Peptides xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Short communication

Peripheral oxytocin treatment affects the rat adreno-medullary catecholamine content modulating expression of vesicular monoamine transporter 2

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ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 4 November 2013
Accepted 4 November 2013
Available online xxx

Keywords:
Oxytocin
Adrenal-medulla
Epinephrine
Norepinephrine
Vesicular monoamine transporter 2

ABSTRACT

The neuropeptide oxytocin has been shown to influence on neuroendocrine function. The aim of the present study was to investigate the effect of peripheral oxytocin treatment on the synthesis, uptake and content of adreno-medullary catecholamine. For this purpose oxytocin (3.6 μg/100 g body weight, s.c) was administrated to male rats once a day over 14 days. In order to assess the effect of peripheral oxytocin treatment on adreno-medullary catecholamine we measured epinephrine and norepinephrine content and gene expression of tyrosine hydroxylase (TH), norepinephrine transporter (NET) and vesicular monoamine transporter 2 (VMAT2) in the adrenal medulla. Our results show a significant increase of epinephrine (1.7-fold, $p < 0.05$) and norepinephrine (1.5-fold, $p < 0.05$) content in oxytocin treated animals compared to saline treated ones. Oxytocin treatment had no effect either on mRNA or protein level of TH and NET. Under oxytocin treatment the increase in VMAT2 mRNA level was not statistically significant, but it caused a significant increase in protein level of VMAT2 (3.7-fold, $p < 0.001$). These findings indicate that oxytocin treatment increases catecholamine content in the rat adrenal medulla modulating VMAT2 expression.

1. Introduction

The neuropeptide oxytocin has been shown to influence a variety of behaviors [18] as well as physiological and endocrine function [22]. In rats, oxytocin treatment induces several long-lasting anti-stress effects. Oxytocin, released during stress, also contributes to the control of other hormones involved in the stress response. The physiological responses to stress are initiated by the activation of the sympatho-adrenal system, resulting in the release of catecholamines and glucocorticoids from the adrenal gland [14]. Subchronic oxytocin treatment lowered plasma corticosterone and adrenocorticotropic hormone [5,15] though its opposite effect was also reported, i.e. an increase in plasma corticosterone and adrenocorticotropic hormone [12]. However, little data are available on adreno-medullar activity in response to oxytocin treatment. Findings obtained from human and animal studies pertaining to the influence of oxytocin on catecholamine release and store are varying. Intranasal oxytocin administration to healthy participants had no effect on plasma catecholamine levels [1,12]. On the other hand, Grewen and Light [7] reported that greater overall oxytocin level in postpartum mothers was related to lower plasma norepinephrine levels. Peripherally administrated oxytocin in rats was described to inhibit catecholamines release. Measurement of plasma catecholamines before and after oxytocin administration revealed a 53% inhibition of epinephrine and 43% inhibition of norepinephrine, suggesting that the inhibition of catecholamines secretion by oxytocin in vivo occurs directly at the adrenal level [5]. It is well known that in the adrenal chromaffin cells, to maintain catecholamine homeostasis, there is a link between catecholamine secretion, synthesis and uptake. Adreno-medullary activity is dependent on the synthesis of catecholamine, as determined by the rate limiting enzyme tyrosine hydroxylase (TH), its reuptake through the norepinephrine transporter (NET), synaptic release, degradation and vesicular transport mediated by the vesicular monoamine transporter 2 (VMAT2). A direct influence of oxytocin on catecholamine synthesizing enzyme and transports has not been studied yet.

In the present study, we have attempted to evaluate possible effects of oxytocin on the rat adreno-medullary catecholamine and to find out whether gene expression of catecholamine synthesizing

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0196-9781/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.peptides.2013.11.001

enzyme and transporters are affected by this treatment. Therefore, we quantified the changes in epinephrine and norepinephrine content, mRNA and protein levels of TH, NET and VMAT2 in the rat adrenal glands after oxytocin treatment.

2. Materials and methods

2.1. Animals

Male Wistar 11-week-old rats, weighing 250–330 g at the onset of experiment, were acclimated to 22 ± 1 °C and synchronized to a 12 h D/L regime. Commercial rat food and water were available ad libitum. The care was taken to minimize the pain and discomfort of the animals according to the recommendations of the Ethical Committee of the "Vinca" Institute, Belgrade based on the Guide for Care and Use of Laboratory Animals of the National Institute of Health (Bethesda, MD, USA).

2.2. Treatment

In the experiment we used 24 animals, which were randomly divided into two groups ($n = 12$ per group), saline- and oxytocin-treated rats. Oxytocin (H-2510, Bachem, Switzerland) was first dissolved in isotonic saline and then subcutaneous administered in dose of 3.6 μg/100 g body weight/day over 14 days. Placebo group of animals received saline. 24 h after last dose of saline/oxytocin treatment rats were decapitated, trunk blood collected the adrenal glands promptly removed and immediately weighed, cortex quickly removed on ice and medulla instantly frozen in liquid nitrogen and stored at −70 °C until analyzed.

2.3. Plasma oxytocin

Trunk blood was collected into cooled polyethylene tubes containing EDTA as anticoagulant and centrifuged immediately at 9000 rpm for 5 min at 4 °C to separate plasma, which was then stored at −20 °C until analyzed. Plasma oxytocin levels were measured by a commercial RIA kit (S-2033, Bachem, Switzerland), according to manufacturer's protocol. Assay sensitivity was 0.02 ng/ml. Intra- and inter-assay coefficient variances were less than 5%.

2.4. Catecholamine concentration in tissue

Adrenal medulla were immersed into cold (4 °C) perchloric acid (0.3 μg of tissue per 30 μl of 0.1 N $HClO_4$), homogenized and the homogenates centrifuged (20 000 rpm, 20 min, 4 °C) and the supernatants (30 μl) used for determination of catecholamines. Catecholamines in the adrenal medulla were determined using the single isotope radioenzymatic assay of Peuler and Johnson [16] based on the conversion of catecholamines to the corresponding O-methylated derivatives by purified catechol-O-methyl-transferase in the presence of S-adenosyl-l-(^{3}H-methyl)-methionine. The O-methylated derivatives were oxidized to ^{3}H-vanilline. Radioactivity was measured with a toluene-based scintillation liquid and with an LKB-Wallac model 1219 scintillation counter (Stockholm, Sweden) at 40% efficiency for tritium. The range of measurement is Window 1 5-320, sensitivity is 20 CPM and interassay is less than 10%.

2.5. RNA isolation and real time RT-PCR

Total RNAs were isolated using TRIZOL reagent (Invitrogen, CA, USA). Reverse transcription was performed using Ready-To-Go You-Prime First-Strand Bead (AP, 90 Biotech) and pd (N)6 primer according to manufacturer's protocol. Real-Time RT-PCR assay was done exactly as previously described [4]. PCR reactions were performed in the ABI Prism 7000 Sequence Detection Systemat 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. TaqMan PCR reactions were carried out using Assay-on-Demand Gene Expression Products (Applied Biosystems, United States) for TH (ID:Rn00562500_m1), for NET (ID:Rn00580267_m1), and for VMAT2 (ID:Rn00565488_m1). A reference, endogenous control, was included in each analysis to correct the differences in the inter-assay amplification efficiency and all transcripts were normalized to cyclophylineA (ID:Rn 00690933) expression.

2.6. Protein isolation and Western blot

Adrenal medulla was homogenized in 0.05 M sodium phosphate buffer pH 6.65 and the homogenates centrifuged (12 000 rpm, 20 min, 4 °C). Protein content was determined in supernantant by the method of Lowry et al. [11]. 30 μg of adrenal medulla protein extract separated by 10% SDS-polyacrylamide gel electrophoresis were transferred to a supported PVDF membrane (Hybond™ P, Amersham Bioscience, GE Healthcare, Buckinghamshire, UK). The membrane was blocked in 5% non-fat dry milk in Tris-buffered saline-Tween (TBST). All following washes and antibody incubations were also performed in TBST at ambient temperature on a shaker. For measuring TH, NET and VMAT 2 protein levels, a polyclonal anti-TH antibody, rabbit (ab51191, dilution 1:1000, Abcam, Cambridge, UK), a polyclonal anti-NET primary antibody, rabbit (ab41559, dilution 1:1000, Abcam, Cambridge, UK) and polyclonal anti-VMAT 2 primary antibody, rabbit (ab81855, dilution 1:5000, Abcam, Cambridge, UK) respectively, were used. Washed membrane was further incubated in the horseradish peroxidase conjugated secondary anti-rabbit antibody for luminol based detection (ab6721, dilution 1:5000, Abcam, Cambridge, UK). Secondary antibody was then visualized by Immobilion Western Chemiluminescent HPR Substrate (Millipore Corporation, Billerica, USA). Western blot analysis was performed as previously described (Gavrilovic et al. [4]).

2.7. Statistical analysis

The results are reported as means ± S.E.M. Significance of the differences in body weight gain, relative and absolute weight of adrenal medulla as well as plasma oxytocin, catecholamine concentration and gene expression levels of the examined catecholamine biosynthetic enzyme and transporters in adrenal medulla of rats subjected to saline or oxytocin-treatment were estimated by one-way ANOVA test. The Tukey post hoc test was used to evaluate the differences between the groups. Statistical significance was accepted at $p < 0.05$.

3. Results

In the present study we examined the effect of chronic oxytocin treatment on animal weight, absolute and relative weight of adrenal gland, plasma oxytocin, epinephrine and norepinephrine content, mRNA and protein level of TH enzyme and transporters in adrenal medulla. One-way ANOVA shows significant difference in relative adrenal gland weight of chronic oxytocin treated animals compared with saline treated ones (1.2-fold, $p < 0.05$). At the end of the treatment, there were no significant differences either in absolute adrenal weight or in body weight gain compared to values at the beginning of the treatment. Chronic oxytocin treatment resulted in a significant increase of plasma oxytocin (4.7-fold, $p < 0.001$). Post hoc analysis of catecholamines content in adrenal medulla showed a significant increase of epinephrine (1.7-fold,

Please cite this article in press as: Jovanovic P, et al. Peripheral oxytocin treatment affects the rat adreno-medullary catecholamine content modulating expression of vesicular monoamine transporter 2. Peptides (2013), http://dx.doi.org/10.1016/j.peptides.2013.11.001