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INHIBITION OF CATECHOL-*O*-METHYLTRANSFERASE (COMT) AS WELL AS TYROSINE AND TRYPTOPHAN HYDROXYLASE BY THE ORALLY ACTIVE IRON CHELATOR, 1,2-DIMETHYL-3-HYDROXYPYRIDIN-4-ONE (L1, CP20), IN RAT BRAIN *IN VIVO*

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Abstract—The orally active iron chelator, 1,2-dimethyl-3-hydroxypyridin-4-one (L1, CP20) proposed for reduction of iron overload in hemoglobinopathic patients, was studied in rats with respect to its ability to interfere with dopamine (DA) and serotonin (5-HT) metabolism. At 100 mg/kg i.p., it reduced the levels of DA, 5-HT, 5-hydroxyindoleacetic acid and particularly homovanillic acid in the rat striatum for several hours. These effects were shown to result from concomitant inhibition of catechol-*O*-methyltransferase (COMT; EC 2.1.1.6), tyrosine [tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating) (EC 1.14.16.2)] and tryptophan hydroxylase [tryptophan, tetrahydropteridine:oxygen oxidoreductase (5-hydroxylating) (EC 1.14.16.4)], with similar time-courses. COMT was inhibited with a threshold dose of about 1 mg/kg i.p. and an ED_{50} of about 10 mg/kg i.p. as determined by the conversion of exogenous L-dihydroxyphenylalanine (L-DOPA) to its O-methylated derivative. Tyrosine and tryptophan hydroxylase activities as measured by the accumulation of DOPA and 5-hydroxytryptophan, respectively, after central decarboxylase inhibition, were inhibited in striatum and cortex, with threshold doses of 3–10 mg/kg and ED_{50}s of about 20–30 mg/kg i.p. or p.o. While COMT inhibition by L1 is probably related to the structural similarity of the latter drug with the normal enzyme substrates, tyrosine and tryptophan hydroxylase inhibition is more likely due to coordination to iron bound to these enzymes. Desferrioxamine at 100 mg/kg i.p. did not show comparable effects. It is not known whether this relates to poor brain and/or cell penetration, or whether multidentate chelators are less suitable as inhibitors of aromatic amino acid hydroxylases.

The iron chelator, 1,2-dimethyl-3-hydroxypyridin-4-one (also known under the code denominations CP20 or L1†) has been proposed for the reduction of iron overload that occurs in hemoglobinopathic patients as a consequence of frequent blood transfusions [1, 2]. Though less effective in removing iron from the body than desferrioxamine [3], the only widely used drug for this purpose, L1 is orally active [1].

On the other hand, L1 is a close structural analogue of 3-hydroxy-4-pyridone (Fig. 1) that was shown long ago to inhibit catechol-*O*-methyltransferase (COMT; EC 2.1.1.6) *in vitro* [4]. COMT inhibitors are thought to be useful as adjuncts in L-dihydroxyphenylalanine (L-DOPA) therapy of Parkinson's disease [5]. The combination of COMT inhibitory and iron chelating properties might make L1 a particularly useful compound for this condition.

Increased basal lipid peroxidation in the substantia nigra (SN) of parkinsonian patients [6] has been related to increased iron concentrations [7, 8]. Melanized dopaminergic SN neurons are particularly susceptible to degeneration [9]. Melanin has a high capacity for binding iron [10] and may play a role in the degeneration process in Parkinson's disease by inducing the formation of radicals at high iron concentrations [11]. It has been argued that removal of iron from the SN should be beneficial, and concomitant intraventricular treatment with desferrioxamine was shown to prevent dopaminergic lesions caused by 6-hydroxydopamine [11]. The neurotoxicity of this agent is believed to involve generation of free radicals under participation of transition metals [12–14].

An orally bioavailable iron chelator like L1 might, therefore, be a useful agent to prevent or halt degeneration of dopaminergic neurons in Parkinson's disease. On the other hand, it has long been known that the key enzyme in dopamine (DA) synthesis, tyrosine hydroxylase [tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating) (EC 1.14.16.2)], is dependent on (bivalent) iron (for example see Ref. 15). Its activity *in vitro* and *in vivo* is markedly inhibited in the presence of iron chelators like bipyridyl [16]. It obviously might present a problem for the therapy of iron overload states if such an effect occurred *in vivo* after administration of an oral iron chelator in relevant doses.

We have investigated the effects of L1 on DA

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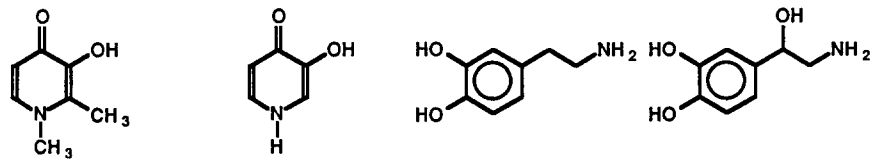
† Abbreviations: COMT, catechol-*O*-methyltransferase; DA, dopamine; DOPA, 3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; 5-HTP, 5-hydroxytryptophan; HVA, homovanillic acid; MAO-A, monoamine oxidase A; 3-MT, 3-methoxytyramine; 3-OMD, 3-*O*-methyldopa; SN, substantia nigra; L1 or CP20, 1,2-dimethyl-3-hydroxypyridin-4-one; SOS, sodium octylsulphate.



1,2-dimethyl-3-hydroxypyridin-4-one | 3-hydroxy-4-pyridone | dopamine | noradrenaline

Fig. 1. Structural formulae of L1, 3-hydroxy-4-pyridone and catecholamines.

synthesis and metabolism in the rat striatum and to some extent in the cortex. Moreover, because the key enzyme in serotonin (5-HT) synthesis, tryptophan hydroxylase [tryptophan, tetrahydropteridine: oxygen oxidoreductase (5-hydroxylating) (EC 1.14.16.4)], is also iron-dependent [17], we have included measurements of the synthesis and concentration of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA).

MATERIALS AND METHODS

L1 was dissolved in one third 0.5% methylcellulose/two thirds H_2O, under addition of 1–2 drops of lactic acid. Desferrioxamine was dissolved in one third 0.5% carboxymethylcellulose/two thirds H_2O. Injection volumes were 2 mL/kg for i.p. administrations and 5 mL/kg for oral treatments. Control animals were treated with vehicle where appropriate (dose–response experiments).

Determination of DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT and 5-HIAA

Female Tif:RAIf(SPF) rats (Tierfarm Sisseln, Switzerland) weighing 160–180 g were killed by decapitation. Striata or cortices were homogenized for 20 sec in 2 mL of the mobile phase used in the HPLC procedure (see below), containing 200 ng/mL α-methyldopa as internal standard, by sonication (Sonifier B-12, Branson, Danbury, CT, U.S.A.; setting 10), and centrifuged for 10 min at 40,000 *g*. The supernatants were frozen overnight and recentrifuged. Two hundred microlitres of the supernatants were injected into the HPLC system.

Determination of 5-hydroxytryptophan (5-HTP) and DOPA accumulation after central decarboxylase inhibition

After treatment with the drugs to be tested, female Tif:RAIf(SPF) rats weighing 160–180 g were injected with 100 mg/kg i.p. of the decarboxylase inhibitor, 3-hydroxybenzylhydrazine (NSD 1015), and killed by decapitation 30 min thereafter. After dissection and weighing, brain areas were frozen on dry ice and stored at −70° until analysed. Striata and cortices were homogenized for 20 sec by sonication in 2 mL of the corresponding mobile phase (see below), and centrifuged for 10 min at 40,000 *g*. One aliquot of 0.5 mL of the supernatant was used for the determination of DOPA, after addition of 400 ng α-methyldopa. A second aliquot of 0.8 mL was used for the determination of 5-HTP, after addition of 100 ng α-methyldopamine as the internal standard. Both aliquots were frozen overnight and recentrifuged. One hundred microlitres of the supernatants were injected into the HPLC systems.

Determination of the accumulation of 3-O-methyldopa (3-OMD) formed from exogenously administered L-DOPA, and L-DOPA itself, in rat striatum

Male Tif:RAIf(SPF) rats weighing 160–200 g were killed by decapitation, and the striata subsequently dissected. Both striata from one animal were homogenized in 2 mL 0.1 M HCl. The homogenates were centrifuged twice at 32,000 *g* for 10 min. Twenty microlitres of the supernatants were diluted with 150 μL of ESA (Environmental Sciences Associates, Inc., Bedford, MA, U.S.A.) buffer CAT-A (pH 2.5) containing the internal standard isoprenaline (8 μg/L). Fifty microlitres were injected into the HPLC system.

HPLC determinations. The basic HPLC systems consisted of an Altex 110 or a Waters 510 pump, an LC 22 temperature controller and an LC 23 column heater [Bioanalytical Systems (BAS), W. Lafayette, IN, U.S.A.]. For the determination of DA, HVA, DOPAC, 5-HT, and 5-HIAA, a Phenomenex Bondclone 10 C_{18} 300 × 3.9 mm (Phenomenex, Torrance CA 90501, U.S.A.) column kept at 27° and a BAS LC4B amperometric detector operated at 0.7 V was used. The mobile phase consisted of 0.15 M chloroacetic acid, pH 3.0, containing 8% acetonitrile, 0.65 mM sodium octylsulphate (SOS) and 0.7 mM Na_2-EDTA. The flow rate was 0.8 mL/min. Typical retention times were: α-methyldopa ~ 6.1 min; DOPAC ~ 5.7 min; DA ~ 9.2 min; 5-HIAA ~ 12.7 min; HVA ~ 14.9 min; 5-HT ~ 22.2 min. 5-HTP was determined using the same apparatus, only the mobile phase contained 1.51 mM SOS. Typical retention times were: 5-HTP ~ 8.6 min; α-methyldopamine ~ 16.4 min. DOPA was determined with the same column and temperature, but using an ESA 5100 A coulometric detector with a 5010 analytical cell (Environmental Sciences Assoc., Bedford, MA, U.S.A.; detector 1 0.15 V, detector 2 off). The mobile phase consisted of 0.15 M chloroacetic acid, pH 2.9, containing 240 μM SOS and 0.2 mM Na_2-EDTA delivered at 1.0 mL/min. Typical retention times were: DOPA ~ 5.9 min; α-methyldopa ~ 9.6 min. DOPA and 3-OMD were determined using a 3 μm ESA catecholamine HR-80 column (100 × 4.6 mm) and a Coulochem ESA 5200 detector (conditioning cell: +100 mV, detector

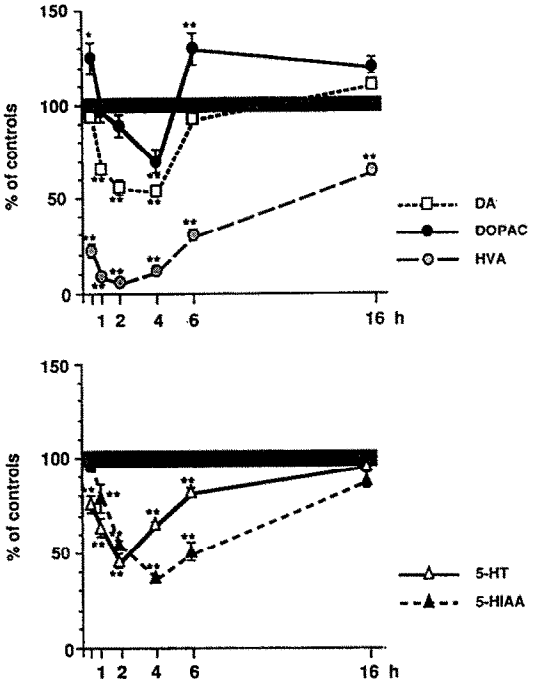


Fig. 2. Time-course of the effects of L1 on the levels of DA, 5-HT and their metabolites in striatum. Groups of six (controls = eight) rats were treated with 100 mg/kg i.p. L1 and decapitated at various intervals thereafter. Concentrations of DA, DOPAC, HVA (upper panel) and, 5-HT and 5-HIAA (lower panel) were determined in the striatum. Data are means ± SEM in per cent of controls. Absolute control values were: DA = 11,262 ± 473 ng/g; DOPAC = 1273 ± 52 ng/g; HVA = 962 ± 31 ng/g; 5-HT = 542 ± 22 ng/g; 5-HIAA = 935 ± 44 ng/g. *P < 0.05; **P < 0.01 vs controls (Dunnett's *t*-test).

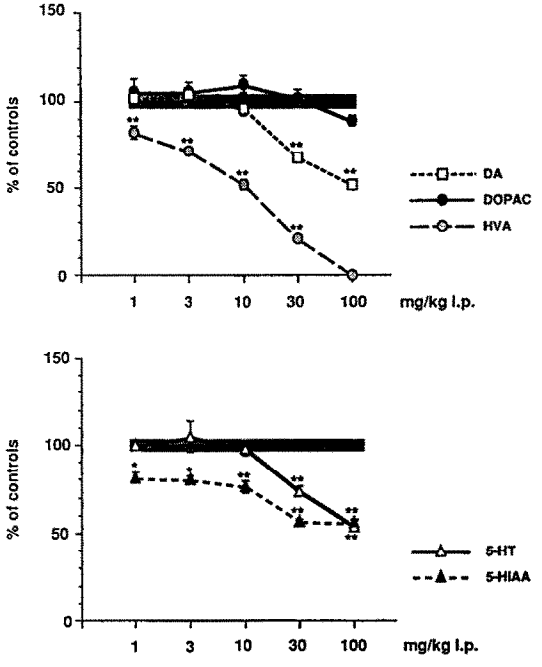


Fig. 3. Dose–response relationships of the effects of L1 on the levels of DA, 5-HT and their metabolites in striatum. Groups of six (controls = eight) rats were treated i.p. with graded doses of L1 and decapitated 2 hr thereafter. Concentrations of DA, DOPAC, HVA (upper panel) and, 5-HT and 5-HIAA (lower panel) were determined in the striatum. Data are means ± SEM in per cent of controls. Absolute control values were : DA = 7962 ± 357 ng/g; DOPAC = 774 ± 36 ng/g; HVA = 719 ± 22 ng/g; 5-HT = 449 ± 17 ng/g; 5-HIAA = 871 ± 48 ng/g. *P < 0.05; **P < 0.01 vs controls (Dunnett's *t*-test).

1: +100 mV, detector 2: +350 mV). The mobile phase (ESA CAT-A buffer pH 2.5 containing 1% MeOH and 0.054 mM SOS) was delivered at 0.8 mL/min. Typical retention times were: L-DOPA ~ 5.9 min; isoprenaline ~ 16.8 min; 3-OMD ~ 19.5 min.

Determination of DA and 3-methoxytyramine (3-MT) in rat striatum

Male Tif:RAIf(SPF) rats weighting 160–200 g were killed by microwave irradiation for 10 sec (2.8 kW operating power, 2450 MHz, 53 W/cm^2, Medical Engineering Consultants, Lexington, MA, U.S.A.). The brain was removed, cooled to 4° and striata were dissected. Both striata from one animal were homogenized in 1 mL 0.1 M HCl containing the internal standard metaraminol (200 μg/L). The homogenate was centrifuged for 5 min at 18,000 *g*. Fifty microlitres of the supernatant were dry-evaporated under vacuum at 34°. Fifty microlitres of pentafluoropropionic anhydride were added to each sample and the mixture reacted at 80° for 40 min. After cooling to 4°, the mixture was extracted with 50 μL of toluol (Vortex-mixed for 30 sec).

Samples of 2 μL were injected into a capillary GC/MS system (Carlo Erba HRGC Mega 2 Series Model MFC 800 gas chromatograph coupled to a Finnigan TSQ 700 mass spectrometer). This system was operated in the chemical ionisation mode with methane as the reagent-gas. Gas chromatographic separation was achieved on a 25 m × 0.3 mm glass capillary column coated with silicon gum SE 54. The oven temperature was raised from 80° at a rate of 8°/min to 270°. Helium was used as carrier gas. The temperatures of the GC/MS interface and the ion source were 250 and 120°, respectively.

Three characteristic MH^+ "quasi-molecular" ions were monitored by multiple ion detection; *m/z* 571 for DA, *m/z* 439 for 3-MT and *m/z* 585 for the internal standard metaraminol. Quantitation was done by relating the peak height ratios (amine/internal standard) to corresponding calibration curves.

RESULTS

Effects of L1 on striatal DA and 5-HT and their metabolites

At 100 mg/kg i.p., the compound caused a very marked, near-total decrease of HVA which was

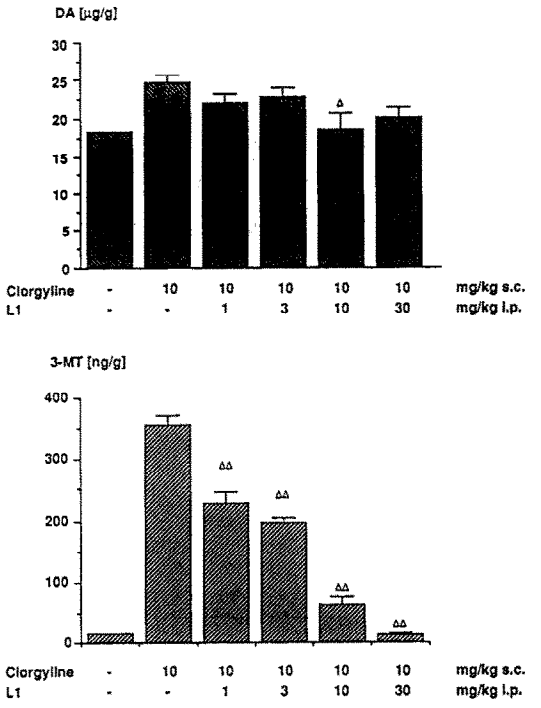


Fig. 4. Effect of L1 on the accumulation of striatal DA and 3-MT after MAO-A inhibition by clorgyline. Groups of five rats were treated i.p. with graded doses of L1 5 min before the s.c. injection of 10 mg/kg clorgyline. One group received clorgyline alone, and two animals received no treatment at all. The animals were killed by microwave irradiation 30 min later and DA (upper panel) and 3-MT (lower panel) were determined in the striatum. Data are means ± SEM in absolute values. $\Delta P < 0.05$; $\Delta\Delta P < 0.01$ vs clorgyline (Dunnett's *t*-test).

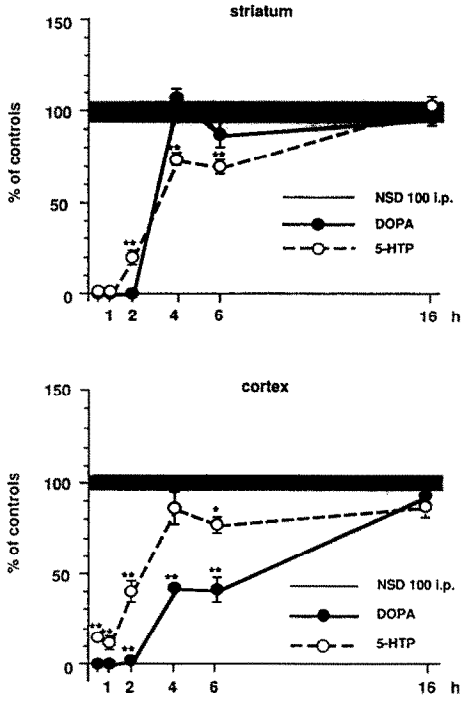


Fig. 5. Time-course of the effects of L1 on the accumulation of DOPA and 5-HTP in striatum and cortex after central decarboxylase inhibition. Groups of six (controls = eight) rats were treated with 100 mg/kg i.p. L1 at various intervals before 100 mg/kg i.p. of the decarboxylase inhibitor, NSD 1015, and decapitated 30 min thereafter. The amounts of DOPA and 5-HTP accumulated during these 30 min were determined in striatum (upper panel) and cortex (lower panel). Data are means ± SEM in per cent of control accumulations. Absolute control accumulations were: in the striatum; DOPA, 1330 ± 80 ng/g/30 min, 5-HTP, 144 ± 4 ng/g/30 min and in the cortex; DOPA, 88 ± 2 ng/g/30 min, 5-HTP, 68 ± 4 ng/g/30 min. $^*P < 0.05$; $^{**}P < 0.01$ vs controls (Dunnett's *t*-test).

maximal at about 2 hr and did not quite vanish within 16 hr (Fig. 2). DOPAC was also reduced after an initial, very short-lasting increase, but much less markedly than HVA; after a maximal decrease at 4 hr, it went above control levels again at 6 hr. After 16 hr, there was no longer any significant effect. DA and 5-HT concentrations were more markedly decreased, maximal effects occurring at 2–4 hr and 2 hr, control levels being reached at 6 and 16 hr, respectively. The decrease of 5-HIAA was similar to that of 5-HT, but the time-course seemed somewhat delayed.

Dose–response curves of these effects 2 hr after i.p. treatment with L1 are shown in Fig. 3. With HVA and 5-HIAA, significant decreases of the order of 20% were obtained with 1 mg/kg; with DA and 5-HT, this was the case at 30 mg/kg, and 50% reductions were obtained at 100 mg/kg. The ED_{50} for the reduction of HVA was 10 mg/kg.

Effects on DA and 3-MT concentrations after clorgyline pretreatment

L1 dose-dependently reduced the accumulation of 3-MT after inhibition of monoamine oxidase A (MAO-A). A marked, significant reduction was already seen with 1 mg/kg i.p. of L1; the ED_{50} was about 3 mg/kg, and a near-total suppression of 3-MT occurred at 30 mg/kg of L1 (Fig. 4 lower panel). Concomitantly, DA levels after clorgyline were also slightly but progressively reduced, becoming significant at 10 mg/kg i.p. of L1 (Fig. 4 upper panel). Due to the small increase of DA levels which MAO inhibitors cause, the effect of L1 on this parameter is not very impressive.

Effects on DA and 5-HT synthesis

As a measure of DA and 5-HT synthesis, the accumulation of their precursors DOPA and 5-HTP after central decarboxylase inhibition by NSD 1015 was chosen [18]. L1 at 100 mg/kg i.p. caused a complete suppression of the accumulation of both precursors in the striatum when given 30 min or 1 hr before the decarboxylase inhibitor (Fig. 5, upper panel). While DOPA accumulation was still totally inhibited at an interval of 2 hr, but had recovered after 4 hr, the inhibition of 5-HTP accumulation began to subside after 1 hr, though more slowly than

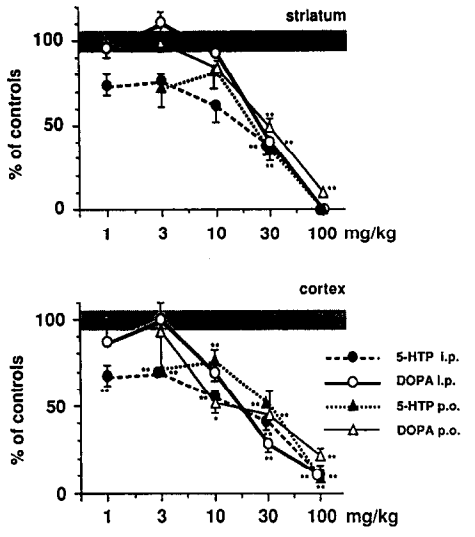


Fig. 6. Dose–response relationship of the effects of L1 on the accumulation of DOPA and 5-HTP in striatum and cortex after central decarboxylase inhibition. Groups of six (controls = eight) rats were treated with graded i.p. or oral doses of L1 1 hr before 100 mg/kg i.p. of the decarboxylase inhibitor, NSD 1015, and decapitated 30 min thereafter. The amounts of DOPA and 5-HTP accumulated during these 30 min were determined in striatum (upper panel) and cortex (lower panel). Data are means ± SEM in per cent of control accumulations. Absolute control accumulations were: in the striatum; DOPA, 1425 ± 57 ng/g/30 min, 5-HTP, 112 ± 9 ng/g/30 min and in the cortex; DOPA, 128 ± 7 ng/g/30 min, 5-HTP, 108 ± 6 ng/g/30 min. *P < 0.05; **P < 0.01 vs controls (Dunnett's *t*-test).

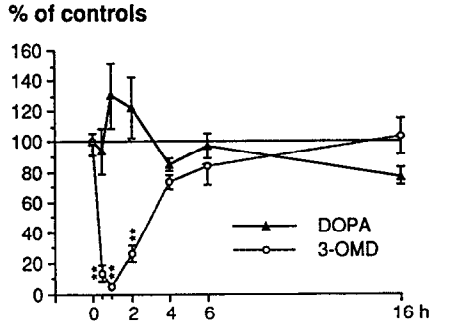


Fig. 7. Time-course of the effects of L1 on the conversion of exogenously administered L-DOPA to 3-OMD in striatum. Groups of five rats were treated with 100 mg/kg i.p. L1 at various intervals before 50 mg/kg i.p. L-DOPA methylester HCl, and decapitated 1 hr thereafter. A control group was treated with L-DOPA methylester HCl alone. The concentrations of DOPA and 3-OMD were determined in the striatum. Data are means ± SEM in per cent of control accumulations. The absolute values of the group treated with L-DOPA methylester HCl alone were: DOPA = 38 ± 4 ng/g; 3-OMD = 1418 ± 74 ng/g. **P < 0.01 vs controls (Dunnett's *t*-test).

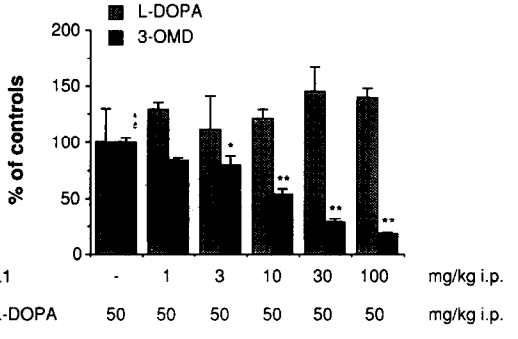


Fig. 8. Dose–response relationship of the effects of L1 on the conversion of exogenously administered L-DOPA to 3-OMD in striatum. Groups of five rats were treated with graded doses of L1 15 min before 50 mg/kg i.p. L-DOPA methylester HCl, and decapitated 1 hr thereafter. A control group was treated with L-DOPA methylester HCl alone. The concentrations of DOPA and 3-OMD were determined in the striatum. Data are means ± SEM in per cent of control accumulations. The absolute values of the group treated with L-DOPA methylester HCl alone were: DOPA = 67 ± 20 ng/g; 3-OMD = 1518 ± 66 ng/g. *P < 0.05; **P < 0.01 vs controls (Dunnett's *t*-test).

that of DOPA accumulation. Control levels were reached after 16 hr. A dose–response curve generated after 1 hr pretreatment with L1 given i.p. or orally shows that the threshold dose is about 10 mg/kg with respect to both DOPA and 5-HTP accumulation; there is no difference between i.p. and p.o. administration (Fig. 6). The ED_{50}s for both DOPA and 5-HTP accumulation are about 30 mg/kg, and 90% inhibition or more is obtained at 100 mg/kg.

In cortex, extent and time-course of L1's effect on 5-HTP accumulation were very similar to those in striatum (Fig. 5, lower panel). The inhibition of DOPA accumulation, however, seemed to be longer lasting in cortex as judged by comparison of the data at 4 and 6 hr. The dose–response relationships were quite similar to those observed in the striatum (Fig. 6, lower panel), although significant effects were already obtained at lower doses than in the cortex.

Effect on conversion of exogenously administered L-DOPA to 3-OMD in striatum

At 100 mg/kg i.p., L1 almost totally suppressed O-methylation of exogenously administered L-DOPA (in the form of its methyl ester hydrochloride) when given 30 min or 1 hr beforehand (Fig. 7). Thereafter, the effect gradually subsided and had vanished after 6 hr. Concomitantly, DOPA levels showed a trend in the opposite direction, but the changes were not statistically significant. In terms of a dose–response relationship, Fig. 8 shows that the inhibitory effect of L1 on the O-methylation of L-DOPA was dose-dependent, already significant at 1 mg/kg i.p., and reached an ED_{50} at about 10 mg/kg i.p. DOPA concentrations were again not significantly increased.

Effects of desferrioxamine on DA and 5-HT synthesis

For comparison, we tested desferrioxamine, an

iron chelator which presumably does not cross the blood–brain barrier, on the synthesis of DA and 5-HT in striatum and cortex. Although it is at least twice as potent as L1 to remove iron from the body, the dose of 100 mg/kg i.p was chosen to be the same. Desferrioxamine did not cause decreases in DOPA and 5-HTP accumulation, neither in the striatum nor in the cortex (Table 1). In contrast, it caused a weak though significant, transient increase of striatal DOPA accumulation and of striatal and cortical 5-HTP accumulation.

DISCUSSION

In the rat, L1 had profound, dose-dependent, but relatively short-lasting effects on striatal monoamine metabolism which demonstrate in the first place that this iron chelator penetrates the blood–brain barrier. The effects were in part compatible with COMT inhibition, particularly the marked decrease of HVA. However, if COMT inhibition was the only action of L1 on DA metabolism, one would have expected a concomitant increase of DOPAC, as has been observed with other COMT inhibitors [19], and no effect on DA levels. Instead, we observed a decrease of DOPAC after an initial increase, and a marked reduction of DA, which both showed a similar temporal profile as the diminution of HVA. Likewise, the marked and dose-dependent decrease of the accumulation of 3-MT after inhibition of MAO-A by clorgyline was accompanied by a reduction in the corresponding DA concentrations, again suggesting an effect of L1 other than, or in addition to, COMT inhibition.

From the effects of L1 discussed above, it was quite obvious that tyrosine hydroxylase inhibition would be a viable explanation. Therefore, we have measured the effects of the compound on a parameter reflecting tyrosine hydroxylase activity *in vivo*, i.e. the accumulation of DOPA after central decarboxylase inhibition [18]. The suspicion was confirmed, L1 did indeed inhibit tyrosine hydroxylation. It was equally potent after oral than after i.p. administration. Interestingly, its effect was somewhat longer lasting in the cortex, but not obviously more potent: only at the intermediate doses of 10 and 30 mg/kg p.o. or i.p., the inhibitory effects were slightly more marked than in the striatum. Concomitantly, approximately synchronously with its effects on DA synthesis and metabolism, and with similar potency, L1 also reduced the levels of 5-HT and its metabolite, 5-HIAA. This was shown to be a consequence of the inhibition of tryptophan hydroxylase, as evidenced by the attenuation of the accumulation of 5-HTP after central decarboxylase inhibition.

In order to obtain concise information on the role of COMT inhibition in the effects of L1 on DA metabolism, we looked at a parameter independent of its action on DA synthesis, i.e. the conversion of exogenous L-DOPA to its O-methylated derivative, 3-OMD. The results clearly demonstrated that L1 impaired this process with a time-course matching those of the synthesis and levels of DA and its metabolites. The dose–response curve was less steep, started at lower doses, and the ED_{50} with about 10 mg/kg i.p. was 3-fold lower than with tyrosine or tryptophan hydroxylation. L1 is thus quite a potent COMT inhibitor in the rat brain *in vivo*, comparable to tropolone or CGP 28014 [19]. It should be noted *inter alia* that tropolone is a good iron chelator [20] while CGP 28014 is not (G. von Sprecher, personal communication). The *in vivo* potency of L1 is perhaps greater than might be expected from its IC_{50} of about 20 μM to inhibit COMT in rat liver homogenates *in vitro* (Erbland and Waldmeier, unpublished). The compound seems to be a substrate, albeit a poor one, of COMT, since its 3-O-methylated metabolite has been found to a minor extent in rat urine [21].

The COMT inhibitory properties of L1 are, in all probability, not related to its ability to chelate iron, but rather a consequence of its catechol structure. On the other hand, its effects on tyrosine and tryptophan hydroxylase may be due to iron complexation. These enzymes are iron-dependent [15, 17], and at least tyrosine hydroxylase has been shown to be inhibited by iron chelators *in vitro* and

Table 1. Effects of desferrioxamine on striatal and cortical DOPA and 5-HTP accumulation

Treatment intervals (hr)	Striatum DOPA ng/g/30 min	5-HTP ng/g/30 min	Cortex DOPA ng/g/30 min	5-HTP ng/g/30 min
Controls	1410 ± 41	168 ± 10	134 ± 10	61 ± 3
0.5	1749 ± 129*	214 ± 13*	138 ± 7	76 ± 3*
1	1518 ± 76	189 ± 10	128 ± 12	72 ± 5
2	1556 ± 90	189 ± 11	134 ± 3	74 ± 3
4	1612 ± 102	196 ± 14	125 ± 6	75 ± 4*
6	1412 ± 37	172 ± 5	107 ± 6	68 ± 5
16	1402 ± 70	170 ± 15	106 ± 4	64 ± 2

Groups of six (controls = eight) rats were treated with 100 mg/kg i.p. desferrioxamine at various intervals before 100 mg/kg i.p. of the decarboxylase inhibitor, NSD 1015, and decapitated 30 min thereafter. The amounts of DOPA and 5-HTP accumulated during these 30 min were determined in striatum and cortex.

Data are means ± SEM of DOPA and 5-HTP accumulation in ng/g tissue per 30 min.

* $P < 0.05$ (Dunnett's *t*-test).

in vivo [16]. Also, both enzymes have been shown to be inhibited by catechol derivatives [22, 23] (for older literature see also references quoted in Ref. 24). Early on, it was suggested that inhibition of tryptophan hydroxylase and phenylalanine hydroxylase, a related enzyme, is due to catechol coordination to the iron centre [25, 26]. In the case of bovine adrenal tyrosine hydroxylase, strong evidence for this was reported by Andersson *et al.* [24]. These authors suggested that two sites on the iron coordination sphere are accessible to catechols. They seem to bind to the (inactive) Fe(III) form of the enzyme, preventing the reduction to the (active) Fe(II) form by the tetrahydropterine cofactor by stabilizing the Fe(III) form or impeding the access of the cofactor to the iron centre. This interaction of catechols with tyrosine hydroxylase may well represent the mechanism of end product inhibition and thus be of physiological relevance [22, 24]. Taking this information into account, it seems more likely that L1 produced the effects reported in this study by binding to iron coordinated to tyrosine and tryptophan hydroxylase in a ternary complex than by removing the metal ion from the enzymes. An interesting issue which awaits clarification is whether, due to the particular sterical situation at the Fe–enzyme complex, bidentate ligands can bind more effectively than multidentate ligands like desferrioxamine.

The fact that desferrioxamine did not show evidence of tyrosine and tryptophan hydroxylase inhibition may be related to poor or absent brain penetration. Although no data are available to document this, its low lipophilicity (P value ≤ 0.01, H. P. Schnebli, personal communication) is a good argument. On the other hand, in consideration of the suggestion by Andersson *et al.* [24] of two "free" sites in the coordination sphere of iron bound to tyrosine hydroxylase, it seems possible that bidentate ligands fit better than multidentate ligands. Taylor *et al.* [16] reported that bipyridil and *o*-phenantroline are better inhibitors of tyrosine hydroxylase *in vitro* than EDTA, while other bidentate ligands such as iron or 8-hydroxyquinoline were rather poor. While these authors thought that the pattern they observed indicated that agents preferentially chelating divalent iron were better tyrosine hydroxylase inhibitors, this seems to be at variance with the conclusion of Andersson *et al.* [24]. One can therefore not exclude at present that bidentate compounds are in principle more suitable inhibitors than multidentate ligands, provided they do not contain groups that hinder their access to iron bound to the enzyme.

COMT inhibition by L1 is not likely to present a problem in the treatment of iron overload in hemoglobinopathic patients with this compound. For the treatment of parkinsonian patients within the context of the iron hypothesis it would even represent an advantage over other iron chelators which do not possess this property. This positive judgement can, however, not be extended to the other properties reported here, i.e. the inhibition of tyrosine and tryptophan hydroxylase. Catecholamines and 5-HT play important roles in CNS function as well as in the periphery and have been implicated in various psychiatric and neurological diseases. The tyrosine hydrolase inhibitor α-methyl-*p*-tyrosine, in doses causing 70–80% inhibition of the enzyme, was reported to cause acute dystonic reactions [27], anxiety [28], and panic attacks [28] in humans. The doses of L1 used in hemoglobinopathic patients are of the order of 50–100 mg/kg/day [2], i.e. clearly in the range of the doses in which we find tyrosine and tryptophan hydroxylase inhibition in the rat. It is therefore possible that these enzymes are inhibited in patients treated with L1 at iron chelating doses. Moreover, in view of the similarities between the aromatic amino acid hydroxylases, it might be advisable to investigate potential effects of L1 on phenylalanine hydroxylase and its impact on phenylalanine metabolism. It may well prove that the lipophilicity of L1 and its congeners, which confers oral bioavailability but also enables cell penetration, ultimately causes unwanted effects by allowing these drugs to inactivate cellular iron enzymes. It will also be interesting to see whether more lipophilic, orally bioavailable multidentate ligands share the effects of L1 on aromatic amino acid hydroxylases.

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