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Modifications of atrial natriuretic peptide and vasopressin peptides in the rat hypothalamic supraoptic nucleus during resistance training

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Summary

Many studies have demonstrated the involvement of atrial natriuretic peptide (ANP) and vasopressin (VP) in the homeostasis of body fluids, but few studies have regarded the hypothalamic magnocellular neurosecretory system during physical exercises. The aim of the present immunohistochemical work is to study the activity of ANP and VP secreting neurons of the hypothalamic supraoptic nucleus during and after resistance training. The study was carried out in Wistar rats trained by a physical resistance-type exercise, using a rung ladder and a varying load fastened to the tail of each rat; the exercise lasted 20 min everyday for periods of 15, 30 and 45 days. Animal groups were sacrificed at the end of each training period and one group was sacrificed after 60 days from the beginning of training, i.e.15 days after completing a 45 day training. The results show that ANP and VP-immunopositivity is at first lesser in the trained rats than in the corresponding controls and then increases from the 15th to the 45th day of training; the increase of the immunopositivity in the trained rats indicates a decreased degranulation of the neurons.
The comparison between VP and ANP-immunopositivity suggests that in the early phase of training VP-release in the bloodstream is higher than ANP-release, therefore the antidiuretic action of VP is expected to prevail on ANP action and an electrolyte unbalance may occur.

Key words

ANP, Vasopressin, neurosecretion, hypothalamus, supraoptic nucleus, physical exercise.

Introduction

There are few studies in the literature about the hypothalamic neurosecretion during physical exercise; prevalently these studies regard oxytocin secretion (Braga et al., 2000; Neumann et al., 2006; Farina Lipari et al., 2008).

The electrolyte concentration of the body fluids is regulated by hormones, particularly vasopressin (VP) and atrial natriuretic peptide (ANP). VP is synthesized by hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei and plays an antidiuretic role; during development its synthesis starts at the 16th day of intrauterine life (Farina Lipari et al., 2001). The ANP is synthesized by the heart but also by hypothalamic SON and PVN and has diuretic and natriuretic effects; during develop-

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ment it is synthesized in SON (Farina Lipari et al., 2005) and suprachiasmatic nuclei (Farina Lipari et al., 2007). The two peptides are involved in the homeostasis of the body fluids and play roles antagonist to each other.

Many studies have reported about the changes in the plasma levels of ANP and VP during exercise. Niessner and coll. (2003) demonstrated that during acute exercise atrial distension, that is caused by increased blood central volume and corresponding raising atrial pressure, induces circulating ANP-increase, while during prolonged exercise (marathon) the ANP-levels are less elevated. Probably this behaviour is due to initially impaired ANP-clearance depending on the decreased blood flow in organs, such as kidney.

Biochemical studies showed that during marathon hyponatremia occurs, that is a life-threatening complication leading to fatal cerebral and pulmonary oedema and is due to inappropriate hypothalamic VP secretion (Siegel, 2006; Siegel et al., 2007).

Since ANP is secreted by the heart but also by SON we have considered important to study possible variations in the immunoreactivity of the SON to understand the role of ANP and VP in the homeostasis of body fluids during physical exercise.

To this aim we carried out an immunohistochemical study on the hypothalamic SON of rats trained by a resistance type exercise for different time periods to verify the changes in VP and/or ANP secretion and their reciprocal correlation.

Material and Methods

Animal care

Forty-five male, six months-old, Wistar rat (315.4±30.3g body weight) were used for the investigations conforming to the Guide for the Care and Use of Laboratory Animals (Clark et al., 1997). Animals were housed in cages and were allowed food and water ad libitum. The daily cycle extended from 7 a.m. to 7 p.m. and the room temperature was maintained at 21.6±0.5°C.

The rats were divided randomly into 5 groups, each including 9 animals:

- group 0 = sedentary controls (SC), which remained in their cages for the entire duration of the experiment;
- group 1= animals trained for 15 days (D15);
- group 2=animals trained for 30 days (D30);
- group 3= animals trained for 45 days (D45);
- group 4=animals trained for 45 days like group 3 and sacrificed 15 days after having stopped training (D 60).
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Nine rats from each experimental group (D15, D30, D45, D60) groups were sacrificed after completing the training; two animals from the SC group were sacrificed simultaneously to those of D15, D30, D45, D60 groups.

Resistance training protocol

Climbing a vertical, 1 m ladder with 2 cm high steps, with a load attached to the rat tail, was used as resistance training. Rats were familiarized with the exercise for 3

days. The first week after familiarization, the exercise was performed with no load. In order to increase the workload, from the second week, increasing loads were attached to the base of the tail with a Velcro strap. The initial load was 50% of the rat body weight (150 g) and was gradually increased throughout the subsequent 6-week training period: at the 30th day, the load weighted 300 g; at the 45th day, the load weighted 360 g. The resistance training consisted of 1 set of 10 repetitions with a 1 min rest interval among the replications, for 5 days/week. When the rats reached the top of the ladder, they were allowed to recover in the test area. Training was performed in the morning.

Immunohistochemistry

Rats were deeply anesthetized with pentobarbital. For immunohistochemistry, the brain was fixed in Bouin's fluid, dehydrated in graded alcohols and embedded in paraffin. Seven micron thick sections were used to perform the ANP or VP-immunostaining, detected with avidin-biotin complex. Endogenous peroxidases were blocked by incubation with PBS containing 0.3% hydrogen peroxide, for 5 min at room temperature. Following blockage of the unspecific binding sites with normal goat serum, the sections were treated with anti-ANP or anti-VP polyclonal antibodies raised in rabbit (Chemicon, Temecula, CA) at serial dilutions 1:500, 1:600 and 1:800 in 0.05 mol/L Tris buffer, pH 7.2, for 12 h at 4°C, subsequently the sections were washed in PBS (three times, for total 15 min). The reaction was demonstrated with amino-c-ethyl-carbazole as substrate. Negative controls were sections treated with pre-immune serum or in which the primary antibody was omitted.

Three sections of the SON were used for counts in each animal; the numbers of ANP- and of VP- immunopositive neurons were counted by two different investigators without previous knowledge of treatment and their percentage on the whole number of neurons in the SON (immunopositive plus immunonegative) was calculated.

Statistical analysis

Standard statistics were applied to calculate the mean and the standard deviation. A one way analysis of variance (ANOVA) was used to evaluate differences among data, which were regarded as significant for $p < 0.05$.

Results

Atrial natriuretic peptide

In the rat SON at the 15th day of training the ANP-immunopositive neurons are in lower number than in controls; from the 30th to 45th day of training the number of ANP-immunopositive neurons increases, nevertheless the number remains lower than in controls. At the 60th day, 15 days after stopping exercise, the number of ANP-immunopositive neurons increases further and becomes higher than that of the relative controls (Figs. 1, 2).

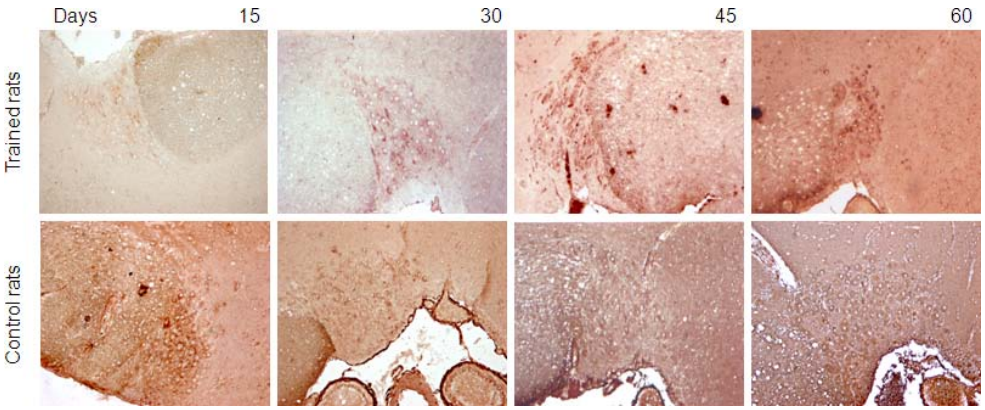

Fig 1 – Supraoptic nucleus. Atrial natriuretic peptide (ANP)-immunopositive neurons 15, 30, 45 days after the start of training and at 60 days, i.e. 15 days after stopping training. Upper row: trained rats; lower row: control rats.

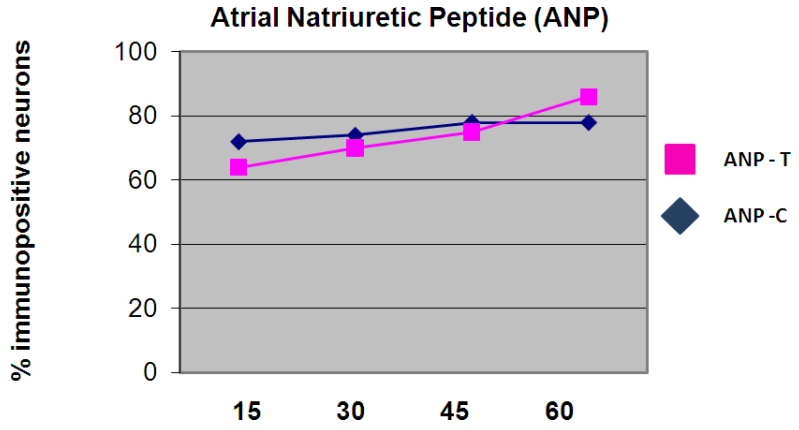

Fig. 2 – Supraoptic nucleus. Percentage of ANP-immunopositive neurons in control and trained rats 15, 30, 45 days after the start of training and at 60 days, i.e. 15 days after stopping training. Control rats (blue), trained rats(red).

Vasopressin

In the SON of rats at the 15th day of training the number of VP immunopositive neurons is lower than in controls; from the 30th to 45th day of training the number of VP-immunopositive neurons increases; precisely at the 30th day of training the number is equal to that of the corresponding controls, while at the 45th day of training it is higher than in corresponding controls. Fifteen days after stopping training, at the 60th day, the number of VP-immunopositive neurons significantly increases over that of the corresponding controls (Figs. 3, 4).

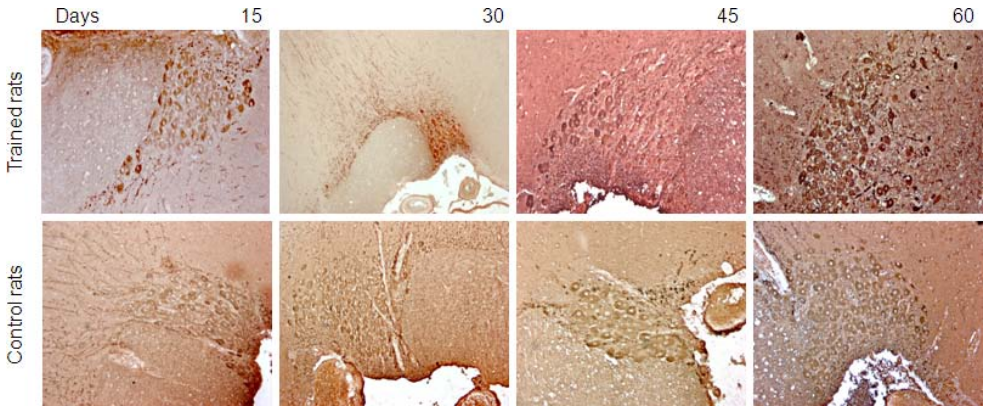

Fig 3 – Supraoptic nucleus. Vasopressin (VP)-immunopositive neurons 15, 30, 45 days after the start of training and at 60 days, i.e. 15 days after stopping training. Upper row: trained rats; lower row: control rats.

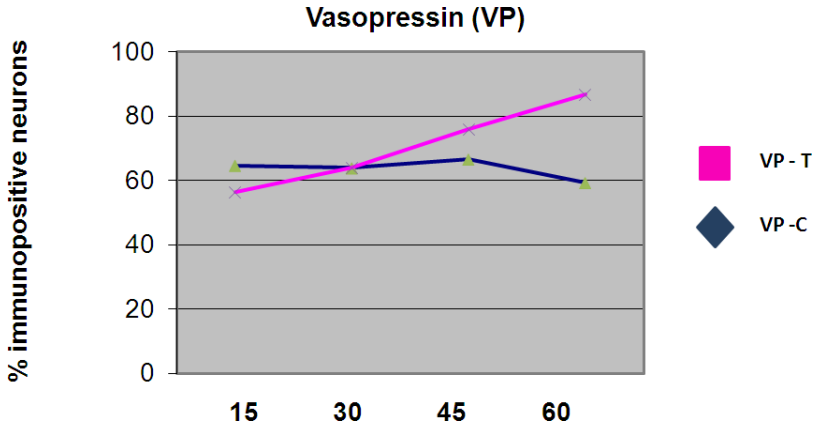

Fig. 4 – Supraoptic nucleus. Percentage of VP-immunopositive neurons in control and trained rats 15, 30, 45 days after the start of training and at 60 days,i.e.15 days after stopping training. Control rats (blue), trained rats (red).

Atrial natriuretic peptide-vasopressin balance

The comparison between the numbers of neurons immunopositive for ANP and those immunopositive for VP shows that from 15 to 45 days the number of ANP-immunopositive neurons is higher than that of VP- immunopositive neurons and that 15 days after stopping exercise, at the 60th day, the number of ANP and VP immunopositive neurons is equal (Fig. 5).

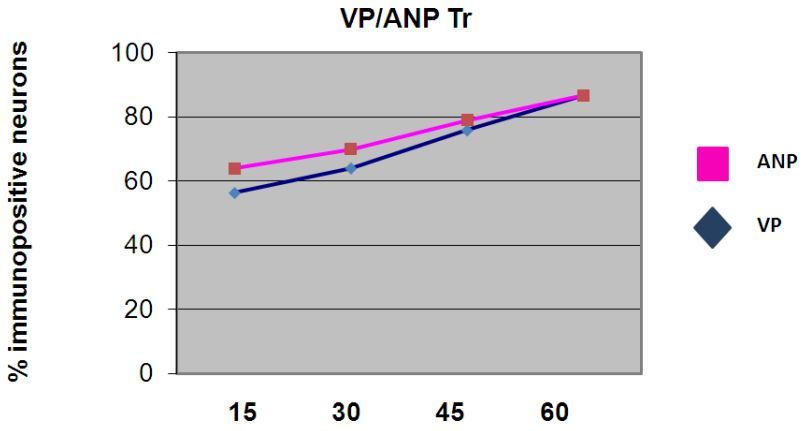

Fig. 5 – Supraoptic nucleus. Comparison between the percentage of ANP and VP-immunopositive neurons in trained rats 15, 30, 45 days after the start of training and at 60 days, i.e.15 days after stopping training. VP (blue), ANP (red).

Discussion

Physical exercise determines a stress to the organism that begins to move different systems to adapt to new conditions. We wanted to investigate, by immunohistochemical methods, on ANP and VP- neurosecretion in the hypothalamic SON of resistance-trained rats.

The results showed that, from the 15th to 45th day of training, the number of the ANP-immunopositive neurons in the SON is lower than in the corresponding controls. This decrease is interpreted as indicating a higher ANP release in the blood stream than in controls.

At the 60th day, 15 days after the stopping training, the number of the ANP-immmunopositive neurons is higher than control; this increase indicates that the ANP release decreases.

The higher level of degranulation of ANP positive neurons, at the beginning of training, is probably related to the stress of exercise that initially is stronger, while successively adaptation determines a reduction in the stress and consequently a decrease in ANP-release.

The results regarding VP-release are taken to indicate that at 15 days of training the degranulation of the VP-ergic neurons is higher than control and hence that the stress of training determines a VP-release higher than in controls. From 30 to 45 days of training the number of VP-ergic neurons increases progressively. This datum is related to the adaptation to the new condition induced by exercise. At the 60th day, 15 days after stopping training, the number of the VP-ergic neurons is like that of ANP-ergic neurons.

The comparison between the curves of VP and ANP-immunopositivity in the SON of resistance- trained rats evidences that both curves have the same behaviour, but during training the number of ANP-immunopositive neurons is at first higher than that of VP-immunopositive neurons, therefore it may be speculated that VP-release in bloodstream is higher than ANP-release. This result is related to the initial

stress by exercise and indicates a unbalance in the neurosecretion of VP and ANP, so that the antidiuretic action of VP is higher than the diuretic and natriuretic action of ANP; this unbalance may cause cerebral and pulmonary complications according to Siegel (Siegel, 2006).

Prolonged exercise induces also a rise in body-temperature and sweating and, because ANP and VP are involved in the homeostasis of body fluids, we hypothesize that the increased degranulation of ANP-ergic neurons induces dilatation (Valentin et al., 1993) of the skin blood vessels and activates the sweat glands (Spreca et al., 2000) to cooperate in lowering of the body temperature.

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