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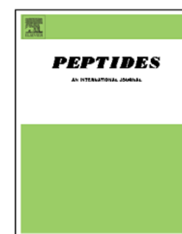

Enzymatic conversion of dynorphin A in the rat brain is affected by administration of nandrolone decanoate

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ARTICLE INFO

Article history:
Received 19 October 2006
Received in revised form
10 December 2006
Accepted 11 December 2006
Published on line 27 December 2006

Keywords:
Anabolic androgenic steroids (AAS)
Nandrolone decanoate
Dynorphin converting enzyme
Rat brain
Enzyme
Dynorphin

ABSTRACT

The misuse of anabolic androgenic steroids (AAS) seems to produce profound effects on the central nervous system, leading to aggressive behavior and increased sensitivity to other drugs of abuse. The present study addresses the effect on the enzymatic transformation, here called dynorphin converting enzyme-like activity. The formation of the mu/delta opioid peptide receptor-preferring Leu-enkephalin-Arg^6 from the kappa opioid peptide receptor-preferring dynorphin A was measured in rats treated with nandrolone decanoate. Significant variations in enzymatic transformation were observed in several brain regions. An altered receptor activation profile in these regions may be one contributory factor behind AAS-induced personality changes.

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1. Introduction

It has been suggested that anabolic androgenic steroids (AAS) serve as a gateway to abuse of other drugs [1]. The misuse of AAS display similar pattern with regard to sociodemographic and personality related factors as the misuse of psychotropic substances [17]. In addition, some AAS users report intoxication as a reason for the use, and there are reports demonstrating that AAS administration can induce euphoric effects as well as experience of a "crash" at cessation, indicating that AAS might affect similar neurobiological systems as psychotropic substances [4,9,16]. In fact, we have earlier reported AAS-induced alterations in the mesolimbic dopaminergic brain reward system, which is under tonic control, inhibitory as well as stimulatory, by the endogenous opioid systems [14,19,23].

In contrast to classical neurotransmitters, which after the release often are subjected to reuptake processes, neuropeptides such as the endogenous opioids are frequently either inactivated by proteases or possibly converted to bioactive fragments exhibiting retained or altered bioactivity (for a review, see Ref. [13]). The enzymatic processing, degradation and conversion mechanisms, are of great importance regarding the modulation of the peptidergic neurotransmission. An interesting aspect in the degradation of prodynorphin-derived peptides is the generation of the Leu-enkephalin-Arg^6 fragment (Leu-enk-Arg^6), which maintains opioid activity although with a different receptor selectivity. Dynorphin

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doi:10.1016/j.peptides.2006.12.011

A(1–17) (Dyn A; Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln), exhibits binding affinity preferentially for the kappa opioid peptide (KOP) receptor [6,7,12]. The N-terminal of Dyn A, which encompasses the penta peptide Leu-enkephalin, determines the overall opioid activity, whereas the C-terminal and central part of the peptide mediate selectivity for the KOP receptor [5,30]. Cleavage of the peptide at the Arg^6-Arg^7-bond yields the hexapeptide Leu-enk-Arg^6, acting on the delta and mu opioid peptide (DOP and MOP) receptors, hence the receptor activation profile is shifted. This conversion is of interest since KOP receptor activation in some cases exerts opposing effects compared to DOP/MOP receptor activation. As an example, rats self-administer DOP- and MOP- but not KOP-receptor agonists [45].

One of the enzymes responsible for dynorphin conversion generating the bioactive Leu-enk-Arg^6 fragment is the dynorphin converting enzyme (DCE). This protease display a high specificity for Dyn A, dynorphin B (Dyn B) and alfa-neoendorphin [31]. DCE has been reported to be present in human and rat CSF, human pituitary and human spinal cord [32,35,39,40]. Furthermore, a negative correlation between the concentration of prodynorphin-derived peptides and the level of DCE in human CSF has been observed [46].

We have previously observed AAS-induced alterations in the dynorphinergic system in brain regions anticipated to be associated with e.g. drug dependence and aggression. Based on these earlier studies, regions were selected for investigation in the present study. Since the opioid peptides are known to contribute in the regulation of the above-mentioned behaviors, the opioid converting enzymes are playing important roles. Enzymatic conversion of prodynorphin-derived peptides in specific brain regions may be one of several possible neurochemical mechanisms that could contribute to the personality changes seen among AAS abusers. Hence, in the present study we investigate the impact of chronic nandrolone decanoate administration on the enzymatic transformation of Dyn A to Leu-enk-Arg^6, here referred as to DCE-like activity.

2. Materials and methods

2.1. Chemicals

The synthetic peptides Dyn A and Leu-enk-Arg^6 were obtained from Bachem (Bubendorf, Switzerland), the nandrolone decanoate (Deca-Durabol®) from Organon (Oss, Netherlands), the sterile arachidis oleum from Apoteket AB (Umeå, Sweden), amastatin, captopril and phosphoramidon from Sigma–Aldrich Sweden AB (Stockholm, Sweden) and α-cyano-hydroxy cinnamic acid (CHCA) was obtained from Bruker Daltonik (Bremen, Germany). All other chemicals and solvents were of analytical grade from commercial sources.

2.2. Animal experiments

Sixteen male Sprague–Dawley rats, 12 weeks of age and weighing 480–520 g at the start of the experiment, were purchased from Alab (Stockholm, Sweden). The animals, housed in air-conditioned rooms at a controlled temperature of 22–24 °C and a humidity of 50–60% with lights on from 6 a.m. to 6 p.m. and with free access to food and water, were randomly divided into two groups. The rats were allowed to adapt to the novel laboratory environment for 2 weeks and during the following 14 days, one group received daily intramuscular (i.m.) injections of the AAS nandrolone decanoate (15 mg/kg) whereas the other group received i.m. injections of the vehicle (sterile arachidonis oleum). The animals were decapitated 1 day after the last injection, and the brains were immediately dissected using a rat brain matrix (Activational Systems Inc., Warren, MI, USA). The dissected brain structures were immediately put on dry ice and stored at −80 °C until further processing. The study was approved by the local ethical committee for animal experimental procedures in Uppsala, Sweden.

2.3. Tissue preparation and enzyme assay

The tissues were thawed on ice and homogenized in 20 mM Tris–HCl (pH 7.4). The homogenates from each tissue were separately centrifuged (Beckman J21) at 8000 × *g* for 20 min whereupon the supernatants were recentrifuged at 10,000 × *g* for 20 min. The new supernatants were collected and analyzed for DCE-like activity by measuring the conversion of Dyn A to Leu-enk-Arg^6 in each individual brain structure. The enzyme solution (30 μl), containing amastatin (20 μM), captopril (10 μM) and phosphoramidon (20 μM), buffered at pH 7.4 with 20 mM Tris–HCl in a final volume of 110 μl, was pre-incubated at 37 °C for 20 min before 0.25 μg (0.12 nmol) Dyn A was added to the incubation vials. The enzyme inhibitors amastatin, captopril and phosphoramidon were used in order to prevent degradation of the released product. Incubations were terminated after 20 and 40 min by adding methanol to withdrawn incubation samples (30 μl). The samples were evaporated in a Speed Vac centrifuge and subsequently analyzed by radioimmunoassay (RIA). For each brain structure, optimizations were made regarding the dilution of the enzyme solution prior to the incubations. Protein concentrations were determined according to the Lowry method, using bovine serum albumin (BSA) as standard [25].

2.4. Labeled peptides

The iodinated Leu-enk-Arg^6 was prepared using the chloramine-T method. Briefly, a solution of 5 μg peptide in 5 μl water was added to 35 μl 0.2 M sodium phosphate buffer (pH 7.4) and 5 μl radioactive iodine (^{125}I). The addition of 10 μl chloramine-T started the reaction, which was carried out for 40 s before termination by adding 100 μl 15% acetonitrile. The reaction solution was injected into a HPLC system equipped with a reversed phase column (Highcrom, Kr 100-5C18). Elution was performed using a linear gradient from 15–40% acetonitrile for 40 min at a flow rate of 0.5 ml/min. The fraction containing the [^{125}I]-labeled Leu-enk-Arg^6 was subsequently diluted, aliquoted and stored at −20 °C until further used.

2.5. Radioimmunoassay

The RIA for Leu-enk-Arg^6 was based on the double-antibody precipitation technique using antibodies raised in rabbits

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