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SHORT COMMUNICATION

Baseline plasma epinephrine levels predict Wisconsin Card Sorting Test scores in healthy volunteers

Christoph Czermak [a,*], Sandra J. Wallner [b], Adelheid Kresse [b], Silvia Schauer [c], Reingard Aigner [d], Gerald Hoefler [c], Michael Lehofer [a], Peter M. Liebmann [b]

[a] *Department of General Psychiatry I, Sigmund Freud Clinics Graz, Wagner Jauregg Platz 1, 8053 Graz, Austria*
[b] *Institute of Pathophysiology and Immunology, Medical University of Graz, Graz, Austria*
[c] *Department of Pathology, Medical University of Graz, Graz, Austria*
[d] *Department of Nuclear Medicine, Medical University of Graz, Graz, Austria*

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Summary The present study was undertaken to further explore the potential neuropsychological information associated with baseline plasma levels of catecholamines and dopamine D3 receptor (DRD3) mRNA expression in peripheral blood lymphocytes (PBL). Baseline plasma norepinephrine and epinephrine levels and PBL DRD3 mRNA expression were compared with performance in the Wisconsin Card Sorting Test (WCST) in $n = 79$ healthy volunteers (mean $\pm$ S.D. age: 24.1 $\pm$ 3.2 years, 34 males). After correction for multiple testing, we found that baseline plasma epinephrine levels predicted WCST total number of errors (Spearman's $\rho = -0.36$, $p < 0.05$), number of perseverative responses (Spearman's $\rho = -0.36$, $p < 0.05$) and percent conceptual level responses (Spearman's $\rho = 0.37$, $p < 0.05$). Plasma norepinephrine levels and PBL DRD3 mRNA expression did not predict WCST scores, but PBL DRD3 mRNA expression correlated negatively with plasma epinephrine levels (Spearman's $\rho = -0.45$, $p < 0.001$). Further studies should be undertaken to explore possible neurophysiological links between plasma epinephrine levels and the neurobiology underlying cognitive performance.

1. Introduction

Previous studies reported that increases in plasma levels of norepinephrine and epinephrine during physical exercise were associated with alterations in cognitive performance in healthy humans (Chmura et al., 1994; McMorris et al., 1999; Winter et al., 2007). In a recent study, we found a negative correlation between dopamine D3 receptor (DRD3) mRNA expression in peripheral blood lymphocytes (PBL) and Cloninger's personality trait of persistence in healthy volunteers (Czermak et al., 2004). In conclusion we and others hypothesized that PBL DRD3 mRNA expression, as well as plasma levels of catecholamines, may to a certain degree reflect monoaminergic neurotransmission in the prefrontal cortex (PFC) (McMorris et al., 1999; Czermak et al., 2004; Genuth, 2004).

* Corresponding author. Tel.: +43 316 2191 2936; fax: +43 316 2191 3211.
E-mail address: christoph.czermak@kages.at (C. Czermak).

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Monoaminergic neurotransmission in the PFC strongly influences executive functions (Arnsten and Li, 2005), which in humans can be assessed by neuropsychological instruments, such as the Wisconsin Card Sorting Test (WCST). The WCST also contains a specific score on cognitive perseverance, describing the tendency to persist in cognitive strategies without further reinforcement (Heaton et al., 1993). This appears similar to Cloninger's personality trait of persistence, describing maintenance of behaviour without further reinforcement (Cloninger et al., 1993).

Based on our previous finding of a correlation between PBL DRD3 mRNA expression and the personality trait of persistence (Czermak et al., 2004), we therefore hypothesized a specific association between PBL DRD3 mRNA expression and the WCST perseveration test score in healthy volunteers. However, based on the hypothesis that plasma levels of catecholamines and PBL DRD3 mRNA expression may more generally reflect monoaminergic neurotransmission in the PFC, we extended our hypotheses to correlations between these peripheral parameters and WCST scores in general.

2. Methods

2.1. Subjects

Seventy-nine healthy subjects (34 males and 45 females) participated in the study. All participants were medical students at the University of Graz, Austria, and recruited by public advertisement. Demographic characteristics for the male subjects were as follows: mean ± S.D. age: 24.4 ± 2.9 years (range: 20–31 years), mean ± S.D. body mass index: 22.3 ± 2.1 kg/m^2 (range: 20.0–27.8). Demographic characteristics for the female subjects were as follows: mean ± S.D. age: 23.7 ± 3.1 years (range: 18–33 years), mean ± S.D. body mass index: 21.7 ± 1.7 kg/m^2 (range: 20.1–26.4). Thirty-two of the female subjects were taking oral contraceptives. Female subjects not taking oral contraceptives were studied during the follicular phase of the menstrual cycle, and in addition, premenstrual dysphoric disorder was regarded as an exclusion criterion for participation in the study.

Any other current or past psychiatric, neurological, cardiovascular, endocrinological, immunological, as well as any current infectious or inflammatory disease, as assessed by medical anamnesis and standard laboratory analyses, were regarded as exclusion criteria. Except for female subjects taking oral contraceptives, all subjects were free of medication. All subjects were non-smokers. All procedures were carried out with the adequate understanding and written consent of the subjects. The study was approved by the local ethics committee (Medical University of Graz, Nr. 14-063 ex 03/04).

2.2. Blood draws and WCST application

All subjects stated that they had no prior knowledge of the WCST. Before application of the test, all subjects were tested for normal colour perception. On the day of the experiment, subjects entered the testing facility at about 09:00 h. Subjects then rested in supine position for about 15 min, before 18 ml of venous blood were drawn from a cubital vein. Thirty minutes after the blood draw, the WCST was applied according to the WCST guidelines (Heaton et al., 1993), in each subject by the same trained and evaluating person (Wallner, S.J.). As WCST output parameters, we used the raw scores of number of errors (perseverative and non-perseverative errors, the latter indicating inattention), number of perseverative responses, percent conceptual level responses (reflecting insight into the correct sorting principles), and failure to maintain set (indicative of the ability to maintain the correct sorting principle).

2.3. Determination of PBL DRD3 mRNA expression

Determination of PBL DRD3 mRNA expression was done as described in detail elsewhere (Czermak et al., 2004). In brief, PBL were obtained from peripheral blood samples using cell separation tubes (9 ml, Becton Dickinson, Austria), washed in calcium–magnesium free buffer, and stored at −80 °C in RNeasy Lysis Buffer (Qiagen, Germany). Total RNA was isolated from PBL by QIAamp RNA Blood Mini Kit (Qiagen) and amount and purity determined by spectrophotometry.

Total RNA was reverse transcribed into first-strand cDNA by using Random Hexamers and MultiScribe™ Reverse Transcriptase (Applied Biosystems, Austria). Real-time polymerase chain reaction (PCR) was carried out in a 5700 AbiPrism Sequence Detection System (Applied Biosystems), using a double-labeled fluorogenic DNA probe to monitor cDNA amplification. PCR was started with uracil-N-glycosylase incubation at 50 °C for 2 min and enzyme activation at 95 °C for 10 min. PCR cycles consisted of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min.

Intron-spanning primers and probes for the DRD3 and β-actin (Table 1), used as housekeeping gene, were designed to avoid known polymorphisms in the respective genes. Measurements were done in duplicate and the mean used for further analysis. For each sample a PCR cycle threshold value was determined from the cycle at which the sample fluorescence met a pre-set threshold. PCR cycle threshold values for the DRD3 were referenced to β-actin values according to the method described by Pfaffl (2001). DRD3 mRNA expression values were obtained in arbitrary units and referenced to the mean value of the entire study sample.

2.4. Determination of norepinephrine and epinephrine plasma levels

Blood samples (9 ml) were collected in chilled tubes containing EDTA anticoagulant. Plasma catecholamines were measured by radioenzymatic technique using a commercial kit (catecholamine—RIA®, Diagnostika GmbH, Hamburg, Germany). All samples were analyzed by the same blinded technician. Assay working ranges were 6–600 pg/ml for epinephrine and 30–3000 pg/ml for epinephrine. The norepinephrine assay intra- and inter-assay coefficients of variation were ∼5.1% and ∼5.8%, respectively. The epinephrine assay intra- and inter-assay coefficients of variation were ∼5.2% and ∼6.3%, respectively.

2.5. Statistical analysis

PBL DRD3 mRNA expression as well as norepinephrine and epinephrine plasma levels were compared with WCST