Effect of tolamolol and other β-adrenoceptor blocking drugs on [^{3}H]haloperidol binding to rat striatal membrane preparations

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The cardioselective β-adrenoceptor blocking drug, tolamolol (Adam, Baird & others, 1974) has been shown to raise plasma prolactin concentrations in man when administered at normal therapeutic doses (Davies, Rawlins & others, 1976; Saxton, in preparation). Propranolol was also tested in one of these studies but did not affect plasma prolactin (Saxton, in preparation).

Noradrenaline (Lawson & Gala, 1975) and 5-hydroxytryptamine (Kamberi, Mical & Porter, 1971) have been implicated in the control of prolactin secretion by the pituitary. However, dopamine appears to exert the major inhibitory influence on prolactin release (Macleod & Lehmeyer, 1974; Sharr & Clemens, 1974) and many dopamine antagonists, e.g. neuroleptic drugs, elevate plasma prolactin in both animals and man (Macleod, 1976).

Recently high affinity receptor-binding sites for haloperidol and dopamine have been found in the pituitary (Brown, Seeman & Lee, 1976) and in dopamine-rich areas of the brain (Creese, Burt & Snyder, 1976). The present study was therefore undertaken to determine whether the tolamolol-induced elevation of prolactin in man could involve blockade of dopamine receptors. The affinities of tolamolol and some other β-blocking drugs for haloperidol binding sites in rat striatal membranes have been compared using [^{3}H]haloperidol as the ligand (Creese, Burt & Snyder, 1975).

Adult male rats, approximately 200 g, were decapitated and the brains removed rapidly. The striata or pituitary from each were dissected, pooled and homogenized in ice-cold 50 mM tris-HCl buffer, pH 7·5. A membrane fraction was collected by centrifugation (50 000 *g*), washed twice with the buffer and resuspended in ice-cold 50 mM tris-HCl buffer containing, 0·1% ascorbic acid, (mM) pargyline, 0·01; NaCl, 120; KCl, 5; $CaCl_2$, 2 and $MgCl_2$, 1 (final pH 7·1 at 4°). The suspension was kept on ice for at least 60 min before use. Assays were in duplicate, each tube containing: membrane suspension (500–600 μg protein), 2 nM [^{3}H]haloperidol and the appropriate concentration of test compound in a final volume of 1 ml. After incubation at 4° for 30 min, duplicate aliquots (400 μl) from each assay tube were rapidly filtered under vacuum through Whatman GF/B filters and washed with 2 × 5 ml ice-cold 50 mM tris-HCl, pH 7·5. The filters were counted in 10 ml Instagel by liquid scintillation spectrometry. Saturable, high affinity, specific binding of [^{3}H]haloperidol was defined as the difference between the ligand bound in the presence and absence of 100 μM dopamine or 0·1 μM (+)-butaclamol. Approximately 40% of the total [^{3}H]haloperidol bound was specific by these criteria. Results are expressed as the concentration of drug giving half-maximal inhibition of specific [^{3}H]haloperidol binding (IC50).

Preliminary studies were made to compare the characteristics of haloperidol binding to pituitary and striatal membranes. The results presented in Table 1 show that haloperidol, metoclopramide and dopamine have similar IC50 values in both preparations, and confirm an earlier report (Brown & others, 1976) of the similarity of pituitary and striatal haloperidol binding sites. Thus in the rat the affinity of drugs for striatal receptors can be taken to reflect their affinity for pituitary receptors. In all subsequent experiments striatal membrane preparations were used for reason of tissue availability.

IC50 values for several dopamine antagonists and for the β-blocking drugs are shown in Table 2. Results for the dopamine antagonists pimozide and haloperidol, and the stereoselective effects of butaclamol and thiothixene, are in good agreement with published values (Burt, Creese & Snyder, 1976). Compared with these dopamine antagonists, none of the β-blocking drugs tested has high affinity for haloperidol binding sites. However, within the relatively low potency range there are some potentially important differences. Practolol, timolol and atenolol are inactive at all concentrations tested (up to 10^{-4} M), whereas propranolol, oxprenolol, labetalol and alprenolol have IC50 values between 10^{-4}–10^{-5} M. Tolamolol is the most active with an IC50 of 9×10^{-6} M. No stereospecific interaction of tolamolol is apparent, the isomers being equiactive. Although tolamolol and labetalol, two of the more active drugs, also possess significant α-adreno-

Table 1. *Effects of various drugs on* [^{3}H]*haloperidol binding to rat pituitary and striatal membranes.* [^{3}H]-Haloperidol binding was assayed as described. Results are the means of duplicate determinations from at least four experiments using a minimum of four concentrations of the drugs indicated. The concentration of drug giving half-maximum inhibition of specific [^{3}H]haloperidol binding was determined by log-probit analysis.

Drug	Half-maximal inhibition of specific [^{3}H]haloperidol binding (IC50M)	
	Pituitary	Striatum
Haloperidol	6×10^{-9}	4×10^{-9}
Metoclopramide	$1{\cdot}2 \times 10^{-8}$	6×10^{-8}
Dopamine	1×10^{-6}	1×10^{-6}

* Correspondence.

Table 2. *Effects of various drugs on [³H]haloperidol binding to rat striatal membranes.* [³H]Haloperidol binding was assayed as described. Results are the means of duplicate determinations from at least four experiments using three different concentrations of the drugs indicated. The concentration of drug giving half-maximum inhibition of specific [³H]haloperidol binding was determined by log-probit analysis. Binding of [³H]haloperidol was independently determined to have a K_D of 6–10 nM.

Drug	Half-maximum inhibition of specific [³H]haloperidol binding (IC50M)
Haloperidol	4×10^{-9}
Pimozide	1×10^{-9}
(+)-Butaclamol	$6{\cdot}5 \times 10^{-9}$
(—)-Butaclamol	6×10^{-6}
cis-Thiothixene	$1{\cdot}4 \times 10^{-8}$
trans-Thiothixene	$2{\cdot}5 \times 10^{-7}$
Tolamolol	9×10^{-6}
Alprenolol	$1{\cdot}5 \times 10^{-5}$
Labetalol	3×10^{-5}
Oxprenolol	6×10^{-5}
Propranolol	1×10^{-4}
Practolol	inactive
Timolol	inactive
Atenolol	inactive
Phentolamine	1×10^{-5}

ceptor blocking activity (Farmer, Kennedy & others, 1972; Adam, Alabaster & Hayden, 1976), it is unlikely that this accounts for the present affinities for haloperidol binding sites in view of the assay conditions (low ligand concentration, brain area) and the low affinity for these sites of phentolamine, the potent α-receptor blocker (Table 2).

The mean plasma concentrations of tolamolol in man following therapeutic doses reached 1–10 μM (Davies & others, 1976; Saxton, in preparation). In addition, tissue distribution studies in animals indicate concentration of tolamolol-related material by the pituitary (D. Stopher, personal communication). Thus, blockade of pituitary dopamine receptors by tolamolol (IC50 against haloperidol binding of 9 μM) could account for the drug-induced elevation of plasma prolactin. We are unaware of data on the effects on prolactin secretion of the other β-blockers with similar affinities to tolamolol for haloperidol receptors. The lack of significant effect of propranolol on prolactin secretion in man found by Saxton (in preparation) could be attributed to its lower affinity for haloperidol receptors and to the lower plasma drug levels achieved; propranolol at high doses has been found to increase plasma prolactin concentrations in animals (Gala, Subramanian & others, 1977). On the basis of our results, practolol, timolol and atenolol would not be expected to influence prolactin secretion by antagonizing the dopamine input to the pituitary. However, effects on prolactin secretion by other mechanisms cannot be ruled out for some of these or other β-blocking drugs. For example, some β-blockers have recently been demonstrated to have high affinity for 5-HT binding sites in rat-brain membranes (Middlemiss, Blakeborough & Leather, 1977).

The possibility of β-blockers having a therapeutic role in mental illness has been raised (Atsmon, Blum & others, 1972; Carlsson & Johansson, 1971). Propranolol at high doses has been shown to have anti-psychotic activity in man (Yorkston, Zaki & others, 1974, 1977) and to modify central dopamine metabolism in animals (Peters & Mazurkiewicz-Kwilecki, 1975; Fuxe, Bolme & others, 1976; Mahon, O'Donnell & Leonard, 1977). Moreover, propranolol is taken up into the brain and concentrated in cortical and limbic structures (Masuoka & Hansson, 1967). Thus these central effects of propranolol could result, in part, from the weak dopamine-receptor blocking activity found in the present study.

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Interaction of 2-[β-(4-hydroxyphenyl)ethylaminomethyl]tetralone (BE-2254: 'HEAT') with catecholamine receptors in rat brain membranes

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2-[β-(4-Hydroxyphenyl)ethylaminomethyl]-tetralone (BE-2254: 'HEAT') has been shown to block peripheral α-adrenoceptors (Benthe, Gothert & Tuchinda, 1972; Baumgarten, Gothert & others, 1972). Indirect pharmacological evidence indicates that BE-2254 administered systemically can also block central catecholamine receptors and impair catecholamine receptor-mediated responses (Clineschmidt, Pflueger & others, 1975a). In this latter study, BE-2254 was found to be approximately equipotent with chlorpromazine in blocking central noradrenaline receptors but was much less active than either chlorpromazine or haloperidol as a blocker of central dopamine receptors. Biochemical examination of the effects of BE-2254 on central catecholamine metabolism also indicated a preference of this compound for noradrenaline rather than dopamine receptors (Clineschmidt, Totaro & others, 1975b).

These studies have now been extended to the *in vitro* level by examining the affinity of BE-2254 for central α-, β- and dopaminergic catecholamine receptors using the following binding assays: α-receptor; binding of [^{3}H]dihydroergocryptine (^{3}H-DHE) (Greenberg & Snyder, 1977) and [^{3}H](2-([2′,6′-dimethoxy]phenoxyethylamino)methyl benzodioxan) (^{3}H-WB-4101) (Greenberg, U'Prichard & Snyder, 1976) to rat cerebral cortical membranes; β-receptor; binding of[^{3}H]dihydroalprenolol (^{3}H-DHA) to rat brain crude synaptosomal membranes (Alexander, Davis & Lefkowitz, 1975); dopamine receptor; binding of [^{3}H]haloperidol (^{3}H-HAL) to rat caudate membranes (Burt, Creese & Snyder, 1976). The ligand concentrations used, total radioactivity bound (d min^{-1} mg^{-1} original wet weight of tissue), total counts bound per assay tube and per cent specific binding were as follows: ^{3}H-DHE; 0·4 nM; 128 d min^{-1}; 1000 c min^{-1}; 70%: ^{3}H-WB-4101; 0·22 nM; 70 d min^{-1}; 530 c min^{-1}; 75%: ^{3}H-DHA; 18 nM; 168 d min^{-1}; 1600 c min^{-1}; 80%: ^{3}H-HAL; 1 nM, 840 d min^{-1}; 1600 c min^{-1}; 50%. Counting efficiency for the ^{3}H-ligand/filter disk complexes in Amersham PCS Scintillation cocktail was 38–40%.

* Correspondence.

^{3}H-DHE and ^{3}H-HAL were obtained from New England Nuclear (Boston, Mass.) and ^{3}H-DHA from Amersham (Clearbrook Heights, Chicago, Ill.). ^{3}H-WB-4101 was custom tritiated by New England Nuclear. Chlorpromazine, haloperidol, phentolamine and propranolol were tested as reference compounds. IC50 values were obtained by examining the compounds at three or four concentrations in triplicate in each experiment. Data thus obtained were analysed using a log concentration-percent response linear regression.

BE-2254 was effective in preventing the binding of ^{3}H-DHE and ^{3}H-HAL to their respective receptors (Table 1). The IC50 for the α-receptor ligand was about 10 times lower than that of the ligand for dopamine receptors. Because of the controversy related to the specificity of ^{3}H-DHE as an α-receptor ligand (Davis, Strittmatter & others, 1977; Tittler, Weinreich & Seeman, 1977), the effects of BE-2254 were also examined using ^{3}H-WB-4101, an α-antagonist. The IC50 of BE-2254 in the WB-4101 assay was 200 times lower than that seen in the ^{3}H-HAL binding assay. Whether this difference in the IC50 values in the α-binding assays represents different classes of binding sites remains to be seen (U'Prichard, Greenberg & Snyder, 1977); however the reported K_D's of the two α-ligands are similar (^{3}H-DHE; 0·8 nM [Greenberg & Snyder, 1977]; ^{3}H-WB-4101; 0·6 nM [Greenberg & others, 1976]). The binding of ^{3}H-DHA was not affected by BE-2254. This profile indicates that BE-2254 binds preferentially to central α-adrenoceptors, but, as previously reported does have some affinity for central dopamine receptors.

Chlorpromazine and haloperidol had, as expected, a high affinity for caudate dopamine receptors. Both compounds also showed a weaker affinity for the α-receptor, chlorpromazine being the more potent of the two. Neither of these neuroleptics affected ^{3}H-DHA binding, although propranolol, a reference β-adrenoceptor blocker, was effective in this assay.

Although BE-2254, haloperidol and chlorpromazine interact with both α-adrenoceptors and dopamine receptors, their ratios between the two binding assays