3H-Rauwolscine Binding in Platelets from Depressed Patients and Healthy Volunteers

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Abstract. 3H-Rauwolscine binds specifically and with high affinity to alpha2-adrenoceptors in human platelets. In a study comparing the binding of 3H-rauwolscine in platelets obtained from 26 control volunteers with 19 hospitalised, untreated, severely depressed patients, the mean maximal binding (Bmax) and mean dissociation constant (Kd) of 3H-rauwolscine binding were found to be identical in both groups. After 7–12 days, treatment with different tricyclic antidepressant drugs there was a significant improvement in the depressive symptoms but no change in the 3H-rauwolscine binding. After an average of 23 days treatment with tricyclic antidepressants and, when the Hamilton Depression Rating Scores had returned to normal, the Kd and Bmax of 3H-rauwolscine binding were still unchanged.

Key words: 3H-Rauwolscine binding — Alpha2-adrenoceptors — Depression — Platelets — Antidepressants — Longitudinal studies

Studies on the biochemical basis of affective disorders have been restricted by the limited number of relevant biochemical markers that can be measured in man. Blood platelets offer the possibility of studying a number of biochemical variables in depressed patients (Sneddon 1973).

The high affinity 3H-imipramine binding sites which have been shown to be associated with serotonin uptake (Langer and Briley 1981) are present in human brain (Rehavi et al. 1980; Langer et al. 1981) and platelets (Briley et al. 1979; Langer et al. 1980). We have recently shown that the density of 3H-imipramine binding sites in the platelets of untreated depressed patients is decreased as compared to healthy volunteers (Briley et al. 1980; Raisman et al. 1981, 1982). This decrease, which has been confirmed in other laboratories (Asarch et al. 1981; Paul et al. 1981), is probably related to the impaired serotonin uptake observed in platelets from untreated depressed patients and may reflect a similar deficiency in central serotonergic neurons. The latter is thought to be associated with certain types of depression (Van Praag 1977).

A deficiency in central noradrenergic transmission has been proposed for a long time as an important factor in certain types of depression (Schildkraut 1965). Alpha2-adrenoceptors in platelets have been found to mediate platelet aggregation (Sneddon 1973) and the inhibition of basal and prostaglandin-stimulated adenylate cyclase activity (Salzman and Neir 1969). Recent results indicate that these receptors correspond to the alpha2 subtype of adrenoceptors (Jakobs 1978; Langer 1980; Elliott and Grahame-Smith 1982). The study of the alpha2-adrenoceptors found on human platelets may give an insight into the noradrenergic aspects of depression in the same way that 3H-imipramine binding possibly reflects alterations in the serotonin system.

Initial studies on alpha2-adrenoceptors in human platelets used the relatively non-selective antagonist ligands, 3H-dihydroergocryptine (Newman et al. 1978; Elliott and Grahame-Smith 1982) or 3H-phenotolamine (Kafka et al. 1977). Subsequently, the preferential alpha2-adrenoceptor agonist 3H-clonidine, was used (Shattil et al. 1981). Using 3H-clonidine as the ligand, Smith and Garcia-Sevilla (1982) demonstrated that a population of untreated depressed patients had a greater density of alpha2-adrenoceptors on their platelets than a group of normal controls. Following treatment with tricyclic antidepressants they observed a significant fall in the number of alpha2-adrenoceptors labelled with 3H-clonidine (Garcia-Sevilla et al. 1981a). The introduction of the selective alpha2-adrenoceptor antagonist ligands, 3H-yohimbine (Motulsky et al. 1980; Daiguji et al. 1981a) and, more recently, 3H-rauwolscine (Perry and U'Prichard 1981), has now made it possible to study platelet alpha2-adrenoceptors in depression using specific alpha2-adrenoceptor antagonist rather than agonist ligands.

We report here the binding of 3H-rauwolscine to the platelets of a group of untreated severely depressed patients in comparison with a group of normal controls. Some of the depressed patients were also studied longitudinally during tricyclic antidepressant medication.

Materials and Methods

Clinical Details of Patients and Volunteers. Depressed patients (27–68 years old; 5 males and 14 females) were suffering from either monopolar endogenous depression (n = 11), or reactive depression (n = 8) of sufficient severity to require hospitalisation. Hamilton scores varied between 41 and 71 (NIMH 1967, 25 items scale) and the mean Hamilton rating score was 55 ± 2 (n = 19).

Endogenous depressed patients fulfilled the criteria for primary major affective disorders and had personal and/or familial history of melancholic and/or manic episodes. Reactive depressions appeared on neurotic patients after clear precipitating events; some of them had previous history of such episodes.
Almost all the patients were sent by general practitioners to the hospital and some had been previously treated with antidepressants without success. At the time of admission to hospital the population of patients was heterogeneous with regard to the length of the evolution of their depressive episode. Nevertheless, all patients had been drug-free for at least 7 days except some who received small doses of benzodiazepines, chloral hydrate or laudanum.

Subsequently, blood samples were taken from some of these patients after 7-10 days of treatment with the tricyclic antidepressants clomipramine (100-200 mg/day) or amitriptyline (100-200 mg/day). Drugs were administered either alone or combined with benzodiazepines. When the patients were considered sufficiently recovered to be discharged from hospital (under continuing antidepressant medication) a third blood sample was taken. This was between 14 and 35 days (mean 23 days) after the initial blood sample.

Control volunteers (21-71 years old; 7 males and 19 females) received no psychoactive medication in the preceding month and were free of symptoms of mental illness. All blood samples were taken between 9 and 10 A.M.

Preparation of Platelet Membranes. Platelet membranes were prepared as described previously (Raisman et al. 1981). Blood (40 ml) was withdrawn by antecubital venopuncture and collected into plastic tubes with citrate anticoagulant (trisodium citrate 93 mM; citric acid 213 mM; glucose 111 mM). Platelets, obtained from platelet-rich plasma by centrifugation at 16,000 g for 10 min., were twice washed with buffer (Tris/HCl 50 mM, Na2EDTA 20 mM, NaCl 150 mM, pH 7.0). Membranes were prepared by hypotonic lysis (Tris/HCl 5 mM, Na2EDTA 5 mM, pH 7.0), homogenisation (Potter) and centrifugation at 39000 g for 10 min. The pellet was washed with buffer (Tris/HCl 50 mM, pH 7.5) and finally resuspended in the same buffer at a concentration of 0.4-0.8 mg protein/ml.

3H-Rauwolscine Binding. 3H-Rauwolscine binding was measured by incubating 400 μl of the membranes from platelets with different concentrations of 3H-rauwolscine (84.4 Ci/m mole, N.E.N. Chemicals) in a total volume of 500 μl for 1 h at 25°C. Following the incubation 200 μl aliquots were filtered using Whatman GF/B glass fibre filters. The filters were washed three times with 5 ml of ice-cold buffer (Tris/HCl 50 mM, pH 7.5), dried at 110°C and the radioactivity counted in toluene, PPO (5 g/l), POPP (0.1 g/l). Specific binding, defined as that inhibited in the presence of 10 μM phentolamine, was 75% at 2.0 nM 3H-rauwolscine.

Protein concentrations were determined by the method of Lowry et al. (1951). Statistical analyses were carried out using the Mann-Whitney non-parametric test.

Results

Characterisation of 3H-Rauwolscine Binding sites in Human Platelets. Specific high-affinity binding of 3H-rauwolscine to human platelets was saturable, giving linear Scatchard plots (Fig. 1). Competition experiments demonstrated a selectivity corresponding to that of an alpha2 subtype of adrenoceptor (Langer 1980). 3H-Rauwolscine binding was inhibited with high affinity by the alpha2-adrenoceptor selective antagonist yohimbine (IC50 0.007 μM), but with relatively low affinity by the alpha1-adrenoceptor selective antagonist prazosin (IC50 7 μM).

Comparison of 3H-Rauwolscine Binding in Platelets from Depressed Patients and from Normal Control Volunteers. The Bmax values for 3H-rauwolscine binding in platelets from control volunteers varied over a wide range (65-326 fmole/mg protein) (Fig. 2) with a mean Bmax value of 147 ± 13 fmole/mg protein (n = 26) (Table 1). The mean value of the apparent dissociation constant (KD) in this group was 2.0 ± 0.2 nM (n = 26).

The binding statistics from the untreated depressed patients are shown in Fig. 2 and Table 1. The mean Bmax value, 147 ± 18 fmole/mg protein (n = 19) (Table 1), is not significantly different from that of the corresponding control population. The mean KD value, 2.3 ± 0.2 nM (n = 19) was also similar to the control population.

When data from patients suffering from endogenous depression was analysed separately from that obtained from patients with reactive depressions, the two groups were found not to differ significantly (endogenous n = 11, HDRS 59 ± 3,