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Effects of an intense training on functional activity of 5-HT$_{1B}$ receptors in human peripheral blood lymphocytes

M. Chennaoui *, C. Drogou, D. Gomez-Merino, C.Y. Guezennec

Département de Physiologie Intégrée, IMASSA, BP 73, 91223 Brétigny-sur-Orge, France

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Abstract

Serotonin (5-HT) is a neurotransmitter and an immune modulator. At the periphery, the serotonergic system appears to possess a regulatory activity via 5-HT$_{1B}$ receptors. The present study investigated the effects of a 5-day military course following 3 weeks of combat training on the functional activity of 5-HT$_{1B/1D}$ receptors in peripheral blood lymphocytes of male soldiers. The results of [^{35}S]GTPγS binding assays showed that h5-HT$_{1B/1D}$ receptors were desensitized after the training program, although serum 5-HT was unchanged. These data suggest the existence of a control on T cells mediated through h5-HT$_{1B/1D}$ receptors leading cytokine production modulation after a physical training.

Keywords: 5-HT$_{1B}$ receptors; Human exercise training; 5-HT-moduline; T lymphocyte

Serotonin (5-hydroxytryptamine) acts roles as both a neurotransmitter and a neuromodulator with functions in brain and in immune peripheral tissues [22]. Moreover, there is now incontrovertible evidence that the nervous and immune systems interact bidirectionnally: the central nervous system (CNS) by mean of its neuropeptides, neurohormones and neurotransmitters interacts with the immune system which, in turn, feeds back both to the brain which then induces changes in behaviour and to the immune system as well [18]. 5-HT can affect immune functions and several findings suggest that modulation of the immune system by serotonin occurs at the lymphocytes level [2,3,15]. However, the precise mechanisms involved are not yet fully understood although 5-HT$_{1A}$, 5-HT$_2$ and 5-HT$_3$ receptors have been identified in immunocompetent cells. The implication of 5-HT$_{1B/1D}$ receptors in 5-HT regulated immune response has not been elucidated due to the lack of selective pharmacological tools. In a study of the role of 5-HT$_{1B}$ and 5-HT-moduline on the activity of immunocompetent cells, Grimaldi and Fillion [13] detected 5-HT$_{1B}$ receptors in rodent lymphocytes and splenocytes and in human lymphocytes (a human T lymphoblastoid cell line, CEM). Sibella-Arguelles [26] found that (i) 5-HT stimulates the proliferation of CEM cells: an effect which could be mimicked by two 5-HT$_{1B/1D}$ receptor agonists (L-694,247 and GR 46611) and (ii) this proliferation induced by 5-HT was subtotally inhibited by a selective competitive 5-HT$_{1B}$ antagonist (SB-224289). In a recent study in rats, we examined the effect of physical training on the sensitivity of brain 5-HT$_{1B}$ receptors. We found that 5-HT$_{1B}$ receptors were slightly desensitized in moderately trained animals but totally desensitized in intensely trained animals. In view of these observations, we investigated here the effect of intense military training on the activity of 5-HT$_{1B}$ receptors in peripheral blood lymphocytes.

A group of 20 male cadets (mean age: 21 ± 2 years) from the French Military Officer School took place in a 4-week military endurance training program. The subjects were trained and in good mental and physical condition. The mean of maximal oxygen uptake (VO_{2max}) was 54.3 ± 1.0 ml min^{-1} kg^{-1}. The study was approved by a French medical ethics committee (Faculty of Medicine, Paris V, France), and the participants had all given voluntary written consent.

The 3-week training and the 5-day combat course took place at the National Center for commando training at Mont-Louis in the Pyrenees mountains in June. The 3-week train-

* Corresponding author. Tel.: +33 1 69 23 77 50; fax: +33 1 69 23 70 02.
E-mail address: mchennaoui@imassa.fr (M. Chennaoui).

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ing included a sea phase (first week) followed by a mountain phase (altitude: 1600 m). During the training, subjects spent most of their time engaged in realistic combat training in rough terrain including heavy physical activities: swimming, walking and running, avoiding roads, lanes and trails while carrying back-packs of 11 ± 1.2 kg. Several parts of the training involved mountain climbing. For the entire training program the total uphill and downhill walking distance covered was 11.2 km.

The 5-day course took place from 6 a.m. on day 1 until 6 a.m. on day 6 and most of the physical activities were at night. Since the cadets slept outside at ambient temperatures during the 3 weeks of training their sleep was disturbed, and since most of the physical activities were at night during the 5-day course, subjects were also sleep deprived.

During the 5-day combat course subjects were estimated to have continuous physical exercise activities corresponding to an average of 35% of maximal oxygen uptake and a daily energy expenditure exceeding 5000 kcal.

Blood sampling were taken before the 3 weeks of the training program and at the end of the 5-days course. The first samples were taken in their Officer School between 7 a.m. and 8 a.m. The second sample, at the end of the course, was taken between 6 a.m. and 7 a.m. during medical and scientific investigations in a military barracks. Thirty millilitres of venous blood were sampled from an antecubital vein after 10-min rest supine. A part was centrifuged for collection of serum in which platelets were not part of the fraction, stored at −80 °C and used for 5-HT subsequent determinations in our laboratory.

Leucosep™ was purchased from Opopharma AG (Zurich, Switzerland). Ficoll-Paque was from Pharmacia (Uppsala, Sweden). [^{35}S]GTPγS (1250 Ci/mmol) was obtained from NEN Life Sciences (Boston, MA, USA). L-694,247 was from Tocris Cookson (Ballwin, MO, USA). L-694,247 was initially dissolved in dimethyl sulfoxide (DMSO) and stored at −20 °C by 10 μl aliquots (10^{-2} M). Membrane protein levels were estimated using the Bio-Rad kit (Hercules, CA, USA). Multiscreen filtration plate (FB glass fibre type) was from Millipore (Bedford, MA, USA). HEPES, GDP salt, ascorbic acid and phenantrolin were purchased from Sigma (St. Louis, MA, USA). All buffered solutions were prepared fresh daily. The liquid scintillation cocktail was from Amersham (Catalog no. NBCS. 104, Buckhinghamshire, UK).

Lymphocytes were isolated from whole blood sample anticoagulated with EDTA according to the method of Boyum [5]. Seven millilitres of fresh blood were added per Leucosep tube containing 3 ml of Ficoll-Paque. After centrifugation at 1100 × *g* for 10 min the plasma layer was removed and lymphocytes were harvested by pipette and transferred in a Falcon tube. Lymphocytes were washed twice in 6 ml of phosphated saline buffer by centrifugation at 500 × *g* for 15 min. The supernatant was discarded and the pellet was stored at −80 °C.

The frozen pellet was resuspended in 2 ml of 20 mM HEPES containing 10 mM EDTA (pH 7.4) and homogenized with an Ultra-Turrax apparatus. Five millilitres of 20 mM HEPES containing 10 mM EDTA were added and the sample was centrifuged for 12 min at 40,000 × *g* at 4 °C. The resulting pellet was resuspended in 20 mM HEPES containing 0.1 mM EDTA, homogenized and centrifuged as described above. The pellet was resuspended in 500 μl of 20 mM HEPES containing 0.1 mM EDTA. A Bradford protein assay was performed and 400–500 μg aliquots were then stored at −80 °C.

Lymphocytes membranes (15 μg of protein in a total volume of 225 μl per well) were incubated in a 20 mM HEPES solution, pH 7.4, containing 100 mM NaCl, 3 m $MgCl_2$, 0.2 mM ascorbic acid, 30 μM GDP and 100 μM phenantrolin for 30 min at 25 °C in MultiScreen filtration plate with increasing concentrations (10^{-10} to 10^{-4} M) of L-694,247. [^{35}S]GTPγS (25 μl, 5 nM) were added for an additional incubation of 30 min. The 96-well filtration plate was rapidly filtered and washed twice with 250 μl of cold 20 mM HEPES containing 3 mM $MgCl_2$. The 96 filtrates were recovered and counted by liquid scintillation.

Serum serotonin concentration was assayed in duplicate by radioimmunoassay using a commercial kit (Labor Diagnostika Nord GmbH & Co. KG, Germany). The adult normal ranges were 40–400 ng/ml. The limit of sensitivity was 10 ng/ml.

Binding experiments were analysed under Prism 3.0 (Graphpad software, San Diego, CA). Binding of [^{35}S]GTPγS measured in lymphocytes membranes of cadets before the training program was stimulated by a specific agonist (L-694,247) in a dose-dependant manner (IC_{50} = 78 ± 16 nM; n = 20). The corresponding binding curve was shifted significantly to the right after the training (IC_{50} = 451 ± 114 nM; n = 20; $p < 0.01$) (Fig. 1).

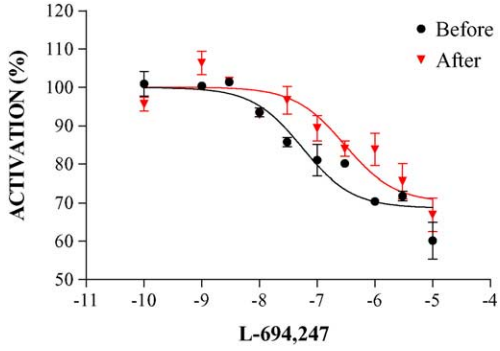

Fig. 1. Effect of military training on 5-HT$_{1B/1D}$ related [^{35}S]GTPγS binding in the peripheral blood lymphocytes of male soldiers population. Homogenates lymphocytes before (●) and after (▼) training program. Lymphocytes membranes (15 μg of protein in a total volume of 225 μl per well) were incubated in a 20 mM HEPES solution, pH 7.4, containing 100 mM NaCl, 3 m $MgCl_2$, 0.2 mM ascorbic acid, 30 μM GDP and 100 μM phenantrolin for 30 min at 25 °C in MultiScreen filtration plate with increasing concentrations (10^{-10} to 10^{-4} M) of L-694,247. [^{35}S]GTPγS (25 μl, 5 nM) were added for an additional incubation of 30 min. The 96-well filtration plate was rapidly filtered and washed twice with 250 μl of cold 20 mM HEPES containing 3 mM $MgCl_2$. The 96 filtrates were recovered and counted by liquid scintillation. Mean ± S.E.M. of triplicate determinations (n = 20 independent experiments).