds were dissolved in distilled water or acetone, and 1 μL of a 3 mg/mL solution was applied to each channel along a line 2 cm above the bottom of the plate. The mobile phase of 200 mL of water or various concentrations of acetone in water was allowed to elute 14 cm from the origin. The developed plates were dried, and the compounds were detected by an ultraviolet illumination. The corresponding R_m values were calculated from the R_f values by means of the equation: $R_m = \log(1/R_f - 1)$. The theoretical values at 0% of

acetone in mobile phase were calculated by the least-squares method from the R_m values in the linearity range of the curves, which were plotted as R_m values versus acetone concentrations.

Solubilization Study. A solution of 2-(hydroxypropyl)-β-cyclodextrin was prepared by dissolving 5 g of the modified starch into 10 mL of cold, degassed, deionized water which had been adjusted to pH 7 with sodium hydrogen carbonate. An excess of the selected tryptophan-CDS was then suspended in the solution and the system was sonicated at 0 °C for 1 h. The mixture was then filtered through 0.45 μm polyvinylidene difluoride membranes and the filtrate frozen on liquid nitrogen. The solid was lyophilized and milled to give a powder which passed through a 60 mesh sieve. Drug incorporation was calculated by UV spectroscopy. Standard curves for the tryptophan derivatives were prepared in methanol and were linear ($r^2 > 0.999$) over the concentration range examined.

Activity Study. Induction of Hypertension in Animals. The antihypertensive activity of tryptophan-CDS compounds was assessed by monitoring changes in systolic blood pressure (BP). To elevate BP, male Sprague-Dawley rats (Blue Spruce Farms), weighing 200–250 g, were administered pentobarbital at 40 mg/kg (ip) and unilaterally (left) nephrectomized. Animals were then implanted with either single 50-mm Silastic tube (Dow Corning; 0.058 in. i.d. and 0.077 in. o.d.) containing deoxycorticosterone acetate (DOCA) or an empty tube (shunt control). After DOCA implantation, blood pressures were monitored weekly by a tail cuff procedure for at least 4 weeks until systolic pressures were ≥150 mmHg, which is an increase of approximately 40 to 50 mmHg.[44] Animals were placed into a 30 °C chamber for 10–15 min to warm the rat to ensure efficient BP recordings. A pressure cuff was then placed at the base of the tail and seven determinations were recorded on a physiograph and averaged. Rats were then returned to their home cages until the next BP recording.

Drug Administration. After obtaining a baseline (0 h) blood pressure, rats (6 per group) received a single tail vein injection of either DL-tryptophan (1; 40.35 mmol/kg), CDSs **5a**, **5b**, **5c**, or ethyl 1-methyl-1,4-dihydronicotinate at doses equimolar to 1. The drug vehicle, 2-(hydroxypropyl)-β-cyclodextrin (HPCD; 40% [w/v]) was administered at a volume of 1 mL/kg. Blood pressures were measured at 1, 2, 3, 4, and 24 h after injection.

Statistical Treatment of Results. The significance of differences among mean values was determined by analysis of variance (ANOVA) and Student-Newman-Keuls tests.[45] The level of probability for all tests was $P < 0.05$.

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In Vivo Intracerebral Microdialysis Studies in Rats of MPP$^+$ Analogues and Related Charged Species

Hans Rollema,*,† E. Anne Johnson,‡ Raymond G. Booth,‡ Patricia Caldera,‡ Peter Lampen,‡ Stephen K. Youngster,§ Anthony J. Trevor,‡ Noreen Naiman,∥ and Neal Castagnoli, Jr.∥

Department of Medicinal Chemistry, University Center for Pharmacy, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands, Division of Toxicology and Department of Pharmacology, University of California, San Francisco, California 94143, Department of Neurology, University of Medicine and Dentistry New Jersey, Piscataway, New Jersey 08854-5635, and Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061. *Received December 18, 1989*

The in vivo dopaminergic neurotoxic properties of 45 MPTP and MPP$^+$ analogues and related compounds were examined by an intrastriatal microdialysis assay in conscious rats. MPP$^+$-like toxicity, as evidenced by the irreversible effects on DA release and enhancement of lactate formation, was observed with a variety of structural types although no compound was more toxic than MPP$^+$. The following global structure–toxicity relationships could be derived: (1) only permanently charged compounds showed neurotoxic effects; (2) with the exception of amino groups, hydrophilic substituents abolished toxicity; (3) activity was enhanced by lipophilic groups although increased steric bulk around the nitrogen atom tended to decrease activity; (4) nonaromatic, quaternary systems (methiodide of MPTP, guanidinium derivatives) were only weakly toxic; and (5) certain bi- and tricyclic systems, including putative metabolites of potential endogenous MPTP-like compounds, were weakly toxic. The lack of toxic effects following perfusions with DA itself confirmed that MPTP dopaminergic neurotoxicity is not likely to be mediated by the MPP$^+$-induced release of DA. With some interesting exceptions, these in vivo data correlate reasonably well with in vitro data on the nerve terminal uptake properties and the inhibitory effects on mitochondrial respiration of these compounds.

Introduction

An understanding of structure–toxicity relationships of compounds related to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **45**) is important for the identification of potential environmental and endogenous neurotoxins and may contribute to the elucidation of the mechanism of action of MPTP. Most in vivo studies have been performed with tetrahydropyridine derivatives[1–3] which, by analogy with the monoamine oxidase (MAO) B

† University Center for Pharmacy.
‡ University of California.
§ University of Medicine and Dentistry New Jersey.
∥ Virginia Polytechnic Institute and State University.

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