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Selectivity of cyproheptadine as assessed by radioligand binding

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Cyproheptadine is widely used experimentally as an anti-5-hydroxytrytaminergic, antihistaminic and anti-acetylcholine drug, these intrinsic properties being well established (Stone et al 1961). More recently, studies on cyproheptadine inhibition of insulin secretion (Donatsch et al 1980) have led to the suggestion that the drug interacts with calcium channels and in so doing produces its in vivo pharmacological effects. Examination of cyproheptadine in radioligand receptor-binding assays showed that it was some five orders of magnitude more active in displacing appropriate radioligands from central histamine, 5-HT-2 and muscarinic cholinergic receptor sites than in inhibiting depolarization dependent calcium fluxes (Donatsch et al 1980).

Radioligand binding to central receptors was measured using membranes prepared from rat brain by described methods as follows (with final radioligand concentration and tissue preparation) muscarinic cholinergic ([^{3}H] quinuclidinyl benzilate ([^{3}H]QNB); 60 pM; rat cortical S_1 fraction; Yamamura & Snyder 1974); α-adrenoceptor ([^{3}H]WB 4101; 0·2 nM; rat forebrain; Greenberg et al 1976); β-adrenoceptor ([^{3}H]-dihydroalprenolol ([^{3}H]DHA); 1·0 nM; rat cortical P_2 fraction; Bylund & Snyder 1976); dopamine ([^{3}H]-apomorphine; 0·2 nM; rat caudate; Seeman et al 1976); neuroleptic ([^{3}H]spiroperidol; 0·1 nM; rat caudate; Burt et al 1976); 5-HT-1 ([^{3}H]5-HT; 4 nM; rat forebrain; Bennett & Snyder 1976); 5-HT-2 ([^{3}H]mianserin; rat forebrain; 0·75 nM; Peroutka & Snyder 1981); 5-HT-1 and 2 ([^{3}H]lysergic acid diethylamide (LSD); 2 nM; rat forebrain; Bennett & Snyder 1976); GABA ([^{3}H]GABA; 10 nM; whole brain crude synaptic membranes; Enna & Snyder 1977); anxiolytic ([^{3}H]diazepam; 1·5 nM; rat cortical P_2 fraction; Squires & Braestrup 1977); adenosine A-1; ([^{3}H]2-chloroadenosine (2-CADO); 1·0 nM; whole brain crude synaptic membranes; Williams & Risley 1980).

Examination of cyproheptadine in eleven receptor binding assays (Table 1), confirmed the acetylcholine-like and 5-HT-ergic properties of the molecule; the compound was approximately equiactive in displacing [^{3}H]QNB from muscarinic cholinergic sites and [^{3}H]-mianserin from 5-HT-2 sites with K_i's of 3–6 nM. Cyproheptadine was about sixty times less active at central 5-HT-1 sites ([^{3}H]5-HT binding) than at 5-HT-2 sites ([^{3}H]mianserin binding). Cyproheptadine also displaced [^{3}H]apomorphine and [^{3}H]spiroperidol from dopaminergic binding sites with about the same efficacy ($K_i \simeq 100$ nM) and had some α-adrenergic activity as evidenced by the displacement of [^{3}H]WB 4101 (K_i = 178 nM). No significant β-adrenergic, GABAenergic, anxiolytic or adenosine A-1-related activity was observed (Table 1). The histaminergic activity of cyproheptadine, while not measured in the present study, has been demonstrated in vitro by Peroutka & Snyder (1981) who found an IC50 of 5·8 nM (K_i = 4·7 nM) using radioligand binding.

It seems unlikely therefore that the demonstrated in vivo pharmacological activity of cyproheptadine (Stone et al 1961) can be ascribed to calcium channel blockade (Donatsch et al 1980).

The finding that cyproheptadine was approximately equiactive in the 5-HT-2, histamine and muscarinic cholinergic radioligand assays indicates that these latter

* Correspondence.

Table 1. Receptor binding profile of cyproheptadine. Results are mean with s.d. for 3–7 separate determinations where cyproheptadine was run at 3–7 concentrations in triplicate. IC50 values were determined by linear regression following log-probit analysis of binding data and K_i's derived by the relationship $K_i = IC50 \div 1 + c/K_d$, where c is the concentration of radioligand used and K_d the dissociation constant determined by Scatchard analysis. QNB = Quinuclidinyl benzilate; DHA = dihydroalprenolol; LSD = lysergic acid diethylamide; 2-CADO = 2-choroadenosine.

Receptor	Radioligand	IC50 (nM)	K_i (nM)
Muscarinic	[^{3}H]QNB	6·38 s.d. 1·42	3·19
α-Adrenergic	[^{3}H]WB 1401*	244·0 s.d. 69·3	178·0
β-Adrenergic	[^{3}H]DHA	> 100 000	56 500
Dopamine	[^{3}H]-Apomorphine	119 s.d. 30	99
Neuroleptic	[^{3}H]-Spiroperidol	100 s.d. 21	97
5-HT-1	[^{3}H]5HT	2826 s.d. 401	1511
5-HT-1 and 2	[^{3}H]LSD	28·2 s.d. 6·93	23·5
5-HT-2	[^{3}H]Mianserin	6·26 s.d. 1·74	5·17
GABA	[^{3}H]GABA	>20 000	>10 000
Anxiolytic	[^{3}H]Diazepam	>100 000	>72 400
Adenosine A_1	[^{3}H]2-CADO	>100 000	>56 500

*2-(*N*-[2,6-dimethoxy phenyloxyethyl]-aminomethyl—1,4-benzodioxane.

two activities should be given consideration when cyproheptadine, instead of other more specific anti-5-HT-ergic agents, is used experimentally to modulate pharmacological and behavioural paradigms (Smialowski et al 1980; Cook & Sepinwall 1980).

In regard to the in vitro α-adrenergic and dopaminergic activities of cyproheptadine, Stone et al (1961) have shown it to be relatively weak at peripheral adrenoceptors while it has no effect on apomorphine-induced stereotypies in rat at doses up to 10 mg kg^{-1} i.p. (data not shown). These additional activities, like the interaction with islet calcium channels (Donatsch et al 1980), appear unlikely to account for the pharmacological properties of cyproheptadine.

The apparent discrepancies between the activity of cyproheptadine in vitro and its lack of effect in these pharmacological test procedures is no doubt a reflection of its relative activity at α-adrenergic and dopaminergic binding sites, some 20–30 times less than that observed at histaminic, muscarinic and 5-HT-2 receptors and also of its potential, as reflected in its bioavailability, to interact with the relevant receptors subserving the in vivo responses. It would appear unlikely that the recognition sites defined by [3H]WB 4101, [^{3}H]apomorphine and [^{3}H]spiroperidol binding are not receptors in view of their thorough pharmacological characterization as such (Greenberg et al 1976; Seeman et al 1976; Burt et al 1976) and also in view of the close correlation between the prominent anti-5-HTergic, antihistaminic and anti-acetylcholine properties of the molecule in vivo (Stone et al 1961) and its high nanomolar affinity for these binding sites in the radioligand assays.

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