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Modulation of Brain β-Endorphin Concentration by the Specific Part of the Y Chromosome in Mice

Michel Botbol1*, Pierre L. Roubertoux2, Michèle Carlier3, Séverine Trabado4, Sylvie Brailly-Tabard5, Fernando Perez-Diaz5, Olivier Bonnot6, Guillaume Bronsard7, Sylvie Tordjman8,9,10


Abstract

Background: Several studies in animal models suggest a possible effect of the specific part of the Y-chromosome (YNPAR) on brain opioid, and more specifically on brain β-endorphin (BE). In humans, male prevalence is found in autistic disorder in which observation of abnormal peripheral or central BE levels are also reported. This suggests gender differences in BE associated with genetic factors and more precisely with YNPAR.

Methodology/Principal Findings: Brain BE levels and plasma testosterone concentrations were measured in two highly inbred strains of mice, NZB/BNJ (N) and CBA/HGnc (H), and their consomic strains for the YNPAR. An indirect effect of the YNPAR on brain BE level via plasma testosterone was also tested by studying the correlation between brain BE concentration and plasma testosterone concentration in eleven highly inbred strains. There was a significant and major effect (P<0.0001) of the YNPAR in interaction with the genetic background on brain BE levels. Effect size calculated using Cohen’s procedure was large (56% of the total variance). The variations of BE levels were not correlated with plasma testosterone which was also dependent of the YNPAR.

Conclusions/Significance: The contribution of YNPAR on brain BE concentration in interaction with the genetic background is the first demonstration of Y-chromosome mediated control of brain opioid. Given that none of the genes encompassed by the YNPAR encodes for BE or its precursor, our results suggest a contribution of the sex-determining region (Sry), carried by YNPAR, to brain BE concentration. Indeed, the transcription of the Melanocortin 2 receptor gene (Mc2R gene, identified as the proopiomelanocortin receptor gene) depends on the presence of Sry and BE is derived directly from proopiomelanocortin. The results shed light on the sex dependent differences in brain functioning and the role of Sry in the BE system might be related to the higher frequency of autistic disorder in males.

Introduction

The Y chromosome includes the YNPAR and the YFAR. The YNPAR is called non-pairing or specific region and is transmitted from father to sons exclusively. The YFAR recombines with the X chromosome at the male meiosis and is called pairing or pseudoautosomal region for this reason. Few functional genes are mapped on YNPAR [histocompatibility Y antigen, RNA binding motif protein, and several other genes contributing to male reproduction such as Sry and genes necessary for the spermatogenesis development and maintenance]. Several lines of evidence suggest a possible effect of YFAR on brain opioid, and more specifically on brain β-endorphin (BE). Neonatal injection of testosterone decreases brain BE concentration and the number of μ receptors in the hypothalamus (μ receptors are receptors of BE) [1,2]. In addition, neonatal injection or exposure to testosterone contributes to the “male pattern” of the ontogenesis of μ receptors in the hypothalamus and to the development of BE innervations in the brain [2–6]. Inversely, intracerebro-ventricular injection of BE decreases plasma Luteinizing Hormone concentration and consequently plasma testosterone concentration; this effect involves the μ receptors and is blocked by the preliminary administration of Naloxone (an antagonist of the μ receptors) [7–10]. Given these previous observations and because YNPAR is involved in plasma testosterone concentration and testicular reactivity to testosterone [11,12], the YNPAR is expected to be associated with brain BE.
This hypothesis of an effect of the Y\textsuperscript{\textsc{par}} on brain BE is also supported by studies in mice models and in humans showing an inhibitory influence of central opioids acting through the \(\mu\) receptors (such as BE) on aggressive behavior [13–20], and an effect of the murine Y\textsuperscript{\textsc{par}} on aggression [21–25]. In addition, Laarakker et al.'s study [26] reporting a contribution of the Y\textsuperscript{\textsc{par}} to anxiety-related behavior in mice, strengthens the hypothesis of an effect of the Y\textsuperscript{\textsc{par}} or brain BE, given that BE is considered as a stress hormone [27–29].

Finally, in humans, and more precisely in autistic disorder, two other arguments support our hypothesis: on one hand, the fourfold higher prevalence of autism in males compared to females [30] (autism is a pervasive developmental disorder for which family and twin studies suggest a genetic contribution [31–33]) could indicate a contribution of the Y\textsuperscript{\textsc{par}}, on the other hand, several studies have reported abnormal central as well as peripheral BE levels in individuals with autism [34].

The present study was designed first to test directly the effect of Y\textsuperscript{\textsc{par}} on brain BE levels in two highly inbred strains of mice, NZB/BINJ (N) and CBA/HGnc (H), and their consomic strains for the Y\textsuperscript{\textsc{par}}. Second, an indirect effect of the Y\textsuperscript{\textsc{par}} on brain BE level via plasma testosterone was tested by studying the genetic correlation between brain BE concentration and plasma testosterone concentration in eleven highly inbred strains of mice.

**Methods**

**Mice and rearing conditions**

Brain β-endorphin and plasma testosterone were measured in a set of inbred strains of mice and of a quartet of parental and their consomic strains for the Y\textsuperscript{\textsc{par}}. The set of inbred strains consisted in 11 inbred mouse strains A/J, XLII, BA, BALB/cBy, C57BL/10Bg, C57BL/6jBy, CPB-K, DBA/1Bg, DBA/2j, CBA/HGnc and NZB/BINJ. The strains were maintained in the laboratory for six or more generations of brother-sister mating regimen. The quartet of parental and their consomic strains for the Y\textsuperscript{\textsc{par}} was developed as follows: we selected two strains of laboratory mice NZB/BINJ (N) and CBA/HGnc (H) and their consomic strains for the Y\textsuperscript{\textsc{par}}. The set of inbred strains consisted in 11 inbred mouse strains A/J, XLII, BA, BALB/cBy, C57BL/10Bg, C57BL/6jBy, CPB-K, DBA/1Bg, DBA/2j, CBA/HGnc and NZB/BINJ. The strains were maintained in the laboratory for six or more generations of brother-sister mating regimen. The quartet of parental and their consomic strains for the Y\textsuperscript{\textsc{par}} was developed as follows: we selected two strains of laboratory mice NZB/BINJ (N) and CBA/HGnc (H) and their consomic strains for the Y\textsuperscript{\textsc{par}}. The strains were maintained in the laboratory for six or more generations of brother-sister mating regimen.

**Methods**

**Statistical analyses**

The effect of the Y\textsuperscript{\textsc{par}} on brain BE level and plasma testosterone concentration was studied using a two-way ANOVA, with genetic background -H versus N- and origin of the Y\textsuperscript{\textsc{par}} -H versus N- as main factors. Effect size was calculated using the \(\theta^2\) statistic and expressed as a percentage of variance [41]. Comparisons between the 11 inbred mouse strains for BE or testosterone variables were performed using ANOVA. The Kolmogorov-Smirnov test indicated that BE and testosterone levels were not normally distributed; thus all ANOVAs were performed using log-transformed BE and testosterone values. Correlations between brain BE levels and plasma testosterone concentrations in the 11 inbred mouse strains (each strain is represented by the mean of five animals for biological variables) were determined by Spearman rank-order correlation analyses. According to Hegmann and Possidente [42], these correlations between biological measures can be considered as an estimation of genetic correlations. The significance level was set at 0.05; however, the usual
level of 0.05 is very conservative when the correlations are computed on mean scores and not on individual scores.

**Results**

**Effect of the Y^NPAR on brain β-endorphin levels**

Brain BE levels were significantly modified by the non-Y^NPAR genotype and the Y^NPAR in interaction with non-Y^NPAR genotype (F(1,17) = 4.80, P<0.04, r^2 = 0.10 and F(1,17) = 27.18, P<0.0001, r^2 = 0.56, respectively). The Y^NPAR alone did not contribute significantly to BE concentration (F(1,17) = 1). Partial comparisons indicated that BE levels were significantly higher in the H strain than in the N strain (t (1.47) = 2.43, P<0.05). The substitution of the Y^NPAR from H by the Y^NPAR from N, in the H strain, reduced significantly the brain BE concentration, and the substitution of the Y^NPAR from N by the Y^NPAR from H, in the N strain, reduced the brain BE level to the lowest concentration (see Figure 1).

**Effect of the Y^NPAR on plasma testosterone concentration**

The non-Y^NPAR genotype, the Y^NPAR region and their interactions modulated significantly plasma testosterone concentration (F (1,17) = 48.42, P<0.0001, r^2 = 18.20; F(1,17) = 109.15, P<0.0001, r^2 = 0.41 and F(1,17) = 72.63, P<0.0001, r^2 = 0.27, respectively). Partial comparisons indicated that testosterone concentration were significantly higher in the N strain than in the H strain (t (5.99) = 11.97, P<0.0001). The substitution of the Y^NPAR from N by the Y^NPAR from H, in the N strain, reduced significantly the testosterone concentration (t (5.99) = 13.37, P<0.001), whereas the opposite replacement of the Y^NPAR from H by the Y^NPAR from N, in the H strain, did not modify the testosterone concentration (see Figure 2).

![Figure 1. Brain BE concentration (mean ± SEM) in NZB and CBA/H and their consomic strains for Y^NPAR. The N.H-Y^NPAR differs only from the NZB by the Y^NPAR from CBA/H, and the H.N-Y^NPAR differs only from the CBA/H by the Y^NPAR from NZB. Partial comparisons with Student’s t test showed that the parental NZB and CBA/H strains differed significantly (P<0.05), and each parental strain differed significantly from its consomic strain (CBA/H vs. H.N-Y^NPAR: P<0.001; NZB vs. N.H-Y^NPAR: P<0.01); n = 5 animals for each strain, except for NZB (n = 6); SEM = standard error of the mean. doi:10.1371/journal.pone.0016704.g001](image1)

![Figure 2. Plasma testosterone concentration (mean ± SEM) in NZB and CBA/H and their consomic strains for Y^NPAR. The N.H-Y^NPAR differs only from the NZB by the Y^NPAR from CBA/H, and the H.N-Y^NPAR differs only from the CBA/H by the Y^NPAR from NZB. Partial comparisons with Student’s t test showed that the parental NZB and CBA/H strains differed significantly (P<0.0001), and the parental NZB strain differed significantly from its consomic strain (NZB vs. N.H-Y^NPAR: P<0.001); n = 10 animals for each strain; SEM = standard error of the mean. doi:10.1371/journal.pone.0016704.g002](image2)
Correlation between \( \beta \)-endorphin and testosterone

The data of brain BE levels and plasma testosterone concentrations (mean ± SEM) in the 11 inbred strains are presented in Table 1. The 11 inbred mouse strains did not differ significantly for BE or testosterone concentrations. In addition, there was no correlation between brain BE levels and plasma testosterone concentration when the analysis was conducted on the eleven different inbred strains including the same N and H males contributing to the present study (\( \rho \) spearman = 0.09, \( P > 0.1 \)).

<table>
<thead>
<tr>
<th>Biological variables</th>
<th>A/J</th>
<th>C57BL/10Bg</th>
<th>C57BL/6</th>
<th>BA</th>
<th>BALB/cBy</th>
<th>CBA/H</th>
<th>CPB-K</th>
<th>DBA/18Bg</th>
<th>DBA/2j</th>
<th>NZB</th>
<th>XLII</th>
<th>( F ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )-endorphin (pmoles/g)</td>
<td>271.5</td>
<td>403.4</td>
<td>143.8</td>
<td>111.8</td>
<td>191.0</td>
<td>351.0</td>
<td>133.4</td>
<td>361.2</td>
<td>342.6</td>
<td>191.8</td>
<td>99.2</td>
<td>1.31</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>±35.4</td>
<td>±250.2</td>
<td>±24.6</td>
<td>±8.7</td>
<td>±17.8</td>
<td>±59.1</td>
<td>±32.1</td>
<td>±260.1</td>
<td>±191.4</td>
<td>±37.42</td>
<td>±16.5</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Note: Comparisons between the 11 strains for \( \beta \)-endorphin and Testosterone concentrations were performed using ANOVA with \( F \) values indicated in the table; \( n = 5 \) animals for each strain, except for NZB/BINJ (\( n = 6 \)); SEM = standard error of the mean.

doi:10.1371/journal.pone.0016704.t001

Discussion

Our main data indicated a significant and major effect of the \( Y \)\(^{\text{YNPAR}} \) in interaction with the genetic background on brain BE levels. The controls to ensure the isogenicity of the background in each parental strain and its consomic strain rule out a possible contribution of residual autosomal alleles from the donor strain to this effect of the \( Y^{\text{YNPAR}} \) in interaction with the genetic background. None of the annotations of the genes carried by the \( Y^{\text{YNPAR}} \) allows us to consider that one of them contributes directly in the BE production [43].

An indirect effect of the \( Y^{\text{YNPAR}} \) in interaction with the genetic background on brain BE levels via plasma testosterone is not supported by our findings. Indeed, there was no correlation in this study between brain BE levels and plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone measured in the parental N and H and their consomic strains inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentration between the 11 inbred strains. In addition, our results on plasma testosterone concentrations, although this research is limited by the absence of variability of BE or testosterone concentra...


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