PERGAMON

Journal of Psychiatric Research 36 (2002) 105–110

JOURNAL OF PSYCHIATRIC RESEARCH

www.elsevier.com/locate/jpsychires

Panic disorder patients have reduced cyclic AMP in platelets

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Received 26 June 2001; received in revised form 28 September 2001; accepted 11 October 2001

Abstract

Little is known about the intracellular mechanisms involved in the pathophysiology of panic disorder (PD). Abnormalities in the cyclic AMP system have been described in several psychiatric disorders but there are no studies in panic patients. We evaluated not only the levels of platelet cyclic AMP, but also cyclic GMP and nitric oxide synthase (NOS) activity in patients with PD at baseline and after treatment with clomipramine and in healthy volunteers. Platelet cyclic AMP was determined by enzymeimmunoassay, cyclic GMP by radioimmunoassay and NOS activity by the conversion of ^{3}H-arginine to ^{3}H-citruline in 17 PD patients before treatment with clomipramine, after remission of panic attacks and in 22 healthy volunteers. Average baseline cyclic AMP of PD patients was lower than after remission of panic attacks ($P<0.005$) and lower than in healthy volunteers ($P<0.005$). Average cyclic AMP after remission of panic attacks was not significantly different than in healthy volunteers. There were no significant differences in cyclic GMP and NOS analysis. Our results suggest that PD patients without treatment have lower platelets cyclic AMP levels than healthy volunteers and that this decrease may be corrected by clomipramine.

Keywords: Panic disorder; Clomipramine; Platelets; Cyclic AMP; Cyclic GMP; Nitric oxide

1. Introduction

The biochemical mechanisms underlying the pathophysiology of panic disorder (PD) remain unclear. Little is known about the role of signal transduction pathways in this disorder, although these processes have been investigated in other several psychiatric disorders. Alterations in cyclic AMP signaling have been described in depression (Shelton et al., 1999); obsessive-compulsive disorder (Perez et al., 2000a); bipolar disorder (Fields et al., 1999; Perez et al., 2000b) and schizophrenia (Tardito et al., 2000). Moreover, chronic administration of antidepressant drugs has been shown to alter this pathway (Nestler et al., 1989; Duman et al., 1997). To our knowledge, there are no studies evaluating cyclic AMP signaling in panic patients.

The cyclic AMP cascade represents an important regulatory mechanism of protein phosphorylation. Cyclic AMP binds to the regulatory subunit of the cyclic AMP-dependent protein kinase (PKA) leading to the dissociation of the catalytic subunit, which will enhance protein (serine/threonine) phosphorylation (Walaas and Greengard, 1991). It is important to emphasize that components of the cyclic AMP pathway are likely to be among a very large number of proteins that have altered expression as a consequence of CREB (cyclic adenosine monophosphate response element-binding protein) activation. In fact, adaptations induced by antidepressants have been reported for several receptors, transporters and other signaling proteins (Hyman and Nestler, 1996; Duman et al., 1997).

Another pathway with possible involvement in PD is nitric oxide (NO) and cyclic GMP system. This second messenger is produced after increase of intracellular calcium, by Ca^{2+} channels or through the liberation of reticulum endoplasmatic calcium. The later is achieved by activation of phospholipase C (PLC) with the production of inositol triphosphate (IP_3), a product of phosphatidylinositol-4,5-biphosphate (PIP_2) hydrolysis.

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0022-3956/02/$ - see front matter
PII: S0022-3956(01)00053-X

Ca^{2+} activates constitutive nitric oxide synthase (NOS), a calmodulin dependent enzyme, producing NO. NO acts on soluble guanylyl cyclase with the formation of cyclic GMP which in turn can activate the cyclic GMP-dependent kinase (PKG) involved in phosphorilation processes (Wang and Robinson, 1995).

Plein and Berk (1999) have investigated platelets Ca^{2+} levels in panic patients after stimulation with serotonin and thrombin, in order to evaluate serotonergic involvement in this disorder. They found Ca^{2+} increase with thrombin but not with serotonin stimulation.

In order to clarify the role of those mechanisms in the pathophysiology of panic disorder, this study explores (1) the involvement of platelets NO, cyclic GMP and cyclic AMP in this clinical condition and (2) the effect of the treatment with clomipramine, an effective drug in this disorder (Gentil et al., 1993).

2. Methods

2.1. Subjects

2.1.1. Panic disorder patients

Twenty-one patients (17 females) aged 18–53 years, meeting DSM-IV (American Psychiatric Association, 1994) criteria for panic disorder with or without agoraphobia were included in the study. In addition to the usual exclusion criteria for clinical trials (pregnancy, any other relevant medical disorder), patients with a history of either psychosis, primary major affective disorder, alcohol or drug abuse were excluded as well as patients with concurrent diagnosis of other anxiety disorders. Patients under any pharmacological treatment were not included. Most patients were not receiving any treatment for panic disorder at screening. Some of them were treated with antidepressants or benzodiazepines but did not reach full remission of panic attacks. For these patients washout was 1 month for benzodiazepines, tricyclic antidepressants and SSRIs; and 2 months for fluoxetine. No cognitive-behavior treatment or exposure instructions were given. Fifteen patients had agoraphobic symptoms; the severity of avoidance was mild (13%); moderate (60%) and severe (27%).

A general physical examination, routine laboratory tests and platelet function, were performed.

2.1.2. Healthy volunteers

Twenty-two healthy volunteers (13 females), aged 18–50 years not using any psychotropic drug during the last 3 years were selected as controls. Subjects selected were those who did not reach cut-off scores in the Self-Report Questionnaire (Harding et al., 1980), and did not meet diagnostic criteria for any psychiatric disorder (DSM-IV criteria) during the last year, as assessed by the semi-structured clinical interview Schedules for Clinical Assessment in Neuropsychiatry (SCAN; Wing et al., 1990) applied by a trained psychiatrist. They were not under any pharmacological treatment and had no relevant medical disorder. Two of them had family history of alcohol abuse; no other established psychiatric diagnosis among relatives was reported.

The study was approved by the hospital's ethics committee and all subjects signed informed consent forms.

2.2. Drugs and experimental design

Patients were treated in an open, flexible dose design, with normal clomipramine form (CMI) until remission of panic attacks. The daily dose was given half in the morning and half at bedtime. Patients received 25 mg/day of CMI for the first week and increments were prescribed according to clinical judgment. Clinical assessments were carried out at baseline (0) and weeks 1, 4, 7, 10, 13 and at remission if it had not occurred until week 13.

The operational criteria for remission was three weeks with no panic attacks as declared by the patients, absence of depressive or anticipatory anxiety symptoms, and significant reduction of avoidance to usual situations. They should not meet criteria for DSM-IV panic disorder, generalized anxiety or major depression.

Blood samples (25 ml) were collected at the morning (8–9 a.m.) for the determination of cyclic AMP, cyclic GMP and NOS at baseline and after remission of panic attacks, which was evaluated through the "Clinical Global Impression" scale (severity of illness, global improvement forms).

Blood collection was done once (8–9 a.m.) for the healthy volunteers.

2.3. Rating scales

The following scales were used: The "Clinical Global Impression" Scale (CGI; Guy et al., 1976)—severity of illness (from zero—normal, to six—extremely ill); global improvement (from zero—very much improved, to six—very much worse); therapeutic effects (from one—unchanged or worse, to four—marked); and side effects forms (from one—none, to four—intense); the "Beck Depression Inventory" (BDI; Beck et al., 1961); the "Hamilton Depression Scale" (HDS; Hamilton, 1960); the "Hamilton Anxiety Scale" (HAS; Hamilton, 1959); the "Bandelow Panic and Agoraphobia Scale" (BPAS; Bandelow, 1992); the 35 item "Patient-Rated Anxiety Scale"—interview and self-rating forms (PRAS; Sheehan, 1983).

The sum of scores for PRAS item number 32 (severity of spontaneous attacks with more than three symptoms), item 33 (severity of limited panic attacks or symptoms) and item 35 (severity of situational panic attacks) were used as an index of "severity of panic" (PRAS-P; Gentil et al., 1993).