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Biochemical and pharmacological activities of zetidoline (DL 308-IT): a new antidopaminergic agent

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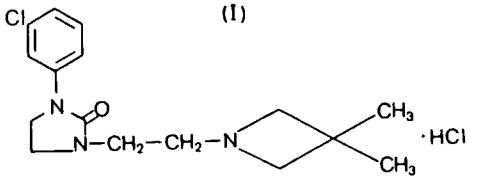
Zetidoline (DL 308-IT, 1-(3-chlorophenyl)-3-[2-3,3-dimethyl-1-azetidinyl)ethyl]imidazolidin-2-one HCl I) is a member of a novel group of dopamine antagonists (Fontanella et al 1981), pharmacologically active in animals (Diena & Allocca 1980) and man (Szabadi et al 1980) in tests predictive of potential antipsychotic activity. We show that DL 308-IT differs fundamentally from some other neuroleptic drugs in lacking the ability to inhibit dopamine-induced stimulation of striatal adenylate cyclase, and in its weaker ability to displace [^{3}H]spiroperidol from cerebral dopamine binding sites in vitro. Yet, in vivo, it displays the characteristic biochemical and pharmacological profile of a potent dopamine antagonist.



Materials and methods

Biochemical studies. Male Wistar rats, 200–230 g, were housed at constant temperature (23 °C) and humidity (60%) with a 12 h light/dark cycle. Groups of 5 or 6 rats, injected i.p. with water or various doses of DL 308-IT, dissolved in water, were decapitated after 1 h and the brains rapidly removed. Left and right striata were dissected on an ice-cold plate, weighed, frozen on dry ice and stored at −80 °C until homogenization. Striatal homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), the dopamine metabolites, were determined by Sephadex G-10 column separation and subsequent fluorometric analysis by the method of Westerink & Korf (1977). Tyrosine hydroxylase activity in vivo was studied as the accumulation of dopa in striatal tissue after the inhibition of dopa decarboxylase by 3-hydroxybenzylhydralazine (NSD 1015: 100 mg kg^{-1} i.p.) given 30 min before killing by microwave irradiation. Dopa was determined by the radioenzymatic procedure of Zürcher & Da Prada (1979). Trunk blood was collected in heparinized tubes, immediately frozen and stored at −20 °C until the determination of plasma prolactin levels by the double antibody radio-immunoassay technique (Niswender et al 1969). The in vitro effect of different concentrations of DL 308-IT on dopamine-sensitive cyclic (c)AMP production was tested in rat striatal homogenates incubated with 100 μM of dopamine by the method of Miller & Tainter (1944). The ability of DL 308-IT to displace [^{3}H]spiroperidol from its specific binding sites was studied in rat striatum in vitro by the method described by Creese & Snyder (1978) and in rat whole brain (minus cerebellum) for [^{3}H]prazosin (Greengrass & Bremner 1979) and [^{3}H]clonidine (U'Prichard et al 1977).

Pharmacological studies. In the pharmacological tests selected to study the interaction of DL 308-IT with cerebral dopaminergic mechanisms in vivo, male Wistar rats, CD_1 mice and mongrel dogs were used. DL 308-IT dissolved in water was always given 30 min before the agonist drugs, while haloperidol suspended in Dow's 0·5% methocel HG 90 in water and chlorpromazine (CPZ) dissolved in water were given 60 min before. At least ten animals per dose of DL 308-IT were used. The antagonism of the hypermotility induced by apomorphine (1 mg kg^{-1} s.c.) in mice (Worms & Lloyd 1979) was assessed in groups of 5 mice/cage by means of an Animex (LKB Sweden) activity counter. The antagonism of the 'jumping' induced in mice by the administration of amphetamine (4 mg kg^{-1} s.c.) plus L-dopa (400 mg kg^{-} i.p., 15 min later) was measured by a modification of the method of Lal et al (1976). The antagonism of the 'running fit' induced by morphine (60 mg kg^{-1} s.c.) in mice was also measured by means of the Animex. Drug-pretreated mice were placed in individual round Plexiglass cages and motility was recorded for 15 min starting 30 min after morphine injection. Antagonism of the behavioural stereotypy induced by amphetamine (15 mg kg^{-1} i.p.) involved the observation of rats housed in single cages 1 h after amphetamine administration; the

Table 1. Inhibition of [^{3}H]spiroperidol, [^{3}H]prazosin and [^{3}H]clonidine specific binding by DL 308-IT and reference compounds. The apparent K_i values were calculated for each ligand from the equation:

$$K_i = \frac{IC50}{1 + \frac{[^3H\text{-ligand}]}{K_d}}$$

The IC50 values were assessed by at least 7 concentrations of the drugs in triplicate.

	K_i (nM) for		
Drug	[^{3}H]Spiroperidol Binding	[^{3}H]Prazosin Binding	[^{3}H]Clonidine Binding
DL 308-IT	69·50	698·0	28 000
Haloperidol	3·21	89·10	>90 000
Chlorpromazine	7·08	9·85	4600
Clozapine	145·60	40·50	693
Sulpiride	136·50	>90 000	>70 000
Metoclopramide	200·10	>90 000	>17 000

* Correspondence.

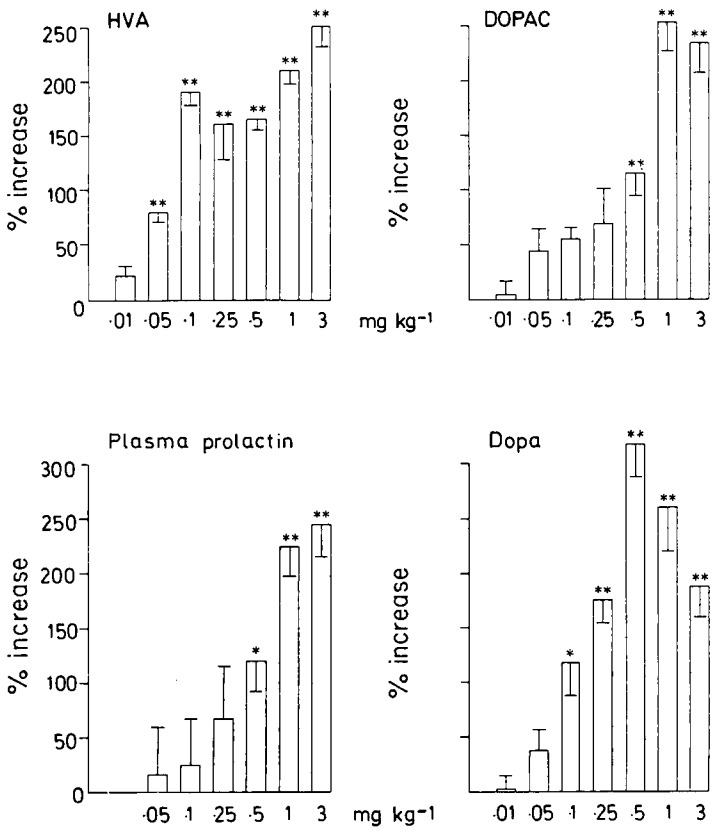


FIG. 1. Effect of DL 308-IT on striatal dopamine metabolism and biosynthesis and on plasma prolactin in the rat. The values are expressed as percent increase above control values which were 676·8 ± 40·6 ng g^{-1} for HVA; 1108·4 ± 75·4 ng g^{-1} for DOPAC; 1085·9 ± 53·6 ng g^{-1} for DOPA; 27·5 ± 6·6 ng ml^{-1} for prolactin. Five rats/group for HVA, DOPAC, dopa and 8 rats/group for prolactin were killed 1 h after administration. Dunnett *t*-test was used for statistical analyses (* $P < 0.05$; ** $P < 0.01$).

rating scale used was the absence or presence of stereotypy movements (licking, sniffing, gnawing and biting). Antagonism of the conditioned avoidance (CAR) in rats (Cook & Weidley 1957), and the antagonism of the apomorphine-induced emesis (0·1 mg kg^{-1} s.c.) in dogs (Chen & Ensen 1950) were also studied.

Results

Biochemical studies. DL 308-IT caused a dose-related increase in rat striatal HVA and DOPAC concentrations and of dopa after inhibition of dopa-decarboxylase by 3-hydroxybenzylhydralazine (Fig. 1). Rat plasma prolactin concentrations were also increased by DL 308-IT in a dose-related manner (Fig. 1). Table 1 shows the ability of DL 308-IT and standard anti-dopaminergic drugs to inhibit the binding of tritiated spiroperidol, prazosin and clonidine to receptors related to the dopaminergic and noradrenergic systems in the rat brain. Haloperidol and CPZ were by far the two most potent displacers of [^{3}H]-spiroperidol, however CPZ at a nearly comparable concentration was also active in displacing [^{3}H]prazosin from α_1-adrenoceptors. The receptors labelled by clonidine were left practically uninfluenced by both compounds. DL 308-IT showed a moderate affinity for the receptors labelled by spiroperidol which was respectively 10 and 20 times lower than those of CPZ and haloperidol. However, DL 308-IT resembled haloperidol more closely than CPZ in its affinity ratio for the three receptors. Interestingly, clozapine had an affinity for the prazosin receptors 3-times higher than for the spiroperidol receptors, and also showed a moderate affinity for the clonidine receptor. Both benzamides tested and clozapine were the least effective in affecting spiroperidol binding; the benzamides, contrary to clozapine, not binding on α_1- and α_2-adrenoceptors. Haloperidol (10 μM) and clozapine (10 μM) completely inhibited dopamine-stimulated cAMP production by striatal homogenates, whereas DL 308-IT (10 μM) had no effect and caused only 76% inhibition at 1mM (Table 2), which was similar to sulpiride and metoclopramide which caused 68 and 81% inhibition, respectively, at 1 mM.

Pharmacological studies. The ED50 values for the antagonism by DL 308-IT, haloperidol and CPZ of a variety of behavioural responses brought about through the activation of dopaminergic mechanisms are listed in Table 3. DL

Table 2. Percent inhibition of dopamine-stimulated cAMP production in striatal homogenates by DL 308-IT and reference compounds. Basal level of cAMP production was 33·4 ± 0·46 pmole/sample (2 mg wet weight) and stimulated level (100 μM dopamine) 65·6 ± 1·2 pmole/sample. Each value is the mean ± s.e. of at least 3 separate incubations.

Drug	Concentration μM	% Inhibition of dopamine-induced control stimulation
DL 308-IT	10	0
	100	20
	1000	76
Haloperidol	1	36
	10	97
	100	100
Chlorpromazine	1	37
	10	100
	100	>100*
Clozapine	1	24
	10	100
	100	>100*
Sulpiride	100	15
	1000	68
Metoclopramide	100	22
	1000	81

* More than 100% inhibition probably due to inhibition of basal cAMP production.

308-IT was more active than CPZ but less active than haloperidol in antagonizing apomorphine-induced hypermotility, amphetamine plus L-dopa-induced jumping, morphine-induced running fits in mice and amphetamine-induced stereotypy and conditioned avoidance response in rats. DL 308-IT and haloperidol were equiactive in preventing apomorphine-induced emesis in the dog and were much more potent than CPZ. Normal behaviour of mice, rats and dogs was not markedly affected by DL 308-IT apart from a cataleptogenic activity in the rat.

Discussion

DL 308-IT possesses potent biochemical and pharmacological properties indicative of dopamine antagonistic activity. Thus the increase in striatal HVA and DOPAC concentrations in the absence of any change of dopamine concentration (unpublished observations) and the increase in dopa level after dopa decarboxylase inhibition are strongly suggestive that DL 308-IT enhances the turnover and synthesis of dopamine. Furthermore, the interference of DL 308-IT with central dopaminergic mechanisms is indicated by its stimulation of prolactin secretion, an observation also made in healthy volunteers after as little as 5 mg orally (Lepetit internal records, unpublished). Paradoxically, DL 308-IT appeared to be more active in vivo especially in mice and dogs than in vitro. Unlike haloperidol and CPZ, it does not show strong in vitro affinity for [^{3}H]spiroperidol-labelled receptors. However, high affinity for spiroperidol-labelled receptors does not necessarily correlate with stimulation of dopamine turnover in vivo. Clozapine and the benzamides are active in vivo, at moderately high doses at increasing the content of DA

Table 3. Interaction with dopaminergic system in vivo. DL 308-IT was given 30 min before the challenge substances; haloperidol (HALO) and CPZ 60 min before.
ED50s were calculated with probit analysis according to Finney (1952) for tests of conditioned avoidance response and stereotypy induced by amphetamine, while a modification of the method of Miller & Tainter (1944) using percent effect was employed for the calculation of the ED50s in all the other tests.

Tests	Route	ED50 (mg kg^{-1}) DL 308-IT	HALO	CPZ
Apomorphine-induced hypermotility (mouse)	i.p.	0·99	0·19	1·76
Amphetamine + L-dopa-induced jumping (mouse)	i.p.	0·63	0·12	—
Morphine-induced running fit (mouse)	i.p.	0·54	—	—
	oral	1·56*	0·10	1·67
Amphetamine-induced stereotypy (rat)	i.p.	2.60	0·26	4·95
	oral	10.00	0·42	14·90
Conditioned Response (rat)	i.p.	1·04	0·10	3·95
	oral	6·85	0·50	11·60
Apomorphine-induced emesis (dog)	i.p.	0·02	0·03	1·05
	oral	0·02	0·03	~1·36

* DL 308-IT given 60 min before.
For details of the procedures see text.

metabolites in the rat brain (Ahtee 1975; Keller et al 1980) and in the frontal cortex (Restelli et al 1975) notwithstanding their poor in vitro affinity for spiroperidol receptors. In the behavioural tests indicative of dopamine antagonistic activity, DL 308-IT was more potent than CPZ, whereas it was only one tenth as potent as CPZ in its ability to displace [^{3}H]spiroperidol from its striatal binding sites. Like haloperidol DL 308-IT was much more selective than CPZ for the dopamine receptor labelled by spiroperidol, CPZ being nearly equally active in displacing both [^{3}H]-prazosin and [^{3}H]spiroperidol. Of all the drugs tested, clozapine was unique in showing a higher affinity for α_1-adrenoceptors labelled by [^{3}H]prazosin than for [^{3}H]spiroperidol. The overall profile of DL 308-IT as it emerges from these studies and particularly from its failure to inhibit dopamine-induced stimulation of adenylate cyclase, which is apparently associated with the so called D_1 receptors (Kebabian & Calne 1979), strengthens the suggestion that DL 308-IT interacts with other DA receptor sites than those so far studied. Further in vitro and in vivo studies using tritiated DL 308-IT as ligand are needed to find a better correlation between the pharmacological and biochemical events induced by the compound. Moreover, the distribution of DL 308-IT into strategic cerebral areas of mice, rats and dogs has to be investigated to explain why mice and dogs seem to be more sensitive to the compound in some behavioural tests. In rats the radiolabelled DL 308-IT and/or its metabolites were found to be distributed almost equally in different brain areas (Lepetit, internal files). Little information is available as yet about the actions of DL 308-IT on other central transmitter systems, although weak peripheral autonomic blocking properties have been described in animal tissues in vitro (Fosbraey et al, 1982) and in volunteers (Szabadi et al 1980).

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Determination of the steady-state volume of distribution using arterial and venous plasma data from constant infusion studies with procainamide

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The apparent steady-state volume of distribution (V_{ss}) of a drug is an important pharmacokinetic parameter. It can be estimated by the following general equation (Chiou 1982a):

$$V_{ss} = \frac{\int_0^\infty X_t\,dt}{\int_0^\infty C_t\,dt} \quad (1)$$

where X_t is the amount of drug in the body and C_t its concentration in plasma at time t, after a dose. Equation 1 is independent of the site of elimination and the route and rate of drug administration. It is also applicable to blood sampling at any site of the body. When a constant infusion is given intravenously and drug elimination is assumed to be proportional to the total body clearance, CL, equation 2 is obtained (Chiou 1982a):

$$V_{ss} = \frac{\int_0^\infty (A_t - CL\,AUC_{0\to t})\,dt}{AUC_{0\to\infty}} \quad (2)$$

where A_t is the total amount of drug infused into the body at time t, CL is the apparent total plasma clearance and AUC is the area under the plasma curve.

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Until recently, relatively little attention has been directed toward the differentiation between arterial and venous (A–V) plasma concentration in pharmacokinetic analysis (Chiou & Lam 1982; Chiou et al 1981). Since the elimination of a drug is assumed to be directly proportional to its concentration in plasma, the potential effect of the plasma source on the kinetic parameters of drugs remains to be assessed. We now report the preliminary results of the influence of A–V differences on the determination of V_{ss} using the infusion method and attempt a rationale for such phenomena.

Method

Three pigmented male rabbits were anaesthetized with urethane 1·0 g kg^{-1} i.p., and the right carotid artery and the jugular vein catheterized with polyethylene tubing. A third catheter was placed in the sacral part of the vena cava via the right femoral vein, the tip of the cannula being positioned at the junction of the femoral branch (confirmed post mortem). The animals were allowed to recover overnight.

Infusion solutions prepared by dissolving varying amounts of procainamide HCl in 0·9% NaCl (saline) infused into the jugular vein by means of a constant infusion