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ORIGINAL ARTICLE

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Effects of physical training on functional activity of 5-HT_{1B} receptors in rat central nervous system: role of 5-HT-moduline

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Abstract The effect of physical exercise was examined on the sensitivity of 5-HT_{1B} receptors and on 5-HT-moduline tissue concentration in the central nervous system of rats.

Rats were trained for 7 consecutive weeks to run on a treadmill. Three groups of animals were selected: group 1, sedentary rats (controls); group 2, animals running for 1 h at 18 m/min for 5 days per week (moderate training) and group 3, animals running for 2 h, at 30 m/min on a 7% grade for 5 days per week (intensive training). The animals were sacrificed 24 h after the last running. Rat brains were dissected out to obtain hippocampus and substantia nigra and kept at –80 °C until use. 5-HT_{1B} receptor activity was determined by studying [^{35}S]GTPγS binding in a substantia nigra membrane preparation from individual animals, after stimulation by a selective 5-HT_{1B} receptor agonist (CP 93,129). 5-HT-moduline tissue content in hippocampus from individual animals was determined by ELISA using a polyclonal anti-5-HT-moduline antibody.

In moderately trained animals (n=5), the CP 93,129-stimulated [^{35}S]GTPγS binding curve was shifted to the right compared with controls (n=6), whereas the binding was totally suppressed in intensely trained animals (n=5). In parallel, 5-HT-moduline tissue concentration in the hippocampus was slightly increased in moderately trained animals (117.3±8.9%) (n=5), whereas it was significantly increased in intensely trained animals (182.6±29.5%) (n=5) compared to controls (100±6.11%) (n=6). These results show that 5-HT_{1B} receptors are slightly desensitized in moderately trained animals and totally desensitized in intensely trained animals; moreover, they suggest that the observed desensitization is related to an increase of 5-HT-moduline tissue content; this mechanism may play a role in various pathophysiological conditions.

Key words Physical exercise · 5-HT_{1B} receptors · 5-HT-moduline · Rat brain · CP 93,129

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Introduction

The serotonin (5-hydroxytryptamine, 5-HT) system plays an important role in the adapted functional response of the brain to environmental stress. Serotonin is known to be involved in a number of human and animal behaviours (Zifa and Fillion 1992). The regulation of the serotonergic transmission involves 5-HT_{1B} autoreceptors located in serotonergic terminals and controlling the release of serotonin via a negative feed-back mechanism (Engel et al. 1986; Hjorth and Tao 1991). Several studies have shown that acute running, prolonged exercise and training enhance the synthesis of 5-HT in brain (Chaouloff et al. 1985, 1986b, 1987). Hypotheses were proposed to explain the effects of physical exercise on 5-HT metabolism such as enhanced tryptophan (TRP) availability (Chaouloff et al. 1986a), and/or a decrease in 5-HT_{1B} receptor sensitivity (Seguin et al. 1998). The sensitivity of 5-HT_{1B} receptors is known to be modulated by a tetrapeptide called 5-HT-moduline; this peptide specifically interacts with 5-HT_{1B} receptors via an allosteric mechanism corresponding to a non-competitive high apparent affinity (EC_{50} 10^{-9} M) (Massot et al. 1996). The interaction was observed in rat and guinea-pig brain tissues as well as in cells transfected with the 5-HT_{1B} receptor gene (Rousselle et al. 1998). The interaction of 5-HT-moduline with 5-HT_{1B} receptors corresponds to a decrease of their functional activity. Indeed, it was previously described that 5-HT-moduline decreases the effect of a 5-HT_{1B} agonist to inhibit the K^+ evoked-release of [^{3}H]-5-HT from rat hippocampal synaptosomes and significantly reduces the inhibitory effect of a 5-HT_{1B} agonist on K^+-induced overflow of [^{3}H] dopamine from rat striatal synaptosomes (Massot et al. 1996; Sarhan

et al. 1999). It was also demonstrated that 5-HT-moduline shows an antagonistic effect on the [^{35}S]GTPγS binding response induced by 5-HT (Rousselle et al. 1998). Moreover, 5-HT-moduline was described as a novel neuropeptide, heterogeneously distributed in mammalian brain and located in neuronal cell profiles (Grimaldi et al. 1997). It was also observed that an acute stress induces a rapid and significant increase in the amount of 5-HT-moduline contained in various brain areas; this increase in the peptide tissue content may be related to the stress-induced 5-HT$_{1B}$ receptor desensitization previously described (Bonnin et al. 1999; Seguin et al. 1997; Bolanos-Jimenez et al. 1995), since the endogenous peptide appears as a regulator of 5-HT$_{1B}$ receptor activity. The present study examined the influence of physical exercise in rat on the sensitivity of 5-HT$_{1B}$ receptors and on brain tissue content in 5-HT-moduline.

Methods

Materials. Synthetic peptides (LSAL) were purchased from BACHEM (Voisins-le-Bretonneux, France). [^{35}S]GTPγS was obtained from NEN Life Science Products and GTPγS from Sigma (St Louis, Mo., USA). 1,4-Dihydro-3-(1,2,3,6-tetrahydro)-4-pyridinyl-5H-pyrrolo [3,2-b]pyridin-5-one (CP 93,129) was kindly provided from Pfizer laboratory, Orsay, France.

Animals and tissue collections. The experiments were performed using male Wistar rats (Centre d'élevage R. Janvier, Le Genest-Saint-Isle, France) weighing 125–130 g at the beginning of the experiments. The animals were housed five per cage under controlled conditions of temperature (20–23 °C), humidity (40%) and light/dark cycle (12 h/12 h, lights on at 7 a.m.) with ad libitum access to food and water. The care and treatment of animals were supervised by the veterinary surgeons of the Institut de Médecine Aérospatiale du Service de Santé des Armées. The animals arrived in the laboratory at least 1 week before the experiments. After the experimental training, rats were sacrificed by decapitation and the brain regions were dissected on ice to obtain the substantia nigra and hippocampus. Brain tissues of each animal were immediately frozen in liquid nitrogen and stored at –80 °C.

Experimental training protocol. The animals were divided randomly into three groups: one sedentary and two exercise training groups (moderate and intensive) (Fig. 1). The experimental training protocols were designed to increase mitochondrial oxidation in skeletal muscle by the 4th week of the training program (Baldwin et al. 1977). The training groups were exercised by running on a motorized treadmill over 7 weeks. Acclimation to running occurred over 2 weeks during which the treadmill speed and running duration were progressively increased. These first 2 weeks of training were considered as part of the training program. At the end of this period, rats were able to run 1 h/day at 18 m/min. After the first 2 weeks, the training groups were subdivided. The moderate training group continued with the same workload of running at 18 m/min on a 0% grade for 60 min per day, 5 days per week for a period of 5 weeks. In the intensive training group, the workload was increased progressively over the following 3 weeks up to 30 m/min on a 7% grade, 120 min per day, 5 days per week. This level of exercise was then maiontained for the remaining 2 weeks of training. Rats were weighed 3 times a week during the experimental training period.

[^{35}S]GTPγS binding studies. Tissues from individual animals (tissues were not pooled) were washed in phosphate-buffered saline (PBS) containing 0.1 mM EDTA (pH 7.4) and centrifuged for 20 min at 48,000 *g*. The pellet was homogenized with a Polytron in 20

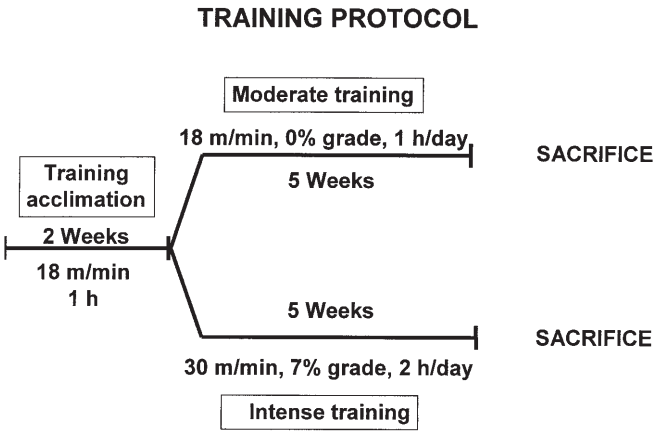

Fig. 1 Training protocol of moderately and intensely trained rats over 7 weeks. The training groups were exercised by running on a motorized treadmill, 5 days/week for 7 weeks. Adaptation to running occurred over 2 weeks. At the end of this period, rats were able to run 1 h/day at 18 m/min. After the first 2 weeks, the two training groups were subdivided. The moderate training group continued at the same workload (18 m/min, 0% grade, 1 h/day, 5 days/week) for 5 weeks. In the intense training group the workload was increased progressively during 5 weeks up to 30 m/min on a 7% grade

mM HEPES containing 10 mM EDTA (pH 7.4). The homogenate was centrifuged at 48,000 *g* at 4 °C for 10 min. The resulting pellet was washed twice in 20 mM HEPES containing 0.1 mM EDTA (pH 7.4) homogenized and centrifuged. The pellet was stored at –80 °C in fractions of 0.8–1.0 mg protein/ml. [^{35}S]GTPγS binding responses were measured essentially as described by Pauwels et al. (1997). Membranes (30–50 µg) were incubated for 30 min at 25 °C with a 5-HT$_{1B}$ receptor agonist (CP 93,129; 10^{-10}–10^{-5} M) in 20 mM HEPES containing 100 mM NaCl, 3 mM $MgCl_2$, 0.2 mM ascorbic acid, 30 µM GDP and 0.1 mM phenanthroline. Then 0.05 nM [^{35}S] GTPγS was added for 30 min at 25 °C. After incubation, a rapid filtration over Whatmann GF/B glass fibre filters using a Brandel harvester was carried out. The filters were rinsed 3 times with 3 ml HEPES buffer, placed in scintillation vials for counting. Basal binding of [^{35}S]GTPγS was measured in the presence of 30 µM GTP. Non-specific binding was measured in the presence of 10 µM unlabelled GTPγS. Maximal stimulation of [^{35}S] GTPγS binding was that observed in the presence of 1 µM CP 93,129. E_{max} values were expressed as a percentage of the maximal response obtained in the presence of 10 µM CP 93,129. EC_{50} values were defined as the concentration of compound inducing 50% of their own maximal stimulatory effect.

Tissue homogenization. Hippocampi from individual animals (tissues were not pooled) were homogenized using a Potter apparatus and gently forced through decreasing diameter (0.45–0.12 mm) needles in 1–2 ml ice-cold extraction buffer [phenylmethylsulphonyl fluoride (PMSF) 2 mM, EDTA 2 mM, (octylphenoxy)polyethoxyethanol (IGEPAL CA-630), 0.5% in PBS]. The resulting homogenate was incubated for 20 min at 4 °C and centrifuged (12,000 *g*) at 4 °C for 15 min. Aliquots of the supernatants were used to determine 5-HT-moduline levels; they were transferred to separate Eppendorf tubes and stored at –20 °C until the assay. Total protein content for each extract was determined according to the method of Lowry et al. (1951).

Measurement of 5-HT-moduline in hippocampus. 5-HT-moduline tissue content was determined by a non-competitive dot-ELISA method as previously described by Bonnin et al. (1999). Briefly, samples were coated onto Ultrabind-US450 membrane disks (Gelman, Ann Arbor, Mich., USA) lying in the wells of PVC-ELISA plates (CML, Nemours, France) in a final volume of 6 µl. On the

same ELISA plate, a set of membrane disks was coated with 6 µl standard solution (synthetic 5-HT-moduline dissolved in PBS) concentration 0–1 µg. Membranes were allowed to dry for 1 h at room temperature and then incubated further for 1 h in buffered medium (150 µl/well; 0.5% ovalbumin dissolved in PBS; Tween 0.2%). Aliquots (100 µl) of the antiserum diluted (1:2,000) in the latter buffer were added to each well; plates were further incubated at room temperature for 1 h. After three 5 min washes in the same buffer (150 µl/well), 100 µl of diluted (1:2,000) peroxidase-coupled goat anti-rabbit IgG (BIOSSYS, Compiegne, France) were added and incubated for 1 h. Plates were washed (3×5 min in the buffer) and reacted with orthophenylenediamine (OPD, 0.4 mg/ml). The enzymatic reaction was stopped after 10 min by the addition of 1 M sulphuric acid (50 µl) and the optical density in each well was measured by an ELISA reader (Dynatech MRX, Chantilly Va., USA) at 492 nm. Each point of individual dot-ELISA assay was performed in quadruplicate and each independent experiment was conducted 3 times.

Mathematical analysis. Binding curves were analysed using Prism 2.0 (GraphPad Software, San Diego, Calif., USA). The other results were analysed by ANOVA using Statistica. The significance of differences observed in the measurement of 5-HT-moduline tissue content was assessed by a two-tailed Student's *t*-test.

Results

Effects of training on sensitivity of 5-HT_{1B} receptors in rat substantia nigra

Binding of [^{35}S]GTPγS measured in sedentary rats was stimulated by a 5-HT_{1B} specific agonist (CP 93,129) in a dose-dependant manner (EC_{50}=29.7±10.6 nM; *n*=6). The corresponding binding curve was shifted to the right in moderately trained rats (EC_{50}=56.8±8.7 nM; *n*=5); the difference between sedentary and moderately trained rats was not significant. The binding of [^{35}S]GTPγS was totally suppressed in intensely trained rats (*n*=5); the difference with the sedentary rats was significant (Fig. 2).

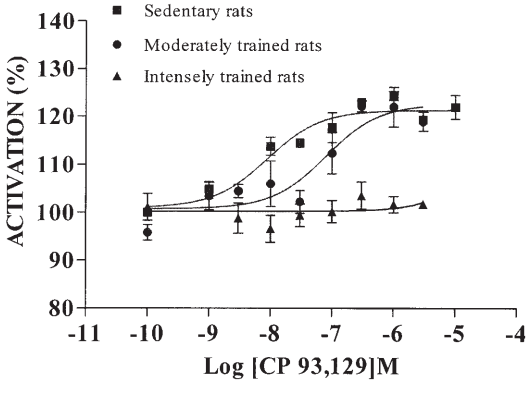

Fig. 2 Effect of training on 5-HT_{1B} related [^{35}S]GTPγS binding in rat brain substantia nigra. Homogenates of substantia nigra were prepared from sedentary (■), moderately trained (●) and intensely trained (▲) rats. Membrane preparations of substantia nigra were obtained as described in Methods. Membranes were preincubated in the presence of CP 93129 (10^{-10}–10^{-4} M) for 30 min. at 25 °C in HEPES buffer containing NaCl (100 mM), $MgCl_2$ (3 mM), ascorbic acid (0.2 mM), GDP (30 µM) and phenanthroline (0.1 mM) and then incubated in the presence of [^{35}S]GTPγS (0.05 nM) for 30 min at 25 °C. Specific [^{35}S]GTPγS binding was determined after filtration. Means±SEM of triplicate determinations; *n*=5 independent experiments

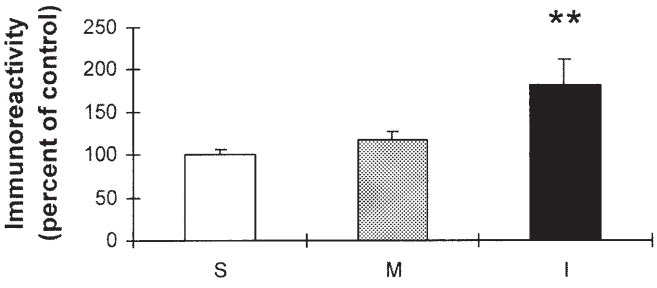

Fig. 3 Effect of training on 5-HT-moduline concentration in rat hippocampus. Quantification of 5-HT-moduline level in hippocampus of sedentary (*S*), moderately trained (*M*) and intensely trained (*I*) rats was carried out using ELISA blots as described in Methods. Mean±SEM, *n*=5–6. ***P*<0.01 vs. sedentary rats

Effect of training on 5-HT-moduline concentration in rat hippocampus

5-HT-moduline tissue concentration measured in hippocampus corresponded to 2.06±0.13 µg/mg protein (100±6.1%; *n*=6) in sedentary animals. 5-HT-moduline tissue concentration slightly increased in moderately trained rats (117.3±8.9%; *n*=5); the difference between sedentary and moderately trained rats was not significant, whereas the increase in 5-HT-moduline tissue content in intensely trained rats (182.6±29.5% of sedentary animals; *n*=5) was significant (*P*<0.05) (Fig. 3).

Discussion

The present results show that physical training decreases the sensitivity of 5-HT_{1B} receptors as measured by [^{35}S]GTPγS binding. These results are in agreement with those previously shown using measurement of adenylate cyclase activity to determine 5-HT_{1B} receptor activity (Seguin et al. 1998). Furthermore, it was shown that moderate and intense physical exercises have different effects on 5-HT_{1B} receptor sensitivity. Moderate exercise decreases the sensitivity of 5-HT_{1B} receptors as evidenced by the shift to the right of the concentration/effect curve of a specific 5-HT_{1B} agonist (CP 93,129) on [^{35}S]GTPγS binding as compared to that observed in sedentary rats used as controls. It should be noted that the observed desensitization is not significant. Interestingly, in moderately trained rats, the maximal effect of the agonist to stimulate 5-HT_{1B} receptors is the same as in sedentary rats although it is observed at slightly higher concentrations of the agonist than in the latter animals. In intensely trained animals, the desensitization of the 5-HT_{1B} receptors is total or quasi total as no significant stimulating effect of the agonist is observed at concentrations which induced a maximal response in sedentary animals. 5-HT_{1B} receptors have been described as autoreceptors located on serotonergic terminals where

they regulate the release of 5-HT itself (Engel et al. 1986; Hjorth and Tao 1991) and as heteroreceptors located on non-serotonergic terminals where they control the release of other neurotransmitters i.e.: noradrenaline (Göthert et al. 1986; Hoyer et al. 1994) glutamate (Li and Bayliss 1998; Boeijinga and Boddeke 1996), γ-aminobutyric acid (GABA) (Stanford et al. 1994), acetylcholine (Ach) (Raiteri et al. 1986; Bolanos-Jimenez et al. 1994), dopamine (Guan and McBride 1989; Sarhan et al. 1999), prolactin (Van de Kar et al. 1994). The desensitization of 5-HT_{1B} receptors is expected to increase the release of 5-HT as these receptors inhibit the release of the amine from the neuron terminals. The hypothesis that 5-HT_{1B} autoreceptors are desensitized under physical exercise is supported by previous reports indicating that physical exercise increases 5-HT-tissue content (Blomstrand et al. 1989; Bailey et al. 1993) and enhances the release of 5-HT in microdialysis studies in various brain areas (Marsden et al. 1979; Kawahara et al. 1993; Meeusen et al. 1996; Kirby et al. 1997; D. Gomez-Mérino, F. Béquet, M. Berthelot, M. Chennaoui, C. Guezennec, unpublished results). It is also supported by the presented observations that the 5-HT_{1B} receptors are desensitized in substantia nigra after physical exercise. It is not known whether or not 5-HT_{1B} heteroreceptors also are desensitized by physical exercise as no measurements of the release of other neurotransmitters were carried out. However, since the [^{35}S]GTPγS binding related to the activity of 5-HT_{1B} receptors is totally inhibited after intense physical exercise, it is suggested that, in the examined brain area, 5-HT_{1B} heteroreceptors as well as autoreceptors were desensitized under these experimental conditions. The mechanism responsible for the desensitization of the receptors is not completely clarified and may originate from various events; however, a plausible hypothesis may be presented corresponding to the existence of a recently characterized endogenous allosteric modulator specifically acting at 5-HT_{1B} receptors and named 5-HT-moduline (Rousselle et al. 1996; Massot et al. 1996). This peptide appears to fulfil many criteria for being a novel neurotransmitter: it has a specific protein target, it is released in a Ca^{2+}- and K^{+}-dependent manner from synaptosomal preparations, it is located in neuronal profiles in the brain (Grimaldi et al. 1997); it is heterogeneously distributed and rapidly degraded by specific peptidases after being released (Plantefol et al. 1999), ultimately, it has physiological activity at molecular and cellular levels and in vivo on animal behaviour (Grimaldi et al. 1999). It was shown that 5-HT-moduline tissue content was affected by acute stress in rat brain (Bonnin et al. 1999). The fact that 5-HT-moduline was clearly demonstrated to desensitize 5-HT_{1B} receptors in vitro as well as in vivo after intracerebroventricular administration (Seguin et al. 1997) favours the hypothesis that it is involved in the increase of 5-HT release observed in acute stress models (Kawahara et al. 1993; Meeusen et al. 1996; Kirby et al. 1997; Vahabzadeh and Fillenz 1995). The increase of 5-HT activity observed after physical exercise may also be related to a similar mechanism involving 5-HT-moduline. The total desensitization of 5-HT_{1B} receptor activity reported in the present experiments after intense physical exercise together with the observed increase in 5-HT-moduline tissue content favours that hypothesis. Moreover, since it was shown that 5-HT_{1B} receptors also exist as heteroreceptors i.e. those located on dopaminergic terminals (Sarhan and Fillion 1999), physical exercise appears to affect the serotonergic activity directly via the 5-HT_{1B} autoreceptors regulating the release of 5-HT and 5-HT_{1B} heteroreceptors regulating other neurotransmitters.

The physiological consequences of the desensitization of 5-HT_{1B} receptors induced by physical exercise are not yet known. However, it may be suggested that this mechanism is related to adaptive responses of the animal to physical exercise. The slight desensitization of 5-HT_{1B} receptors induced by moderate training may increase the release of 5-HT and other neurotransmitters in various cerebral areas to maintain the homeostasis of the brain. It should be noted that under moderate physical exercise, the slight right-shift of the response-curve to 5-HT_{1B} receptor agonist indicates that 5-HT_{1B} receptors still possess the capacity to inhibit the release of the neurotransmitters, although operating at slightly higher concentrations of 5-HT. On the contrary, intense physical exercise induces a total desensitization of 5-HT_{1B} receptors which do not allow the control of the release of 5-HT or that of other neurotransmitters. Under these extreme conditions, adaptive responses are not met and physiological disorders may be expected. Preliminary observations are in agreement with the latter hypothesis since intensely trained animals show deleterious behavioural changes (P. Liscia, C. Guezennec, unpublished results).

In conclusion, the present experimental data indicate that physical exercise alters the sensitivity of 5-HT_{1B} receptors possibly through the interaction of a recently characterized endogenous allosteric modulator specific of these receptors. The induced desensitization of 5-HT_{1B} receptors may participate to adaptive changes to physical exercise.

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