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The pharmacological effect of citalopram resides in the (S)−(+)-enantiomer

Short Communication

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Summary. The enantiomers of citalopram and N-demethylcitalopram have been investigated. Based on the inhibition of 5-HT uptake in vitro and the potentiation of l-5-HTP in vivo the pharmacological activity resides in the (+)-enantiomers (the eutomers*) with the 1-(*S*) absolute configuration. In the 5-HT uptake test eudismic ratios of 167 and 6.6 are obtained for the enantiomers of citalopram and N-demethylcitalopram, respectively. The pharmacological profile of the eutomers of citalopram and N-demethylcitalopram very much resembles the profile of the respective racemates.

Keywords: Citalopram, enantiomers, 5-HT uptake.

Introduction

The selective 5-HT-uptake inhibitor citalopram (Hyttel, 1982) is a racemate. Recently the enantiomers of citalopram (Bøgesø and Perregaard, 1990) and the primary metabolite (N-demethylcitalopram) have been synthesized with high purity (> 99.5%) in quantities sufficient for pharmacological characterization. The absolute configuration of the active enantiomer of citalopram has been established to be 1-(*S*) (personal communication, S. Larsen, Dept. of Chemistry, University of Copenhagen).

Material and methods

Animals

Male Wistar rats (Mol: Wist, SPF, 170–270 g) and male mice (NMRI/BOM, SPF, 18–25 g) were used. Rats (five animals per Macrolon III cage) and mice (20 animals per plastic cage)

* The more active enantiomer is called the eutomer, the less active enantiomer is called the distomer. The eudismic ratio is a measure of degree of stereo-selectivity i.e. the ratio of activities of a pair of enantiomers

were housed conventionally in animal rooms with automatic control of temperature (21 ± 1 °C), relative humidity (55 ± 5%), air exchanges (16 times/hr), and day/night cycle (light on 6 a.m.-6.p.m.). They had free access to a commercial pelleted diet (New Rostock diet, KFK, Aarhus) and tap water.

Uptake studies

Inhibition of the accumulation of ^{3}H-DA, ^{3}H-5HT and ^{3}H-l-NA into rat brain synaptosomes. Inhibition of the accumulation of ^{3}H-DA into rat striatal synaptosomes and of ^{3}H-5-HT into rat whole brain synaptosomes was estimated as described by Hyttel (1978 a, b, respectively). Inhibition of the accumulation of ^{3}H-l-NA into synaptosomes from rat frontal plus temporal cortex was estimated analogously to the above-mentioned methods. The final concentration of ^{3}H-l-NA was 10 nM. Samples were incubated for 30 min at 37 °C, then filtered. Nonspecific binding and passive transport were determined in the presence of 100 µM benztropine, 10 µM citalopram, or 10 µM talsupram for the uptake of DA, 5-HT, or NA, respectively.

l-5-HTP potentiation in mice. Thirty min after administration of test compound mice were given l-5-HTP (460 µmol/kg = 100 mg/kg, s.c.). After 15 min the animals were evaluated during a 15 min observation period with respect to the following symptoms: Head weavings (lateral head movements), tremor, and hind limb abduction. One point was given for each symptom being present. A total of 5–10 mice were used per dose.

ED_{50}-values were calculated for each symptom separately by means of log-probit analysis. Furthermore, the sum of scores obtained for the individual symptoms were calculated and the ED_{50}-value corresponding to half the maximal score was calculated by means of log-probit analysis.

Drugs

Drugs were dissolved in water (in vitro) or saline (in vivo). l-5-hydroxytryptophan (l-5-HTP), MW 220; benztropine-mesylate, MW 404 (MSD, Denmark). The following test drugs were synthesized at H. Lundbeck A/S: Citalopram HBr, MW 405, (*S*)-(+) and (*R*)-(−)-citalopram oxalate, MW 414.5; N-demethylcitalopram HCl, MW 346.9; (*S*)-(+)- and (*R*)-(−)-demethylcitalopram oxalate, MW 400.4; talsupram HCl, MW 348. l-[7,8-^{3}H]noradrenaline (spec. act. 37–45 Ci/mmol) and [G-^{3}H]5-hydroxytryptamine creatinine sulphate (spec. act. 18 Ci/mmol) were obtained from Amersham International (Buckinghamshire, England); 3,4-[ring-2,5,6-^{3}H]dopamine hydrochloride (33 Ci/mmol) was obtained from DuPont NEN (Dreieich, FRG).

Results

In vitro effects

The eutomer (the more potent enantiomer) (*S*)-(+)-citalopram is equipotent with citalopram on 5-HT uptake in vitro (Table 1). The ratio IC_{50}NA/IC_{50} 5-HT is 1700 for (*S*)-(+)-citalopram, slightly lower than the ratio of 3400 for the racemic mixture.

Like citalopram itself, (*S*)-(+)-citalopram is without effect on uptake of DA.

The distomer (the less potent enantiomer) (*R*)-(−)-citalopram is considerably weaker than the eutomer on 5-HT uptake giving eudismic ratio (potency or affinity ratio) of 167. (*R*)-(−)-citalopram is slightly weaker than the eutomer on NA uptake and equally non-effective on DA uptake.

Table 1. Inhibition of the accumulation of ^{3}H-labelled amines into rat brain synaptosomes in vitro

	Citalopram			N-Demethylcitalopram		
	(*R*, *S*)−(±)	(*S*)−(+)	(*R*)−(−)	(R, S)−(±)	(*S*)−(+)	(*R*)−(−)
5-HT uptake	1.8	1.5	250	14	9.9	65
NA uptake	6100	2500	6900	740	1500	500
DA uptake	40000	65000	54000	28000	34000	25000
Ratio NA/5-HT	3400	1700	28	53	150	7.7
Ratio DA/5-HT	22000	43000	220	2000	3400	380

Results are expressed as IC_{50}-values in nM (logarithmic means). Two full concentration-response curves were measured using 5 concentrations of test drug in triplicate (covering 3 decades). In a series of 100 determinations the variance of the log ratio (log R) between the double determinations ($\sum(\log R)^2/2n$) was determined. In case the log ratio was greater than corresponding to 3 × sd (99% confidence interval) extra determinations were performed and outliers were discarded. The following antilog (sd)'s were obtained: 5-HT, 1.4(n = 100), NA, 1.6(n = 100) and DA, 1.7 (n = 100)

Table 2. Effect of the racemate and enantiomers of citalopram on l-5-HTP-potentiation in mice

	Citalopram		
	(*R*, *S*)−(±)	(*S*)−(+)	(*R*)−(−)
Head weaving	0.61 (0.41−0.92)	0.85 (0.65−1.1)	> 48
Tremor	0.66 (0.51−0.86)	1.5 (1.2−2.0)	> 48
Hind limb abduction	> 12	3.7 (2.5−5.6)	> 48
Full syndrome	1.8 (1.2−2.7)	1.7 (1.4−2.0)	> 48

Shown are ED_{50}-values in μmol/kg (s.c.). 95% confidence intervals in parenthesis

The eutomer of N-demethylcitalopram, (*S*)-(+)-N-demethylcitalopram is slightly more potent than the racemic mixture on 5-HT uptake (Table 1). However, the eudismic ratio for N-demethylcitalopram is considerably smaller than that of citalopram (6.6). For NA and DA uptake the (*R*)-(−)-enantiomer of N-demethylcitalopram is slightly more potent than the (*S*)-(+)-enantiomer.

In vivo effects

In the l-5-HTP potentiation tests (*S*)-(+)-citalopram is equipotent with or slightly more potent than citalopram whereas the distomer (*R*)-(−)-citalopram is inactive (Table 2).

Discussion

The highly selective inhibitor of 5-HT uptake citalopram (Hyttel, 1978 b) with proven clinical efficacy in depressed patients (Milne and Goa, 1991) is a racemic

mixture. Recently the enantiomers of citalopram and N-demethylcitalopram have been synthesized with high enantiomeric purity in quantities sufficient for pharmacological characterization. Based on the inhibition of 5-HT uptake in vitro and on the potentiation of l-5-HTP in vivo we have found that the pharmacological activity resides in the (+)-enantiomers (the eutomers) with the 1-(*S*) absolute configuration. The purity of the enantiomers is at least 99.5%. If some of the impurity in the distomer of citalopram is in fact the eutomer, this would contribute to the inhibition of 5-HT uptake. Thus the eudismic ratio of 167 for citalopram enantiomers must be regarded as a minimum value.

(*S*)-(+)-citalopram potently inhibits 5-HT uptake in vitro in concentrations equal to those of citalopram. This is reflected in vivo where citalopram and (*S*)-(+)-citalopram potentiate l-5-HTP in mice in equal doses. The selectivity for inhibition of 5-HT uptake versus the inhibition of uptake of NA and DA is retained in (*S*)-(+)-citalopram and (*S*)-(+)-N-demethylcitalopram (Table 1). Like citalopram the enantiomers of citalopram and N-demethylcitalopram have very weak affinity for a series of neurotransmitter receptors (D_1, D_2, 5-HT_{1A}, 5-HT_2, α_1, α_2, β, H_1, muscarine) (results not shown).

In summary the effects of citalopram and N-demethylcitalopram in these in vitro and in vivo tests are mainly – or solely – expressed by the eutomers, (*S*)-(+)-citalopram and (*S*)-(+)-N-demethylcitalopram, with negligible contribution of the distomers.

As the inhibition of 5-HT uptake seems to be the only mechanism of action to explain citalopram's pharmacological and clinical effects it is assumed that the presence of the distomer in the racemate (citalopram) probably does not contribute to the antidepressive effect of citalopram.

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