Psychiatry Research 179 (2010) 231–234

Contents lists available at ScienceDirect

Psychiatry Research

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

Associations between the angiotensin-converting enzyme insertion/deletion polymorphism and monoamine metabolite concentrations in cerebrospinal fluid

Kristina Annerbrink [a,*], Erik G. Jönsson [b], Marie Olsson [c], Staffan Nilsson [d], Göran C. Sedvall [b], Henrik Anckarsäter [e], Elias Eriksson [a]

[a] Department of Pharmacology, University of Gothenburg, S-405 30, Gothenburg, Sweden
[b] Department of Clinical Neuroscience, Karolinska Institutet, S-171 77, Stockholm, Sweden
[c] Apoteket AB, S-413 45, Gothenburg, Sweden
[d] Department of Mathematical Statistics, Chalmers University of technology, S-412 96, Gothenburg, Sweden
[e] Institute of Clinical Sciences, Lund University, S-205 02, Malmö, Sweden

ARTICLE INFO

Article history:
Received 19 February 2008
Received in revised form 17 March 2009
Accepted 9 April 2009

Keywords:
Angiotensin
ACE I/D
Serotonin
5-HIAA
Dopamine
HVA

ABSTRACT

Angiotensin II has been suggested to influence central dopamine and serotonin turnover. Since the angiotensin-converting enzyme (ACE) plays a key role in angiotensin regulation by converting inactive angiotensin I to active angiotensin II, we hypothesised that the functional insertion/deletion (I/D) polymorphism in the ACE gene, which has previously been suggested to be associated with, depression and panic disorder, may influence monoamine activity. A well-established technique for assessing brain monoamine turnover in humans is to measure concentrations of monoamine metabolites in the cerebrospinal fluid (CSF). We thus investigated possible associations between the ACE I/D polymorphism and CSF monoamine metabolite concentrations in a population of healthy male subjects. After having found such an association between the ACE I/D polymorphism and CSF levels of the dopamine metabolite homovanillic acid and the serotonin metabolite 5-hydroxyindoleacetic acid in this sample, I carriers displaying lower levels, we tried to replicate this observation in a population of violent male offenders from which also both CSF and DNA were available. Also in this sample, the same associations were found. Our results suggest that the ACE I/D polymorphism may play a role in the modulation of serotonergic and dopaminergic turnover in men.

1. Introduction

Serotonin, dopamine and noradrenaline are monoaminergic neurotransmitters of importance for numerous psychiatric disorders. Measurement of cerebrospinal fluid (CSF) levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), the dopamine metabolite homovanillic acid (HVA), and the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) has been used in many studies as an indirect way to assess the central turnover of these compounds (Agren, 1980; Asberg and Traskman, 1981). Studies of human twins indicate that CSF levels of 5-HIAA, HVA and MHPG are under both genetic and environmental influence (Oxenstierna et al., 1986). In addition, in rhesus monkeys (Higley et al., 1993) and baboons (Rogers et al., 2004), CSF levels of 5-HIAA, HVA and MHPG are shown to be largely, but not exclusively, determined by genetic factors.

The renin angiotensin system (RAS) appears to exert a complex influence on brain dopaminergic transmission. In rat striatum, where dopaminergic neurons express angiotensin II (Ang II) type 1 (AT_1) receptors (Allen et al., 1992), administration of Ang II induces an increase in extracellular levels of dopamine (in the presence of a dopamine reuptake inhibitor), as well as in the formation of the dopamine metabolites HVA and DOPAC, suggesting Ang II to exert mainly a stimulatory influence on dopamine transmission (Jenkins, 2008; Jenkins et al., 1997a). On the other hand, the same authors have shown angiotensin-converting enzyme (ACE) inhibitors, which should reduce the formation of Ang II, to enhance dopamine release and turnover (Jenkins, 2008; Jenkins et al., 1997b), effects that may have a bearing on the mood-lifting (Gard, 2004) and anti-Parkinson (Reardon et al., 2000) actions that have been attributed to these antihypertensive agents. Moreover, in addition to exerting acute effects on dopamine release and turnover, angiotensin II has been reported not only to facilitate the formation of dopaminergic nerve cells (Rodriguez-Pallares et al., 2004), but also to promote oxidative stress-induced degeneration of dopamine neurons (Munoz et al., 2006).

The interaction between angiotensin and serotonin is less well studied. It has however been shown that angiotensin II reduces central serotonin release in rats (Tanaka et al., 2003; Voigt et al., 2005), and that an AT_1 receptor antagonist enhances serotonin formation (Jenkins, 2008).

* Corresponding author. Department of Pharmacology, Göteborg University, POB 431, SE 405 30 Göteborg, Sweden. Tel.: +46 31 7733448; fax: +46 31 821085.
E-mail address: kristina.annerbrink@pharm.gu.se (K. Annerbrink).

0165-1781/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.psychres.2009.04.018

A polymorphism in the ACE gene constituted by an insertion (I)/deletion (D) of 287 nucleotides in intron 16 has been reported to account for about half of the phenotypic variance in serum ACE levels. Subjects homozygous for the long allele (I/I) display the lowest concentrations; subjects homozygous for the short allele (D/D) display the highest; and heterozygous subjects (I/D) display ACE concentrations in between (Rigat et al., 1990). Whereas numerous studies have been published suggesting the ACE I/D polymorphism to be associated with cardiovascular function and dementia (Sayed-Tabatabaei et al., 2006), an increasing body of evidence suggests that it may also be associated with psychiatric conditions such as panic disorder (Olsson et al., 2004), depression (Baghai et al., 2006), schizophrenia (Crescenti et al., 2009) and suicide (Sparks et al., 2009), hence emphasising the importance of possible interactions between RAS and monoaminergic transmission. To our knowledge, no studies have however been published assessing the possible association between the AC I/D polymorphism and endophenotypes reflecting monoaminergic activity.

Prompted i) by the studies suggesting the ACE I/D polymorphism to be associated with serotonin- and dopamine-related psychiatric disorders, ii) by the animal experiments revealing interactions between RAS on the one hand, and serotonin and dopamine transmission on the other and iii) the tentative mood-lifting effect of drugs counteracting angiotensin activity, we deemed it of interest to explore to what extent the ACE I/D polymorphism may influence monoamine metabolite concentrations in lumbar CSF. To this end, we first investigated possible associations between this polymorphism and CSF concentrations of 5-HIAA, HVA and MHPG in a population comprising healthy male subjects. After having found significant associations in this group, we tried to replicate this observation using a sample comprising violent male offenders from which also both CSF and DNA were available. The associations found in the first sample were also present in the second one.

2. Methods

2.1. Ethical considerations

All investigations were carried out in accordance with the Declaration of Helsinki. The Ethics Committee of the Karolinska Hospital, Stockholm, approved the part of the study concerning healthy volunteers, and the Ethics Committee of Göteborg University, Göteborg, approved the part concerning violent offenders. Informed consent was obtained from all participants after the nature of the procedures had been fully explained.

2.2. Subjects

2.2.1. Healthy males

Healthy Caucasian men ($n=46$) were recruited predominately among students and hospital staff in the Stockholm area. Subjects who fulfilled Diagnostic and Statistical Manual of Mental Disorders, third edition, revised (DSM-III-R) criteria for any psychiatric disorders according to a structured interview performed 8–19 years after the lumbar puncture (LP) were excluded from the first analysis in line with the protocol of the study. All participants were drug free at LP. The mean age at LP was 28.2 years (range 18–43). For further information on this population, see a previous paper by Jonsson et al. (2000).

2.2.2. Violent male offenders

In an attempt to replicate the observation made in healthy volunteers, we used a sample comprising arrested violent offenders from which also both CSF and DNA had been obtained. All individuals who had been the subject of inpatient forensic psychiatric investigations in Gothenburg during 1997 ($n=113$) had been screened for participation in a research project aimed at evaluating neuropsychiatric functioning. Inclusion required that the subject be charged with a severe crime (homicide, attempted homicide, aggravated assault, arson, rape or sexual violation of minors), that he had no history of treatment for any major mental disorder, and that no clinical signs suggested the onset of such a disorder. Psychiatric diagnoses according to DSM-IV criteria were made by a forensic psychiatric specialist in consensus with a clinical psychologist, a psychiatric social worker and ward staff on the basis of clinical interviews, neuropsychological tests, personality and psychiatric assessments, physical and neurological examinations, extensive file reviews and close observation on the ward. Both genotype and CSF data were available for 16 of the offenders. Ten subjects fulfilled DSM-IV criterion for alcohol and/or substance abuse or dependence, five for anxiety disorders and two for major depression. Nine subjects did not receive any medication, whereas three were taking antidepressants, two anxiolytics/hypnotics and one antipsychotics. The mean age at LP was 30.3 (range 17–67). Further information regarding this population has been previously published in a paper by Soderstrom et al. (2001).

2.3. CSF sampling procedure

2.3.1. CSF concentrations in healthy males

All subjects had at least 8 h of bed rest in the hospital, abstaining from food and smoking, before LP was performed between 8 and 9 a.m.; 12.5 ml of CSF were obtained with the subjects in either sitting or recumbant position. Samples were stored at below −20 °C and analysed within a few months. 5-HIAA, HVA and MHPG concentrations were measured by mass fragmentography with deuterium-labelled internal standards (Sedvall et al., 1980).

2.3.2. CSF concentrations in violent male offenders

LP was performed between 8 and 9 a.m. with the subjects lying down and fasting since midnight. Twelve-millitre CSF was collected and stored at below −80 °C. Determination of the monoamine metabolites was performed by means of high-performance liquid chromatography with electrochemical detection (see Soderstrom et al., 2001).

2.4. Molecular genetics

Venous blood was collected from each subject, and genomic DNA was isolated using the QIAamp DNA blood Mini Kit (Qiagen, Valencia, CA, USA). The region of interest was amplified in a Perkin–Elmer 9700 thermal cycler. Reactions were carried out using HotstarTaq polymerase from Qiagen in a total volume of 15 µl containing 1.5 mM MgCl2, 0.3 µM primers (5′CTGGAGACCACTCCCATCCTTTCTS3′, 5′GATGTGGCCATCACATTCGT-CAGAT3′), and approximately 50 ng genomic DNA. After an initial 15 min denaturation step at 95 °C, 30 cycles were performed including 30 s at 94 °C 30 s at 58 °C and 30 s at 72 °C. Polymerase chain reaction products for ACE I/D (490 base pairs or 190 base pairs) were separated on 2% agarose gels supplemented with ethidium bromide and visualised by ultraviolet transillumination. This PCR protocol has previously been described by Rigat et al. (1992). Since mistyping of the DD genotype may occur, we confirmed each DD genotype by using an insertion-specific primer (5′TTTGAGACG-GAGTCTCGCTC3′) (Shanmugam et al., 1993).

2.5. Statistics

Linear regression was used for analysing associations between the ACE polymorphism and CSF levels of monoamine metabolites. Correlation measures between the genotype and metabolite levels and partial correlations to control for possible influences of one metabolite on the other were also performed. In the first sample, two-tailed *P*-values were used, and in the second sample, that was investigated to confirm the observations made in the first sample, one-tailed *P*-values were used. *P*-values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Healthy males

There was no significant deviation from the Hardy–Weinberg equilibrium (data not shown). The ACE I/D polymorphism was significantly associated with CSF levels of 5-HIAA ($F=12.4$, $R^2=0.22$, $P=0.001$) and HVA ($F=5.9$, $R^2=0.12$, $P=0.02$), but not MHPG ($F=1.9$, $R^2=0.04$, $P=0.17$) (Table 1). 5-HIAA and HVA concentrations correlated significantly with each other ($F=84.955$, $R^2=0.659$, $P<0.001$). When we correlated ACE I/D with 5-HIAA and controlled for HVA using partial correlation, the correlation remained significant ($P=0.02$). When we correlated ACE I/D with HVA and controlled for 5-HIAA using partial correlation, the correlation did not remain significant ($P=0.6$).

Table 1
Associations between the ACE I/D polymorphism and monoamine metabolite concentrations in CSF in healthy male volunteers.

Variables	I/I ($n=10$)	I/D ($n=23$)	D/D ($n=13$)	*P*-values
5-HIAA	67.8 ± 25.3	86.0 ± 30.5	114.5 ± 39.4	**0.001**
HVA	123.8 ± 42.6	158.3 ± 53.5	192.9 ± 101.5	**0.02**
MHPG	40.6 ± 7.6	41.9 ± 7.1	44.7 ± 7.2	0.17

Mean values ± standard deviations. *P*-values refer to two-tailed linear regression.