Studies on the dopamine agonist properties of 8-amino-2-methyl-4-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline, a derivative of nomifensine

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A pre-requisite for optimal dopamine-like activity of many compounds from the phenethylamine, aporphine and 2-amino-1,2,3,4-tetrahydronaphthalene series is the presence of a catechol moiety (Costall, Naylor & Pinder, 1974; McDermed, McKenzie & Phillips, 1975). Nomifensine, an isoquinoline derivative (Hoffmann, 1973), has recently been shown to possess powerful dopamine-like activity to modify motor function (Braestrup & Scheel-Kruger, 1976; Costall & Naylor, 1977), although a catechol grouping is conspicuously absent from its structure. Three identified metabolites of nomifensine have a single hydroxyl function in the 3 or 4 position but this is apparently insufficient to confer direct dopamine-like receptor stimulant properties (Braestrup & Scheel-Kruger, 1976; Costall & Naylor, 1977). In the present study we investigate the dopamine-like activity of a new derivative of nomifensine having hydroxyl functions in both the 3 and 4 positions, 8-amino-2-methyl-4-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (hereafter referred to as 3,4-diOH-nomifensine).

Male Sprague–Dawley rats, 200–300 g were used between 08.00 and 18.00 h in a sound-proofed room, maintained as close as possible to holding room temperature, 21 ± 1°, and diffusely illuminated. Animals for the circling experiments were prepared by injecting 6-hydroxydopamine (8 μg/4 μl) unilaterally into the nigrostriatal pathway at the level of the lateral hypothalamus (Ant. 4·6, Vert. −2·7, Lat. −1·9) (De Groot, 1959) (see also Costall, Naylor & Pycock, 1975 for details). Circling behaviour was measured by placing animals in a circular cage (diameter 40 cm) and manually recording the number of complete revolutions made in 1 min. Rats chosen showed circling of 10–15 rev min^{-1}, contralateral to the side of denervation, to a challenge of 0·25 mg kg^{-1} (s.c.) apomorphine. For injections into the nucleus accumbens, bilateral stainless steel guide cannulae, 0·65 mm diameter, were stereotaxically implanted according to Costall & Naylor (1977). Injection units, 0·3 mm diameter, deposited drug/solvent at the centre of the nucleus accumbens at Ant. 9·4, Vert. 0·0, Lat. ±1·6 (De Groot, 1959). Where nialamide was used this was given as a 2 h pretreatment (100 mg kg^{-1}, i.p.). Animals were used once only, 14 days after surgery. Hyperactivity was measured in Perspex boxes fitted with photocells (see Costall & Naylor, 1977 for details), or by using an Animex recorder.

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3,4-DiOH-nomifensine (dissolved in a minimum quantity of *NN*-dimethylformamide made up to volume with distilled water) given subcutaneously at 1 mg kg^{-1} caused only a weak stereotyped behaviour which could not be shown to be dose-dependent using the usual sytems for stereotypy assessment (Costall & Naylor, 1977). No response was seen at 3·7 mg kg^{-1}. At 7·5 mg kg^{-1} approximately 20% of animals exhibited weak stereotyped movements of the head and limbs (onset 20–30 min, duration approx. 2·5 h). At 15 and 30 mg kg^{-1} all animals responded with repetitive head and limb movements (onset 10–20 min, duration 3·5–4 h). Animals exhibiting stereotyped behaviour appeared alert but a 'true' hyperactivity was not noted using either an Animex recorder or photocell cages (recordings obtained were considered to reflect the stereotyped head and limb movements). Aceperone (5 mg kg^{-1}, i.p.), propranolol (5 mg kg^{-1}, i.p.), haloperidol (0·2 mg kg^{-1}, i.p.) and methysergide (1 mg kg^{-1}, i.p.), were given 45 min after 3,4-diOH-nomifensine (30 mg kg^{-1}, s.c.). α-Methyl-*p*-tyrosine (α-MT) (250 mg kg^{-1}, i.p.) was given as a 6 h pretreatment. Of these agents, only haloperidol (onset 15 min) and α-MT specifically abolished all motor components: an antagonism by aceperone could not be differentiated

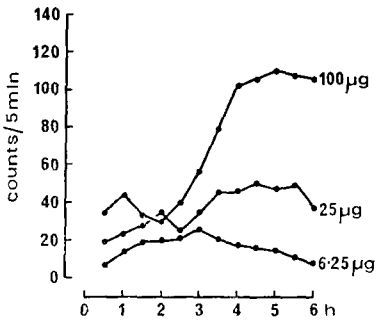

FIG. 1. Hyperactivity induced by 3,4-diOH-nomifensine on bilateral intracerebral injection into the nucleus accumbens (pretreatment with nialamide, 100 mg kg^{-1}, i.p. 2 h). Control animals (receiving intracerebral solvent) gave recordings of up to 15 counts/5 min during the first 30 min only; in subsequent periods control animals remained quiet or went to sleep, giving a recording of 0–5 counts/5 min only. See text for methodology. n = 6–8. s.e.s. < 16% mean values. Ordinate: Counts/5 min. Abscissa: Time (h).

from non-specific changes in motor function, in that this drug caused sedation at effective doses (whilst haloperidol and α-MT did not) as assessed in control animals.

Ipsilateral circling behaviour was induced by 15 and 30 mg kg^{-1} (s.c.) 3,4-diOH-nomifensine. The onset was delayed for 30–45 min, and the behaviour was of weak intensity (ipsilateral asymmetry apparent, but active circling of 2–3 rev min^{-1} only occurred when animals were disturbed). The response at both doses persisted for 2–2·5 h. No asymmetry/circling was observed at 7·5 mg kg^{-1}. Circling behaviour was subject to the same antagonism studies as described above for stereotyped behaviour: propranolol and methysergide failed to antagonize either the asymmetry or the mild circling apparent when animals are disturbed; aceperone prevented the circling (and not the asymmetry) but only at doses which caused sedation, whilst haloperidol and α-MT prevented both the asymmetry and weak circling responses.

The bilateral injection of 3,4-diOH-nomifensine directly into the nucleus accumbens of nialamide pretreated rats caused a dose-dependent hyperactivity in a dose range 6·25–100 μg (Fig. 1).

A dose-dependent hyperactivity was also produced in the absence of nialamide: generally, the onset of effect was the same and the duration was significantly ($P < 0·001$) reduced at all doses, e.g. at 6·25 μg, maximum intensity was 10–15 counts/5 min ($P > 0·05$), maximum duration was only 2 h ($P < 0·001$); at 25 μg maximum intensity was 35–40 counts/5 min ($P > 0·05$), maximum duration was 5 h ($P < 0·001$) (comparison with data on Fig. 1, n = 6–10, s.e.s <14% means). The marked hyperactivity induced by 3,4-diOH-nomifensine, 100 μg, in the presence of nialamide was not antagonized by aceperone (5 mg kg^{-1}, i.p.) or propranolol (5 mg kg^{-1}, i.p.), but was abolished by haloperidol (0·2 mg kg^{-1}, i.p.).

On peripheral administration, nomifensine can effectively induce changes in the motor behaviour of normal rats, hyperactivity, stereotypy and circling in rats with unilateral disruption of the nigrostriatal system. These motor effects have been related to an enhanced cerebral dopamine-like activity, and the modification of the nomifensine molecule to include the dihydroxy substitution, and thus render the phenethylamine component of the molecule closer to dopamine in structure, could theoretically be expected to enhance such effects. However, for peripheral administrations the reverse was found for 3,4-diOH-nomifensine.

Whilst the 3,4-dihydroxy derivative induced a distinct but modest ipsilateral circling in rats with unilateral 6-hydroxydopamine lesions, in normal rats the stereotyped movements were restricted to the head and limbs and the intensity of stereotypy, as assessed over a four-fold dose range, was independent of the dose administered. Although the animals appeared alert after drug treatment, and could easily be distinguished from vehicle-treated controls, an increase in 'locomotor' activity could not be detected with the photocell apparatus. The count recorded using the Animex was considered to be due to repetitive head and limb movements. Nevertheless, the induction of circling and stereotypy and the inability of α- and β-adrenoceptor blocking agents, aceperone and (±)-propranolol, and the 5-HT antagonist, methysergide, to specifically antagonize the behaviours, whilst haloperidol blocked all effects, could indicate a dopaminergic component. The direction of circling ipsilateral to the side of the lesion could indicate that the compound enhances dopamine function via a presynaptic action (Costall, & others, 1975). The abolition of effect by α-MT pretreatment, sufficient to disrupt catecholamine synthesis, supports this hypothesis. Whether this action involves an inhibition of dopamine re-uptake, similarly to nomifensine (Schacht, Leven & Backer, 1977), or an enhanced biogenic amine release, remains to be determined.

A number of factors could account for the reduced activity of 3,4-diOH-nomifensine. Firstly, the introduction of hydroxyl functions is known to reduce the ability of many compounds to pass the blood brain barrier. Secondly, the introduction of the catechol function (as in the phenethylamine and aporphine series) may allow a rapid peripheral methylation, and it may be significant that the methylated derivative (4-methoxy-3-hydroxy)nomifensine has low efficacy (Costall & Naylor, 1977) to cause dopamine-like behavioural effects.

To elucidate the first factor, 3,4-diOH-nomifensine was injected directly into the nucleus accumbens where it produced powerful dopamine-like effects. The marked dose-dependent hyperactivity was specifically blocked by haloperidol and by pretreatment with α-MT. The established specificity of this motor response within the nucleus accumbens for dopamine and like agents (Costall, Naylor & others, 1977) suggests that 3,4-diOH-nomifensine is acting in this area by enhancing presynaptic dopamine mechanisms. The failure of this agent to increase locomotor activity after peripheral administration could therefore reflect its failure to penetrate the blood brain barrier. When injected directly into cerebral tissue it was more effective than the methylated metabolites (3-methoxy-4-hydroxy- and 3-hydroxy-4-methoxy-), although the limited solubility of the 4-hydroxy-derivative does not allow a direct comparison (Costall & Naylor, 1977). Nevertheless, the present studies indicate that a catechol grouping enhances dopamine-like action within a series of potential agonists.

The authors are indebted to Dr K. Schmitt, Hoechst, Frankfurt, for the synthesis of 3,4-diOH-nomifensine. The studies were supported, in part, by the Medical Research Council.

January 30, 1978

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Pre- and postsynaptic α-adrenoceptor antagonism by indoramin in isolated tissues of the rat

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There is considerable evidence that adrenergic nerve terminals carry α-adrenoceptors which play an inhibitory role in transmitter release. It has been suggested that the presynaptic and postsynaptic α-adrenoceptors differ and that certain antagonists show specificity for one or other type of receptor (Starke, Borowski & Endo, 1975; Borowski, Ehrl & Starke, 1976).

We have compared indoramin hydrochloride, an antihypertensive agent with α-adrenoceptor antagonist activity (Alps, Hill & others, 1972) with phentolamine mesylate (Ciba) and thymoxamine hydrochloride (Warner) for presynaptic effects on the rat field-stimulated vas deferens preparation using a modification of the method of Drew (1977).

Desheathed vasa deferentia from sexually mature rats were suspended in a 6 ml organ bath in Krebs solution at 37° and bubbled with 5% CO_2 in oxygen. Platinum ring electrodes were positioned above and below the tissue for field stimulation, the stimulus parameters being 0·1 Hz, 1 ms pulse width at supramaximal voltage (Square wave stimulator, Scientific and Research Instruments Ltd.). Twitch responses were recorded isotonically (Harvard Apparatus Smooth Muscle Transducer) with a 0·5 g loading. Clonidine hydrochloride was used as the α-adrenoceptor agonist and cumulative concentration-response curves were constructed for the inhibition of twitch obtained with clonidine in the range 0·125 to 4 ng ml^{-1}. After washing out clonidine, the twitch response quickly recovered and an antagonist was then introduced into the Krebs reservoir. Clonidine concentration-response curves were repeated 90 min after introduction of the antagonist. The concentrations of clonidine producing 50% inhibition of twitch before and after introduction of antagonist were obtained and the dose-ratio for clonidine was calculated. Molar concentrations of antagonists used were: indoramin 10^{-6}, 10^{-5} and 5×10^{-5}, thymoxamine 10^{-5}, $2{\cdot}239 \times 10^{-5}$ and 5×10^{-5} and phentolamine 10^{-7}, $2{\cdot}239 \times 10^{-7}$, 5×10^{-7} and 10^{-6}. At least four preparations were used at each concentration.

None of the antagonists inhibited the twitch response. Both phentolamine and thymoxamine produced concentration-dependent antagonism of the clonidine response.

These results were plotted in the manner described by Arunlakshana & Schild (1959) and the values of pA_2 and slope were calculated. Indoramin produced no antagonism at 10^{-6} M, and a mean pA_2 of 5·13 ($n = 5$) at 10^{-5} M (pA_2 values calculated from individual dose-ratios assuming a plot slope of unity). Preparations showed spontaneous contractions when exposed to 5×10^{-5} M indoramin and the effects of clonidine could not be assessed. A summary of results is shown in Table 1. This table also includes data previously published from this laboratory (Collis & Alps, 1973) showing the activity of these antagonists against noradrenaline at postsynaptic α-adrenoceptors in the isolated perfused rat mesenteric vasculature preparation.

These results suggest that indoramin possesses little antagonist activity at presynaptic α-adrenoceptors in contrast to its marked competitive antagonism at the postsynaptic site. Thymoxamine resembles indoramin in having some selectivity for postsynaptic receptors but the slope of the Schild plot is not that expected for a simple competitive antagonism at presynaptic receptors.

Table 1. *Plot slopes and pA_2 values for α-adrenoceptor antagonists on the field-stimulated rat vas deferens and on the perfused rat mesenteric vasculature preparations derived according to Arunlakshana & Schild* (1959).

	Rat vas deferens		Rat mesenteric vasculature	
Antagonist	pA_2	Slope	pA_2	Slope
Indoramin	5·13*	—	8·05	−1·06
Phentolamine	7·90	−1·07	7·84	−0·91
Thymoxamine	4·85	−1·67	6·47	−1·00

* Mean value calculated from dose-ratios obtained at 10^{-5} M indoramin assuming a plot slope of unity ($n = 5$).

* Correspondence.