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Characterization of calf brain dopamine receptors

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The characteristics of brain dopamine receptors using tritiated agonists and antagonists have been reported from several laboratories. From such binding studies Creese, Burt & Snyder (1975) proposed a two-state convertible receptor whereas, more recently, Nagy, Lee, Seeman & Fibiger (1978) proposed that there were two receptors, i.e. pre- and postsynaptic receptors. In order to test the above hypothesis we have compared the potencies of several dopamine agonists and antagonists to displace [^{3}H]-dopamine, [^{3}H]-apomorphine and [^{3}H]-spiroperidol binding from calf caudate membranes in the presence and absence of saturating concentrations of non-radioactive dopamine agonists and antagonists.

Calf caudate membranes were prepared and incubated for 30 min at 25° in the manner described by Titeler, Weinreich & Seeman (1977) except that the incubation volume was 1.0 ml. To measure 'antagonist' potencies membranes (20 mg wet weight) were incubated with [^{3}H]-spiperone (0.25 nM, 23.6 Ci/mmole) in the presence and absence of dopamine (1 μM). To measure 'agonist' potencies membranes (20 mg wet weight) were incubated with [^{3}H]-dopamine (1.5 nM, 10 and 7 Ci/mmole) or [^{3}H]-apomorphine (1.5 nM, 10.2 Ci/mmole) in the presence and absence of fluspirilene (1 μM). The membranes were recovered by rapid filtration through GF/B filters followed by two 5 ml washes with fresh, ice-cold, incubation medium. Specific binding of [^{3}H]-spiperone was defined as that radioactivity displaced by (+)-butaclamol (1μM) and specific binding of [^{3}H]-dopamine and [^{3}H]-apomorphine as that radioactivity displaced by 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (1μM, ADTN).

The compounds tested fell into three categories in their relative potencies to displace the tritiated ligands used. The neuroleptic drugs, such as spiperone, chlorpromazine and fluspirilene were effective at nanomolar concentrations to displace [^{3}H]-spiperone (IC_{50}^S = 3.5 nM, 78 nM and 95 nM respectively) but were only effective at micromolar concentrations to displace [^{3}H]-dopamine (IC_{50}^S = 11.3 μM, 27 μM and >100 μM) and [^{3}H]-apomorphine (IC_{50}^S = 13.4 μM, 14.4 μM and >100 μM). Conversely dopamine agonists, such as dopamine, apomorphine and ADTN, were effective at nanomolar concentrations, to displace [^{3}H]-dopamine (IC_{50}^S = 1.8 nM, 4.0 nM and 3.7 nM) and [^{3}H]-apomorphine (IC_{50}^S = 2.0 nM, 3.4 nM and 3.0 nM) but were only effective at micromolar concentrations to displace [^{3}H]-spiperone (IC_{50}^S = 47 μM, 1.1 μM and 8.0 μM).

The *in vivo* dopamine agonists, bromocriptine and dihydroergocriptine however, were both more potent to displace the binding of [^{3}H]-spiperone (IC_{50}^S = 240 nM and 68 nM) than either the binding of [^{3}H]-dopamine (IC_{50}^S = 1347 nM and 254 nM) or the binding of [^{3}H]-apomorphine (IC_{50}^S = 1121 nM and 317 nM). Thus there appears to be two classes of dopamine agonist, one having higher affinity for [^{3}H]-dopamine and [^{3}H]-apomorphine binding sites and the other having affinity for [^{3}H]-spiperone binding sites. These results are not consistent with the two-state convertible receptor hypothesis.

The addition to the incubation media of saturating concentrations of dopamine or fluspirilene had little or no effect on total binding in the absence of drugs nor did it significantly affect the potencies of dopamine agonists and antagonists. Therefore, occupancy of the sites to which (^{3}H]-spiperone binds has no effect on the binding of [^{3}H]-dopamine and [^{3}H]-apomorphine. Conversely, occupancy of the sites to which [^{3}H]-dopamine and [^{3}H]-apomorphine bind has no effect on the binding of [^{3}H]-spiperone. These results are also not consistent with the two-state convertible receptor hypothesis.

The above data support the hypothesis that in the calf caudate, there are two populations of dopamine receptors. One of these receptor populations may be labelled by [^{3}H]-dopamine and [^{3}H]-apomorphine and the other labelled by [^{3}H]-spiperone and probably by [^{3}H]-dihydroergocriptine (Titeler *et al.*, 1977). Whether these two populations represent pre- and postsynaptic receptors remains to be resolved.

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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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