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ORIGINAL RESEARCH

Adenylate-cyclase activity in platelets of patients with obsessive-compulsive disorder

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Abstract: Although the main biological hypothesis on the pathophysiology of obsessive-compulsive disorder (OCD) is centered on the serotonin system, indications are available that other neurotransmitters, and even second messengers, particularly the cyclic adenosine monophosphate (cAMP) signaling, may be involved, though effective data are few. Therefore, the aim of the present study was to evaluate and compare the basal and isoprenaline (ISO)-stimulated velocity of adenylate-cyclase (AC) in human platelet membranes of patients with OCD and healthy control subjects. The results showed that the basal and ISO-stimulated AC activity, as well as the dose-response curves of ISO by using agonist concentrations ranging between 0.1 nM and 10 μM, were not different in the two groups. However, OCD patients showed lower EC_{50} and higher E_{max} values than healthy subjects. These findings suggest the presence of supersensitive β-adrenergic receptors in platelets of OCD patients.

Keywords: obsessive-compulsive disorder, norepinephrine, second messengers, adenylate-cyclase, platelets, isoprenaline, β-adrenergic receptors

Introduction

The most consistent hypothesis on the pathophysiology of obsessive-compulsive disorder (OCD) is focused on the serotonin (5-HT) system and, to a lesser extent, on dopamine (DA).[1] However, few indications are available on the involvement of other neurotransmitters, such as norepinephrine (NE), peptides and, more recently, glutamate.[2–4] For NE, decreased levels of tiramine, a precursor of catecholamines, and of homovallinic acid, one of their metabolites, in the cerebrospinal fluid of OCD patients have been reported.[5] Other support for a role of NE in OCD has been provided by the blunted growth hormone response to clonidine, an α_2-adrenoreceptor antagonist, which would suggest hypersensitive presynaptic or hyposensitive postsynaptic α_2 receptors,[6] but a negative finding has also been reported.[2] Similar controversies are present in the literature on the direct evaluation of the binding parameters of α_2-adrenoreceptors in platelets. In fact, when labeled by [^{3}H]rauwolscine, they showed a similar density in OCD patients and control subjects,[7] while [^{3}H]clonidine revealed a greater density of α_2-adrenoreceptors in OCD patients.[2] The possible role of α_2-adrenoreceptors is indirectly supported by the evidence that mirtazapine, an α_2-adrenoreceptors antagonist, may reduce the onset of citalopram response in OCD patients.[8]

Blood platelets represent a reliable, peripheral model of presynaptic serotonergic neurons; in particular, they possess a 5-HT reuptake transporter similar to that present in the brain which has been deeply investigated in different psychiatric disorders including

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OCD.[9–10] Besides the 5-HT transporter, platelets carry 5-HT1A and 5-HT2 receptors, but also structures belonging to the catecholamine systems, such as the DA transporter and α- and β-adrenergic receptors.[11] More recently, much interest has been devoted to intracellular mechanisms following receptor activation and there is a preliminary evidence for the involvement of cyclic adenosine monophosphate (cAMP) signaling in OCD, as shown by the lower protein kinase A (PKA) activity.[12–13]

In the present study, we aimed to explore and compare the basal adenylate cyclase (AC) activity in platelets of OCD patients and healthy control subjects, and that after stimulation by isoprenaline (ISO), a synthetic catecholamine which behaves as a β_1-/β_2-adrenergic receptor agonist, in both the absence and the presence of α- and β-adrenoreceptor antagonists.

Methods

Subjects

Twenty patients (10 men and 10 women, aged between 22 and 31 years; mean ± SD: 24.1 ± 6.5), recruited at the outpatient unit of the "Clinica Psichiatrica, Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie" at Pisa University, were included in the study. All met DSM IV-TR criteria for OCD, were not currently depressed, or had any history of mood disorders or any other comorbid conditions. Diagnoses were made by experienced psychiatrists, following the Structured Clinical Interview for DSM IV (SCID).[14] No patients had ever taken psychotropic drugs in the past, except three who had taken benzodiazepines 1 year previously and one who had undergone supporting psychotherapy.

The subjects' age (mean ± SD) at the onset of OC symptoms was 17 ± 5 years and the duration (mean ± SD) of the disorder was 5 ± 1 years.

The severity of OC symptoms was evaluated by means of the Yale Brown Obsessive-Compulsive Scale (Y-BOCS).[15] The total score (mean ± SD) was 26.4 ± 6.3 (range: 25 to 40); the obsession subtotal score (mean ± SD) was 14.2 ± 4.5 (range: 13 to 20); the compulsion subtotal score (mean ± SD) was 14.5 ± 4.9 (range: 12 to 20). The most commonly reported obsessions by the Y-BOCS Check-List were "contamination" (66.7%), "order and symmetry" (25.5%) and "others" (23.3%) comprising the need to remember certain things, the fear of saying certain things, the fear of being inexact, intrusive images, meaningless sounds or words or musical themes, fortunate/unfortunate numbers, and meaningful colors. The most common compulsions were cleaning (53.3%), checking (53.3%), and repetition (53.3%). The degree of insight (mean ± SD) was 1.5 ± 1.5 (range: 0 to 4). Eight patients only had one obsession and/or compulsion, while the remaining 12 had more than one.

The severity of depression was rated by means of the Hamilton Rating Scale for depression (HRSD):[16] the total score (mean ± SD) was 4 ± 1 and 3 ± 2 in, respectively, OCD patients and healthy controls.

Twenty healthy volunteers (10 men and 10 women, aged between 21 and 32 years, mean age ± SD: 24.2 ± 7.1) were selected for this study according to the following criteria: all subjects were without familiar or personal history of major psychiatric disorders, as assessed by the SCID, and had no current medical complaints and were physically healthy, as ascertained by their medical history and physical examination. No woman of both groups took contraceptive pills.

All subjects gave their written informed consent to participate in the study which was approved by the Ethics Committee at Pisa University.

Chemicals

The radio-labelled compounds [^{32}P]α-ATP (specific activity: 30 Ci/mmol) and [^{3}H] cAMP (specific activity: 27 Ci/mmol) were purchased from Perkin Elmer Life Science (Boston, MA, USA). Creatine kinase and creatine phosphate were obtained from Roche Molecular Biochemicals (Mannheim, Germany). All other reagents were from Sigma-Aldrich Chemical Co. (Milan, Italy) and of the highest grade available.

Methods

Thirty mL of blood were collected from fasting subjects between 8.00 and 10.00 AM to avoid circadian rhythm interference. Samples were immediately put into Falcon tubes containing sodium citrate anticoagulant (1:6 dilution), composed by 2.2% sodium citrate, 1.2% citric acid, and gently mixed. Platelet-rich plasma (PRP) was obtained by blood low-speed centrifugation at 200 × g for 20 min at room temperature. The PRP was then centrifuged at 2000 × g for 15 min at room temperature to precipitate platelets.

Adenylate cyclase assay

Platelets were washed once in 40 to 50 mL isotonic solution, 0.9% NaCl, by centrifugation at 15,000 × g for 15 min at 4 °C. The resulting pellet was resuspended in a strongly hypotonic buffer containing 5 mM Hepes-NaOH, pH 7.4 at 25 °C, 1 mM EGTA and protease inhibitors (bacitracine 20 mg/100 mL; benzamidine 16 mg/100 mL, soybean trypsine inhibitor, 2 mg/100 mL). Samples, kept on ice, were then homogenized by ultraturrax for 5 to 10 sec. After a 10- to 15-min incubation

at 0 °C to remove endogenous compounds, samples were then centrifuged at 40,000 × g at 4 °C for 15 min. The final pellet was frozen in liquid nitrogen and stored until assay, performed within no more than 2 weeks. On the day of assay, membrane pellets were thawed and suspended in 10 mM Hepes buffer pH 7.4, containing 1 mM EGTA, to obtain a protein concentration of about 0.8 to 1.8 mg/mL: protein estimation was performed by means of the Bradford Bio-Rad kit,[17] using γ-globulin as the standard. AC activity was measured in the reaction mixture, as described previously,[18] with slight modifications, such as the presence in the mix buffer of 0.33 mM EGTA, 0.005 mM pargyline and the use of GTP at the concentration of 0.01 mM.

Basal enzyme activity was also evaluated in the absence or presence of various concentrations (0.1 nM to 10 μM) (-)ISO. Some experiments were performed by adding the β antagonist propranolol (1 μM) and the α_2 antagonist rauwolscine (10 μM). The reaction was started by the addition of 50 μL of membrane suspension and carried out by incubating samples for 15 min at 30 °C in a final assay volume of 150 μL. [^{32}P]α-AMPc and [^{3}H]AMPc were further purified through a double-step Dowex-Alumina chromatography.[13]

Data analysis

All experiments performed herein were carried out in duplicate and presented as mean ± SEM. Statistical significance was preset at p = 0.05. Data analysis and statistics were performed by GraphPad Software Inc., version 3.00, San Diego, CA, USA. The equation of Salomon[12] was used to evaluate AC activity (pmol cAMP/min/mg protein). Concentration-response curves of ISO on basal AC were analyzed in order to estimate EC_{50} (potency, concentration of drug causing 50% of the maximal effect on enzyme activity, nM) and E_{max}, (efficacy, maximal inhibition, %) values. Data were shown as percentage (%) of basal AC activity in the presence of ISO vs basal AC activity without the agonist (considered to be 100%). Two-tailed paired (within group) or unpaired *t*-tests (between group) were used to compare platelet AC velocities or ISO dose-response parameters in the two groups.

Results

As shown in Table 1, the platelet basal AC activity was similar in OCD patients and healthy subjects. The addition of 10 μM ISO enhanced significantly ($p < 0.05$) platelet basal AC in both groups, without intergroup differences.

The different concentrations of ISO led to dose-response curves with EC_{50} and E_{max} values (mean ± SEM), different in the two groups: OCD patients showed significantly lower

Table 1 Basal and 10 μM ISO-stimulated adenylate cyclase activity in platelet membranes from healthy subjects and OCD patients

	Basal AC	+ ISO 10 μM
Healthy subjects	14.00 ± 1.72	20.61 ± 2.50
OCD patients	13.60 ± 1.59	22.99 ± 3.48

Note: Data are presented as mean ± SEM of *n* experiments each performed in duplicate.
Abbreviation: ISO, isoprenaline.

(t = 4.78, p = 0.01) mean EC_{50} and higher (t = 6.46, p = 0.01) mean E_{max} than healthy subjects (Table 2, Figure 1).

In previous work, carried out in intact human platelets, the ISO stimulation was generally determined in the presence of the α-adrenergic antagonist phentolamine to avoid platelet α_2 inhibition of AC at the tested agonist concentrations.[19–21] In the present study, we observed a stimulatory response after ISO in all subjects even without α-antagonists. Figure 2 shows the dose-response curves of ISO alone and in the presence of 10 μM rauwolscine, 1 μM propranolol, and both antagonists. The addition of rauwolscine provoked a small leftward shift of the curve, whereas the addition of propranolol, a β-blocker, abolished the stimulation and revealed a weak ISO-mediated inhibition of AC at its higher concentrations (1 to 10 μM). The concomitant addition of ISO, rauwolscine and propranolol led to a partial AC stimulation by ISO (EC_{50} and E_{max} of about 2.5 μM and 25%, respectively), while displaying a dose-response curve shifted towards the right.

In the presence of rauwolscine the ISO EC_{50} (mean ± SEM, n = 3) was 68.67 ± 26.91 nM and the E_{max} was 161.3 ± 8.57%. In the same experiments without rauwolscine, the ISO EC_{50} (mean ± SEM, n = 3) was 88.33 ± 16.59 nM and the E_{max} 147.7 ± 1.45%, not statistically different.

No correlation between biochemical parameters and clinical characteristics of the disorder was detected.

Discussion

The main findings of the present study were the following. First, we did not detect any difference in the basal AC activity

Table 2 ISO stimulation parameters measured in platelet membranes of healthy subjects and OCD patients

	EC_{50} (nM)	E_{max} (%)
Healthy subjects	85.62 ± 18.82	151.70 ± 4.40
OCD patients	53.80 ± 23.26*	177.40 ± 9.51**

Notes: Data are presented as mean ± SEM of 3 experiments each performed in duplicate.
*significant, t = 4.78, p = 0.01.
**significant, t = 6.46, p = 0.01.
Abbreviation: ISO, isoprenaline.

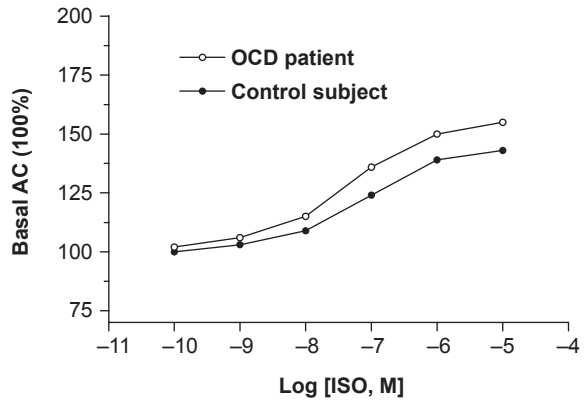

Figure 1 Isoprenaline dose-response curve in one OCD patient and control subject.
Abbreviation: ISO, isoprenaline

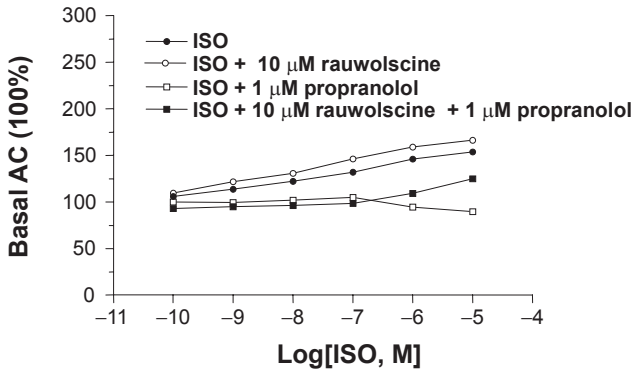

Figure 2 Dose-response curve of isoprenaline alone and in the presence of rauwolscine, propanolol and both the compounds.
Abbreviation: ISO, isoprenaline

in platelet membranes of healthy subjects and OCD patients. The addition of 10 μM ISO to platelet membranes provoked a significant stimulation of the enzyme activity of the same magnitude in the two groups. When different concentrations of ISO were used, the dose-response curve showed a significant decrease of the agonist EC_{50} and increase of E_{max} values in OCD patients, as compared with those of control subjects. The β-blocking compound (−)propranolol (1 μM) removed the ISO response, while revealing also a weak degree of inhibition of AC at ISO concentrations >1 μM. In the presence of rauwolscine 10 μM, the ISO-stimulating response showed a shift of EC50 of about 1.5-fold. Previous studies in whole platelets showed that the presence of α-antagonists, such as phentolamine, can provoke the ISO-stimulating response.[22] In our study, carried out in platelets membranes and in the presence of an optimal mix of reaction, we observed a significant stimulation via ISO, even in the absence of the α-antagonists. OCD patients and healthy controls were compared by using a lower range of concentration of the agonist and by eliminating the highest concentrations of ISO, when the activation of the inhibiting α_2 receptors was detected. These findings suggests that, under our experimental conditions, there is an inhibitory component of ISO effect on platelet AC, due to the agonist interaction with α_2-receptors, at its higher concentrations (>1 μM).

These results suggest the presence of a condition of supersensitive β-receptors in platelets of OCD patients that, perhaps, could reflect (or provoke) alterations of the intracellular signaling system mediated by the cAMP and of the related kinases. This is consistent with recent findings showing a decreased activity of PKA in OCD patients.[23]

The concomitant evidence of increased PKC activity, triggered by the hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PI), in patients suggest that, perhaps, this condition might be characterized by an imbalance between the two main second-messenger pathways (cAMP and PI), with a prevalence of the second, given the cross-talk between the two main second messengers at the level of different effectors.[12]

In conclusion, the results of the present study suggest that abnormal second messenger pathways in OCD may be also related to cathecolamine system disturbances, which might open new therapeutic strategies especially in resistant patients who do not respond to serotonergic medications.

Disclosures

The authors disclose no conflicts of interest.

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