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Stereospecific Opiate Binding in Human Erythrocyte Membranes and Changes in Heroin Addicts

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STEREOSPECIFIC OPIATE BINDING IN HUMAN ERYTHROCYTE MEMBRANES AND CHANGES IN HEROIN ADDICTS

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Stereospecific opiate binding has been demonstrated in human erythrocyte membranes, having a K_d of $9 \cdot 10^{-9}$ M. In most respects the binding characteristics resemble those of snyaptic membranes. These included the correlation of binding affinity and pharmacological potency of opiates; competition by naloxone; inhibition by Ca^{2+} and Na^+; and sensitivity to phospholipases and trypsin. A comparison of stereospecific opiate binding in control human subjects and heroin addicts revealed a 43% increase in the addict group.

Key words: opiate binding, heroin addicts, erythrocytes, membranes

In recent years, there has been considerable interest in the identification of the "opiate receptor" in brain tissue, and in the attempt to correlate the binding affinity of opiates to their pharmacological action (Goldstein et al., 1971; Pert and Snyder, 1973; Simon et al., 1973). Our interest in opiate binding developed from the observation that phosphatidyl serine not only exhibited stereospecific binding for opiates (Abood and Hoss, 1975), but its addition to neural membrane preparations significantly enhanced stereospecific binding (Abood and Takeda, 1976). Since erythrocyte membranes contain a significant amount of phosphatidyl serine (Pasternak and Snyder, 1974), while the total lipid extract exhibits opiate binding, a study was undertaken to determine whether high affinity opiate binding occurred in the membranes. Initial attempts to demonstrate stereospecific opiate binding to intact erythrocytes were unsuccessful, largely due to the fact that the hemoglobin, which leaked out into the incubation medium, was inhibitory to binding. The present study established that human erythrocyte membranes exhibit high affinity stereospecific binding for opiates, and that the binding site has many characteristics of the sites present in the brain tissue. In addition, a comparison was made of opiate binding in erythrocyte membranes of control subjects and heroin addicts.

Freshly drawn heparinized human blood was diluted with 5 volumes of 10 mM ice-cold Tris-EDTA, pH 7.5, and after 30 min was centrifuged at 100,000 g for 30 min. The pellet was homogenized in five times the original blood volume of 10 mM Tris-EDTA and recentrifuged. The washing procedure was repeated four times or until the hemoglobin had been removed. Stereospecific opiate binding was measured by the technique of Pert and Snyder (1973). A typical incubation medium contained the following in a final volume of 1.2 ml: 0.05 M Tris, pH 7.5, 0.05 μC of ^{3}H-dihydromorphine (^{3}H-DHM), specific activity 71 curies/mmole, and either 10^{-7} M levorphanol or dextrorphan. After 15-min incubation at 35°C, the samples were filtered by suction through a Whatman GF/B glass fiber filter, which was washed twice with 5 ml of ice-cold Tris buffer and transferred to a liquid scintillation vial for measuring radioactivity. All of the addicts used in the study were presently on heroin and had been on the drug for at least 1 year. The group consisted of seven males and three females ranging in age from 21 to 35 years.

In an effort to compare the opiate binding characteristics of erythrocyte membranes to those of brain tissue, the effect of a number of parameters known to influence the latter were examined (Table I). All values were expressed in terms of specific binding, which represented the difference in counts/min of the bound ^{3}H-DHM in the presence of 10^{-7} M dextrorphan from that in the presence of the experimental agent. At a concentration of 10^{-7} M hydromorphone produced complete inhibition and morphine 85%,

TABLE I. Effect of Various Treatments on Binding of ^{3}H-DHM to Erythrocyte Membranes

	^{3}H-DHM bound	Specific binding, cpm	Specific binding*	
			% change	% brain change
Dextorphan	845	—	—	—
Levorphanol	602	243	—	—
Hydromorphone	586	259	−106	−100
Morphine	638	207	− 85	− 90
Codeine	786	59	− 25	− 45
Naloxone	713	45	− 18	− 95
10^{-3} M CaCl$_2$	590	255	−104	− 95
0.05 M NaCl	580	265	−109	−100
Philpase A	610	235	− 97	− 90
Philpase C	603	242	−100	− 50
Trypsin	552	293	−120	−100
P-Serine	705	140	− 58	+35

Membranes were exposed to various treatments or drugs 30 min prior to the addition of ^{3}H-DHM. Incubation medium described in text; each tube containing 1.3 mg membrane protein. Amounts of phospholipase A (bee venom), phospholipase C (B. cereus) and trypsin (crystalline, treated with diphenyl carbamyl choline) was 1 unit, 2 units, and 0.2 mg respectively. Phosphatidyl serine was 99% pure (bovine brain) and used at a concentration of 100 μ/mg protein. See (5) for details on use of enzymes and phosphatidyl serine.

*Specific binding: $\dfrac{(243-\text{column 2}) \cdot 100}{243}$.

The values represent the mean of three separate experiments run in triplicate, and the variation from the mean was less than 6%.

values comparable to those obtained with rat brain synaptic membranes. Codeine, a weaker opiate, produced a 35% inhibition in erythrocytes as compared with 45% in the brain. On the other hand, the opiate antagonist naloxone had only a small effect on erythrocytes at a concentration completely inhibitory to brain. Both $CaCl_2$ (10^{-3} M) and NaCl (0.05 M) produced complete inhibition in both preparations, as did exposure to phospholipase A and trypsin. The erythrocyte appeared to be more sensitive to treatment with phospholipase C. Upon the addition of phosphatidyl serine (100 μg/mg membrane protein), binding was inhibited 58% as compared to a 35% enhancement of synaptic membranes.

A Scatchard analysis was performed on the stereospecific opiate binding of erythrocyte membranes from control human blood (Fig. 1). The K_d was calculated to be 9 · 10^{-9} M. This value compares with the lower affinity binding of brain tissue which exhibits a biphasic Scatchard plot with K_d s of 5 · 10^{-10} M and 8 · 10^{-9} M (Abood and Takeda, 1976; Pasternak and Snyder, 1975).

A preliminary study was performed on the stereospecific binding of erythrocytes obtained from a group of ten heroin addicts and an equal number of control subjects (Table II). The mean value of the control group was 130 ± 25, with the range being 115–180. This compares with a mean of 202 ± 38 in the addicts, with a range of 160–225. The mean is 43% greater in the addict than in the control group, the difference being significant at P < 0.01 (t test). It should be emphasized that the population of both control subjects and heroin addicts is too small to conclude that opiate binding increases during drug addiction; however, the data do encourage a more extensive study.
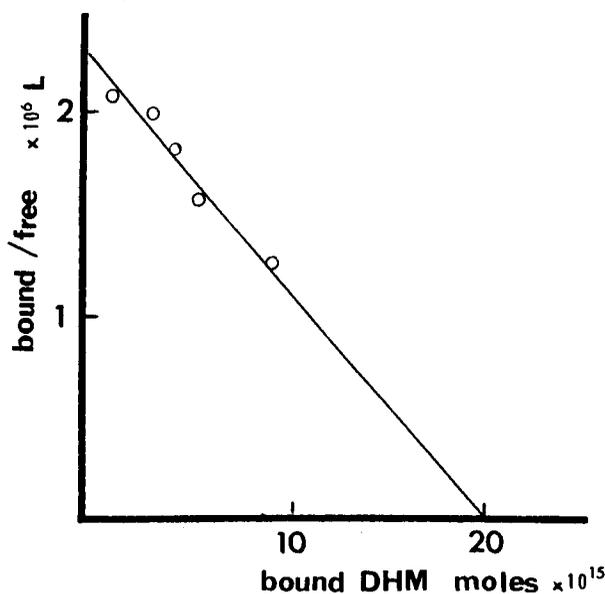

Fig. 1. Scatchard plot of ^{3}H-dihydromorphine binding to human erythrocyte membranes. All assays were run in triplicate and represent two separate experiments. Binding is stereospecific, representing the difference in the presence of 10^{-7} M levorphanol and dextorphan. Each tube contained 1 mg membrane protein in 0.05 M Tris, pH 7.5, and varying concentrations of ^{3}H-dihydromorphine. The points are an average of three separate experiments run in triplicate; the variation from the mean being no more than 6%.

TABLE II. Stereospecific Opiate Binding in Erythrocyte Membranes of
Control Human Subjects and Heroin Addicts

	No. of subjects	^{3}H-DHM binding	Range	% Increase
		cpm		
Controls	10	130 ± 25	115–180	–
Addicts	10	202 ± 38	160–225	43*

Stereospecific binding was expressed as difference in cpm of bound
^{3}H-DHM in the presence of 10^{-6} M dextrorphan from that in the
presence of 10^{-7} M levorphanol. Values are expressed as mean ±
standard deviation counts/min/mg protein

*Significant at $P < 0.01$ (t test).

The significance of the present study in terms of the pharmacological action of
opiates is difficult to assess. It is, however, apparent that the characteristics of the opiate
binding in erythrocyte membranes are very similar to those of brain tissue. Opiate bind-
ing in both tissues is inhibited by phospholipase A or C as well as by trypsin and NaCl.
The opiate antagonist naloxone is antagonistic to ^{3}H-DHM binding in both preparations;
however, the erythrocyte is only one-fifth as sensitive as synaptic membranes. Both Ca^{2+} and
Na^+ are completely inhibitory to both types of membranes. There appears to be a rela-
tionship between the affinity of various opiates and their pharmacological potency, hydro-
morphone being the most potent opiate and codeine the least. A major difference was the
effect of added phosphatidyl serine which significantly enhanced opiate binding in brain
tissue but was actually inhibitory to that in erythrocytes. Erythrocytes already contain a
comparatively large proportion of phosphatidyl serine, as much as 20% of the total phos-
pholipid (Rouser et al., 1968); consequently, all binding sites evidently have their comple-
ment of this lipid. As discussed elsewhere (Abood and Takeda, 1976; Abood et al., in
press), stereospecific opiate binding in neural membranes is significantly enhanced by
phosphatidyl serine, a finding which suggests that phosphatidyl serine may be a compo-
nent of the binding site.

Although the observed increase in opiate binding in the erythrocyte membranes of
heroin addicts is based on only a few subjects, chronic drug usage is generally associated
with a variety of adaptive mechanisms. The observation is also in conformity with the
report that chronic administration of opiates to rats increases the number of opiate bind-
ing sites in the brain (Pert and Snyder, 1976).

A major difference was the inability of naloxone to compete for the erythrocyte
binding site. At present the only plausible explanation for this difference is that the
presence of the N-allyl substituent on the opiate molecule tends to interfere with binding
to the erythrocyte membrane. This conclusion is supported by the finding that N-allyl-
morphine and levalorphan, which are also morphine antagonists, at concentrations of
10^{-7} M do not interfere with ^{3}H-DHM binding. It would appear that the reversal of
opiate binding by antagonists, which is so characteristic of the brain receptor, is absent
from erythrocytes.

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