Brain Levels and Metabolism of the Dopaminergic Agonist 2-Amino-6,7-dihydroxytetrahydronaphthalene After Administration of Various Prodrugs

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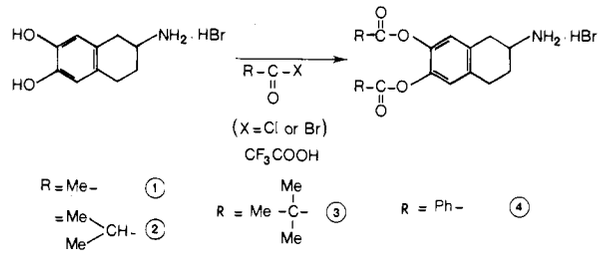
The synthesis of four prodrug diesters (diacetyl, diisobutyryl, dipivaloyl, and dibenzoyl) of the potent dopaminergic agonist 2-amino-6,7-dihydroxytetrahydronaphthalene (6,7-ADTN) is described. The effects of prodrug structure on the levels of 6,7-ADTN in the rat corpus striatum and cerebellum, as well as the levels of the metabolite, 6-hydroxy-7-methoxy-2-aminotetralin, in the corpus striatum, have been determined after intraperitoneal administration. In addition, the striatal levels of 6,7-ADTN after administration of the dibenzoyl analogue via intraperitoneal, subcutaneous, and oral routes have been measured. These prodrugs produce a significant improvement in the penetration and accumulation of 6,7-ADTN in the brain.

Although the dopaminergic agonist 2-amino-6,7-dihydroxytetrahydronaphthalene (6,7-ADTN) is potently active in in vitro test systems[1,2] and after intracerebral injections,[3,4] it is known to be inactive after intraperitoneal (ip) administration.[5] It was therefore thought that 6,7-ADTN might be a good substrate for the application of prodrug methodology, since the problem was clearly that of passage through the blood–brain barrier. Of the two functional groups in the molecule, i.e., the catechol and amino functions, we decided to prepare diesters of the former, rather than amides of the latter, due to our expectation that simple amides might be hydrolyzed at too slow a rate to be pharmacologically useful.[6] A small group of four diesters (diacetyl, diisobutyryl, dipivaloyl, and dibenzoyl) was prepared (Scheme I), and the concentrations of 6,7-ADTN in two brain areas after various time intervals and modes of administration were determined. In addition, the concentrations of the metabolite, 6-hydroxy-7-methoxy-2-aminotetralin[7,8] (7-MeO-ADTN), were measured in the rat striatum after injections of the four prodrugs (Scheme II).

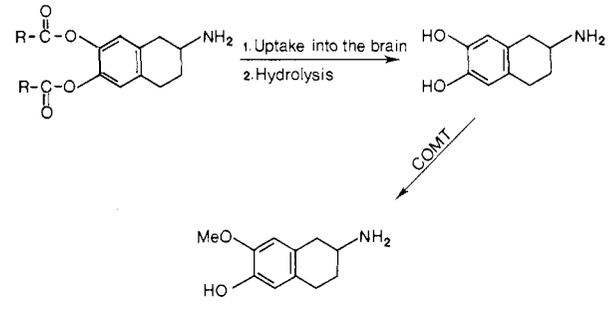
Chemistry. 6,7-ADTN was prepared using our previously described method.[9] The diesters were synthesized in one step using the method of Borgman et al.[10] (Scheme I). The acyl bromides or chlorides were used depending on the commercial availability of these acylating agents. The yields were in the range of 60–80%. The synthesis of the metabolite, 6-hydroxy-7-methoxy-2-aminotetralin, has been published.[8]

Neurochemistry. The brain levels of 6,7-ADTN and its 7-methoxy metabolite were determined with an HPLC system using reverse-phase elution and an electrochemical detector.[7,11]

Scheme I



Scheme II



Results

In the DA-rich corpus striatum (Figure 1), the 6,7-ADTN time–concentration curves can be clearly divided into two groups, i.e., those arising from the diacetyl and diisobutyryl derivatives, which produced rapid peak concentrations in the first 5–15 min, and the dibenzoyl and dipivaloyl analogues, which gave peak levels at 3 h and generally resulted in plateau-like levels over many hours. A somewhat similar effect was seen in the cerebellum, a brain area having very low DA levels, although here the overall levels of 6,7-ADTN were generally lower (Figure 2). The effect of the mode of administration on striatal 6,7-ADTN levels was studied using the dibenzoyl derivative (Figure 3). At a dose of 100 μmol/kg, peak levels were reached after 3 h with a ip injection, while there was little difference between the levels of the 3- and 6-h points after subcutaneous administration. After oral administration, the parent drug was barely detectable after 6 h. Following an iv dose of 25 μmol/kg of Bz_2-6,7-ADTN, the striatal concentration of 6,7-ADTN after 3 h was 5.4 ± 0.84 nmol/g (mean ± SEM, n = 6). The four prodrugs gave similar levels of the main metabolite, 6-hydroxy-7-methoxy-2-aminotetralin, in the corpus striatum after a dose of 100 μmol/kg (Figure 4). The shapes of the curves were quite

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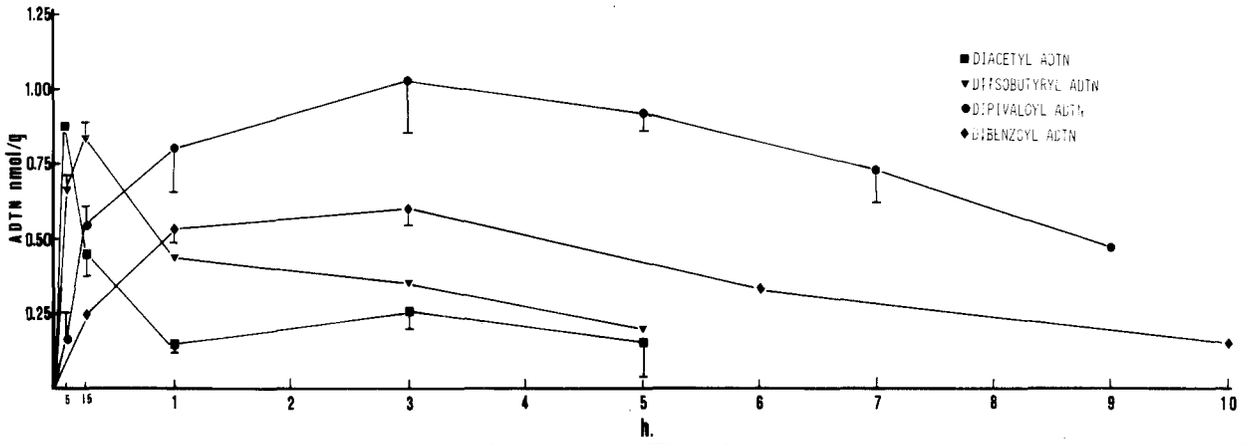

Figure 1. Concentration–time curves for the accumulation of 6,7-ADTN in the rat corpus striatum after ip injections of 100 μmol/kg solutions of the various prodrugs. The values have been corrected for a recovery of 86% and are shown as means ± SEM, $n = 6$. The first two points refer to 5 and 15 min, respectively.

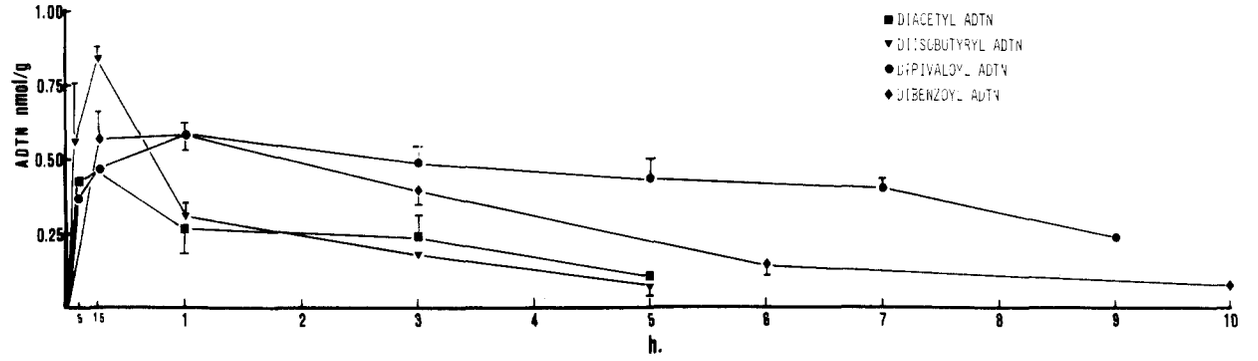

Figure 2. Concentration–time curves for the accumulation of 6,7-ADTN in the rat cerebellum after ip injections of 100 μmol/kg solutions of the various prodrugs. The values have been corrected for a recovery of 86% and are shown as means ± SEM, $n = 3$. The first two points refer to 5 and 15 min, respectively.

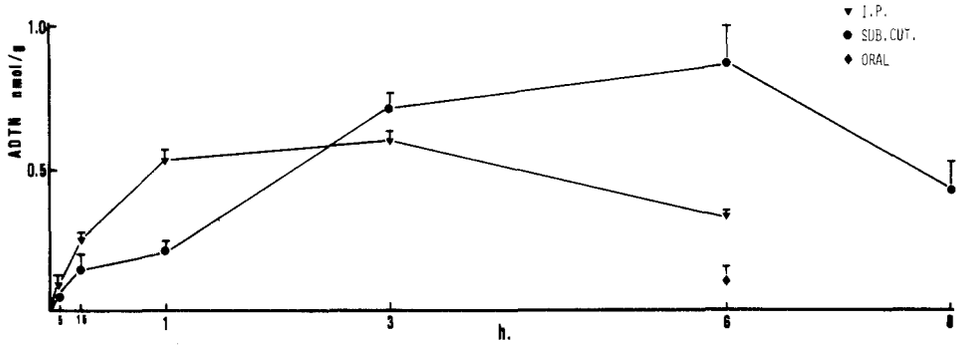

Figure 3. The effects of the mode of administration, i.e., ip, sc, and po, on the accumulation of 6,7-ADTN in rat corpus striatum after administration of 100 μmol/kg of Bz-6,7-ADTN (4). The values have been corrected for recoveries, which were ip, 86%; sc, 80%, and po, 80%. The values are shown as means ± SEM, $n = 6$. No 6,7-ADTN could be detected at an earlier time point than 6 h after an oral administration of the prodrug. The first two points refer to 5 and 15 min, respectively.

different from those found for the levels of the parent drug (cf. Figures 1 and 4).

All the prodrugs produced a characteristic hypomotive or "sedative" effect after ip administration in which the animals assumed a very flat posture with their hind legs extended. There was a striking difference in the effects on behavior following the different modes of administration, i.e., a long-lasting hypomotility was seen after the ip injections but not after the subcutaneous and oral routes. However, the iv route of administration also produced a sedative-like effect.

Stability of the Prodrugs. The stability of the prodrugs in water was determined (Table I). Apart from the slight instability of the diacetyl compound, the other three diesters were all found to be quite stable. A similar pattern of stability was found for solutions of the prodrugs in the vehicle (ethanol–PEG 400–water) and during the extraction of 6,7-ADTN from the brain samples (results not shown).

Table I. Stability of the Prodrugs in Water[a]

	% hydrolysis	
no.	6 h	24 h
1	1.64	3.74
2	0.06	0.23
3	0.01	0.02
4	0.03	0.06

[a] The prodrugs were dissolved in distilled water at a concentration of 1 mg/100 mL and left at room temperature for 6- and 24-h periods. Samples were then taken, and the 6,7-ADTN concentrations were determined using an HPLC C_{18} reverse-phase chromatography system fitted with a rotating electrochemical detector. The values shown are the means of two determinations.

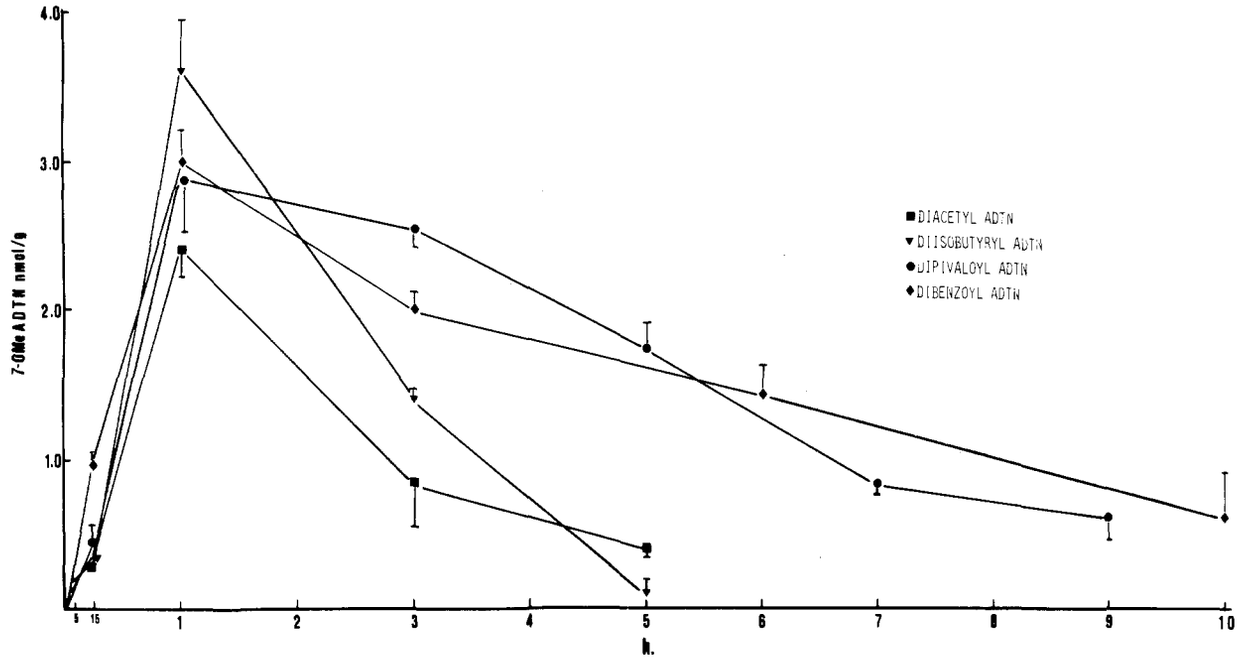

Figure 4. Concentration–time curves for the accumulation of 6-hydroxy-7-methoxy-2-aminotetralin in the rat corpus striatum after ip injections of 100 μmol/kg solutions of the various prodrugs. The values have been corrected for a recovery of 73% and are shown as means ± SEM, $n = 6$. The first two points refer to 5 and 15 min, respectively.

Discussion

Diester prodrugs of dopamine (DA) and related analogues have been reported by various groups in recent years.[12-15] However, with the excepton of DA itself, the problem is not strictly comparable to that with 6,7-ADTN, i.e., other dopaminergic analogues, such as apomorphine, N,N-dialkylated dopamines, and *m*-tyramines, are able to quite readily pass the blood–brain barrier. That this is not the case with 6,7-ADTN is shown by the fact that doses as high as 279 μmol/kg (50 mg/kg) ip produce no apparent CNS effects in rats.[5] In contrast, an intraventricular injection of 0.154 μmol in mice produces a very long-lasting increase in spontaneous locomotor activity.[3] The chief disadvantage with diester prodrugs of DA is that they are substrates for MAO (Horn et al., unpublished observations).

The penetration into the brain of diester prodrugs of 6,7-ADTN is clearly significantly better than that of 6,7-ADTN itself. We have previously shown that the peak striatal concentrations of 6,7-ADTN after giving the dibenzoyl analogue were almost 6 times as high as after an equimolar does of the parent drug.[11] On chemical grounds[16] it was expected that the dipivaloyl ester would be more resistant to hydrolysis, both chemical and enzymatic, than the diacetyl compound. The expectation was borne out in the concentration–time profiles for the four prodrugs. In fact, the profile for the diacetyl analogue is very similar to that of a normal drug, i.e., a rapid peak followed by a slow decline. Unlike other workers,[17] we therefore felt that the diacetyl prodrug was not the best choice for further detailed investigation. When the dibenzoyl ester was used, it was demonstrated[11] that one can obtain a selective accumulation of 6,7-ADTN in DA-rich brain areas, such as the corpus striatum and olfactory tubercle. This is probably due to the fact that 6,7-ADTN is an excellent substate for the neuronal dopamine transport system.[18]

The brain levels of 6,7-ADTN are, however, not only governed by the lipophilicity and rate of hydrolysis of the prodrugs but also by the activity of the enzyme COMT, which has been shown to methylate 6,7-ADTN at the 7-OH group.[7,8] There were only small differences in the profiles for the metabolite levels in the corpus striatum after ip injections of the four prodrugs.

The fact that the concentration–time curve for 6,7-ADTN in the corpus striatum following an ip injection of the dibenzoyl analogue (Figure 3) was much steeper than after a subcutaneous administration is probably due to the more rapid absorption of the prodrug following the former mode of application. The shape of the curve and the higher peak concentrations due to a subcutaneous injection can be ascribed to the probable lack of a significant first-pass effect coupled with a slower rate of absorption. The very low levels following the oral route are most certainly the outcome of both enzymatic and chemical hydrolysis in the GI tract, followed by further hydrolysis in the plasma and liver together with catechol O-methylation in the latter organ.

Following ip injections the prodrugs produced a characteristic hypomotility. As a result of detailed investigations,[19] it has become apparent that this may not only be due to a possible interaction with central presynaptic DA receptors but also to a stimulation of central α_2 receptors, which are thought to be responsible for clonidine's sedative effects.

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In conclusion, we have shown that ester-prodrug methodology can be a useful method for increasing a drugs ability to pass the blood-brain barrier. These results have led to a better understanding of the factors controlling the brain penetration of 6,7-ADTN.

Experimental Section

Melting points were determined in open glass capillaries on a Büchi Tottoli apparatus and are uncorrected. Elemental analyses were performed in the Department of Chemistry, University of Groningen. Where elemental analyses are indicated, results obtained were within ±0.4% of the theoretical values. Infrared spectra were run on a Beckman Acculab 2. Mass spectra were obtained using a Finnigan 3300.

2-Amino-6,7-diacetoxy-1,2,3,4-tetrahydronaphthalene Hydrobromide (1). Under a nitrogen atmosphere, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide[9] (0.520 g, 0.002 mol) was added to a solution of acetyl bromide (0.786 g, 0.0064 mol) in trifluoroacetic acid (8.0 mL). The solution was stirred at room temperature for 1.5 h. The excess acetyl bromide and trifluoroacetic acid was then removed under reduced pressure, and 50 mL of dry ether was added. The resulting white precipitate was filtered off to yield 0.570 g (83%) of the HBr salt. After crystallization from methanol–ether, a white crystalline solid was obtained: mp 198–199.5 °C; IR (KBr) 1755 (C=O) cm^{-1}; MS, *m/e* 263 (M^+). Anal. ($C_{14}H_{17}NO_4$·HBr) C, H, N, Br.

2-Amino-6,7-bis(isobutyryloxy)-1,2,3,4-tetrahydronaphthalene Hydrobromide (2). Under a nitrogen atmosphere, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide[9] (0.520 g, 0.002 mol) was added to a solution of isobutyryl chloride (0.680 g, 0.0064 mol) in trifluoroacetic acid (8.0 mL), and using the same general procedure as above, 0.640 g (80%) of a white HBr salt was obtained. Crystallization from methanol–ether produced a white crystalline solid: mp 210–211 °C; IR (KBr) 1765 (C=O) cm^{-1}; MS, *m/e* 319 (M^+). Anal. ($C_{18}H_{25}NO_4$·HBr) C, H, N, Br.

2-Amino-6,7-bis(pivaloyloxy)-1,2,3,4-tetrahydronaphthalene Hydrobromide (3). When the same general method was used, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide[9] (0.520 g, 0.002 mol) and pivaloyl chloride (0.770 g, 0.0064 mol) in trifluoroacetic acid (8 mL) yielded, after workup, 0.520 g (61%) of a white crystalline solid, mp 275–276 °C; IR (KBr) 1755 (C=O) cm^{-1}; MS, *m/e* 347 (M^+). Anal. ($C_{20}H_{29}NO_4$·HBr) C, H, N, Br.

2-Amino-6,7-bis(benzoyloxy)-1,2,3,4-tetrahydronaphthalene Maleate (4). 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide[9] (1.0 g, 0.0038 mol) and benzoyl bromide (2.26 g, 0.0122 mol) in trifluoroacetic acid (12 mL) under the above standard reaction conditions yielded an oily semisolid HBr salt that could not be induced to crystallize. This material was dissolved in 50 mL of water and carefully made basic to litmus paper by the addition of 1 N $NaHCO_3$. This solution was then extracted with ether (4 × 150 mL), and the ether extract was then dried over anhydrous $MgSO_4$. The dried ether extract was then evaporated under reduced pressure to yield an oil. An equivalent amount of maleic acid in isopropyl acetate was added to the oil, and this produced a white solid: 1.60 g (82.5%). The maleate was crystallized from methanol–ether to produce a white crystalline solid: mp 148–150 °C; IR (KBr) 1740 (C=O) cm^{-1}; MS, *m/e* 387 (M^+). Anal. ($C_{24}H_{21}NO_4$·$C_4H_4O_4$) C, H, N.

Neurochemistry. Female Wistar albino rats (C.D.L. Groningen) weighing 150–200 g were used in all experiments. They were injected with solutions of the prodrugs prepared in a mixture of polyethylene glycol 400–ethanol–water (4:3:3, v/v). After various time intervals, the rats were sacrificed, their brains were removed, and the corpus striatum and cerebellum were dissected on dry ice. Tissue samples were then stored at −80 °C until assayed for 6,7-ADTN and 6-hydroxy-7-methoxy-2-aminotetralin levels. Details of the experimental procedure for the isolation and subsequent analysis of these two substances using an HPLC C_{18} reverse-phase chromatography system linked to a rotating electrochemical detector have already been published.[7,8,11] All reported values have been corrected for recovery, which was determined by spiking brain samples from untreated rats.

Angiotensin Converting Enzyme Inhibitors: Modifications of a Tripeptide Analogue

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Modified nonhydrolyzable tripeptide analogues of (*S*)-1-[5-(benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline (1), designed to impart oral angiotensin converting enzyme (ACE) inhibitory activity, were made and evaluated in vivo and in vitro. The *N*-methyl and C_5-methyl analogues of 1 were inactive. Insertion of heteroatoms (O, S, NH) into the C–C chain of 1 gave a series of compounds with high in vitro activity in the guinea pig serum ACE assay. The O-analogue was the most potent with an $IC_{50} = 4.4 \times 10^{-9}$ M compared to 1 with an $IC_{50} = 3.2 \times 10^{-9}$ M. The structure–activity relationships in this series of compounds lead one to speculate that the heteroatom provides an additional binding site to the surface of the enzyme; however, these compounds were inactive when tested for antihypertensive activity in the renal hypertensive rat at 30 mg/kg by the oral route (captopril is active at 1.0 mg/kg po).

Angiotensin converting enzyme (ACE) inhibitors hold great promise in the treatment of hypertension.[1] Recently, the efficient synthesis[2] of a ketomethylene analogue (1)[3] of the tripeptide Bz-Phe-Gly-Pro was described, which showed potent in vitro ACE-inhibitory activity and in vivo activity during continuous iv infusion. This analogue was less active in vivo when given either by iv bolus injection or by the oral route. It was suggested that limited in vivo activity by iv bolus injection may be due to rapid metabolic

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