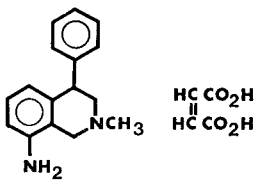
Nomifensine: a new potent inhibitor of dopamine uptake into synaptosomes from rat brain corpus striatum

Biogenic amines released into the synapse by neuronal activity are in large part re-captured by a very efficient re-uptake process across the pre-synaptic membrane. Inhibitors of this process provide a useful tool for investigating the mechanistic aspects of synaptic events. The re-uptake of 5-hydroxytryptamine (5-HT) and noradrenaline is blocked by a variety of pharmacological agents, but notably by the "imipramine-like" tricyclic antidepressants, whose clinical effects, moreover, have been correlated with their inhibitory activity (Carlsson, Corrodi & others, 1969; Kannengiesser, Hunt & Raynaud, 1973). The tricyclic compounds do not inhibit the re-uptake of dopamine (Horn, Coyle & Snyder, 1971; Fuxe & Ungerstedt, 1968), for which, among the most active inhibitors so far reported, are amphetamine and the powerful anti-acetylcholine compound, benztropine (Coyle & Snyder, 1969).



Nomifensine (Hoe 984)

A new inhibitor of dopamine uptake, nomifensine [8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline, hydrogen maleate, Farbwerke Hoechst AG, Frankfurt/Main Höchst], is more active than both amphetamine and benztropine in an *in vitro* preparation of synaptosomes.

Nomifensine has antidepressant and some central stimulating properties (Hoffmann, 1973) and in contrast to benztropine has only weak anti-acetylcholine activity, at least in the periphery. Other biochemical characteristics of the compound have recently been described (Schacht & Heptner, 1974).

The corpus striatum was dissected from the brain of an immature female rat (19–21 days), Sprague-Dawley strain, and homogenized in 0·32 M sucrose (3 ml) according to Whittaker (1969). The supernatant from the 900*g* debris centrifugation was diluted five times with 40 mM Na phosphate buffer pH 7·0 containing (mM) NaCl 100, KCl 4, D-glucose 11 and 0·2 mg ml^{-1} Na ascorbate. Aliquots (1·5 ml) of this suspension were incubated with [ring (G)]-[^{3}H]dopamine (500 mCi $mmol^{-1}$; Radiochemical Centre, Amersham, Bucks) and inhibitors as described. The incubation was terminated by cooling in ice. An aliquot was removed for radioactivity measurement and the remainder was centrifuged (Ecco, type E2/12 centrifuge) at 7000 *g* for 20 min at 4° which eliminates the use of high-speed centrifugation. The rinsed pellet was extracted with 0·4 N $HClO_4$ (0·3 ml) and after removal of precipitated proteins by centrifugation, the radioactivity in the extract was measured.

The preparation retains the characteristics of the re-uptake process as previously demonstrated for 5-HT (Kannengiesser & others, 1973). That is, dopamine is concentrated by a saturable*, temperature-dependent process, closely linked to the activity of the Na^+, K^+-ATPase system.

Nomifensine, benztropine and (±)-amphetamine were tested as inhibitors of [^{3}H]dopamine uptake into the crude synaptosome pellet by measuring the incorporation of radioactivity at various concentrations of inhibitor. The concentration (IC50) of compound producing 50% inhibition of uptake was estimated using log-probit paper as shown in Fig. 1.

Nomifensine (IC50 ± s.e. = $1{\cdot}45 \pm 0{\cdot}15 \times 10^{-7}$ M) proved to be approximately

* A kinetic constant ($K = 1{\cdot}52 \pm 0{\cdot}13 \times 10^{-7}$ M) for the overall uptake of dopamine was estimated as for 5-HT (Kannengiesser & others, 1973).

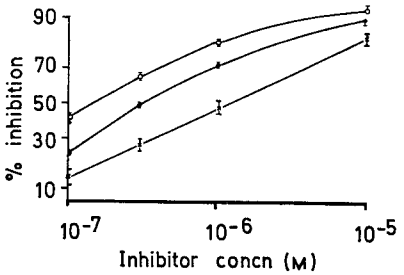


FIG. 1. Percentage inhibition of [^{3}H]dopamine uptake as a function of inhibitor concentration: (○) Nomifensine, (●) benztropine, (×) (±)- amphetamine. [^{3}H] Dopamine at 5×10^{-8} M, with or without inhibitor, was incubated for 5 min at 37° with the crude synaptosomal preparation. Inhibitors were at 10^{-7}, 3×10^{-7}, 10^{-6} or 10^{-5} M. The curves represent an average of 6 determinations in 3 separate experiments.

twice as active an inhibitor as benztropine (IC50 ± s.e. = $3{\cdot}1 \pm 0{\cdot}3 \times 10^{-7}$ M). In the same system, (±)-amphetamine (IC50 ± s.e. = $1{\cdot}2 \pm 0{\cdot}3 \times 10^{-6}$ M) was less active than benztropine.

Nomifensine is also an effective inhibitor of noradrenaline uptake in a preparation of synaptosomes from rat hypothalamus, having an activity of the order of nortriptyline (Schacht & Heptner, 1974). However, in view of its powerful inhibition of dopamine uptake, it is possible that nomifensine owes some of its antidepressant properties to this latter activity. That dopamine as well as 5-HT and noradrenaline may possibly be implicated in disorders of the affective state is suggested by the likely association of the reserpine depressive syndrome with the dopaminergic system (Ross & Renyi, 1967; Carlsson & Lindqvist, 1967).

In spite of certain central stimulating properties (Hoffmann, 1973), nomifensine does not resemble amphetamine insofar as it has no apparent "releasing" effect on either noradrenaline (Schacht & Heptner, 1974) or dopamine (Kannengiesser, unpublished observations) in an *in vitro* system.

Having apparently only weak anti-acetylcholine properties, the compound may provide a means of testing the theory of Coyle & Snyder (1969) that the anti-parkinsonian activity of certain anti-acetylcholine drugs such as benztropine is due, in part, to inhibition of dopamine uptake. This theory is based on the assumption that re-uptake inhibition potentiates transmitter activity at the synaptic level.

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