Physiol. Res. 48: 129-133, 1999

Serum Opioid Activity After Physical Exercise in Rats

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Received February 9, 1998
Accepted November 27, 1998

Summary

In order to study the effect of exercise on the total serum opioid activity, female rats were trained for 3 weeks on a motor-driven treadmill and the experiment was ended by a final strenuous run until exhaustion. The serum samples were taken immediately after the final run and were analyzed by radioreceptor assay. Despite considerable interindividual variations, serum opioid activity, expressed in met-enkephalin equivalents (ME eq ± S.D.), was significantly higher in the exercising group (74.5±50.5 pmol ME eq/ml) than in the control group (35.7±20.2 pmol ME eq/ml). Because of the much lower molar levels of β endorphin and met-enkephalin, this result suggests that many other opioid peptides might be involved in that increase.

Key words

Exercise • Opioid peptides • Radioreceptor assay • Rat • Serum opioid activity

Introduction

Physical exercise is a physiological stress situation that requires the organism to adapt to physical effort. Thus, as well as increasing the heart rate, blood pressure, tidal volume and breathing frequency, exercise has been shown to alter the levels of various hormones and metabolites. The release of opioid peptides, in particular of β endorphin and met-enkephalin, has often been pointed out, although its exact significance remains unclear.

It has to be stressed that β endorphin and met-enkephalin account for only a little part of total serum opioid activity. Indeed, most reports in man indicate that the circulating levels of β endorphin vary between 1 and 10 fmol/ml (Rakhila *et al.* 1988, Goldfarb *et al.* 1990, Glämsta *et al.* 1993, Heitkamp *et al.* 1993), whereas the circulating levels of met-enkephalin (ME) vary between 25 and 250 fmol/ml (Clement-Jones *et al.* 1980, De Sommers *et al.* 1989). These values have to be compared with the higher rate of total activity estimated by Boarder *et al.* (1982) in man (63.2 pmol Leu-enkephalin equivalents/ml). From this comparison we can conclude that the major part of opioid activity is due to opioid peptides other than β endorphin and met-enkephalin. As far as we know, opioid activity during exercise has always been studied by β endorphin and met-enkephalin assays using radioimmunological techniques that are very sensitive but specific for one molecule only. The purpose of the present study in rats was to investigate the development of overall opioid activity during physical exercise. To achieve this, we decided to use radioreceptor assay (RRA). This technique measures all molecules binding to the same type of receptor, in the present case

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the μ opioid receptor, which is correlated with the pharmacological activity of opioid peptides. It is therefore well-adapted to overall evaluation of serum opioid activity.

Methods

Animals

A standardized experimental model has been developed in order to control the variability of factors such as age, sex, physical fitness or environmental conditions. Thirty-six female Wistar rats weighing 159±9 g were housed 6 per cage with food and water *ad libitum*. The room was maintained at 20±2 °C on a 12/12 hour light-dark cycle with lights on at 07:00 h. The study described in this article was conducted in compliance with the guidelines of Council Directive 86/609/EEC of November 24, 1986, regarding accommodation and care of animals.

Training

Rats were randomly divided into an exercising group (R for runners) and a control (C) group (n=18 each). Training consisted of running on a motor-driven treadmill set at 5 % grade. It was performed twice a day, (5 days a week) and lasted for 3 weeks. The training program was carried out as follows: After a one-minute warming up period at 20 $m.min^{-1}$, the velocity was increased to 40 $m.min^{-1}$ for an acute run whose duration was progressively increased from 3 to 6 min, and finally a one-minute run at 20 $m.min^{-1}$ was allowed for active recovery.

Final acute run

The final run at the end of the training protocol was always done at the same time of the day (11:00 h) to avoid circadian variations. Rats from the R group performed a final run consisting of an acute run to exhaustion. The initial velocity (40 $m.min^{-1}$) was progressively increased until a maximum of 60 $m.min^{-1}$.

Sample preparation

Immediately after the final run, the rats were killed by decapitation. Their blood was collected into glass tubes, was centrifuged at 4 °C and the serum was removed. Samples were stored at −20 °C until further analysis. Prior to the assay, a solid phase extraction was done after acidification of the samples with 100 μl 1N HCl per ml. C18 cartridges were prewetted with methanol and washed with 4 % acetic acid. After application of each sample, the cartridge was washed twice with acetic acid and then eluted with methanol. The eluates were dried under nitrogen and the dried samples were reconstituted with Tris HCl buffer at pH 7.6.

Total opioid activity assay

RRA is based on the principle that opioid peptides in the serum compete for the binding of [^{3}H] DAMGO to the μ opiate receptor in the rat brain. As reported by Mansour *et al.* (1995) most opioid peptides bind to the μ opiate receptor with a high affinity.

Membrane preparation

Male Wistar rats (150-200 g) were exsanguinated and their brains were immediately removed and placed on ice. Whole brains without cerebella were homogenized (Ultra Turax tissue homogenizer) in 10 volumes of Tris HCl buffer (50 mM, pH 7.4, 4 °C). The homogenate was centrifuged at 800x*g* for 10 min, the pellet was discarded and the supernatant centrifuged at 35 000x*g* for 15 min. The pellet (P2) was resuspended in the same buffer and preincubated (37 °C for 20 min) to remove endogenous opioids. The homogenate was centrifuged and resuspended again so that the protein content was 0.3-0.35 %.

Radioreceptor assay procedure

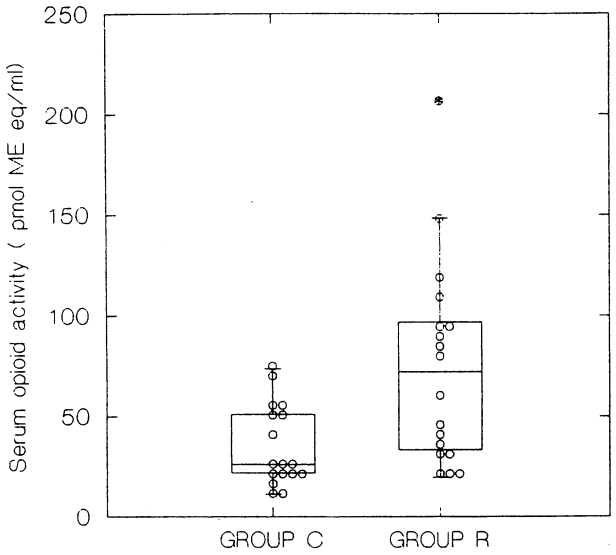
The assays were carried out with minor modifications according to a protocol by Swain *et al.* (1992). The binding assays were performed in polypropylene tubes in a total volume of 1 ml containing 50 μl of [^{3}H] DAMGO (specific activity 63 Ci/mmol), 250 μl of reconstituted extracts, 100 μl of membrane suspension and 600 μl of 50 mM Tris HCl buffer pH 7.4 containing a peptidase inhibitor cocktail. The samples were incubated in triplicate for one hour at 25 °C and were afterwards immediately filtered under reduced pressure through Whatman GF/B filters prewetted with a 0.1 % polyethylene imine solution, using three washes of 5 ml ice-cold buffer. The filters were placed in vials with 5 ml scintillation liquid. Radioactivity representing bound [^{3}H] DAMGO was counted on a Beckman L 1800 liquid scintillation counter. Specific binding was determined by subtracting non-specific binding (in the presence of 20 μM levallorphan) from the total binding (in the absence of levallorphan). A methionine-enkephaline standard curve was plotted logarithmically against the percentage inhibition of DAMGO binding. Serum opioid activity, finally expressed in picomoles of methionine-enkephaline equivalents per milliliter of

serum, was obtained by linear regression from the percentage inhibition of DAMGO binding.

Statistical analysis

As the data were not normally distributed and the variances were unequal, we used the non-parametric Wilcoxon two-sample test. Mean values, standard deviation (S.D.) and the medians were calculated for each group.

Fig. 1. *Dallal representation of serum opioid activity in group C (control) and group R (runners).*



Results

The body weight of the exercising rats increased by 58±22 g during the entire training period, while the increase in the control group was 60±13g. The difference between the two groups was not statistically significant (Student's t test, p=0.05). The levels of serum opioid activity for each animal are shown in Figure 1 (Dallal representation). These levels range from 11.3 to 73.7 pmol ME eq/ml for the control group (C) and from 19.7 to 206.9 pmol ME eq/ml for the exercising group (R) after the final run. The medians were 26.15 pmol ME eq/ml for group C and 72.25 pmol ME eq/ml for group R and are represented on the Dallal graph by horizontal lines inside the boxes.

The means of serum opioid activity were 35.7±20.2 pmol ME eq/ml for the control group C and 74.5±50.5 pmol ME eq/ml for the exercising group R after the final run, respectively. The value of the R group was 109 % higher than the value of the C group. This difference was statistically significant (Wilcoxon two-sample test, p=0.0139)

Discussion

The mean serum opioid activity measured at rest in the C group was 35.7 pmol/ml ME equivalents. This result is consistent with the value published by Swain *et al.* (1992) that was 41.6 pmol ME eq/ml. The identity of most of the molecules generating this serum opioid activity was not specified. The basal levels of plasma endorphin in rats is estimated by Fisher *et al.* (1984) as 35.5 fmol/ml, by Houghten *et al.* (1980) as 75 fmol/ml and by Perhonen *et al.* (1995) between 69 and 101 fmol/ml. All these values were higher than in man. According to Swain *et al.* (1992) the met-enkephalin levels were for rats below 50 pg/ml, i.e. 87 fmol/ml. These molar amounts are lower compared to the serum opioid activity measured by Swain *et al.* (1992) (41.6 pmol ME eq/ml) and by us (35.7 pmol ME eq/ml). This discrepancy confirms the results in man obtained by Boarder *et al.* (1982) who reported the existence of multiple forms of peptides with different molecular weights in human plasma. These peptides may belong to the classical opioid families that are enkephalins, dynorphins and endorphins, but may also belong to other families such as hemorphins which are yielded by degradation of hemoglobin (Brantl *et al.* 1986). These more recently discovered peptides might exert considerable influence on opioid activity. For instance, according to Glämsta *et al.* (1993), even though hemorphin-7 has a thousandfold lower affinity for μ opioid receptor than β endorphin, the much higher levels could be counterbalanced by this lack of affinity .

After the final run, serum opioid activity appears to be 109 % higher in the R group than in the C group. Boarder *et al.* (1982) briefly studied the total serum opioid activity in relation physical effort. Their study was carried out in a single subject that was exposed to light physical stress for three days. These authors reported that the activity after stress was two orders of magnitude higher than at rest. This result is comparable to the 109 % higher activity observed in the R group, although the work performed by the rats was more intensive. To our knowledge, apart from this study, overall serum opioid activity during physical exercise has not yet been studied. Hence, a direct comparison of our results with others is impossible. Nevertheless, we can draw an analogy parallel with β endorphin, the only opioid peptide that has been widely studied. Thus, the marked interindividual variability, which we observed for serum opioid activity, is comparable to that reported in studies concerning β endorphin. Most investigators have found that physical exercise is associated with a rise

in plasma β endorphin. However, the individual levels are very scattered around the mean value. Studies by Gambert *et al.* (1981) and Glämsta *et al.* (1993) display levels of β endorphin ranging between 2 to 32 fmol/ml and 2 to 12 fmol/ml, respectively. According to previous studies in humans, the levels of β endorphin rose by 100 to 600 % after physical exercise (Glämsta *et al.* 1993, Goldfarb *et al.* 1990, Heitkamp *et al.* 1993, Rakhila *et al.* 1988), whereas the release of met-enkephalin is much more uncertain, although De Sommers *et al.* (1989) found a 30 to 98% increase and Howlett *et al.* (1984) a maximum of 400% increase. Lately, Glämsta *et al.* (1993) reported that following long distance running there is a significant rise in hemorphin-7 which correlated with β endorphin. These results were obtained in man and cannot be systematically applied to rats. However, it seems unlikely that the considerable increase in serum opioid activity could only be generated by increased rates of β endorphin and met-enkephalin. The possibility must be considered that other peptides are involved and that their levels are also increased. The rise in circulating opioid peptides might be due to their increased release into the blood as well as to decreased degradation.

In conclusion, the results from this study have demonstrated that strenuous physical exercise induces in rats a significant increase of serum opioid activity. This increase might be partly caused by a rise in β endorphin and met-enkephalin levels but many other opioid peptides are probably involved. The identification of these peptides will be a further step toward a more accurate understanding of the relationship between physical exercise and the opioid system.

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Reprint requests

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