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The purported dopamine agonist DPI inhibits [^{3}H]noradrenaline release from rat cortical slices but not [^{3}H]dopamine and [^{14}C]acetylcholine release from rat striatal slices in-vitro

†*J. C. VAN OENE, **P. SMINIA, **A. H. MULDER AND *A. S. HORN

*Department of Medicinal Chemistry, Pharmaceutical Laboratories, State University of Groningen, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands, **Department of Pharmacology, Free University, Medical Faculty, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands

The effects of the purported dopamine (DA) receptor agonist (3,4-dihydroxyphenylimino)-2-imidazolidine (DPI) upon the in-vitro K^+-induced release of [^{3}H]DA and [^{14}C]acetylcholine from rat neostriatal slices, and of [^{3}H]noradrenaline from rat neocortical slices have been investigated and compared with those of the DA receptor agonist TL-99 and the α-adrenoceptor agonist clonidine, respectively. The rapid decomposition of the catechol compounds DPI and TL-99 in the Krebs-Ringer bicarbonate superfusion medium was shown to be inhibited by both the chelating agent EDTA and the reducing agent ascorbic acid. The results suggest that in-vitro DPI is unable to stimulate striatal DA receptors, whereas it is effective in stimulating cortical α_2-adrenoceptors (EC50 = 61 nM). It is concluded that DPI should be considered as a mixed α_1/α_2-adrenoceptor agonist and that the designation of DPI as a DA receptor agonist should be abandoned.

Many studies have been concerned with the pharmacological actions of the imidazolidine-derivative (3,4-dihydroxyphenylimino)-2-imidazol-idine (DPI) since this compound was reported to possess dopamine (DA) like properties in snails (Struyker Boudier et al 1975). In that species DA may provoke either neuronal excitations or inhibitions and it has been postulated that these responses reflect the activation of different types of DA receptors (Struyker Boudier et al 1974). As DPI was found to specifically induce the inhibitory type of responses, it also was thought to be a useful tool in investigating a possible divergence of DA receptor types in mammals. Following direct intracerebral administrations, DPI was reported to mimick DA-induced inhibitions in the central nervous system of both rats and cats (Cools et al 1976a,b) and it was concluded that in the mammalian brain DA receptors may be divided in a similar manner to those occurring in snails i.e. excitation-mediating (DAe) and inhibition-mediating (DAi) receptors (Cools & van Rossum 1976). The latter concept, however, has recently been questioned by other investigators and their criticism mainly applies to the nature of the DPI elicited responses on which this hypothesis has been based (Costall & Naylor 1981).

† Correspondence.

We have previously demonstrated that DPI was able to significantly reduce rat striatal DA turnover after a peripheral route of administration (Van Oene et al 1982a) and that this effect could not result from a stimulation of striatal DA autoreceptors, but rather appeared to be due to a stimulation of α_2-adrenoceptors. Although it was found that DPI did not readily pass the blood-brain barrier, we suggested that the α-adrenoceptors involved might be possibly located within the central nervous system. However, the ineffectiveness of peripherally administered DPI in reducing rat central noradrenaline (NA) turnover, a process thought to be mediated via stimulation of central presynaptic α_2-adrenoceptors, argued against the possibility of central α-adrenoceptors being stimulated by peripherally administered DPI. Therefore we thought it useful to assess whether DPI was able to stimulate central α-adrenoceptors under circumstances where pharmacokinetic parameters did not interfere with the results. This can be carried out by investigating the influence of DPI upon the in-vitro depolarization-induced [^{3}H]NA release from rat cortical slices, a reduction of which is generally believed to reflect presynaptic α-adrenoceptor stimulating potency (Starke 1979; Mulder et al 1980; Langer 1982).

The influence of DPI upon the depolarization-induced release of ^3H and ^{14}C from rat striatal slices, previously labelled with [^{3}H]DA and [^{14}C]choline, was also investigated as a measure of its stimulating potency towards DA autoreceptors and postsynaptic DA receptors, respectively (Stoof et al 1979, 1980). In the latter experiments the aminotetralin TL-99, which is known to act as a potent agonist at rat central DA receptors (Goodale et al 1980; Horn et al 1982), was included as a reference compound. It appeared that, without preventive measures, the catechol compounds DPI and TL-99 were rapidly decomposed under the in-vitro conditions used. However, a combination of ascorbic acid and EDTA was effective in preventing this decomposition.

MATERIALS AND METHODS

Stability of the catechol compounds in the medium

Glass vials used for the release studies, were filled with 50 ml of Krebs-Ringer bicarbonate (KRb) medium, whose composition (mM) was: NaCl (118), KCl (1·85), KH_2PO_4 (1·15), $MgSO_4$ (1·15), $CaCl_2$ (1·2), $NaHCO_3$ (25), D(+)-glucose (11·1), pH 7·2–7·4. The vials were placed in a water bath, kept at 37 °C, and the medium was saturated by bubbling through with a mixture of 95% O_2 and 5% CO_2. L(+)-Ascorbic acid, EDTA or both were added to the medium just before the addition of the catechol compounds, in a volume of 50 µl, final concentration in the medium was: ascorbic acid $1·1 \times 10^{-4}$ M and EDTA $2·7 \times 10^{-5}$ M. One of the catechol compounds, TL-99 or DPI, was added to the medium, dissolved in a volume of 50 µl 0·01 M HCl. Immediately and at several time points thereafter, 1 ml aliquots were taken out of the vials and transferred to ice-cooled glass tubes. A 50 µl volume of a solution containing ascorbic acid, EDTA or both was added to the tubes to obtain the same concentration of these agents in all samples. Quantitative analysis of the catechol compound content was made by injecting 50 or 100 µl volumes of the samples directly into a high performance liquid chromatographic system equipped with a reverse-phase column (150 × 4·6 mm, filled with Nucleosil 5 C18, Chrompack). Detection was performed using a highly sensitive electrochemical detector flow cell (Oosterhuis et al 1980); the potential of the working electrode versus the reference electrode was: +0·5 V. Phosphate/citrate (McIlvaine) buffers containing 0·1 mM EDTA were used as eluents. For the elution of TL-99 a buffer of pH 4·0 plus 10% v/v methanol, and for DPI a buffer of pH 5·5 plus 8% v/v methanol was used (Van Oene et al 1982a) and the elution rate was adjusted to 1 ml min^{-1}. Under these conditions elution volumes of 6·1 and 5·7 ml were found for TL-99 and DPI, respectively, at a calculated number of theoretical plates of $2·8 \times 10^3$ and $2·1 \times 10^3$. Detection sensitivities amounted to about 1·3 and 1·0 kA mol^{-1} for TL-99 and DPI, respectively.

Samples were analysed the same day and for each vial the concentration measured at a given time point was expressed as a percentage of the initial concentration.

Tissue preparation, incubation and superfusion

Male albino Wistar rats (140–180 g) were decapitated, the brains were removed and the corpora striata or pieces of the neocortex were dissected. Tissue slices of 0·3 × 0·3 × 2 mm in size were prepared using a McIlwain tissue chopper and 200–250 mg of the slices were suspended in 2·5 ml KRb medium (for composition see above) containing ascorbic acid ($1·1 \times 10^{-4}$ M) and EDTA ($2·7 \times 10^{-5}$ M). Following a 10 min preincubation in a Dubnoff metabolic shaking incubator at 37 °C, 5 µCi [^{3}H]NA was added to the cortical slices (final concentration: $6·6 \times 10^{-8}$ M) or 5 µCi [^{3}H]DA and 2µCi [^{14}C]choline were added to striatal slices (final concentrations: $4·9 \times 10^{-8}$ M and $1·4 \times 10^{-5}$ M, respectively) and the slices were subsequently incubated with agitation for 15 min at 37 °C in an 95% O_2/5% CO_2 atmosphere. After rinsing, the slices were transferred to each of 24 superfusion chambers so that each chamber contained about 4 mg of striatal slices or 7 mg of cortical slices. The volume of the chambers was adjusted to 0·25 ml, the temperature was maintained at 37 °C and the slices were superfused (0·25 ml min^{-1}) with O_2/CO_2 (95%/5%)-saturated KRb medium containing ascorbic acid and EDTA at the above mentioned concentrations. After a 30 min (cortical slices) or 40 min (striatal slices) equilibration period, 4 successive 10 min fractions were collected. Drugs were dissolved in 0·01 M HCl and added to the medium 5 min before starting the collection of fraction 1 (Fig. 1). During the first 5 min of the collection of fraction 2, neuronal depolarization was effected by elevating the K^+-concentration of the medium to 15 mM (cortical slices) or 20 mM (striatal slices). In order to maintain isomolarity, the elevation of the K^+-concentration was performed by substituting KCl for NaCl in the KRb medium. At the end of the experiment the radioactivity remaining in the slices was extracted with 0·1 M HCl.

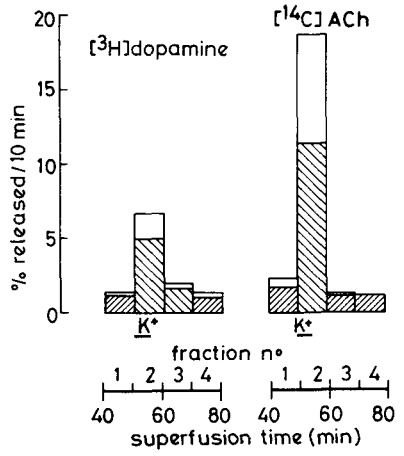

FIG. 1. Typical experiment showing the influence of TL-99 (10^{-7} M; hatched area; n = 4) upon the basal efflux of radioactivity and on 20 mM K^+-induced transmitter release from rat neostriatal slices. For each fraction the release is expressed as a percentage of the total amount of ^3H or ^{14}C present in the tissue at the onset of collection of that fraction.

Measurement of the radioactivity and calculation of the results

Radioactivity was assayed by liquid scintillation counting. Using an external standard channel ratio method the efficiencies for the counting of the disintegrations varied from 56 to 63% for [^{14}C], from 17 to 26% for [^3H] in the presence, and from 24 to 34% for [^3H] in the absence of [^{14}C].

For each superfusion fraction obtained from a certain chamber, the radioactivity measured was expressed as a percentage of the total amount of radioactivity present in the tissue at the onset of collection of that fraction. The total percentage stimulated (K^+-induced) ^3H-release in excess of basal efflux was calculated by subtracting the sum of the percentages of radioactivity in fractions 1 and 4 from the sum of the percentages in fractions 2 and 3. The percentage stimulated ^{14}C release was calculated by subtracting the mean percentage of radioactivity in fractions 1 and 3 from the percentage in fraction 2 (for illustration see Fig. 1).

The percentage of radioactivity found in fraction 1 was taken as a measure of the basal efflux.

Experiments were carried out in triplicate or quadruplicate and were repeated at least twice. In each experiment release obtained in the presence of drugs was expressed as a percentage of drug-free control release. The data obtained in the various experiments were added together and are presented as means ± s.e. (n = number of measurements). The statistical significance of the data was evaluated using Dunnett's multiple comparison test (two-tailed).

Chemicals and Drugs

(−)-[7,8-^3H]Noradrenaline (NA), [7,8-^3H]-dopamine (DA) and (methyl-^{14}C]choline were purchased from the Radiochemical Centre (Amersham), specific activities were 30, 40 Ci $mmol^{-1}$ and 58 mCi $mmol^{-1}$, respectively. The inorganic salts and disodium edetate dihydrate were of analytical grade (Merck). D(+)-Glucose monohydrate and L(+)-ascorbic acid were also obtained from Merck (Darmstadt) and yohimbine hydrochloride from Sigma. Clonidine hydrochloride was a generous gift from Boehringer (Ingelheim, FRG).

NN-Dimethyl-2-amino-6,7-dihydroxytetrahydronaphthalene hydrochloride (TL-99) and (3,4-dihydroxyphenylimino)-2-imidazolidine hydrochloride (DPI) were synthesized in the laboratory in Groningen by Mr D. Dijkstra and Dr H. A. Houwing, respectively.

RESULTS

Stability of TL-99 and DPI in the medium

In 95% O_2/5% CO_2-saturated KRb-medium, both TL-99 and DPI were readily decomposed. As illustrated for DPI (Fig. 2), the decomposition of

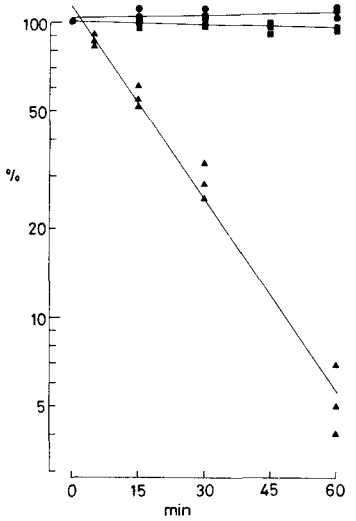

FIG. 2. Recoveries of DPI (3×10^{-6} M), expressed as a percentage of its initial concentration at various times after starting the incubation in 95% O_2/5% CO_2-saturated KRb medium at 37 °C either alone (▲), in the presence of EDTA ($2{\cdot}7 \times 10^{-5}$ M) (■) or in the presence of ascorbic acid ($1{\cdot}1 \times 10^{-4}$ M) and EDTA ($2{\cdot}7 \times 10^{-5}$ M) (●). The equation of the regression line (log y = 0·0215x + 2·05 r = −0·99 and the coefficient of correlation (r) apply to the points obtained in KRb medium alone (▲; 15 measurements). Significance of regression: $P < 0{\cdot}001$ (Student's *t*-test).

both drugs appeared to obey first order kinetics. The half-lives t½ (95% confidence limits) were calculated from the expression $t½ = -\log 2/k$, where k represents the slope of the regression line, and amounted to 23·2 (19·7–28·1) min for TL-99 (initial concn 10^{-6} M), 13·9 (12·9–15·1) min for DPI (initial concn 3×10^{-6} M) and 14·7 (13·3–16·6) min for DPI (initial concn 3×10^{-7} M).

If ascorbic acid was added to the KRb medium (initial concentration: $1·1 \times 10^{-4}$ M) 5 min before DPI, the latter compound was protected against decomposition for half an hour after which time DPI concentrations started to decline. Although under the present chromatographic conditions the concentration of ascorbic acid in the medium could not be adequately measured because of the appearance of its peak in the solvent front of the chromatogram (Fig. 3), the diminution of the broadness of this front that was found as time went on, strongly suggested that ascorbic acid itself was rapidly broken down in the medium. After 1 h practically none appeared to be left in the medium while DPI concentrations were reduced to 36·3 ± 9·1% (n = 3; results not shown).

If EDTA was added to the KRb medium (concentration in the medium: $2·7 \times 10^{-5}$ M) hardly any decomposition of DPI could be observed over 1 h (Fig. 2). If both ascorbic acid and EDTA were present in the concentrations used throughout the release studies, i.e. $1·1 \times 10^{-4}$ and $2·7 \times 10^{-5}$ M, respectively, the decomposition of TL-99 and DPI was fully prevented. From the chromatograms it could be concluded that under these conditions there was also no significant breakdown of ascorbic acid (results not shown).

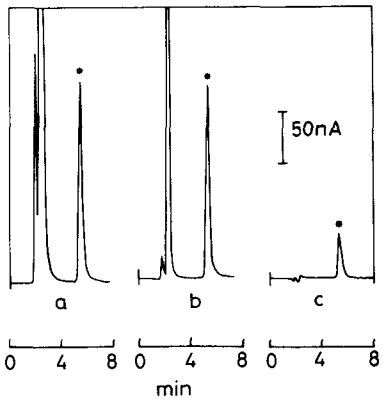

FIG. 3. High performance liquid chromatograms, showing the recovery of DPI (3×10^{-6} M) from 37 °C. 95% O_2/5% CO_2-saturated KRb medium in the presence of ascorbic acid (initial concentration: $1·1 \times 10^{-4}$ M) at 0 (a), 30 (b) or 60 (c) min after starting the incubation. Note the diminution of the solvent front with time, which is suggestive of a rapid breakdown of ascorbic acid in the medium. A 50 µl injection loop was used; * denotes the DPI peak.

Influence of TL-99 and DPI upon [^{3}H]DA and [^{14}C]acetylcholine release from striatal slices

It has been demonstrated that the K^+-stimulated overflow of [^{3}H] and [^{14}C] (in excess of the basal efflux of radioactivity) from striatal slices previously labelled with [^{3}H]DA and [^{14}C]choline is calcium-dependent and validly reflects depolarization-induced release of [^{3}H]DA and [^{14}C]acetylcholine, respectively (Mulder et al 1974; Hadhazy & Szerb 1977; Stoof et al 1980).

As shown in Table 1, TL-99 significantly reduced the 20 mM K^+-induced release of [^{3}H]DA at both concentrations used (10^{-7} and 10^{-6} M). On the contrary, the higher concentration of DPI (3×10^{-6} M) did not change, while the lower concentration of DPI (3×10^{-7} M) significantly increased the stimulated release of [^{3}H]DA. Basal [^{3}H]efflux was shown not to be influenced by TL-99 or DPI except for the higher DPI concentration (3×10^{-6} M), which caused a slight though significant increase

Table 1. Influence of TL-99 and DPI upon the basal efflux of radioactivity and on 20 mM K^+-induced (stimulated) release from rat striatal slices previously labelled with [^{3}H]dopamine and [^{14}C]choline. The data represent the means ± s.e. of n determinations as a percentage of the control drug-free release. Between brackets: range of control release between the various experiments, expressed as a percentage of the total amount of [^{3}H] or [^{14}C] present. * denotes a statistically significant difference versus the corresponding control group ($P < 0·01$); NS = not significantly different from control ($P < 0·05$).

			Release as a percentage of control			
			^{3}H		^{14}C	
		n	Basal	Stimulated	Basal	Stimulated
Control		20	100 ± 1·3 (1·4–2·7%)	100 ± 2·2 (5·9–13·5%)	100 ± 1·3 (2·2–4·0%)	100 ± 1·2 (14·5–17·1%)
TL-99	10^{-7} M	14	96·9 ± 2·5 NS	82·6 ± 3·7*	79·0 ± 2·5*	64·9 ± 2·0*
TL-99	10^{-6} M	12	102·1 ± 3·3 NS	68·9 ± 2·8*	80·2 ± 1·5*	63·9 ± 1·8*
DPI	$3·10^{-7}$ M	12	98·7 ± 1·3 NS	125·4 ± 4·2*	102·5 ± 2·0 NS	104·4 ± 2·3 NS
DPI	$3·10^{-6}$ M	10	108·4 ± 2·3*	98·4 ± 3·5 NS	105·5 ± 2·7 NS	100·1 ± 1·8 NS

(Table 1). Both concentrations of TL-99 reduced the basal [^{14}C] efflux as well as the stimulated [^{14}C] acetylcholine release by about 20 and 35%, respectively, whereas DPI had no influence upon either the basal efflux of [^{14}C] or the stimulated [^{14}C]acetylcholine release (Table 1).

Influence of DPI alone and in combination with yohimbine, upon the [^{3}H]NA release from cortical slices

Other studies have shown that the K^+-stimulated overflow of ^{3}H from brain slices, previously labelled with [^{3}H]NA, is calcium-dependent and reflects depolarization induced [^{3}H]NA release (Taube et al 1977; Dismukes et al 1977; Wemer et al 1979). As shown in Fig. 4 DPI concentration-dependently reduced the 15 mM K^+-induced release of [^{3}H]NA. Assuming that a maximal reduction in [^{3}H]NA release was obtained at 3×10^{-6} M DPI, i.e. to about 55% of controls, the half-maximally effective concentration (EC50) of DPI was found to be $6{\cdot}1 \times 10^{-8}$ M. Clonidine (10^{-6} M) which was included as a reference, reduced [^{3}H]NA release to 52·0 ± 3·0% (n = 19) of controls (Fig. 4). Yohimbine (10^{-6} M), which itself increased [^{3}H]NA release by about 50%, shifted the log dose-effect curve of DPI to the right in a roughly parallel manner (Fig. 4). In the presence of this concentration of yohimbine, DPI was about 20× less effective, and had an EC50 of $1{\cdot}3 \times 10^{-6}$ M.

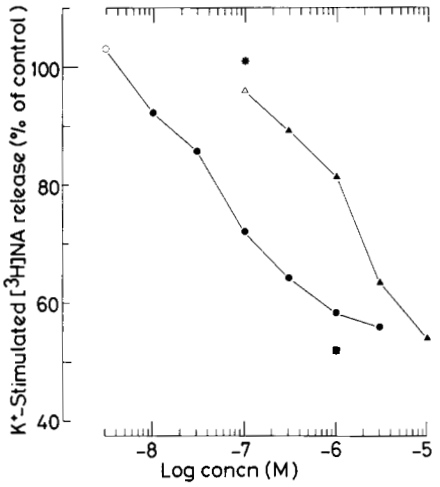

FIG. 4. Influence of DPI alone (circles) or in combination with yohimbine (10^{-6} M; triangles) upon the 15 mM K^+-induced [^{3}H]NA release from rat neocortical slices. The results are expressed as a percentage of control; standard errors ranged from 1·1 to 4·6% of control. Open symbols represent the mean of 5 determinations and are not significantly different from control. ● and ▲ represent the mean of 9–10 determinations and differ significantly from control ($P < 0{\cdot}05$). The clonidine point (square), which is the mean of 19 determinations, also differs significantly from control ($P < 0{\cdot}01$). * △ and ▲ are expressed as a percentage of the release obtained with yohimbine (10^{-6} M) as a control, which amounted to 147·8 ± 5·6% (n = 3) of drug-free controls.

Assuming a competitive antagonism of yohimbine as suggested by the parallel displacement of the DPI log dose-response curve, the pA_2-value of yohimbine can be calculated from the equation

$$pA_2 = \log([D_A]/[D_o] - 1) - \log[A];$$

where [A] = concentration of the antagonist and $[D_A]$ and $[D_o]$ represent the concentration of the agonist in the presence and absence of the antagonist, respectively. By substituting the above EC50-values in this equation the pA_2 of yohimbine was found to be 7·3, which correlates well with the one reported for the yohimbine antagonism of the effect of NA in the same test system (i.e. pA_2 = 7·4; Wemer et al 1979).

None of the compounds used in this study appeared to affect the basal ^{3}H efflux from cortical slices labelled with [^{3}H]NA (results not shown). Among the various experiments basal [^{3}H]efflux varied between 1·5 and 2·5% per 10 min while the 15 mM K^+-induced [^{3}H]NA release in drug-free controls ranged from 13·2 to 20·6% of the total amount of radioactivity present.

DISCUSSION

Although the in-vitro instability of catechol compounds has been known for a long time, many studies using these compounds have been made without regard to the possibility of their rapid breakdown. In oxygenated aqueous solutions of relatively high temperature and pH such a breakdown is especially likely to occur and oxidation of the catechol nucleus, catalysed by heavy metal ions, has been reported to be another factor (e.g. Iversen 1972; Gergely et al 1981).

The observation that DPI was protected against decomposition as long as a sufficient amount of the reducing agent ascorbic acid was present in the medium, stresses the importance of oxidation, while the finding that the chelating agent EDTA protected DPI and TL-99 (and to some extent also ascorbic acid) against decomposition is in line with the proposed role of heavy metal ions in the oxidative breakdown of these compounds. Therefore, the presence of EDTA in the KRb medium appears to be critical in order to prevent the decomposition of compounds that are readily oxidized.

Numerous studies have shown that the depolarization-induced release of (radiolabelled)

NA from brain slices is subject to presynaptic α-adrenergic modulation (Starke 1979; Mulder et al 1980; Langer 1982). The receptors involved appear to belong to the α_2-type, since α_2-adrenoceptor agonists, such as clonidine, inhibit [^{3}H]NA release, whereas α_1-agonists like phenylephrine are ineffective. Furthermore, α_2-adrenoceptor antagonists, such as yohimbine, increase [^{3}H]NA release, conceivably by blocking released endogenous NA that partially activates presynaptic α_2-adrenoceptors, but α_1-antagonists such as prazosin have no effect on release (Hedler et al 1981; Wemer et al 1982; Frankhuyzen & Mulder 1982). The present study shows that DPI inhibition of K^+-induced [^{3}H]NA release from cortex slices was concentration-dependent and that this inhibitory effect was competitively antagonized by yohimbine, indicating that DPI is able to stimulate α_2-adrenoceptors. Stimulation of rat cortical α-adrenoceptors by DPI has also been reported in an electrophysiological study with DPI (Bevan et al 1979).

Thus it appears that the ineffectiveness of peripherally administered DPI in reducing rat cortical NA utilization (Van Oene et al 1982a), a process that is most probably also mediated via cortical presynaptic α_2-adrenoceptor stimulation (Andén et al 1978), does not result from a lack of stimulating potency of DPI towards these receptors, but may rather be due to its poor brain penetration (Van Oene et al 1982a).

Dopamine and DA receptor agonists have been demonstrated to inhibit the release of radiolabelled DA and acetylcholine by stimulating DA autoreceptors and postsynaptic DA receptors, respectively (Reimann et al 1979; Hertting et al 1980; Stoof et al 1979, 1980; Helmreich et al 1982). In a recent study we have shown that TL-99 also strongly inhibits the release of both [^{3}H]DA and [^{14}C]acetylcholine and that these effects were antagonized by the DA-receptor antagonists spiperone and fluphenazine (Horn et al 1982). The present data indicate that, unlike TL-99, DPI is unable to stimulate DA autoreceptors as well as postsynaptic DA receptors. In fact, the lower concentration of DPI (3×10^{-7} M) significantly increased K^+-induced [^{3}H]DA release, for which we have no ready explanation at the moment.

Previously we have reported that DPI was unable to reduce rat striatal DA metabolism following direct intrastriatal administration (Van Oene et al 1982a). This in-vivo result agrees well with the above mentioned ineffectiveness of DPI in reducing depolarization-induced [^{3}H]DA release from rat striatal slices in-vitro, as both processes are thought to be mediated via striatal DA autoreceptors. It must be stressed, however, that it is not clear whether the DA autoreceptors modulating striatal DA synthesis and metabolism are the same as those thought to modulate the release of DA.

Additional evidence against a striatal DA receptor interaction of DPI comes from binding studies, where it was found that DPI was only poorly effective in displacing [^{3}H]DA and [^{3}H]haloperidol from rat striatal binding sites (Blackburn et al 1978). Furthermore it was reported that DPI lacked effectiveness in stimulating striatal DA-sensitive adenylate cyclase activity, a test model in which catecholic DA agonists have been found to be particularly effective (Woodruff & Sumners 1979). Although DA agonists have been reported to induce hypomotility and hypothermia by stimulating central DA receptors (Di Chiara et al 1976; Brown et al 1978), the hypomotility and hypothermia induced by DPI were found not to result from DA receptor stimulation, but to be due to a stimulation of both α_1- and α_2-adrenoceptors (Van Oene et al 1982b).

On the other hand the reduction of drug-induced hypermotility of rats, observed following intra-accumbens administration of DPI (Pijnenburg et al 1976; Costall et al 1979), has been ascribed to a stimulation of DA receptors (Cools et al 1976a).

As we have pointed out previously (Van Oene et al 1982b), the latter effect cannot be accounted for by DPI's α-adrenoceptor stimulating properties (Pijnenburg et al 1976; Costall et al 1979) but appears to be compatible with a 5-hydroxytryptamine receptor, rather than a DA receptor, involvement (Costall et al 1979). The above considerations therefore lead us to conclude that, until now, there is no firm evidence to indicate that DPI is able to stimulate DA receptors in the rat central nervous system.

It is known that DPI can stimulate peripheral DA receptors, however, this only occurs at high doses and when both α- and β-adrenoceptors are blocked (ED50 = $1{\cdot}5 \times 10^{-3}$ M, Schmidt et al 1982). Thus this effect appears to be only of theoretical importance since under normal i.e. non-pretreated conditions, the strong α-adrenoceptor stimulating potency of DPI would greatly outweigh its weak DA receptor stimulating activity.

As a conclusion we feel that, in view of its potent stimulating properties at both α_1- and α_2-adrenoceptors, DPI has to be designated as a mixed α_1/α_2-adrenoceptor agonist and that, as there is only limited evidence in favour of a DA receptor stimulation at a very high dose, the designation of DPI as a DA agonist should be abandoned.

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