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Letters to the Editor

The pharmacological characterization of 3,4-dihydroxyphenylimino-2-imidazolidine (DPI) as a potent mixed α_1/α_2-adrenoceptor agonist rather than as a dopamine receptor agonist

J. C. VAN OENE, A. S. HORN*†, *Biochemical Neuropharmacology Section, ETB, NINCDS, National Institutes of Health, Bethesda, MD 20205, USA and †Pharmaceutical Laboratories, Department of Medicinal Chemistry, State University of Groningen, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands*

In the past decade many studies have been carried out on the pharmacological actions of the imidazoline derivative DPI (3,4-dihydroxyphenylimino-2-imidazolidine) because it has been proposed as a selective agonist at a postulated subtype of dopamine (DA) receptor. According to the first publication on this concept of multiple DA receptors, authored by Cools & van Rossum (1976), mammalian DA receptors can be divided into excitation-mediating (DAe) and inhibition-mediating (DAi) receptors. DAe receptors appeared to coincide with 'classical' DA receptors i.e. those that can be selectively stimulated with apomorphine and inhibited with haloperidol, whereas the newly postulated DAi receptors could be selectively stimulated with DPI and inhibited with ergometrine (Cools & van Rossum 1976, 1980). Though interesting from a conceptual point of view, the idea of the existence of DAi receptors in the mammalian brain was rather speculative, and Cools and coworkers consequently put much effort into attempting to provide it with an experimental basis. They found that in some test models DPI and ergometrine had indeed opposing effects, and they explained these results as being in strong support of the presence of DAi receptors and thus of the validity of the DAe/DAi concept (Cools et al 1976; Cools 1977). In spite of the latter conclusion, however, these authors have repeatedly revised their original concept, i.e. by the additional postulations of the 'alpha-like norepinephrine receptor' (Cools & van Rossum 1980) and of the 'DAi/e receptor' (Cools 1981).

Due to the claims that DPI is a selective agonist at a certain DA receptor subtype, we have attempted to quantify the effects of this compound in a variety of test models for DA agonistic activity, but we have found no effects of DPI other than those mediated by a stimulation of α_1- and/or α_2-adrenoceptors (Houwing et al 1983; Van Oene et al 1982a, b, 1983). In a recent Letter to the Editor of this Journal, Struyker Boudier & Cools (1984) remark that 'Van Oene et al fail to recognize that in certain preparations DPI behaves as a DA receptor stimulant'. The reason for this failure, however, becomes quite clear if the available literature data on DPI are considered critically: there is no evidence for a DA receptor stimulatory action of DPI in the mammalian brain. We shall briefly resume the arguments for the latter statement.

(1) Considering the work of Cools et al, it should be emphasized that these authors have made the assumption that the effects of ergometrine in some of their experiments were due to a blockade of DA receptors. Apart from the absence of supportive evidence for this supposition (see below), it is known that ergometrine behaves more or less as a 'chameleon' amongst the pharmacological agents that interfere with monoaminergic neurotransmission, since it exhibits agonistic as well as antagonistic properties at DA receptors (Bell et al 1974; Woodruff 1979), α-adrenoceptors (Kalkman et al 1982) and 5-HT receptors (Cerletti & Doepfner 1958; Muller-Schweinitzer 1980). The choice of ergometrine as a 'selective DAi antagonist' could therefore be considered, at least as rather unfortunate.

Two purported DAi receptor-mediated effects of DPI have been especially studied by Cools et al, and these are the ipsilateral head turning of anaesthetized cats following direct administration of DPI into the caudate nucleus (Cools et al 1976) and inhibition of ergometrine-enhanced locomotion of rats induced by direct intra-accumbens administration of DPI (Cools & Oosterloo 1983). Measurement of the former effect is severely complicated by the fact that it can only be accomplished during certain months of the year and at definite times of the day (Cools et al 1978); to our knowledge Cools et al are the only ones who have used this effect as a test model for dopaminergic activity. The latter effect has been measured by other investigators, but they ascribed ergometrine-induced locomotion of rats to a stimulation, rather than an inhibition, of DA receptors (Elkhawad et al 1975; Pijnenburg et al 1976). Dose-dependency of the DPI-induced reduction of drug-enhanced locomotion of rats has been assessed by Costall et al (1979a, b) who showed that the effects of DPI could be mimicked by 5-HT and antagonized with

* Correspondence.

5-HT antagonists, thus suggesting an interaction of DPI with 5-HT rather than dopaminergic mechanisms (Costall et al 1979b). These and further difficulties with the reports of Cools and collaborators have been discussed in more detail by Van Oene (1984) and lead us to conclude that Cools et al have not succeeded in presenting sound evidence for an interaction of DPI with any type of DA receptor.

(2) Another argument used by Struyker Boudier & Cools (1984) in favour of a DA receptor stimulatory action of DPI, has been that DPI specifically mimicked DA-induced neuronal inhibitions in ganglia of the snail *Helix aspersa* (Struyker Boudier et al 1975). However, the question as to what extent results obtained in molluscan preparations can be extrapolated to the mammalian nervous system, is presently still open to debate. This is illustrated by the observation that DPI was ineffective in mimicking DA-induced neuronal inhibitions in another molluscan species i.e. *Aplysia californica* (Gospe & Wilson 1981). Actually only a single short report has been dedicated to the actions of DPI as a 'specific potent agonist at inhibitory dopamine receptors' in snails (Struyker Boudier et al 1975) and this has led others to conclude that 'indeed the basis of the "specificity" is not clear' (Costall & Naylor 1981).

(3) Following on from the work of Cools et al, other researchers have also attributed some of the pharmacological effects of DPI to a stimulation of DAi receptors, but their reports all suffer from the fact that the involvement of DA receptors is 'proved' by antagonism with ergometrine and/or the investigators neglected to critically rule out the involvement of α-adrenoceptors (Van Oene 1984). In only one case was it possible to conclude that there may be some evidence for a stimulation of mamalian DA receptors by DPI. Under conditions of α- and β-adrenoceptor blockade, DPI was able to relax dose-dependently the prostaglandin $F_{2\alpha}$-contracted rat isolated perfused kidney, an effect that could be inhibited by haloperidol (Imbs et al 1979; Schmidt et al 1982). This effect with DPI was, however, only reached at a very high dose (EC50 = 1·5 mM) and it remains to be elucidated why the DPI curve had an apparently steeper slope than the other dose-response curves in these studies. Nevertheless, the results suggest that DPI is able to stimulate certain peripheral DA receptors, albeit at a comparatively high dose.

(4) On the other hand, there is overwhelming evidence that DPI is without effect in test models that are believed to selectively reflect a stimulation of DA receptors. Thus DPI appeared to be weakly, if at all effective, in striatal dopaminergic binding assays (Blackburn et al 1978; Lehmann et al 1983; Closse et al 1984), DPI was ineffective in inhibiting the stimulation-evoked release of DA or acetylcholine from striatal slices of rabbits, cats or rats (Vizi et al 1977; Lehmann et al 1983; Starke et al 1983; Van Oene et al 1983) and DPI exhibited no effectiveness in stimulating striatal DA-sensitive adenylate cyclase activity (Woodruff & Sumners 1979; Horn unpublished observations). Though effectiveness in the latter test model is believed to reflect stimulating potency at a certain subtype of DA receptors i.e. D-1 receptors according to the classification scheme of Kebabian & Calne (1979), the ineffectiveness of DPI, being a catecholic compound, in this test model is highly suggestive of the absence of DA receptor stimulating properties, since many catecholic DA agonists are known to be quite potent in this test system (e.g. Miller et al 1974). In-vivo DPI was found to be ineffective in reducing the rate of central DA synthesis in rats treated with γ-butyrolactone (Haubrich & Pflueger 1982; Van Oene et al 1982a), a model thought to reflect selectively stimulating potency at DA autoreceptors (Walters & Roth 1976). An effect of DPI in this model, however, is obscured by its lack of sufficient brain penetration (Van Oene et al 1982a). DPI was also ineffective in mimicking the blood pressure reducing effects of DA and DA agonists in anaesthetized guinea-pigs and rats, an effect produced by a stimulation of peripheral DA receptors (Woodruff & Sumners 1979; De Jonge et al 1982).

(5) In a number of non-selective dopaminergic test models, DPI was found to mimic the actions of DA and DA agonists; however, the effects of DPI could be antagonized with α-adrenoceptor antagonists but not with DA receptor antagonists, whereas the reverse appeared to be true for DA and DA agonists. Thus the DPI-induced excitation of rat single cortical neurons as well as its potent inhibition of sympathetic neurotransmission in the rabbit ear artery appeared to be due to a stimulation of α-adrenoceptors, instead of DA receptors (Bevan et al 1979; Brown et al 1979; Hieble and Pendleton 1979). DA agonists have been found to reduce central DA turnover rate, to inhibit overall animal motility, and to decrease body temperature in many mammalian species, but the DPI-induced reduction of central DA turnover appeared to be due to a stimulation of peripheral α_2-adrenoceptors (Van Oene et al 1982a; Van Oene 1984), while the DPI-induced hypomotility and hypothermia appeared to rely upon a stimulation of both α_2- and α_1-adrenoceptors (Costall et al 1981; Van Oene et al 1982).

In fact there is abundant evidence indicating that DPI acts as a potent α-adrenoceptor agonist in both in-vitro and in-vivo experiments (for a review of the literature, see Van Oene 1984). Like many imidazoline derivatives, DPI has a high degree of selectivity for α-adrenoceptors, but unlike most other imidazolines DPI has been found to act as a full, instead of a partial, α-adrenoceptor agonist (Ruffolo et al 1979). Both α_1- and α_2-adrenoceptors can be effectively stimulated by

DPI, though presynaptic α_2-adrenoceptors may be preferentially stimulated at low doses (Hieble & Pendleton 1979; Hieble et al 1982). In contrast to many other imidazoline derivatives, including clonidine. DPI was found to be ineffective in stimulating histamine H_2 receptors (Malta et al 1980). In addition, DPI had no influence on histamine H_1 receptors (Malta et al 1980), but it did appear to produce a stimulation of β-adrenoceptors at the higher doses (McCulloch et al 1980). Thus, the most pronounced pharmacological features of DPI known so far, are its stimulating properties at α_1- and α_2-adrenoceptors; however, at high doses DPI may additionally produce a stimulation of β-adrenoceptors and possibly some peripheral DA receptors.

As a final remark in their letter, Struyker Boudier & Cools (1984) state that 'disregarding DPI's DA receptor stimulating potential is similar to denying that DA is a DA receptor agonist'. Probably some misunderstanding is involved about the implications of the word 'agonist'. In our view, the designation of a compound as a DA receptor agonist requires that its stimulating properties at DA receptors are more pronounced than its stimulating or blocking properties at other receptor types, the difference in potency being preferably one order of magnitude or more. Since it is known that DA is able to stimulate α- and β-adrenoceptors in addition to DA receptors, much can be said in favour of labelling exogenously applied DA as a mixed DA receptor/adrenoceptor agonist. Otherwise, the influence of DA upon mammalian blood pressure (i.e. a decrease at low doses due to a stimulation of DA receptors, followed by an increase at higher doses due to adrenergic effects) clearly indicates that DA does have some selectivity for DA receptors, and thus permits one to designate it as a DA receptor agonist. However, following systemic administration of DPI only an increase in blood pressure has been observed (e.g. Woodruff & Sumners 1979), illustrating once again the inability of DPI to stimulate DA receptors selectively.

It may therefore be concluded that the designation of DPI as a potent mixed α_1/α_2-adrenoceptor agonist still reflects its pharmacological character in the most appropriate manner. Since DPI does not penetrate into the brain to any significant amount following a systemic route of administration (Van Oene et al 1982a), it can be recommended as a tool of choice for in-vivo experiments that require the selective stimulation of peripheral α-adrenoceptors. Finally, we feel that Cools and van Rossum should be congratulated on the fact that their idea of a multiplicity of DA receptors may have stimulated other workers to consider this general concept as a possible explanation for a vast amount of interesting yet confusing neuropharmacological data. However, it should be now abundantly clear that DPI is simply not a DA receptor agonist in the normal sense of the word and therefore the original classification of DA receptors into DAe and DAi types can no longer be defended.

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The determination of yield values using a BP plate plastometer

JOHN D. TOPHAM, GARY P. MARTIN*, *Departments of Pharmacy, Portsmouth Polytechnic PO1 2DZ and *Brighton Polytechnic, Moulsecoomb, Brighton BN2 4GJ, UK*

The BP 1980 Appendix VJ, A79, describes a method determining the yield values of viscoelastic gels. The test relies upon four separate 0·1 g aliquots of the gel being compressed between two glass plates, the upper plate weighing 100 g. After allowing the plastometer to stand for 10 min, the resulting zone diameter of each of the four samples is measured. The Appendix states that a yield value (τ) in N m^{-2} can be calculated from the expression:

$$\tau = \frac{2{\cdot}943 \times 10^3}{d^3} \quad (1)$$

where d = the mean zone diameter in centimetres of the four samples.

The equation appears to be derived from that given by Voet & Brand (1950) for a single spread sample:

$$f = \frac{12PV}{\pi d^3} \quad (2)$$

where f is the yield value (interchangeable with the symbol τ of the BP), P is the force applied to the sample by the top plate of the plastometer in dynes, V is the volume of the sample in cm^3, d is the diameter of spread in cm.

When the BP plastometer specifications are inserted in equation 2, it becomes:

$$f = \frac{12 \times 100 \times 981 \times 0{\cdot}1}{4\pi d^3} = \frac{2{\cdot}943 \times 10^4}{\pi d^3}$$

This assumes a density for the gel of 1 g cm^{-3}. Conversion of the yield value from dynes cm^{-2} to N m^{-2}, results in equation 3 (d still being measured in cm):

$$f = \frac{2{\cdot}943 \times 10^3}{\pi d^3} \quad (3)$$

Equation 3 indicates that the BP equation (equation 1) has omitted division by π. In addition a dimensional analysis of the units of measurement of equation 2 casts doubt upon the validity of the whole equation.

Voet & Brand (1950) state that they derived equation 2 from the work of Howink (1934). His work contains equation 4 which relates the effective weight of the top plate (W), to the radius (r) of the zone and the distance (h) between the plates of the plastometer:

$$W = \frac{2\pi}{3} \cdot \frac{fr^3}{h} \quad (4)$$

This can be rearranged to give:

$$f = \frac{12Wh}{\pi d^3} \quad (5)$$

Assuming that spread occurs uniformly when the sample is compressed, then h can be expressed in terms of the volume of a cylinder, i.e. $h = (4V/\pi d^2)$. When this is substituted in equation 5 and W is replaced by the downward force, P, then the BP symbol τ will replace f for the yield value:

$$\tau = \frac{48PV}{\pi^2 d^5} \quad (6)$$

* Correspondence.