J. Pharm. Pharmacol. 1984, 36: 411–413
Communicated November 16, 1984

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Effects of nomifensine and its metabolites on dihydropteridine reductase

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Nomifensine and three of its metabolites were studied as potential inhibitors of dihydropteridine reductase. Purified enzyme preparations from human liver and the P_2 fraction of rat striatal synaptosomes were used as enzyme sources. Nomifensine and its 3′-hydroxyl derivative inhibit this enzyme from both sources at 1·3 to 3·5 × 10^{-4} M (I50 values). 4′-Hydroxylated nomifensines, however, non-competitively inhibited this enzyme with K_i values of 2·8 to 4·4 × 10^{-5} M. Dihydropteridine reductase regenerates tetrahydrobiopterin, the required cofactor for the hydroxylation of tyrosine and tryptophan, from quinonoid dihydrobiopterin. Inhibition of this enzyme could reduce the availability of the biopterin cofactor for the synthesis of dopamine and 5-hydroxytryptamine.

The tetrahydroisoquinoline, nomifensine, is a relatively new antidepressant drug acting primarily by inhibiting the re-uptake, into presynaptic neuronal terminals, of both dopamine (Hunt et al 1974; Maj et al 1976; Tuomisto 1977; Algeri et al 1982) and noradrenaline (Schacht & Heptner 1974; Maj et al 1976; Tuomisto 1977). Although nomifensine does not directly affect the release of dopamine from rat brain synaptosomes (Schacht & Heptner 1974; Raiteri et al 1979), some studies have shown that it enhances (Braestrup & Scheel-Kruger 1976), and others that it inhibits (Dembiec 1980; McKillop & Bradford 1981), synaptosomal dopamine release. Nomifensine and its 4′-hydroxyl metabolite are inactive as dopaminergic agonists (Poat et al 1978), as opposed to 3′,4′-dihydroxynomifensine, a putative precursor of the 3′- and/or 4′-methoxyl metabolites, which is a potent dopamine receptor agonist (Poat et al 1978; Costall & Naylor 1978) and a partial dopamine vascular agonist (Kohli & Goldberg 1980).

Studies on the effects of nomifensine on several neurotransmitter-related enzymes have shown that at high concentrations (10^{-3} M), nomifensine reduces the activity of monoamine oxidase B (MAO-B) from rat brain by 35% (Izumi et al 1976), but at 10^{-4} M it inhibits neither rat brain nor liver MAO activities (Schacht et al 1977). Nomifensine has no effect on dopamine-β-hydroxylase from bovine adrenal medulla (Izumi et al 1976) and does not alter endogenous dopamine (Keller et al 1982) or noradrenaline (Maj et al 1976) concentrations in rat brain. Raiteri et al (1982) however, have demonstrated its apparent activation of dopamine synthesis in rat brain. This evidence indicates that nomifensine may increase postsynaptic dopamine availability and/or activity by a variety of mechanisms.

Previous studies in our laboratories have demonstrated that dopamine-derived tetrahydroisoquinolines and compounds containing an hydroxyphenyl moiety markedly inhibit dihydropteridine reductase activity (Shen et al 1982; Shen 1983; Shen & Abell 1983). Dihydropteridine reductase (NADH: 6,7-dihydropteridine oxidoreductase, EC 1.6.99.10) catalyses the formation of tetrahydrobiopterin from quinonoid dihydrobiopterin, and possibly also plays a role in the de novo synthesis of tetrahydrobiopterin (Gal et al 1979), an essential cofactor for tyrosine hydroxylase (Kaufman & Fisher 1974). This enzyme may also play an important role in reversal of the dopamine-induced feedback inhibition of tyrosine hydroxylase activity (Nagatsu et al 1964; Udenfriend et al 1965). Inhibition of this enzyme may limit dopamine synthesis and/or increase the sensitivity of tyrosine hydroxylase to end product inhibition.

The purpose of this study was to determine if nomifensine and its metabolites affect dihydropteridine reductase activities in preparations purified from human liver and in rat striatal synaptosomes.

Materials and methods

Nomifensine maleate (Merital, HOE 984, 8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline), 8-amino-2-methyl-4-(4′-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline maleate (M_1), 8-amino-2-methyl-4-(4′-hydroxy-3′-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (M_2), and 8-amino-2-methyl-4-(3′-hydroxy-4′-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (M_3) were generously supplied by Hoechst-Roussel Pharmaceuticals, Inc. (Somerville, NJ). 2-Amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine ($DMPH_4$) was obtained from Aldrich Chemical Company (Milwaukee, WI). Horseradish peroxidase, maleic acid, and NADH were obtained from Sigma Chemical Company (St Louis, MO). Solutions of nomifensine and its metabolites were prepared fresh in 0·05 M Tris-HCl (pH 6·8) and degassed immediately before use.

Human liver dihydropteridine reductase was purified by ammonium sulphate precipitation and sequential chromatography (on a naphthoquinone affinity, a DEAE-Sephacel, and a second naphthoquinone affinity column) according to the procedures described by Shen

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& Abell 1981, Shen et al 1982, and Firgaira et al 1981. The enzyme preparation used in this study was form B, which had a specific activity of 422 U mg^{-1} protein (a 2720-fold purification from crude extract). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis revealed a single band corresponding to a subunit mol. wt of 26 000. Kinetic studies indicated K_m (Michaelis constant) values of 25 μM for quinonoid 2-amino-6,7-dimethyl-4-hydroxydihydropteridine ($qDMPH_2$) and 19 μM for NADH.

Rat striatal synaptosomes were prepared by the method of Gray & Whittaker (1962) with slight modification. Male Sprague-Dawley rats (220–280 g) were decapitated and their striatal tissue was removed and homogenized in 10 ml of 0·32 M sucrose solution (pH 7·4) with a motor-driven Teflon pestle-glass homogenizer (0·13–0·18 mm clearance). Striatal homogenates were centrifuged at 1000*g* for 15 min. The resulting supernatant was centrifuged at 17 000*g* for 20 min to sediment the P_2 fraction, which was then resuspended in 15 vol (w/v) of a buffered medium containing (in mM concn) 50 Tris-HCl (pH 7·4), NaCl 125, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, and glucose 10. Dihydropteridine reductase activity in intact synaptosomes was assayed at 50 μM of each substrate. Kinetic studies indicated that dihydropteridine reductase in rat striatal synaptosomes had K_m values of 17 μM for $qDMPH_2$ and 10 μM for NADH.

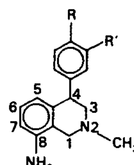
Dihydropteridine reductase activity was determined spectrophotometrically by measuring the rate of disappearance of NADH at 340 nm at 25 °C, according to the method of Nielsen et al (1969), using $qDMPH_2$ as substrate (Shen & Abell 1981; Shen 1983).

The reaction mixture in the presence and absence of inhibitors has been described (Shen & Abell 1983). Methods for obtaining the dissociation constants of the enzyme-inhibitor complexes (K_i values) and the inhibitor concentration that gives 50% inhibition of the enzyme activity (I50 values) have also been described (Shen 1983).

Results and discussion

Table 1 summarizes K_i and I50 values of nomifensine and its metabolites as inhibitors of dihydropteridine reductase in purified human liver preparations and in rat striatal synaptosomes. Nomifensine and metabolite M_3 are weak inhibitors of dihydropteridine reductase derived from both biological sources. However, metabolites with 4′-hydroxyl substituents, such as M_1 and M_2, are potent inhibitors of this enzyme, with K_i values in the range of 30 to 40 μM. Metabolite M_3, which contains a *m*-phenolic structure, is a less effective inhibitor, and nomifensine itself, which is devoid of hydroxyl substitution, demonstrates little or no inhibition of this enzyme. These results agree with our previous findings that compounds containing *p*-phenolic moieties are more potent than those with a *m*-phenolic structure (Shen

Table 1. K_i and I50 Values of nomifensine and its metabolites as inhibitors of dihydropteridine reductase. Purified human liver enzyme (6·6 mU or 17 ng protein) or rat striatal synaptosomes (6·5 mU or 100 μg P_2 protein) were incubated 10 min with each inhibitor at 25 °C. Residual enzyme activity was assayed at 50 μM of each substrate to obtain I50 values, and at different $qDMPH_2$ concentrations (20 to 50 μM) while the concentration of NADH was kept constant (50 μM), to obtain K_i values. All values are expressed in μM.



R	R′	Compound	Human liver I50	Human liver K_i	Rat striatal synaptosomes I50	Rat striatal synaptosomes K_i
H	H	8-Amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (nomifensine maleate)	345		192	
OH	H	8-Amino-2-methyl-4-(4′-hydroxyphenyl)-1,2,3,4-tetrahydro-isoquinoline (M_1)	45	35	35	44
OH	OCH_3	8-Amino-2-methyl-4-(4′-hydroxy-3′-methoxy-phenyl)-1,2,3,4-tetra-hydroisoquinoline (M_2)	48	28	36	30
OCH_3	OH	8-Amino-2-methyl-4-(3′-hydroxy-4′-methoxy-phenyl)-1,2,3,4-tetra-hydroisoquinoline (M_3)	241		131	
		Maleic acid (1 mM)	No effect		No effect	

1983; Shen & Abell 1983). Since nomifensine and M_1 were obtained in maleate forms, we also tested maleic acid (1 mM) as an inhibitor of dihydropteridine reductase and found that at 1 mM it had no effect on enzyme activity.

Lineweaver-Burk plots of the kinetics of inhibition of dihydropteridine reductase by nomifensine metabolites indicate noncompetitive inhibition with respect to either $qDMPH_2$ or NADH (data not shown). The I50 values for these compounds, which are comparable to their K_i values, also support the interpretation that these are non-competitive inhibitors (Cheng & Prusoff 1973).

The major metabolites of nomifensine in human and animal species are M_1, M_2, and M_3 (Kruse et al 1977; Heptner et al 1978). The 3′,4′-dihydroxyl metabolite, i.e. 8-amino-2-methyl-4-(3′,4′-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (M_5), along with three other minor metabolites, altogether account for less than 1% of the total metabolites in human urine (Heptner et al 1978). However, M_5 may be a precursor of M_2 and/or M_3 (Poat et al 1978), and it is a potent dopamine agonist (Poat et al 1978; Costall & Naylor 1978; Kohli & Goldberg 1980). We have found that dopamine (Shen et al 1982; Shen 1983) and dopamine agonists, such as apomorphine and its analogues (Shen et al 1983) are potent non-competitive inhibitors of dihydropteridine reductase.

The relevance of these in-vitro studies to the action of nomifensine and its metabolites in-vivo should be interpreted with caution. Our studies suggest that the 4'-hydroxy-3'-methoxy- and 4'-hydroxy-metabolites of nomifensine can significantly inhibit dihydropteridine reductase, an effect which could reduce the availability of the biopterin cofactor for tyrosine hydroxylation.

This work was generously supported by US Public Health Service (NICHD) grant DHHS HD-14635, the Hogg Foundation, and the Multidisciplinary Research Program on Schizophrenia. The authors thank Dr Constance B. Denney for helpful comments on the manuscript.

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