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The protein kinase A in platelets from patients with panic disorder

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Abstract

Although previous studies suggested that dysfunctions in the protein kinase A (PKA) and in some of its substrates are associated with several psychiatric disorders, there is no evidence regarding the possible involvement of such components in panic disorder (PD). Thus, the aim of the present study was to investigate the levels of PKA and Rap1 in platelets from patients with such disorder. Twenty-four drug free patients with PD and 24 healthy volunteers participated to the study. Employing the Western Blot analysis, immunostaining and computer-assisted imaging, the levels of the regulatory (R, type I and type II) and the catalytic (C) subunits of PKA, and those of Rap1 were assessed in platelets from the two groups. The data show that patients with PD have significantly higher levels of platelet RI and C subunits of PKA than controls, whereas the levels of RII were unchanged. No significant differences were found in the immunolabelling of Rap1 between groups. These findings may provide clues toward understanding the involvement of cAMP signalling in anxiety disorders.

Keywords: Panic disorder; cAMP signalling; PKA; Rap1; Platelets

1. Introduction

The cAMP signalling pathway is implicated in the intracellular events mediated by various neurotransmitters and neuropeptides. Previous studies have shown that PKA, a key element of the cAMP signalling, and some of its substrates are involved in the regulation of motor and emotive behaviour, memory, stress and also in the action of psychotropic medications (Abel et al., 1997; Adams et al., 1997; Brandon et al., 1998; Silva et al., 2000; Perez et al., 2000; Feliciello et al., 2001). Furthermore, clinical investigations have reported that abnormalities in such components are present in peripheral cells and postmortem brains of patients with affective disorders (Perez and Tardito, 2001; Stewart et al., 2001).

Panic disorder (PD) is a common mental illness, characterized by recurrent panic attacks which consist of episodes of intense fear or anxiety accompanied by symptoms of cognitive and autonomic arousal. Epidemiological data of PD have reported lifetime prevalence ranging from 1.6 to 3% and a higher risk for females (Weissman et al., 1997).

The biochemical mechanism(s) underlying the pathophysiology of this disorder remain still unclear. However, an increasing body of evidence suggests that dysfunctions of neurotransmitters and neuropeptides including monoamines, GABA, acetylcholine and cholecystokinin could be associated with PD (Sullivan et al., 1999; Grove et al., 1997; Goddard et al., 2001; Battaglia et al., 2001; Kennedy et al., 1999). Furthermore, functional alterations in upstream components of the cAMP signalling as well as lower levels of cAMP have been reported in PD patients (Charney et al., 1989; Maddock et al., 1993; Gurguis et al., 1997; Marcourakis et al., 2002). Although these alterations may have associated effects on downstream elements of

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cAMP signalling, no evidence is available regarding the involvement of such components in PD. Thus, the aim of this study was to assess the levels of PKA and Rap1 in patients with PD.

2. Experimental procedures

2.1. Subjects

This study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Patients, admitted to the San Raffaele Hospital (Milan, Italy), that met the full DSM-IV criteria for PD were recruited. At the baseline assessment, the patients were required to have a total score ≥20 on the Hamilton Rating Scale for Anxiety (HAM-A) and a total score of ≥18 on the Panic Associated Symptoms Scale (PASS). Those with a history of important acute or chronic medical illness, or alcohol or psychotropic drug abuse were excluded from the study. Lifetime diagnoses were assigned by trained psychiatrists and supervised by an independent senior psychiatrist on the basis of unstructured clinical interviews and medical records, according to DSM-IV criteria. Clinical information was collected directly from each patient and from at least 1 close relative as a coinformant. Medical health was documented by the medical history, physical examination, electrocardiography, blood and serum chemical analyses (including hepatic and renal profiles), and thyroid function tests.

The severity of symptomatology was evaluated using HAM-A and PASS, administered by trained psychiatrists, who had a good inter-rater reliability.

After providing written informed consent, 24 outpatients (six male and 18 female, mean age 35.2±10.6 years) entered into the study. The mean age at onset was 29.8±8.5 years. Patients had been free of all medications for at least 1 month before blood specimens were drawn.

The comparison group included 24 age and sex comparable healthy consenting volunteers (eight male and 16 female; mean age 36.0±11.6 years), who had no personal or familial history of mental disorders, alcoholism, or drug abuse and no active medical problems, as determined by clinical interview. The healthy subjects had been free of all medications for at least 3 months before entering the study. None of the subjects were part of previous studies.

2.2. Platelets isolation and immunoblot analysis

A morning blood specimen was obtained from healthy volunteers and patients by venipuncture and placed in tubes containing sodium citrate as anticoagulant. Platelets were isolated, prepared, and then analyzed by personnel unaware of the diagnosis as previously described (Perez et al., 1999).

Aliquots of platelet homogenates with equal amount of proteins (5 μg) from patients with PD and their respective controls were subjected in duplicate or triplicate to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK). Immunoblotting experiments were performed as previously described (Perez et al., 1999). Briefly, the membranes were blocked for 1 h in Tris buffered salina-Tween 20 buffer–1% bovine serum albumin (BSA). Then, the membranes were incubated for 90 min with monoclonal antibodies against the RI, the RII and the C subunits of PKA, Rap1 and actin, a cytoskeletal protein used as an internal control, diluted in blocking buffer. The monoclonal antibodies against RI, C and Rap1 were purchased from Transduction Laboratories (Lexington, KY, USA); the anti RII was from Biomol (Plymouth Meeting, PA, USA), and the monoclonal anti actin (clone DC40) was from Sigma–Aldrich (St. Louis, MO, USA). Next, the membranes were incubated with a secondary antibody conjugated with horseradish peroxidase in blocking buffer. Several washes with TBST were performed between the steps. The Western blots were developed with the ECL™ (Amersham Pharmacia Biotech) and exposed to films. Quantitation of the immunoreactivities was performed by densitometric scanning of the autoradiograms using an image analysis system. An aliquot of pooled standard platelet proteins was run on one lane of every gel to minimize the inter-assay variation, as previously described (Perez et al., 1999). The optical density units obtained from each immunoreactive band were normalized against those from the pooled platelet standard.

2.3. Statistical analysis

Comparisons between groups were performed using two-tailed Student's *t*-test. Results are expressed as means±S.D. A significance level of 0.05 was used for all comparisons.

3. Results

The PKA subunits, Rap1 and actin immunoreactivities were assessed in whole platelet proteins from 24 drug free patients with panic disorder and from 24 age and sex comparable healthy volunteers.

As shown in Fig. 1, the immunolabelling of the RI subunit of PKA was significantly higher in platelets of patients with PD than in control subjects ($P<0.001$, $t=6.92$; df=46).

The analysis revealed significant differences also in the levels of the C subunit of the enzyme (Fig. 2). In particular, patients with PD displayed significantly higher levels of this protein with respect to controls ($P<0.001$, $t=6.92$; df=46).

No significant differences were observed in the immuno-