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H.K. Strüder · W. Hollmann · P. Platen · R. Wöstmann
H. Weicker · G.J. Molderings

Effect of acute and chronic exercise on plasma amino acids and prolactin concentrations and on [^{3}H]ketanserin binding to serotonin$_{2A}$ receptors on human platelets

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Abstract The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been shown to modulate various physiological and psychological functions such as fatigue. Altered regulation of the serotonergic system has been suggested to play a role in response to exercise stress. In the present study, the influence was investigated of acute endurance exercise and short-term increase in the amount of training on the concentrations of the 5-HT precursor tryptophan (TRP), of prolactin (PRL) and of branched-chain amino acids (BCAA) in the blood, as well as on the binding of [^{3}H]ketanserin to the serotonin-2A (5-HT$_{2A}$) receptors on platelets. Nine healthy endurance-trained men were tested the day before (I) and after (II) a 9-day training programme. Samples of venous blood were drawn after an overnight fast and following 5 h of cycling. Fasted and post-exercise plasma concentrations of free TRP, BCAA and free TRP:BCAA ratio did not differ between I and II. A significant decrease of plasma BCAA ($P < 0.01$) and significant augmentations of plasma free TRP, free TRP:BCAA ratio and PRL ($P < 0.01$) were found post-exercise. The increase in plasma PRL was smaller in II compared with I. Acute endurance exercise reduced the density of platelet 5-HT$_{2A}$ receptor [^{3}H]ketanserin binding sites at I and II ($P < 0.05$). The basal density of the binding sites and the affinity of [^{3}H]ketanserin for these binding sites were unaffected by an increase in the amount of training. The present results support the hypothesis that acute endurance exercise may increase 5-HT availability. This was reflected in the periphery by increased concentration of the 5-HT precursor free TRP, by increased plasma PRL concentration, and by a reduction of 5-HT$_{2A}$ receptors on platelets. It remains to be resolved whether these alterations in the periphery occur in parallel with an increase in the availability of 5-HT in the brain.

Key words Endurance exercise · Amino acids
Serotonin · Central fatigue · Overtraining

H.K. Strüder (✉)
Institute of Sports Games, German Sport University,
Carl-Diem-Weg 6, D-50933 Cologne, Germany

W. Hollmann · P. Platen · R. Wöstmann
Institute of Cardiology and Sports Medicine,
German Sport University,
Carl-Diem-Weg 6, D-50933 Cologne, Germany

H. Weicker
Department of Pathophysiology and Sports Medicine,
Ruprecht-Karls-University,
D-69117 Heidelberg, Germany

G.J. Molderings
Institute of Pharmacology and Toxicology,
University of Bonn, Reuterstrasse 2b, D-53111 Bonn, Germany

Introduction

Excessive endurance training has been related to symptoms such as reduced performance capacity, prolonged fatigue, altered mood states, sleep disturbance, loss of appetite and increased anxiety as well as changes in neuroendocrine function (Kuipers and Keizer 1988; Lehmann et al. 1993). Since some of these symptoms have also been found when the brain serotonin (5-hydroxytryptamine, 5-HT) concentration is altered (Young 1986), it has been speculated that exercise-induced changes of the activity of the serotonergic system in the brain could be involved in the development of overtraining syndrome (Newsholme et al. 1991). Brain 5-HT concentrations have been shown to depend largely on free tryptophan (TRP) availability in the brain, since enzymes involved in 5-HT synthesis are not saturated with substrate (Fernstrom and Wurtman 1971). Plasma free fatty acids (FFA) have been shown to displace TRP from binding to serum albumin, leading to an increase in free concentration of TRP which competes with branched-chain amino acids (BCAA) for transport through the blood-brain barrier (Curzon et al. 1973). It has been shown in animal studies that the increase of plasma FFA during acute endurance exercise induces an increase of plasma free TRP and, thereby, increases brain 5-HT synthesis (Blomstrand et al. 1989; Chaouloff 1989, 1997). In humans, it has been demon-

strated that an increase in the amount of training increased plasma FFA concentration (Costill et al. 1971). Hence, it has been suggested that an elevated basal plasma free TRP:BCAA ratio, reflecting the possibility of an enhanced brain 5-HT synthesis, might be a consequence of acute and chronic endurance exercise (Tanaka et al. 1997).

Since the synthesis of 5-HT and the activity of the serotonergic system in the brain cannot be easily measured in living subjects, surrogate parameters have to be determined which reflect the state of central serotonergic activity. Prolactin (PRL) release from the pituitary is, among others processes, regulated by the activity of the 5-HT system. Therefore, a change in the plasma concentration of PRL has been used as a peripheral marker of the activity of the 5-HT system in the brain (Yatham and Steiner 1993). It has been shown that PRL secretion in response to a 5-HT agonist was lower in endurance trained subjects, suggesting that a downregulation of the central 5-HT receptor system might have occurred as an adaptation to training (Jakeman et al. 1994). Moreover, in a study by Kaufman et al. (1985) it has been found that training reduced the plasma PRL response to endurance exercise. Further peripheral biochemical indices of the serotonergic system are the densities of the 5-HT transporters and the serotonin-2A (5-HT_{2A}) receptors on platelets. In accordance with the view that exercise leads to an increase in 5-HT availability, it has recently been found that the platelet 5-HT transporter density was increased in endurance-trained men (Strachan and Maughan 1998). Platelet 5-HT_{2A} receptors may also provide a suitable peripheral model for evaluation of the regulation of central 5-HT_{2A} receptors and the serotonergic system influencing these receptors in the brains of living human subjects, because the pharmacological properties have been shown to be similar to those of brain 5-HT_{2A} receptors (McBride et al. 1983; Geaney et al. 1984; Da Prada et al. 1988; Elliott and Kent 1989). Both platelet and frontal cortex 5-HT_{2A} receptors have been shown to be related to the phosphoinositide second-messenger system, and to have identical nucleotide sequences (De Courcelles et al. 1985; Conn and Sanders-Bush 1986; Cook et al. 1994). Moreover, a positive correlation between interindividual 5-HT_{2A} receptor binding characteristics in brain cortex and platelets has been observed in mood disorders in humans (for review, see Spigset and Mjörndal 1997).

Thus, the aim of the present study was to determine whether acute endurance exercise and a short-term increase in the amount of training would affect the plasma free TRP:BCAA ratio, plasma PRL concentration and radioligand binding to platelet 5-HT_{2A} receptors, as three different parameters of 5-HT availability.

Methods

Subjects

Nine healthy men [mean age: 25.7 (SD 2.9) years, body mass 76.6 (SD 6.7) kg, height 182.7 (SD 4.8) cm] were recruited for the investigation. The criterion for selection was a similarity in training habits prior to the study. The average duration of normal training for all the subjects was 100 min, three times a week, at an intensity which corresponded to 1–2 $\text{mmol} \cdot \text{l}^{-1}$ blood lactate as measured during incremental cycle ergometry. All the athletes were well trained [mean maximal oxygen uptake ($\dot{V}O_{2max}$) 62.3 (SD 5.6) $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$] and had been actively competing in cycle races. They were free of drugs and did not show clinical signs of overtraining (Kuipers and Keizer 1988). The subjects were informed about the risks and stresses of the investigation. The study was approved by the Ethics Committee of the German Sport University.

Protocols

During the week prior to the training programme and on the last day of the training programme the subjects exercised in an incrementally graded cycle ergometer test until exhaustion. The initial exercise intensity on the cycle ergometer was 100 W and this was increased by 40 W every 5 min. The concentration of lactate was determined in capillary blood from the hyperaemized earlobe at each level of intensity. Heart rate was recorded continuously using a Vantage NV watch (Polar, Büttelborn, Germany). Oxygen uptake was measured from respiratory gas exchange using an Oxycon Alpha System (Jaeger, Würzburg, Germany). The mean work load at the 4 $\text{mmol} \cdot \text{l}^{-1}$ lactate threshold [286.3 (SD 37.9) W vs 297.4 (SD 36.7) W] and the corresponding mean heart rate [179.0 (SD 11.2) $\text{beats} \cdot \text{min}^{-1}$ vs 179.4 (SD 3.9) $\text{beats} \cdot \text{min}^{-1}$] did not differ significantly between tests before and after the training programme.

Main test procedure

The subjects were tested the day immediately before (I) and after (II) the training programme. Participants reported to the laboratory at 7.30 a.m. after an overnight fast. Samples of venous blood were taken from an antecubital vein with the subjects lying in a supine position. The subjects then received a standard breakfast (two roles with cheese, water ad libitum). At 9 a.m. the participants cycled for 5 h on a cycle racing track. Each subject was advised to maintain his heart rate at a level corresponding to 2 $\text{mmol} \cdot \text{l}^{-1}$ lactate concentration as measured during incremental cycle ergometry. This type of exercise was chosen because earlier findings have shown that this intensity and duration present a stimulus for PRL secretion (Strüder et al. 1997). Heart rate was monitored every 15 s during exercise. In I the mean heart rate was 152 (SD 5.0) $\text{beats} \cdot \text{min}^{-1}$. In II mean heart rate was lower [144.6 (SD 6.4) $\text{beats} \cdot \text{min}^{-1}$] due to the subjects' inability to maintain the advised intensity level. Average food intake (400 g carbohydrates, 14 g protein and 17.5 g fat) during exercise did not significantly differ between I and II. Water was allowed ad libitum. Immediately after the cessation of exercise, samples of venous blood were drawn with the subjects in a supine position. Plasma concentrations of PRL, free TRP, total TRP, BCAA and total protein (P), as well as binding of [^{3}H]ketanserin to 5-HT_{2A} receptors on platelets, were determined from all venous blood samples.

Blood analysis

The samples of venous blood were drawn in prechilled EDTA-containing vacutainers, cooled for 10 min in ice water and then centrifuged at 3000 rpm for 10 min at 4°C. Plasma was divided into fractions. Separation of free TRP from albumin-bound TRP was carried out immediately by an ultrafiltration method according to Bloxam et al. (1977). The ultrafiltrate and fractions of the original plasma were stored at −80°C until analysis. Prior to analysis for free amino acid concentrations an internal standard (Norvalin) was added. The plasma was deproteinized by acetonitril and centrifuged (10 min, 2000 rpm). The liquid phase was transferred to an autosampler vial. A precolumn derivation was performed automatically by an high performance liquid chromatography (HPLC) autoin-

jector (HPLC HP1090, Hewlett Packard, Waldbronn, Germany) with *o*-phthalaldehyde. The fluorescence detector was used at excitation and emisson wavelengths of 230 and 455 nm, respectively, for detection of primary amino acids. Free TRP was directly analysed from the ultrafiltrate by precolumn derivation and HPLC fluorescence detection. Total protein was analysed by a colorimetric method (Unimate 7 TP, Hoffmann La Roche, Basel, Switzerland; biuret method) using a Cobas-Bio centrifuge analyser (Hoffmann La Roche, Basel, Switzerland). The PRL was determined using the Enzyme-Imunoassay-Automate ES 300 and corresponding kits from Boehringer (Mannheim, Germany). The blood lactate concentration was determined enzymatically using a Lactate-Analyser 5060 (Eppendorf, Hamburg, Germany).

Isolation of platelets was carried out as described by Mellerup and Langer (1990). Samples of 20 ml blood were collected in EDTA-containing vacutainers. After gently mixing, they were centrifuged at 200 *g* for 20 min at room temperature. Six millilitres of platelet-rich plasma were collected and centrifuged at 10 000 *g* for 10 min at 4°C. The supernatant was discarded. Platelet-rich plasma preparations were then mixed with half their original volume of a hypotonic medium (5 mM Tris-HCl, pH 7.5) and homogenized for 15 s with a Teflon-glass homogenizer at its highest speed. The lysed preparation was centrifuged at 45 000 *g* for 20 min at 4°C, the supernatant decanted and the membrane pellet washed twice in 50 mM Tris-HCl (pH 7.5), then resuspended in 50 mM Tris-HCl (pH 7.5; incubation buffer), homogenized, diluted to give a protein concentration of about 1 mg · ml^{-1} and stored at −80°C until used.

Binding assay

A 150 µl aliquot of the membrane suspension was incubated for 60 min with 25 µl [^{3}H]ketanserin (0.1–46 nM; specific activity 66.4 Ci · mmol^{-1}) at ambient temperature in a final volume of 250 µl. Non-specific binding was defined as [^{3}H]ketanserin binding in the presence of 10 nM mianserin. The reaction was stopped by rapid vacuum filtration with a Brandel cell harvester through GF/B filter strips (presoaked in 1% bovine albumin and dried) followed by rapid washing of the incubation tubes and filters with 10 ml ice-cold incubation buffer. The filters were placed in 6 ml scintillation fluid, shaken overnight and the radioactivity determined by liquid scintillation counting at 44% efficacy. The mean value for specific binding was 24.1 (SEM 3.6) % at a [^{3}H]ketanserin concentration of 4.6 nM. Data from the saturation experiments were analysed by the least-squares fitting programme GraphPadInPlot (GraphPad Software Inc.). All the experiments were carried out in triplicate. Final protein concentration was measured by the method of Bradford (1976) with bovine serum albumin as standard.

Training

The 9-day training programme was identical for all the subjects and carried out under supervision. During this period, the subjects stayed together in a hotel to ensure identical diets and life-styles.

Table 1 Exercise intensity during the 9-day training programme expressed as a percentage of exercise duration ($t_{\%}$) at different levels of intensity. Values were received by relating the heart rate – which was assessed continuously during exercise in the training programme – to the respective blood lactate (*La*) concentration which had been obtained during incremental cycle ergometry to exhaustion

La	[mmol · l^{-1}]	>4	3–4	2.5–3	2–2.5	1.5–2	1–1.5	<1
$t_{\%}$	Mean	0.5	1	1.7	4.7	13.4	62.5	16.2
	SD	0.5	0.8	1.1	2.4	5.7	6.4	12.4

The duration of training was reduced during the first (154 min) and last day (144 min). The mean amount of training during the other days was 395 (SD 76) min. The exercise intensity was monitored during the whole training programme from continuous measurement of heart rate, and related to the blood lactate concentration measured during incremental cycle ergometry to exhaustion prior to the training camp (Table 1).

Data analysis

The BCAA were calculated by summing the concentrations of leucine, isoleucine and valine. With the exception of the binding data, multiple-factorial analysis of variance with repeated measurement and Newman-Keuls post hoc tests were applied. To investigate whether the [^{3}H]ketanserin binding parameters were changed by acute endurance exercise or an increase in amount of training, respectively, values were compared by using the Student's *t*-test for paired data. All groups of data were normally distributed, so Students test was suitable for analysis of the data. The level of significance for all analyses was $P < 0.05$.

Results

Plasma amino acids and PRL concentrations

Fasted and post-exercise plasma concentrations of free TRP, BCAA, TRP, free TRP:BCAA ratio and P did not differ between I and II (Table 2). After 5 h of cycling, plasma BCAA and TRP concentrations were significantly decreased ($P < 0.01$) and plasma PRL and free TRP concentrations and free TRP:BCAA ratio significantly increased ($P < 0.01$) (Table 2). The increase in mean plasma PRL concentration during acute exercise

Table 2 Plasma total tryptophan (*TRP*), free tryptophan (*free TRP*), branched-chain amino acids (*BCAA*), total protein (*P*) and prolactin (*PRL*) concentrations and free TRP:BCAA ratio after an overnight fast (*B*) and after 5 h of cycling (*E*) before (*I*) and after (*II*) 9 days of intensified training

		I				II			
		B		E		B		E	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total TRP	[µmol · l^{-1}]	96.9	15.6	73.4	14.2	90.9	7.3	74.1	8.7
Free TRP	[µmol · l^{-1}]	8.6	1.3	14.9	5.9	8.9	0.5	12.2	3.3
BCAA	[µmol · l^{-1}]	362.9	47.1	303.1	49.2	374.8	29.8	294.2	45.1
Free TRP:BCAA		0.024	0.003	0.051	0.021	0.024	0.003	0.041	0.013
Total P	[g · dl^{-1}]	6.89	0.56	6.82	0.37	6.79	0.46	6.89	0.52
PRL	[ng · ml^{-1}]	8.9	1.9	14.1	1.5	8.2	1.9	11.6	1.9

was significantly lower in II [45.4 (SD 25.3) %; $P < 0.01$] compared with I [63.5 (SD 32.8)%].

[^{3}H]Ketanserin binding

Figure 1 shows a representative experiment of the equilibrium specific binding of [^{3}H]ketanserin to human platelet membranes as a function of the radioligand concentration. In all samples the best-fit equation obtained by non-linear regression analysis describes the reaction of [^{3}H]ketanserin with one binding site. At the two basal conditions before exercise (Table 3, I B, II B, respectively) the affinity of [^{3}H]ketanserin for its specific binding site (K_d) and the density of the binding sites (B_{max}) did not differ significantly. However, acute endurance exercise significantly reduced ($P < 0.05$) B_{max} during I and II (Table 3). The affinity of [^{3}H]ketanserin was significantly increased ($P < 0.02$) by acute exercise during pre-examination; the increase in affinity during post-examination did not reach the level of significance (Table 3). We also used analysis of variance to examine whether the observed changes of K_d and B_{max} after exercise were significantly different. Again, significant decreases of B_{max} were found after acute endurance exercise in I and II, whereas the changes of K_d did not reach the level of significance.

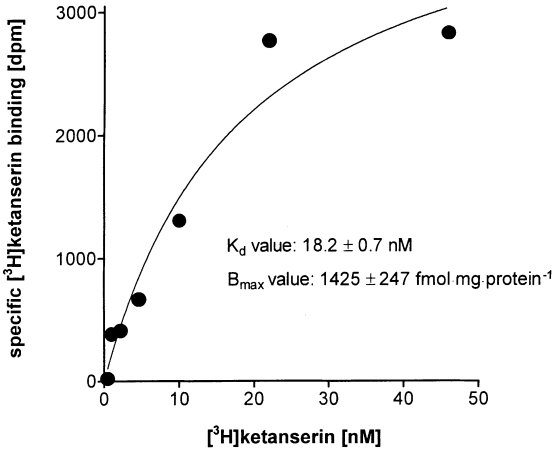

Fig. 1 Saturation curve for specific [^{3}H]ketanserin binding. Membranes from human platelets were incubated for 60 min at room temperature with increasing concentrations of [^{3}H]ketanserin. The graph shows one representative experiment performed in triplicate. For definitions see Table 3. Values are mean ± SEM

Discussion

From preliminary evidence it has been concluded that the brain serotonergic system might be involved in the aetiology of fatigue during acute endurance exercise and

Table 3 Binding of [^{3}H]ketanserin to serotonin-2A receptors on pre-frozen platelet membrane preparations in all subjects (1–9). Values are given for the density of specific binding sites (B_{max}) and affinity of [^{3}H]ketanserin for these specific binding sites (K_d) in fasted state (*B*) and post-exercise (*E*) before (*I*) and after (*II*) 9 days of increased training. *n.m.* not measured

	I				II			
	B		E		B		E	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
B_{max} [fmol · mg · protein^{-1}]								
1	1968	699	564	239	1454	441	1235	148
2	n.m.	n.m.	n.m.	n.m.	4100	3618	1797	323
3	371	236	0	–	1425	247	0	–
4	971	571	0	–	0	–	0	–
5	1704	393	0	–	329	217	0	–
6	3276	1880	2495	310	418	90	61	27
7	0	–	0	–	575	138	0	–
8	718	79	74	26	905	175	375	117
9	1198	305	1526	233	3389	1690	655	67
Mean	1275.8	594.7	582.4	202.0	1399.4	827.0	458.1	136.4
SEM	969.5	558.4	877.8	106.0	1343.6	1163.8	618.2	102.1
K_d [nM]								
1	84	42	10	9	34	19	20	9
2	n.m.	n.m.	n.m.	n.m.	55	78	26	15
3	3	5	–	–	18	7	–	–
4	23	29	–	–	–	–	–	–
5	37	28	–	–	14	23	–	–
6	65	62	20	9	17	9	4	6
7	–	–	–	–	28	14	–	–
8	32	7	0,9	2	41	18	43	23
9	59	23	18	9	44	38	6	2
Mean	43.3	28.0	12.2	7.3	31.4	25.8	19.8	11.0
SEM	25.6	18.3	7.5	3.0	13.8	21.7	14.3	7.3

disturbances associated with chronic excessive exercise stress (Newsholme et al. 1991). It has been suggested that endurance exercise may alter the plasma free TRP:BCAA ratio which affects brain 5-HT synthesis (Blomstrand et al. 1989; Chaouloff 1989). The [^{3}H]ketanserin binding to platelets and the plasma PRL response to stimuli have been shown to be peripheral models which may reflect some changes in the brain 5-HT system (Biegon et al. 1987; Yatham and Steiner 1993; Jakeman et al. 1994).

It has been suggested that a trained muscle develops the potential for an increased capacity for BCAA oxidation and transamination reactions (Graham et al. 1997). Several studies have described increased leucine oxidation or turnover, respectively, as a consequence of training programmes (Dohm et al. 1977; Henderson et al. 1985; Lamont et al. 1990). However, in the present study plasma BCAA concentrations at rest and post-exercise were not affected by the training programme, nor did the increase in the amount of training change plasma free TRP:BCAA ratio. Hence, it is unlikely that 5-HT synthesis in the brain was modified because of an altered availability of free TRP in the brain. The present findings confirmed a recent report which showed that a 40% increase in the amount of training was not accompanied by changes in basal plasma concentration of free TRP and BCAA (Tanaka et al. 1997).

In accordance with previous reports (Kaufmann et al. 1985; Lehmann et al. 1992), the present data revealed a decrease in plasma PRL concentration after the short-term training programme. Previously, Jakeman et al. (1994) have reported that central serotonergic receptor function, assessed via the plasma PRL concentration response to buspirone (a partial agonist for 5-HT$_{1A}$ receptors) challenge, was reduced by endurance training. In addition, Barron et al. (1985) have demonstrated a reduced plasma PRL concentration response to insulin-induced hypoglycaemia in overtrained athletes. Therefore, it may be assumed that endurance training induces a decrease in PRL secretion because certain 5-HT receptors in the central nervous system (CNS; at least the 5-HT$_{1A}$ receptors in the pituitary gland) are less excitable. It is conceivable that acute exercise-induced augmentation of 5-HT synthesis may lead to a down-regulation of 5-HT receptors in the CNS.

To substantiate further the hypothesis of an increased 5-HT availability by exercise we investigated whether the density of and the affinity to 5-HT$_{2A}$ receptors on platelets were altered by acute or chronic exercise. Under the present experimental conditions, binding of [^{3}H]ketanserin was specific, saturable and of high affinity. These features fulfil the criteria for the identification of a specific binding site. Comparison of the [^{3}H]ketanserin binding parameters with those reported in the literature revealed that the mean affinity of [^{3}H]ketanserin in the present study was about 5–10 times lower (see Biegon et al. 1987) and the number of binding sites was higher than has been found in previous radioligand binding experiments (Biegon et al. 1987; Sheline et al. 1995; Spigset and Mjörndal 1997). This difference might be explained by the fact that in the previous studies, only [^{3}H]ketanserin concentrations up to 2.5 nM have been used at which saturation of the binding sites was not complete (Biegon et al. 1987). Under this condition, computer-aided fitting of a binding isotherm to the data underestimates the binding density, and as a consequence the K_d value is overestimated. Moreover, a distinct interindividual variance has been reported for 5-HT$_{2A}$ receptors in human platelets (Spigset and Mjörndal 1997) which probably also contributes to the large range of the B_{max} values observed in the present and previous studies. In this context it is interesting to note that in previous studies when evaluating [^{3}H]ketanserin binding to platelets in humans, platelets were derived from untrained volunteers, whereas in the present study platelets were taken from endurance athletes. This difference in subjects might have contributed to the differences in platelet [^{3}H]ketanserin binding site density. Finally, due to the scatter of our data (Table 3) the difference between the mean values in the present study and those reported in the literature was not statistically different (data not shown). Hence, the difference might also have been caused by chance.

Since tritiated ketanserin and unlabelled mianserin, which was used to define specific binding, bind relatively specific to 5-HT$_{2A}$ receptors in the concentration range used, it would appear justifiable to assume that in the present study [^{3}H]ketanserin labelled mainly the 5-HT$_{2A}$ receptor population in the platelet membranes. The present study revealed that acute exercise (5 h of cycling) decreased density of the 5-HT$_{2A}$ receptors on the platelets. It is conceivable (albeit speculative) that acute long-lasting exercise may have led to an increase in 5-HT concentration which, in consequence, induced a receptor downregulation. The decrease in the number of 5-HT$_{2A}$ receptors on platelets brought about by acute endurance exercise represents a robust effect, since it is found in spite of an intraindividual variability which has been reported for the density of 5-HT$_{2A}$ receptors on human platelets (Spigset and Mjörndal 1997). In contrast, the increase in affinity of [^{3}H]ketanserin observed after acute endurance exercise in I is probably an artefact of the fit due to the decreased density of the specific binding sites (see above). This view was supported by the finding that analysis of variance did not reveal a significant difference to the basal values. The increase in the amount of training did not alter basal [^{3}H]ketanserin binding to platelet 5-HT$_{2A}$ receptors. In a study by Dey et al. (1992), exercise training of normal rats was shown to result in enhanced sensitivity of brain 5-HT$_2$ receptors along with subsensitivity of 5-HT$_{1A}$ autoreceptors. Although rapid downregulation by 5-HT$_2$ receptor agonists has been clinically established, the duration and intensity of the training programme in the present study might not have been sufficient to affect the density of platelet 5-HT$_{2A}$ receptors.

The question as to whether platelet 5-HT$_{2A}$ receptor density can be changed by an exercise-induced increase

in platelet count (10–30% increase; see, for example, Banfi et al. 1995; Gleeson et al. 1995) has not been addressed in the present study. However, a marked influence on platelet 5-HT_{2A} receptor density should not occur, since the platelet distribution width, which describes the composition of the platelet population, has been found not to be affected or even reduced (i.e. a more homogenous population) by exercise (van Wersch and Janssen 1989; Long et al. 1990; Banfi et al. 1995). Nevertheless, a minor contribution of the increased platelet count to the changes in platelet 5-HT_{2A} receptor density by exercise cannot be completely ruled out at present.

In summary, the main new finding of the present study was that acute endurance exercise induced a reduction of 5-HT_{2A} receptors on platelets. Assuming that this receptor downregulation was due to an increased 5-HT concentration in blood and that a corresponding increase in 5-HT concentration also occurred in the brain, it is conceivable that such changes in the activity of the serotonergic system in the brain might be involved in the genesis of *central* fatigue during acute prolonged exercise. In line with this hypothesis, the reduced plasma PRL response to exercise may point towards a downregulation of brain 5-HT_1 receptors by repeatedly increased 5-HT availability during chronic exercise stress.

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