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Rapid communication

TRICYCLIC ANTIDEPRESSANT DRUG TREATMENT DECREASES α_2-ADRENORECEPTORS ON HUMAN PLATELET MEMBRANES

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Chronic administration of amitriptyline decreases the number of α_2-adrenoreceptors which are quantitated by the binding of [^{3}H]clonidine to neural membranes isolated from specific areas of the rat brain (Smith et al., 1980). This effect appears to be the molecular mechanism by which the long-term administration of tricyclic drugs increases the neuronal release of noradrenaline. This increase in noradrenaline release was previously shown to be secondary to the development of presynaptic α_2-adrenoreceptor subsensitivity (Crews and Smith, 1978, 1980). Since desensitization of the α_2-adrenoreceptor might be relevant to the clinical effectiveness of tricyclic drugs, the assessment of the function of this receptor in a readily available peripheral tissue is of both theoretical and practical value. The alpha adrenoreceptors on human platelets appear to be of the α_2-subtype (Hoffman et al., 1979). By use of the highly specific radioligands, [^{3}H]clonidine and [^{3}H]yohimbine, we have shown that α_2-adrenoreceptors on human platelets resemble those found in the rat brain (García-Sevilla et al., 1980 and in preparation). We now report that the chronic administration of various tricyclic drugs to psychiatric patients reduces the number of α_2-binding sites on blood platelet membranes.

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Blood was obtained from psychiatric patients who were drug free for at least two weeks and again from the same patients after treatment with tricyclic antidepressants. Acid-citrate-dextrose (ACD) solution was used as the anticoagulant. After centrifugation ($160 \times g$ for 10 min) the platelet-rich plasma was titrated to pH 6.5 with ACD solution and recentrifuged ($5100 \times g$ for 15 min). The platelet pellet was washed twice with 5 ml of Tyrode buffer and recentrifuged. The pellet was lysed by homogenization in 2 ml of hypotonic buffer (Tris-EDTA, 5 mM; pH 7.5). After centrifugation ($39\,000 \times g$ for 10 min) the platelet membranes were resuspended in the 50 mM Tris-HCl buffer (pH 7.5) used in the assay. Total [^{3}H]clonidine binding was measured in 1 ml aliquots of the membranes which were incubated in duplicate for 20 min at 25°C with the labelled ligand (5×10^{-10} to 6.4×10^{-8} M, specific activity 23.8 Ci/mmol, N.E.N., Boston). Nonspecific binding, assessed in the presence of unlabelled clonidine, 10^{-5} M, was not affected by drug treatment. Specific binding, the difference between total and nonspecific binding, represented 90% of the total binding at 4×10^{-9} M. Incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters. After washing with 20 ml of incubation buffer, the filters were air dried and counted for radioactivity.

Chronic administration of imipramine led to a significant decrease (35 ± 7%) in the

TABLE 1

[^{3}H]Clonidine binding to human platelet membranes before and after chronic imipramine treatment [a].

Patient [b] No.	Imipramine HCl		Drug-free		Treated		Paired *t*-test	
	Dose (mg/day)	Duration (weeks)	K_D (nM)	B_{max} (fmol/mg protein)	K_D (nM)	B_{max} (fmol/mg protein)	ΔK_D	ΔB_{max}
1	175	4	9.7	48	4.3	37	−5.4	−11
2	175	2	4.5	29	2.6	18	−1.9	−11
3	200	4	2.8	37	2.6	28	−0.2	−9
4	75	2	4.7	28	3.9	13	−0.8	−15
5	50	7	4.4	21	1.0	16	−3.4	−5
	Mean ± S.E.M.		5.2 ± 1.2	33 ± 5	2.9 ± 0.6	22 ± 4	−2.3 [c] ± 0.9	−10 [d] ± 1.6

[a] K_D and B_{max} were calculated by Scatchard analysis of full saturation curves for platelets from each subject.
[b] Diagnoses include endogenous depression (Patients 1, 2 and 3) and agoraphobia with panic attacks (Patients 4 and 5).
[c] $t = 2.49$, $P < 0.05$.
[d] $t = 6.25$, $P < 0.005$.

number of high affinity binding sites (B_{max}) for [^{3}H]clonidine (table 1). The K_D for [^{3}H]clonidine binding was also decreased slightly. Treatment of an endogenously depressed patient with nortriptyline (75 mg/day for 5 weeks) also resulted in a decreased B_{max} (20%) for the high affinity binding site. Binding parameters for membranes from drug-free patients did not differ from those determined with a normal control population ($K_D = 5.3 \pm 0.4$ nM; $B_{max} = 35 \pm 3$ fmol/mg protein; $n = 15$). In vitro, tricyclic antidepressant drugs also inhibited the binding of [^{3}H]clonidine to platelet membranes. However, this inhibition was competitive (K_D increased) and was not associated with a change in the number of binding sites (B_{max}) (García-Sevilla et al., 1980 and in preparation).

These results indicate that α_2-adrenoreceptors on human platelets, like those on neurons in the rat brain, are decreased in number after the chronic administration of tricyclic antidepressant drugs. The specific binding of radiolabelled α_2-receptor ligands to human platelet membranes might be a simple and useful laboratory test to monitor therapeutic responsiveness not only in endogenous depression but in other clinical disorders which might involve altered noradrenergic neuronal function.

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