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SEROTONIN RECEPTORS OF LYMPHOCYTES: A RADIORECEPTOR STUDY

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UDC 612.112.94.014.46:577.175.823

KEY WORDS: lymphocytes; serotonin receptors; ^{3}H-serotonin; radioreceptor method.

According to data in the literature several receptors for neurotransmitters exist on the surface of lymphocytes. For instance, the use of the radioreceptor method has shown the presence of β-adrenoreceptors [4] and acetylcholine receptors [7] on immunocompetent cells and evidence has been obtained of the existence of opiate receptors of lymphocytes [2] and of nonopiate receptors for β-endorphin [8]. Data also have been published on the effect of adrenomimetics [4] and of methioineenkephalin [2] on the adenylate cyclase system of lymphocytes.

The existence of sites of high-affinity specific binding of ^{3}H-spiroperidol [6], a marker of brain serotonin receptors [11], on lympocytes has recently been demonstrated. However, doubts have recently been expressed on the existence of serotonin receptors on lymphocytes, for specific binding of ^{3}H-spiroperidol was neither stereochemically specific nor saturating. Binding sites of this ligand also were characterized by high values of inhibition constants of serotonin and other drugs with high affinity for brain serotonin receptors [5].

Despite the proven action of serotonin on development of the immune response [1], direct proof of the presence of serotonin receptors on lymphocytes thus has not so far been obtained.

Accordingly, in the investigation described below an attempt was made to obtain proof of the existence of serotonin receptors of human lymphocytes by radioreceptor analysis of binding of ^{3}H-serotonin (^{3}H-5HT) with these cells.

EXPERIMENTAL METHOD

Lymphocytes were isolated from heparinized peripheral blood from healthy donors by centrifugation in a Ficoll—Verografin gradient. To isolate the coarse membrane fraction of lymphocytes the cells were disintegrated by ultrasoudn [9]. The suspension of disintegrated lymphocytes was centrifuged at 700g for 20 min. The supernatant was kept and the residue resuspended in mM 50 Tris-HCl (pH 7.4) on a "Type 302 Homogenizer" (Poland) with Teflon-glass attachment, at 2000 rpm (1.5 μ), and then centrifuged again. The resulting supernatants were

Institute of Psychiatry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhenevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 52-53, July, 1982. Original article submitted January 28, 1982.

 0007-4888/82/9407-0914$07.50

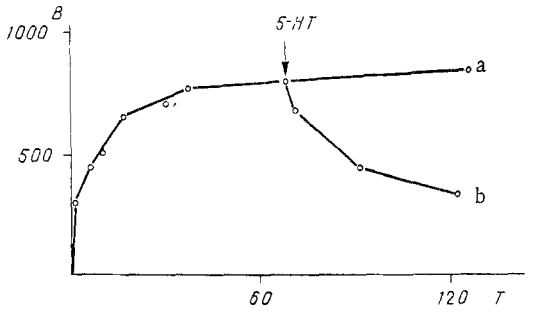

Fig. 1. Specific binding of ^{3}H-5HT (B) as a function of incubation time (a) and times to displace excess of unlabeled serotonin after incubation for 1 h with label (b). Abscissa, incubation time (in min); ordinate, specific binding (in cpm). Composition of reacting medium: 0.1-0.2 mg protein of coarse membrane fraction of lymphocytes, ^{3}H-5HT in final concentration of 12 nM, pargyline in concentration of 1 μM, 50 mM Tris-HCl (pH 7.4). Incubation at 18°C. Volume of sample 1 ml.

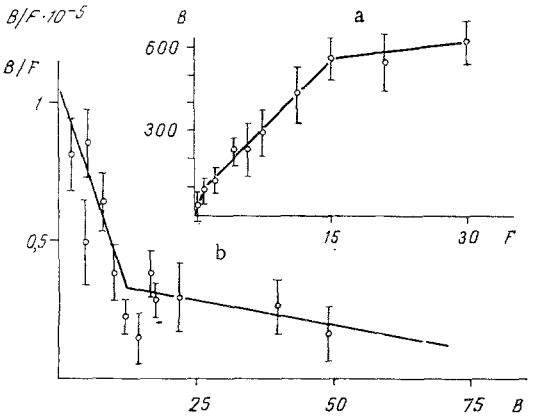

Fig. 2. Specific binding of ^{3}H-5HT (B) as a function of ^{3}H-5HT concentration (a) and ratio between amounts of specifically bound (B) ^{3}H-5HT and free ^{3}H-5HT (F) not bound by coarse membrane fraction of lymphocytes as a function of specific binding (B) (b). a: Abscissa, concentration of label (in nM); ordinate, specific binding (in cpm). b: Abscissa, specific binding (in femtomoles); ordinate, ratio between specifically bound label and unbound label; regression lines correspond to equations $y = 419.1 - 0.052x$ and $y = 968.2 - 0.45x$, with coefficients of correlation $r = 0.90$ and $r = -0.65$ respectively. Composition of reacting mixture: 0.1-0.2 mg/ml protein of coarse membrane fraction of lymphocytes, ^{3}H-5HT in final concentration of 0.5 to 60 nM, paragyline in final concentration of 1 μM, unlabeled serotonin in final concentration of 2 μM, 50 mM Tris-HCl (pH 7.4). Incubation of 18°C. Volume of sample 1 ml.

pooled and centrifuged at 6000g for 25 min. The new supernatant was centrifuged at 25,000g for 40 min. The residue thus formed was resuspended in the necessary volume of 50 mM Tris-HCl (pH 7.4) and used without delay to study binding of ^{3}H-5HT. All procedures for isolation of the membranes were carred out at 2°C. Protein was determined by Lowry's method after preliminary incubation in 1 N NaOH for 10 min. The yield of protein was 10% of that determined in the cell suspension.

The reaction mixture contained (in a volume of 1 ml): 50 mM Tris-HCl (pH 7.4), 0.1-0.2 mg protein of the coarse membrane fraction of the lymphocytes, ^{3}H-5HT in concentrations of 0.5 to 60 nM, and pargyline in a concentration of 1 μM. The reaction was carried out for 40 min at 18°C.

Specific binding of ^{3}H-5HT was determined as the difference between binding in the presence and absence of unlabeled serotonin in the incubation medium in a concentration of 2 μM. Bound and unbound label were separated by rapid filtration, for not more than 10 sec per sample, through type GF/B glass filters (Whatman, England). The filters were washed twice, each time with 3 ml of 50 mM Tris-HCl (pH 7.4), cooled to 0°C, and transferred to flasks for measurement of radioactivity. Each flask contained 7 ml of Lipoluma scintillator. The filters were kept in the scintillator for 12 h at 18°C.

The level of radioactivity was determined on a type LS-7000 counter (Beckman, England).

The following reagents were used: ^{3}H-serotonin (specific activity 16.6 Ci/mmole, from the Radiochemical Centre, Amersham, England); serotonin from Reanal, Hungary; pargyline from Sigma, USA; Ficoll/400 from Pharmacia, Sweden; Tris from Serva, West Germany; Verografin from Spofa, Czechoslovakia; Lipoluma scintillator from Lumac System AG, Switzterland.

The regression line in the Scatchard plot was drawn by the method of least squares.

EXPERIMENT RESULTS

The study of interaction between ^{3}H-5HT and lymphocyte membranes revealed specific bindind of this ligand to the extent of about 60% of the total. Analysis of the kinetics of specific binding of ^{3}H-5HT showed that equilibrium conditions at 18°C were reached after 30 min of incubation (Fig. 1a). Introduction of an excess of unlabeled ligand to the medium led to partial reversibility of specific ^{3}H-5HT binding (Fig. 1b). The reversible component of binding amounted to 60%.

The next experiment demonstrated the saturating character of interaction (Fig. 2a). Analysis of the saturation curve in Scatchard coordinates (Fig. 2b) reavealed the presence of two types of specific binding sites of ^{3}H-5HT on the lymphocyte membranes. High-affinity sites of presumptive receptor interaction were characterized by an equilibrium dissociation constant of 2 nM, low-affinity sites by a constant of 66 nM.

The value obtained for the equilibrium dissociation constant for high-affinity binding sties of ^{3}H-5HT with lymphocyte membranes, namely 2 nM, is close to the value of these characteristics for mammalian brain serotonin receptors [10, 11].

This investigation thus revealed the presence of high-affinity, saturating, partially reversible specific binding of ^{3}H-5HT in the coarse membrane fraction of human lymphocytes, and it can accordingly be postulated that serotonin receptors are present on these cells.

In light of data in the literature on close interaction between the number of psychotropic drugs and serotonin receptors [11] and the role of biogenic amines in the pathogenesis of mental diseases [3], the discovery of serotonin receptors of lymphocytes indicates a method of developing an extracerebral model for the intravital study of these receptors in man under normal and pathological conditions. Investigation of the serotonin receptors of lymphocytes is also interesting from the point of view of the study of mechanisms of humoral regulation of the immune response.

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