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Affinity of butriptyline and other tricyclic antidepressants for α-adrenoceptor binding sites in rat brain

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Butriptyline is a tricyclic compound possessing a neuropsychopharmacological profile in animals similar to that of various tricyclic antidepressants (Voith & Herr 1969; Herr et al 1971) and is a clinically effective antidepressant agent (Ambrus 1971; Levinson 1974; Kapadia & Smith 1976; Brodie et al 1978; Burrows et al 1979). The drug does not appreciably block noradrenaline (NA) uptake in mouse and rat heart in vivo (Lippmann 1969, 1971) or NA and 5-hydroxytryptamine (5-HT) uptake in rat brain in vivo (Pugsley & Lippman 1974). In vitro, butriptyline inhibits [^{3}H]-dopamine uptake in rat corpus striatum, the drug being similar in activity to maprotiline, trimipramine, iprindole, mianserine and the classical tricyclics and about 50 times less potent than nomifensine (Randrup & Braestrup 1977). Butriptyline and the other abovementioned drugs exhibit only weak ability to inhibit in vitro [^{3}H]5-HT uptake in rat whole forebrain and [^{3}H]NA uptake in rat occipital cortex preparations in comparison with the classical antidepressants, e.g. chlorimipramine and desipramine, respectively (Randrup & Braestrup 1977).

U'Prichard et al (1978) have shown that the affinities of the classical tricyclic antidepressants for rat brain α-adrenoceptors, as judged by their ability to displace the postsynaptic α-adrenoceptor antagonist [^{3}H]-WB-4101 2-(*N*-[2′,6′-dimethoxyphenoxyethyl]) aminomethyl-1,4-benzodioxane [phenoxy-3-^{3}H (N)], correlate directly with the ability of these agents to relieve psychomotor agitation and to induce sedation and hypotension and inversely with their tendencies to elicit psychomotor activation. We have therefore determined the ability of butriptyline to interact with rat brain α-adrenoceptors labelled by [^{3}H]WB-4101 and to compare the affinity of butriptyline with that of the classical antidepressants amitriptyline, imipramine and desipramine.

The α-adrenoceptor binding activity of the test drug was determined essentially as described previously (Greenberg et al 1976). Two rats were decapitated and the brains quickly removed. The cerebellum and brain stem were excised and the remainder of the brain homogenized in 20 volumes of ice-cold 50 mM Tris-HCl (pH 7·7; 25 °C) with a Brinkman Polytron PT-10 for 10 s at setting No 6 and then centrifuged at 50 000 *g* for 10 min. The homogenate was washed once by resuspension and centrifugation; the final suspension was in 50 volumes of cold 50 mM Tris-HCl buffer (pH 7·7; 25 °C). In the standard binding assay, incubation tubes each received 1·0 ml of tissue suspension (20 mg, wet weight of original tissue), 100 μl (4·4 nM) of [^{3}H]WB-4101 to give a final concentration of 0·22 nM, 100 μl of various concentrations of test drug prepared freshly in 0·1% ascorbic acid, 0·7 ml of Tris-HCl buffer (pH 7·7; 25 °C) and 100 μl ascorbic acid. The extent of non-specific binding of [^{3}H]WB-4101 was determined from parallel assay tubes which contained a large excess of (−)-NA (2 mM) in 100 μl of 0·1% ascorbic acid. Assays were conducted in triplicate; tubes were incubated for 15 min at 25 °C with constant shaking. After the incubation, each sample was rapidly filtered, under reduced pressure, through a Whatman GF/B glass fibre filter. The incubation tube was washed twice with 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7·7; 25 °C) and each wash filtered. Each filter was placed in a vial containing 10 ml of Aquasol and after shaking for 1 h, the radioactivity content was measured in a liquid scintillation spectrometer. Specific binding for [^{3}H]WB-4101 is defined as the total binding minus the binding obtained in the presence of 100 μM (−)-NA.

The IC50 (concentration producing 50% inhibition of the specific binding of [^{3}H]WB-4101) for the test drug and the Hill coefficient were calculated by linear regression of the line obtained by plotting the log of [% B_{max}/100%−%B_{max}] versus the log of the concentration of inhibitor. B_{max} is taken as the specific binding occurring in the absence of displacing drug. Binding occurring in the presence of displacing drug is expressed as % B_{max}. The IC50 value is the point at which log of [% B_{max}/100%−%B_{max}] is 0 and the Hill coefficient is the slope of the line.

Butriptyline hydrochloride (Evadyne) was from Ayerst Laboratories. Desipramine hydrochloride (Pertofrane; Ciba-Geigy Ltd), imipramine hydrochloride (Tofranil; Ciba-Geigy Ltd) and amitriptyline hydrochloride (Elavil; Merck, Sharpe & Dohme, Ltd) were gifts from the respective companies. [^{3}H]WB-4101

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(spec. act. 25·4 Ci $mmol^{-1}$) and Aquasol were purchased from New England Nuclear and (−)-NA bitartrate from Sigma Chemical Co. Male Sprague Dawley albino rats (140–160 g) from Canadian Breeding Laboratories, St Constant, Quebec, Canada, were used.

The specific binding of [^{3}H]WB-4101 to rat brain membranes showed a single component as determined by Scatchard analysis with an apparent dissociation constant, K_D, of 0·42 nM and maximal binding, B_{max}, of 5·05 pmol g^{-1} of wet tissue; the values were similar to those reported by Greenberg et al (1976). Inhibition of [^{3}H]WB-4101 binding (expressed as IC50) by the α-adrenoceptor antagonists examined was in the following order: prazosin (0·65 nM) > phentolamine (4·8 nM) > yohimbine (847·1 nM) (Table 1). Each exhibited a Hill coefficient of about 1 indicating binding to a single population of binding sites.

Each of the antidepressants tested, i.e. butriptyline, amitriptyline, imipramine and desipramine, inhibited the specific binding of [^{3}H]WB-4101 with butriptyline exhibiting a relatively low affinity (Table 1). The IC50 values for butriptyline, amitriptyline, imipramine and desipramine were 2097, 47·0, 162·4 and 398·7 nM, respectively. Hill coefficients were calculated for the displacement curves for all of the drugs and were near unity, consistent with the binding being to a single population of binding sites.

In the present study, butriptyline was demonstrated to possess a weak affinity for postsynaptic α-adrenoceptors labelled with [^{3}H]WB-4101 in comparison to that of amitriptyline, imipramine and desipramine. This indicates an important difference between the tricyclic antidepressant butriptyline and the classical tricyclic antidepressants. The affinity order and the relative potency of the α-adrenoceptor antagonists and the classical tricyclic antidepressants observed in the present study are in accord with those reported by Greenberg et al (1976) and U'Prichard et al (1978), respectively.

Clinically, in depressed patients, the secondary amine tricyclic antidepressants, like desipramine, tend to cause psychomotor activation and are less likely to cause sedative and hypotensive side-effects and are useful in treating retarded depressions; tertiary amines, like amitriptyline, cause little psychomotor activation and are of use in the treatment of anxious, agitated depression and also tend to elicit the highest incidence of sedation and hypotensive side-effects (see Carlsson et al 1969a, b; Benesova & Nahunek 1971). More recently, U'Prichard et al (1978) have shown that the affinities of tricyclic antidepressant drugs for α-adrenoceptors correlate well with the ability of these agents to relieve psychomotor agitation and to induce sedation and hypotension in depressed patients.

Butriptyline, like amitriptyline, has been shown to relieve psychomotor agitation in depressed patients (see introduction for references) and to exhibit a lower incidence of sedation and hypotensive side effects than amitriptyline (Brodie et al 1978). Thus, the weak affinity of butriptyline for α-adrenoceptors in comparison to amitriptyline, is correlated well with its low incidence of sedation and hypotensive side effects, but is not correlated with the observed ability of butriptyline to relieve psychomotor agitation in depressed patients. Therefore, butriptyline appears to be an exception to the findings found by U'Prichard et al (1978) that the affinities of tricyclic antidepressants for α-adrenoceptors correlates with their relief of psychomotor agitation. In this regard, other actions of butriptyline (Lippmann 1969, 1971; Voith & Herr 1969; Pugsley & Lippmann 1974, 1979; Randrup & Braestrup 1977) may be of possible relevance to its mode of action.

Table 1. α-Adrenoceptor affinities of butriptyline, amitryptiline, imipramine and desipramine. Rat brain membrane samples were incubated in triplicate with 0·22 nM of [^{3}H]WB-4101 and 4 concentrations of the test drug. The IC50 is the concentration of the test drug required to inhibit by 50% the specific binding of [^{3}H]WB-4101. IC50's and Hill coefficients were calculated by linear regression from plots of log of [% B_{max}/ 100%−% B_{max}] versus the log of the concentration of inhibitor. Number of separate determinations is indicated in brackets. r is the correlation coefficient of best fitted lines calculated by linear regression.

Drug	IC50 (nM ± s.e.)	Hill Coeff. ± s.e.	Corr. Coeff. (r)
Butriptyline	2097·0 ± 2·8 (3)	1·00 ± 0·02	0·98
Amitriptyline	47·0 ± 4·7 (6)	1·04 ± 0·04	0·99
Imipramine	162·4 ± 10·4 (6)	0·96 ± 0·08	0·99
Desipramine	398·7 ± 35·1 (4)	1·10 ± 0·09	0·97
Phentolamine	4·8 ± 0·5 (4)	1·00 ± 0·08	0·99
Prazosin	0·65 ± 0·06 (12)	1·11 ± 0·03	0·99
Yohimbine	847·1 ± 11·1 (3)	1·07 ± 0·04	1·00

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An attempt to freeze-dry haemoglobin in the presence of macromolecules

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Macromolecules are widely used as adjuvants in freeze-drying (Regner 1979). In the course of our research into compounds that prevent the oxidation of haemoglobin to methaemoglobin during desiccation (Labrude & Vigneron 1980a,b), we have examined the anti-oxidant effect of some synthetic or natural polymers and proteins of different molecular weights.

Materials and methods

Haemoglobin solution was prepared from blood that had reached the end of its storage time. Washed red blood cells were haemolysed in demineralized water, and the stromata were eliminated by two centrifugations at 25 000 *g* for 30 min, followed by decantation. Finally the solution was dialysed against demineralized water for 15 h at 4 °C. The concentration of haemoglobin was 80 $\pm$ 5 g litre^{-1}.

The following compounds were examined: freeze-dried human serum-albumin more than 95% pure (Centre régional de Transfusion sanguine et d'-Hématologie de Nancy); the gelatin hydrolysate used in the synthesis of 'Polygéline' (Hoechst); dextrans T 15–20 and 60–90 (Sigma); 10, 20, 40, 70, 110, 150, 250, and 500 (Pharmacia); hydroxyethylstarch (Polysciences); Polyvidone (Prolabo); Ficolls 70 and 400 (Pharmacia); and polyoxyethylene glycol 10 000 (Merck) (preliminary assays with some PEG of molecular weights 400 to 40 000 showed that PEG 10 000 was the best for methaemoglobin). Each compound was dissolved in 5 ml of demineralized water, and the resulting solution was mixed with 5 ml of haemoglobin solution. The final concentrations of macromolecules were 50 and 100 g litre^{-1}. We went on to study the relationship between the oxidation of the haem and the concentration of protecting substance from 5 to 100 g litre^{-1} with the Ficolls which were the only macromolecules initially found to be effective. Freeze-drying was carried out in an experimental Kreel apparatus (manufacturer at Nancy), in the following conditions: freezing at —40 °C, primary desiccation at —10 °C for 16 h, and secondary desiccation at 5 °C for 8 h. The apparatus was opened to the air, and the flasks, stoppered in air, were immediately analysed.

Analyses were made of the colours and dissolution times of the freeze-dried materials in 10 ml of demineralized water; of the oxyhaemoglobin saturation (using the Hemoximetre OSM 2 Radiometer), of the methaemoglobin concentrations (Evelyn & Malloy 1938); and of the haemoglobin dissociation curves (D.C. Analyzer, Radiometer, as described by Teisseire et al (1975).

Results

The unprotected lyophilizates were brown and poorly soluble, with a methaemoglobin content of 49 $\pm$ 10% (n = 30) and a corresponding low oxygen saturation; the oxygen-dissociation curve lost its sigmoidal shape and the p50 was less than 10 torr.

The lyophilizates containing the macromolecules had colouration ranging from brown for the most denatured to red-orange. They dissolved rather slowly; the least soluble were those obtained with hydroxyethylstarch. The samples prepared with polyoxyethylene glycol freeze-dried poorly, showed traces of melting and remained turbid after dissolution.

Table 1 summarizes the methaemoglobin and oxyhaemoglobin rates obtained with the different macro-

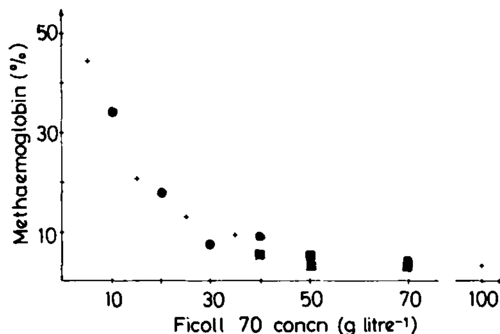

FIG. 1. Relationship between methaemoglobin percent and Ficoll 70 concentrations in freeze-dried haemoglobin samples for three different experiments. Similar results were obtained with Ficoll 400. Without protection, the mean methaemoglobin level is 49%.

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