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Binding of [^{3}H]-dihydroergocryptine to α-adrenoceptors on intact human platelets

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[^{3}H]-Dihydroergocryptine ([^{3}H]-DHE) has been shown to bind to α-adrenoceptors in both nervous (Greenberg & Snyder, 1977) and non-nervous (Williams, Mullikin & Lefkowitz, 1976; Guellaen, Yates-Aggerbeck, Vauquelin, Strosberg & Hanoue, 1978) tissue. The existence of α-adrenoceptors on the human platelet has been shown indirectly by the actions of adrenaline which induces platelet aggregation (O'Brien, 1963; Mills & Roberts, 1967) and inhibits PGE_1-induced cAMP production (Marquis, Becker & Vigdahl, 1970; Jakobs, Saur & Schultz, 1976) *in vitro*. This communication describes the direct binding of [^{3}H]-DHE to α-adrenoceptors on intact human platelets.

Platelet rich plasma from male volunteers (age 24–38) was prepared by centrifugation at 20°C. This was centrifuged at 1700 g for 5 min at 10°C to produce a platelet pellet, which was gently resuspended in incubation buffer (0.1% EDTA, 150 mM NaCl, pH 7.5) to a final cell density of approximately 0.8×10^8 platelets/ml. One ml aliquots were incubated for 20 min at 37°C with 0.5–18 nM [^{3}H]-DHE. Incubations were terminated by centrifugation at 1200 g for 1 min in an Eppendorf 5412 centrifuge to separate cells and supernatant. Individual platelet pellets were washed and sonicated in 500 μl distilled water; 400 μl aliquots were counted in a liquid scintillation spectrometer for total radioactivity. Specific binding was calculated as the total radioactivity bound at a given free concentration minus the non-specific binding which occurred at that free concentration in the presence of phentolamine (5 μM) (see below).

Specific binding of [^{3}H]-DHE has an exponential onset ($T_{\frac{1}{2}}$ = 7.5 min for 2.4 nM DHE) and offset ($T_{\frac{1}{2}}$ = 16 min). If binding were of the simple Langmuir type these values would imply that the rate constants for association (k_1) and dissociation (k_2) were 3.2×10^5 M^{-1} s^{-1} and 0.74×10^{-3} s^{-1}. The equilibrium binding curve showed a simple hyperbolic (Langmuir) form (Scatchard analysis, 5 subjects) with an affinity constant $K_a = 3.48 \times 10^8$ M^{-1} and binding capacity C = 72 fmol/10^8 platelets. This is equivalent to 433 molecular binding sites per platelet. The binding estimate of K_a agrees closely with the value derived from the kinetic measurements ($K_a = k_1/k_2 = 4.34 \times 10^8$ M^{-1}). [^{3}H]-DHE binding in a single subject showed little variability during a five week period, $K_a = 3.046 \pm 0.170 \times 10^8 M^{-1}$ (mean ± s.e. mean), capacity = 70.31 ± 5.15 f mol 10^{-8} platelets (mean ± s.e. mean, n = 5). [^{3}H]-DHE is inhibited stereospecifically by adrenaline and noradrenaline, with (−)isomer being at least ten times more potent than the (+)isomer. In comparison isoprenaline, dopamine and serotonin are poor inhibitors. Phentolamine and yohimbine at high concentrations (greater than 10^{-5}M) appear to displace non-specifically bound [^{3}H]-DHE. Between 10^{-6}M and 10^{-5}M phentolamine [^{3}H]-DHE binding is constant, therefore a concentration of 5 μM phentolamine was used to define non-specific binding.

These results resemble those obtained on platelet lysates (Newman, Williams, Bishopric & Lefkowitz, 1978; Alexander, Cooper & Handin, 1978) except that the dose-dependent effects of phentolamine have not been reported.

We conclude that the binding of [^{3}H]-DHE on intact human platelets occurs at α-adrenoceptor sites and that this technique may be used to monitor the platelet α-adrenoceptor characteristics of subjects in a variety of clinical conditions.

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Binding studies on alpha-adrenoceptors and muscarinic cholinoceptors in rat heart ventricle: effect of chemical sympathectomy

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Noradrenaline release in the peripheral nervous system is regulated through a negative feed-back mechanism mediated by presynaptic alpha-adrenoceptors. In addition a number of other presynaptic receptors including inhibitory muscarinic cholinoceptors have been described (Langer, 1977; Starke, 1977).

Rat heart ventricle possesses a rich noradrenergic innervation with mainly postsynaptic beta$_1$ adrenoceptors. Postsynaptic alpha-adrenoceptors mediating a positive ionotropic effect have also been reported (Wagner & Brodde, 1978).

In the present experiments the binding of two alpha-adrenoceptor ligands, [3H]-dihydroergocryptine ([3H]-DHE) and [3H]-WB 4101 (2-([2',6'-dimethoxy] phenoxyethylamine-methylbenzodioxan), and the muscarinic cholinoceptor ligand, [3H]-quinuclidinyl benzilate ([3H]-QNB), to rat heart ventricular membranes were studied in normal animals and animals in which the noradrenergic nerve terminals were destroyed with 6-hydroxydopamine (6-OHDA) pretreatment for two weeks.

The two adrenoceptor ligands, [3H]-DHE and [3H]-WB 4101, each bound with a single high affinity component with apparent dissociation constants (K_D) of 2.3 ± 3.0 nM and 2.0 ± 0.6 nM respectively. The maximal binding (Bmax) of [3H]-DHE and [3H]-WB were 374.2 ± 36.3 fmoles/g tissue and 275.7 ± 50.5 fmoles/g tissue respectively. Membranes prepared from sympathectomized animals showed decreases in maximal binding of 59.4% for [3H]-DHE ($P<0.002$) and 24.3% for [3H]-WB 4101 ($P<0.25$) when compared with those prepared from control animals.

The loss of alpha-adrenoceptor binding sites after sympathectomy suggests that some of these sites are located presynaptically on noradrenergic nerve terminals in the rat heart ventricle.

The muscarinic cholinoceptor ligand, [3H]-QNB, also showed high affinity, single component, binding with a Kd of 0.8 ± 0.1 nM and Bmax 646.2 ± 99.2 fmoles/g tissue. After 6-OHDA treatment the maximal binding was not significantly altered ($P>0.25$).

Our finding of unchanged muscarinic cholinoceptor binding after sympathectomy is in contrast to the recent report of Sharma & Banerjee (1978) who described a significant decrease in [3H]-QNB binding after 6-OHDA treatment. They interpreted their results as a loss of presynaptic cholinoceptors localized in noradrenergic nerve endings. The unchanged ([3H]-QNB binding seen in the present experiments suggests however that muscarinic cholinoceptors in the rat heart ventricle are localized mainly postsynaptically.

In summary the present results support a presynaptic location for alpha-adrenoceptors regulating noradrenaline release. Furthermore preliminary experiments have shown that the displacement of the alpha-adrenoceptor ligands from heart ventricle membranes by yohimbine and prazosine is different in normal and 6-OHDA treated animals.

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