Adenylate Cyclase Activity in Postmortem Brain of Suicide Subjects: Reduced Response to β-Adrenergic Stimulation

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Background: *Biochemical research on the etiopathogenesis of affective disorders has focused on transduction mechanisms beyond receptors, such as adenylate cyclase activity.*

Methods: *Adenylate cyclase activity (AC) was measured in postmortem frontal cortex samples from 11 suicide victims with a firm antemortem diagnosis of major depressive disorder and 11 matched control cases. We analyzed the basal activity of the enzyme and that following stimulation with forskolin, guanine nucleotides, and the β_1-adrenoceptor agonist xamoterol.*

Results: *A significant negative correlation between the period of tissue storage and the response of AC to the different stimuli assayed was observed. No difference was found in the levels of basal, forskolin-, and GTPγS-stimulated activity between control and major depressive disorder cases, both in the drug-free and the drug-treated subgroups. In contrast, we found a significant lower response to β_1-adrenoceptors agonist-stimulated AC activity in the major depressive disorder group ($p < .01$). This pattern of reduced response was also found in the subgroup of patients with negative toxicology for antidepressants.*

Conclusions: *These results, directly obtained from the brain of depressed patients, reinforce the involvement of noradrenergic neurotransmission in depressive illness. They also support the relevance of cyclic adenosine monophosphate signaling pathways in the etiopathogenesis of affective disorders.* Biol Psychiatry 2003;54: 1457–1464 © *2003 Society of Biological Psychiatry*

Key Words: cAMP, adenylate cyclase, β-adrenergic receptors, major depressive disorder

Introduction

Biochemical research about the etiopathogenesis of affective disorders has been focused, with respect to the events involved in signal transduction, at the level of primary messenger (neurotransmitters, particularly noradrenaline and serotonin) to the level of the synaptic receptors (for review, see Leonard 2000). More recently, an increasing interest has focused on transduction mechanisms beyond receptors, such as adenylate cyclase activity (AC; adenosine triphosphate pyrophosphate lyase cyclizing, EC 4.6.1.1; Cowburn et al 1994; Dowlatshahi et al 1999; Lowther et al 1996; Stewart et al 2001; Young et al 1993) phosphoinositide metabolism (Pacheco et al 1996), and guanine nucleotide binding proteins (G-proteins; Cowburn et al 1994; Dowlatshahi et al 1999; Friedman and Wang 1996; Pacheco et al 1996; Stewart et al 2001; Young et al 1993). An alteration in the balance of second messenger function may be involved in the pathophysiology of depression (Wachtel 1989); however, abnormalities of these mechanisms are not yet well understood. In fact, the possible existence of modifications in the density of G-proteins associated to depressive states is a matter of debate. Increases (Cowburn et al 1994; Friedman and Wang 1996; García-Sevilla et al 1999; Pacheco et al 1996; Young et al 1993), decreases (Ozawa et al 1993; Pacheco et al 1996), and lack of changes (Cowburn et al 1994; Dowlatshahi et al 1999; Pacheco et al 1996) have been reported for $G_{\alpha s}$ and $G_{\alpha i}$ subunits in brain cortical samples from depressed suicides. No changes in the density of the subunit $G_{\alpha o}$ or $G_{\beta\gamma}$ have been found in the cortex from major depression cases (Friedman and Wang 1996; García-Sevilla et al 1999; Young et al 1991).

Regarding chemical transmission in the brain, AC is one of the most relevant enzymatic effectors linked to G protein–mediated activity (Nestler and Duman 1999). The physiologic responses induced by noradrenaline, one of the neurotransmitters strongly suggested to play a role in the pathogenesis of depressive illness, are mediated through the modification of AC. Several lines of evidence

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support the involvement of this neurotransmitter, through the β adrenergic receptor subtype, in depression. First, long-term antidepressant administration results in a reduction of both the density of β-adrenergic receptors and β-adrenergic receptor-mediated production of cyclic adenosine monophosphate (cAMP; desensitization) in rat brain (for review see Hudson et al 1993). Second, pre- and postsynaptic modifications have been reported in biological samples from depressive patients (see Schatzberg and Schildkraut 1995). With respect to β-adrenoceptors, the results obtained are not consistent. Although several studies have reported an increase in the density of these sites in frontal cortex samples from suicide victims compared with age-matched control cases (Arango et al 1990; Biegon and Israeli 1988; Mann et al 1986), other authors could not confirm this finding (De Paermentier et al 1991; Klimek et al 1999; Stockmeier and Meltzer 1991). It must be taken into account that many of these studies have examined suicide cases, without an exhaustive diagnostic identification (Arango et al 1990; Biegon and Israeli 1988; Mann et al 1986; Stockmeier and Meltzer 1991).

Taking into account the relevance of the chemical signaling mediated by AC in major depression and the involvement of the noradrenergic neurotransmission, it is of interest to know the status of AC activity, both basal and after β-adrenergic stimulation, in the brain of depressed patients. In this regard, only a few studies have addressed the issue of AC activity, including basal and forskolin- or guanosine 5′-O-(3-thiotriphosphate) (GTPγS)-stimulated activity, in human brain tissue from suicide victims, and those studies have reported contradictory results (Cowburn et al 1994; Dowlatshahi et al 1999; Lowther et al 1996; Stewart et al 2001; Young et al 1993). With respect to noradrenergic activation, a reduction in cAMP levels following β-adrenoceptor activation has been reported in white blood cells from patients with major depression compared with control subjects (Extein et al 1979; Halper et al 1984, 1988; Mann et al 1985, 1990, 1997; Mazzola-Pomietto et al 1994; Pandey et al 1985); however, no data are available in human brain with respect to AC response to β-adrenergic stimulation in brain tissue from depressed patients.

The aim of this study was to examine, in the same group of tissue samples from a well-characterized sample of patients with antemortem diagnoses of depression, the basal activity of brain AC, as well as that after the stimulation of the enzyme either directly or via β_1 adrenoceptor activation.

Methods and Materials

Materials

Except as noted, all reagents are from Sigma-Aldrich (Boston, Massachusetts).

Subject Selection

Brain samples were obtained at autopsy from 11 suicide victims (depressed) and 11 control subjects. Each suicide case was carefully matched for gender, age, postmortem delay, and time of freezing storage with a control subject who did not die from causes involving the central nervous system and who was without documented or pathologic evidence of mental or neurologic illness. The frontal cortex (Brodmann's areas 8 and 9) was dissected macroscopically on unfrozen tissue. Tissue was then frozen at –70°C and stored at –20°C until preparation of membranes. Table 1 summarizes the demographic characteristics and the drug history of the subjects studied.

All the cases were subjected to retrospective search for antemortem clinical diagnosis and pharmacologic treatment using examiner and clinicians' information and records of general and psychiatric units. This searching was blind to biochemical findings. Only subjects with an established diagnosis of major depressive disorder were included in the study. The diagnosis of depression was carried out according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R and DSM-IV; American Psychiatric Association 1987). This group included nine patients with a definitive diagnosis of major depression (cases 1–9) and two patients with bipolar disorder (cases 10 and 11). Postmortem drug screening for antidepressants and other psychotropic drugs and alcohol was carried out in blood samples. Control subjects with a positive screening test were excluded from the study. Medical records revealed that an antidepressant treatment had been prescribed in six suicide cases. Laboratory screening was positive for antidepressants in three of them (see Table 1).

Membrane Preparation

Frozen brain tissues from control and suicide cases were homogenized (1:20 wt/vol) in ice cold buffer I, comprising 20 mmol/L Tris HCl pH 7.5, .32 mol/L sucrose, 5 mmol/L ethylene glycol bis-2-aminoethyl ether-N,N,N′,N′-tetraacetic acid (EGTA), 2 mmol/L ethylenediamine tetraacetate (EDTA), 1 mmol/L dithiothreitol, and 25 μg/mL leupeptin, using a motor-driven Teflon and glass tissue grinder (10 strokes at 800 rotations per minute). The homogenate was centrifuged at 500 *g* for 5 min at 4°C for removing nuclei. The pellet was discarded, and the supernatant was centrifuged at 12,000 *g* for 15 min at 4°C. The supernatant was discarded, and the pellet was homogenized in ice-cold buffer-II comprising 20 mmol/L Tris HCl pH 7.5, 6 mmol/L $MgCl_2$, 1.2 mmol/L EGTA, 3 mmol/L dithiothreitol, and 25 μg/mL leupeptin (1:60 wt/vol). The membranes were used immediately after preparation.

Adenylate Cyclase Assay

Membranes were diluted in ice-cold buffer II up to a protein concentration of 6–12 μg/assay; 25 μL of membrane suspension were then preincubated for 15 min on ice in 150 μL of reaction buffer (53 mmol/L N-[2-hydroxyethyl]piperazine-N′[2-ethanesulfonic acid] pH 7.4, .3 mmol/L EGTA, 5 mmol/L $MgCl_2$, .1 mg/mL bovine albumin, 1 mmol/L dithiothreitol, .5 mmol/L

Table 1. Demographic, Storage, and Postmortem Delay Characteristics and Toxicologic Data of Control Subjects and Suicide Victims

Case	Sex	Age (Years)	PMD (h)	FST (m)	Cause of Death	Antidepressant Screening
Control						
C1	F	68	30	45	Neoplasia	Negative
C2	F	54	18	44	Neoplasia	Negative
C3	F	62	25	87	Cardiac arrest	Negative
C4	F	34	24	75	Motor vehicle accident	Negative
C5	F	67	4	69	Neoplasia	Negative
C6	F	50	32	9	Motor vehicle accident	Negative
C7	M	67	61	24	Cardiac arrest	Negative
C8	M	35	24	75	Motor vehicle accident	Negative
C9	F	74	21	6	Motor vehicle accident	Negative
C10	M	22	14	16	Motor vehicle accident	Negative
C11	M	41	20	8	Laboral accident	Negative
Mean		52.2	24.8	41.6		
± SD		17.1	14.2	30.8		
Suicide (major depressive disorder)						
S1	F	72	49	45	Jumping from a height	Negative
S2	F	53	17	39	Jumping from a height	Negative
S3	F	64	48	78	Jumping from a height	Negative
S4	F	35	11	55	Caustic intoxication	Negative
S5	F	74	10	49	Hanging	Amitriptyline, paroxetine
S6	F	58	27	20	Hanging	Negative[a]
S7	M	73	60	27	Gunshot wound (chest)	Citalopram
S8	M	35	12	85	Jumping from a height	Negative
S9	F	88	9	11	Jumping from a height	Paroxetine, maprotiline
S10	M	23	14	21	Jumping from a height	Negative[b]
S11	M	42	19	10	Jumping from a height	Negative[c]
Mean		56.1	23.4	40.0		
± SD		20.3	18.7	25.4		

PMD, postmortem delay; FST, freezing storage time at −20°C; h, hours; m, months.
[a]Medical records indicated prescription of citalopram.
[b]Medical records indicated prescription of paroxetine.
[c]Medical records indicated prescription of amitriptyline.

3-isobutylmethylxanthine, and the nucleoside triphosphate regeneration system of 5 mmol/L creatine phosphate, 50 units/mL creatine phosphokinase), and 20 μL of water (basal activity), GTPγS (1 μmol/L or 100 μmol/L), forskolin (1 μmol/L or 100 μmol/L), or the specific β_1-adrenergic agonist xamoterol (Tocris Cookson, Bristol, United Kingdon; 1 μmol/L, 10 μmol/L, or 100 μmol/L). Three replicates were measured for each condition. The reaction was started by the addition of .5 mmol/L Mg-ATP and incubated at 30°C for 10 min. Reactions were stopped by boiling the tubes for 4 min. Samples were then centrifuged at 13,000 *g* for 5 min at 4°C. For cAMP content, 50-μL aliquots of supernatant were assayed using a commercial protein-binding assay kit (cAMP [^{3}H]assay kit TRK 432; Amersham International, Amersham, United Kingdom), combining the high specificity and affinity for cAMP of a high purified and stabilized binding protein with an improved charcoal separation step. Results are expressed as percentage of the basal AC activity. Samples from each suicide case and its matched control were assayed concurrently.

Protein Determination

Membrane protein concentrations were determined using the Bio-Rad Protein Assay Kit (Bio-Rad, Munich, Germany) using γ-globulin as the standard.

Statistical Analysis

Results are expressed as means ± SEM. Demographic parameters of suicide (depressed) and control cases were compared by Student's *t* test. Because of the paired design of the study, data from groups were not independent; therefore, repeated-measures analysis of variance (ANOVA) was used for the statistical evaluation of the differences in AC activity (cAMP levels). When this analysis showed significant differences, post hoc comparisons between suicide and control cases were made with paired *t* test (Arango et al 1990; Sokal and Rohlf 1990). Two-way ANOVA test was used to compare the effects of different concentrations of nucleotides and xamoterol on cAMP levels. The level of significance was chosen at $p < .05$. Pearson's

coefficient of correlation was calculated to evaluate the relationship between the values of the biological variables of interest (age, postmortem delay, and freezing storage time) for each control–suicide pair, as well as that between these variables and the levels of cAMP.

Results

Influence of Demographic Variables on AC Activity

Suicide (depression) and control cases were well matched with respect to the different variables analyzed (gender, age, postmortem delay, and tissue storage time). There were no significant differences between suicide and control cases for gender (seven female and four male cases in both groups), age (52.2 ± 17.1 years for control cases and 56.1 ± 20.3 years for suicide cases; $t = .50$, *ns*), postmortem delay (24.8 ± 14.2 hours for control cases and 23.4 ± 18.7 hours for suicide cases; $t = .21$, *ns*), or freezing storage time (41.6 ± 30.8 months for the control group and 40.0 ± 25.4 months for the suicide groups; $t = .14$, *ns*). Furthermore, a significant correlation was found between control and suicide cases for age ($p < .001$), postmortem delay ($p < .01$), and storage time ($p < .001$) values, confirming the appropriate pairing of these variables between the two groups. Regarding the influence of these biological variables on AC activity, no significant correlation was found between either age or postmortem delay and the levels of cAMP production under the different conditions analyzed (basal, stimulated by forskolin, GTPγS, and the β_1-adrenoceptor agonist) in both control and depressed cases. In contrast, there was an inverse correlation between the freezing storage time and the basal level of AC activity and that stimulated by forskolin, GTPγS, and xamoterol. This tendency reached statistical significance in the control group following stimulation by 100 μmol/L forskolin ($p < .05$) and in both groups after incubation with the β_1-adrenoceptor agonist xamoterol ($p < .01$ for control and suicide cases; Table 2).

Basal, Forskolin-, and Nucleotide-Stimulated Activity in Major Depressive Disorders

No significant differences were found between the control and depressed suicide groups in the basal cAMP levels (86.2 ± 10.9 and 83.0 ± 7.9 pmol/min/mg, respectively, for control and depression cases). The effects of forskolin and GTPγS on AC activity in control and depression cases are shown in Figure 1A. A dose-dependent increase in cAMP levels was observed in both the control and depressed groups ($n = 11$), following incubation with forskolin (control: $F = 78.4$, $p < .001$; depressed: $F = 39.7$, $p < .001$) and GTPγS (control: $F = 19.6$, $p < .001$; depressed: $F = 8.4$, $p < .01$). No significant differences were found between both groups regarding the degree of stimulation induced by forskolin and GTPγS on AC activity (Figure 1A). This lack of differences was also observed when the subgroup of drug-free patients ($n = 8$) was analyzed separately (Figure 1B). In this regard, no significant differences in the level of AC stimulation by forskolin and GTPγS were found in suicide patients under antidepressant treatment ($n = 3$) when compared with those drug-free cases, although a slight tendency to the increase was observed (data not shown).

Table 2. Relationship between Adenylate Cyclase Activity[a] and Tissue Storage Time at −20°C (in months) in Control and Major Depressive Groups

	Control		MDD	
Drug (μM)	r^b	p	r^b	p
Basal	−.44	.17	−.27	.42
F 1	−.45	.16	−.24	.48
F 100	−.64	.03	−.28	.40
GTPγS 1	−.53	.10	−.50	.12
GTPγS 100	−.56	.07	−.48	.13
X 1	−.75	.01	−.74	.01
X 10	−.77	.01	−.71	.01
X 100	−.64	.03	−.70	.01

F, forskolin; GTPγS, guanosine 5′-O-(3-thiodiphosphate); MDD, major depressive disorder; X, xamoterol.
[a]Cyclic adenosine monophosphate, in pmoles/mn/mg of protein.
[b]Pearson's coefficient of correlation.

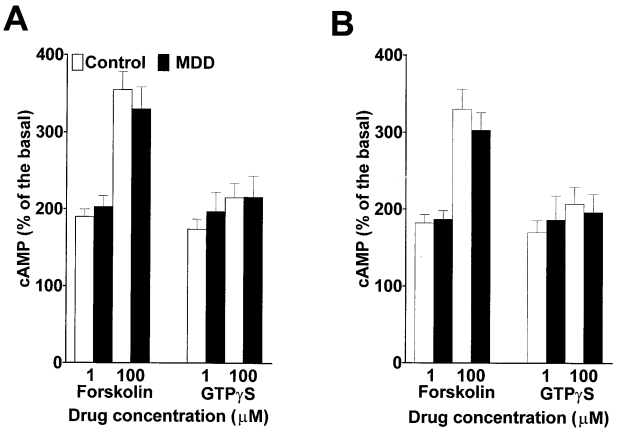

Figure 1. Effect of increasing concentrations of forskolin (1 and 100 μM) and guanosine 5′-O-(3-thiotriphosphate) (GTPγS; 1 and 100 μM) on cyclic adenosine monophosphate (cAMP) levels (expressed as mean ± SEM of the percentage of increase over the basal) in crude membranes from postmortem human frontal cortex of control (open bars) and depressed suicide cases (closed bars). **(A)** total group ($n = 11$); **(B)** antidepressant-free group ($n = 8$); see Methods and Materials.

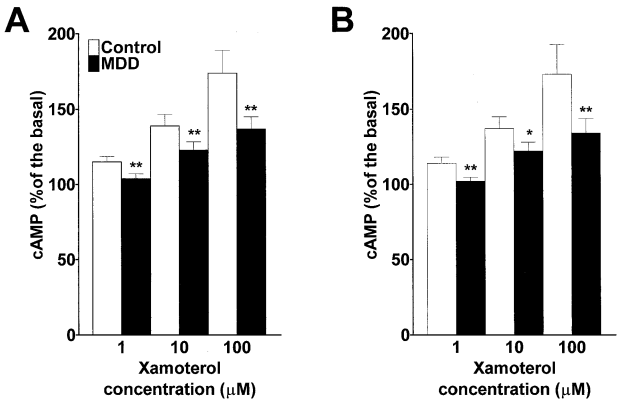

Figure 2. Effect of increasing concentrations of xamoterol (1, 10, and 100 μM), on cyclic adenosine monophosphate (cAMP) levels (expressed as mean ± SEM of the percentage of increase over the basal) in crude membranes from postmortem human frontal cortex of control (open bars) and depressed suicide cases (closed bars). **(A)** total group ($n = 11$); **(B)** antidepressant-free group ($n = 8$). *$p < .05$, **$p < .01$ post hoc paired t test after repeated-measures analysis of variance (see Methods and Materials).

β-*Adrenoceptor-Induced AC Activity in Major Depressive Disorders*

The incubation with increasing concentrations of the β_1-adrenoceptor agonist xamoterol resulted in a dose-dependent increase of cAMP production in both control ($F = 14.1$, $p < .001$) and suicide cases ($F = 11.3$, $p < .001$) (Figure 2A); however, the response of AC (cAMP production) to xamoterol was significantly lower in the depressed group for all concentrations tested ($F = 10.3$, $t = 3.5$, $p < .01$; $F = 20.3$, $t = 3.5$, $p < .01$; and $F = 21.9$, $t = 4.6$, $p < .01$, respectively, for 1, 10, and 100 μmol/L). The marked reduction in the degree of increase of cAMP production in response to xamoterol was fully maintained in the drug-free group ($F = 9.3$, $t = 2.7$, $p < .01$; $F = 15.8$, $t = 2.4$, $p < .05$; and $F = 12.7$, $t = 2.1$, $p < .01$, respectively, for 1, 10, and 100 μmol/L; Figure 2B). In this regard, in the brain samples from patients with positive laboratory screening for antidepressants, the level of reduction in the response of AC to the β-adrenoceptor agonist was also present, although it did not reach significance because of the low number of cases ($n = 3$; data not shown).

Discussion

Information is limited regarding the production of cAMP (AC activity) in postmortem human brain from patients suffering affective disorders (Stewart et al 2001) and has yielded contradictory results, probably because of the heterogeneity in the diagnosis of the subject groups, as well as to the influence of other biological variables. This study is the first in which receptor-mediated AC activity (noradrenergic stimulation) measurement is included. Furthermore, only samples from patients with a firm antemortem diagnosis of major depressive disorder and from control cases carefully matched for several parameters were studied, allowing us to discard the influence of the presence of antidepressants in these patients.

The use of human autopsy material presents several problems when comparing control and diseased brains (Greenamyre and Maragos 1993; Pazos and Palacios 1989), and a careful matching between both groups is always required. Age-related decreases of β-adrenoceptor-stimulated AC activity in human lymphocytes (Halper et al 1984) and in rat brain (Araki et al 1995) have been reported. The prolonged freezing storage time has also been suggested to induce modifications in several neurochemical parameters (Rodríguez-Puertas et al 1996). All these data highlight the need for a careful matching between control and suicide cases. In this study, each suicide case was compared with a control individually matched for gender, age, postmortem delay, and period of storage time, and each tissue pair was assayed concurrently.

A relative stability of AC activity in postmortem human tissue, without a significant adverse influence of postmortem interval, has been reported (Cowburn et al 1994; Dowlatshahi et al 1999; Lowther et al 1996). In agreement with that, we found no significant influence of either age or postmortem delay on AC activity in the frontal cortex from control or suicide groups. In contrast, our results indicate that the period of freezing tissue storage can significantly affect the degree of response of AC to both direct and receptor-induced stimulation. In agreement with previous reports, these results show that it is possible to carry out biochemical studies in human postmortem samples stored at –20°C, but autolytic phenomena are present after long periods of freezing (Rodríguez-Puertas et al 1996). Our results emphasize the importance of assessing all possible sources of variability when using human autopsy material.

In the present study we found no significant difference in the levels of basal, forskolin- and GTPγS-stimulated AC activity in frontal cortex between control and suicide cases. In previous studies, both unaltered (Dowlatshahi et al 1999; Lowther et al 1996; Young et al 1993) or decreased (Cowburn et al 1994) basal AC activity in brain from suicide cases have been reported. Regarding the degree of AC stimulation induced by forskolin, conflicting results have been reported; increases (Young et al 1993), decreases (Cowburn et al 1994), and a lack of changes

(Dowlatshahi et al 1999; Lowther et al 1996) have been found. A similar degree of disagreement is also observed in the results regarding GTPγS-stimulated AC activity in postmortem brain of depressed subjects (Cowburn et al 1994; Young et al 1993). Several factors might account for these discrepancies, including the lack of a complete assessment of psychiatric diagnoses of the suicide patients, the heterogeneity of mood states across the subject groups, and the variable influence of the antidepressant treatments. The results of our study are in good agreement with those reported in an extensive study by Dowlatshahi et al (1999), who did not find any modification in cerebral cortex, except for a nonsignificant trend toward decreased forskolin-stimulated AC activity in depressed patients. In fact, our data also show a slight tendency to decrease following stimulation induced by 100 μmol/L forskolin.

Because of the relative inaccessibility of human brain in live subjects, AC activity has frequently been tested in platelets from depressed patients. In general terms, and in agreement with our results, no clear differences have been reported for basal, Gpp(NH)p-, and forskolin-stimulated AC (García Sevilla et al 1990; Karege et al 1992; Newmann et al 1992), although a lower degree of stimulation by forskolin was found by Menninger and Tabakoff (1997).

It has been suggested that the modifications reported in several studies in basal AC activity, as well as in that following forskolin- or GTPγS-induced stimulation, could be due to the influence of antidepressants treatment, because these drugs have been shown to alter AC activity (Hudson et al 1993), mainly by enhancing G-protein stimulated adenylate cyclase activity (Chen and Rasenick 1995). In our study, the existence of laboratory screening records for these drugs has allowed us to rule out their presence in the subjects' blood. On the other hand, in the small subgroup of patients who had been prescribed antidepressant drugs and presented a positive drug test ($n = 3$), we obtained essentially the same findings as in the drug-free subgroup (absence of changes), although the response to forskolin and GTPγS stimulation was slightly higher in these cases, compared with drug-free subjects. Because of the small sample size of the subgroup and the variety of drugs used, it is not possible to draw any conclusions from our data regarding antidepressant influence on basal and stimulated brain AC activity.

The most interesting finding of this study is the lower response of AC to the β_1-adrenoceptor agonist xamoterol in the suicide group. This is the first study analyzing the response of AC to noradrenergic stimulation in postmortem brain from depressed patients. Previous studies carried out in blood mononuclear cells from depressed subjects have shown a lower response to isoproterenol (Extein et al 1979; Halper et al 1984, 1988; Mann et al 1985, 1990, 1997; Mazzola-Pomiett et al 1994; Pandey et al 1985). This decrease was not always accompanied by changes in β-receptor density or affinity (Mann et al 1985), is normalized with electroconvulsive therapy (Mann et al 1990) or antidepressant drugs (Pandey et al 1985), and has been related to the severity of the depression (Mazzola-Pomietto et al 1994). The exact pharmacologic nature (β_1 or β_2 receptor) of this peripheral response remains to be elucidated.

In this study, xamoterol, a (partial) agonist with a high degree of selectivity for the β_1-adrenoceptor subtype (Nuttall and Snow 1982) was used. Therefore, the specific involvement of this receptor subtype in the alteration of the AC response in depressed patients is suggested. Although the localization of β_1-adrenoceptors in the central nervous system is mainly neuronal, their existence on glial cells has also been demonstrated (Tanaka et al 2002). Taking into account that there is an increasing interest in the possible role of glial cells in depression, the cellular localization of the β-adrenoceptors mediating the reduced cAMP response is of special interest, but it goes beyond the goals of our work. Further studies would be required to clarify this issue. Furthermore, the relevance of our findings is reinforced by the fact that they have been obtained in postmortem brain samples.

There are several potential mechanisms that could lead to this functional β-adrenergic subsensitivity in major depressive disorders. Our results could be explained in terms of either a receptor-specific alteration or a modification at a more distal level of the transductional axis. With respect to receptor properties, a change in the density or the affinity could account for the modification in AC response: in this regard, contradictory results have been reported regarding the status of β-adrenoceptors in brain samples from depressed patients, and both increases, decreases and lack of changes have been shown (Biegon and Israeli 1988; Arango et al 1990; De Paermentier et al 1991; Stockmeier and Meltzer 1991), thus making it difficult to ascribe the modifications in cAMP production to an established pattern of receptor change. A reduction in the density of β-adrenoceptors could explain the lower AC response found in our study. On the other hand, a dysregulation in resting catecholamine levels has been reported in depressive illness (Potter 1984); because catecholamines are one of the major regulators of adrenoceptor function (Feldman et al 1983), this finding could be also of relevance; however, neither the density of β-adrenoceptors nor the levels of plasma noradrenaline appear to present any correlation with the blunting of β-adrenergic responsivity (cAMP levels) found in major depression in peripheral blood cells (Halper et al 1988; Mann et al 1997). Therefore, the possibility exists that the reduction in the production of cAMP following β-adrenergic stim-

ulation is independent of modifications in the density or affinity of β-adrenoceptors.

In this regard, an alteration in the process of coupling of the β-adrenoceptor-G protein complex could also explain the results of our study. In fact, several groups have reported changes in the density of G protein subunits in major depression cases, leading to modifications in effector activity (Cowburn et al 1994; Dowlatshahi et al 1999; Friedman and Wang 1996; Pacheco et al 1996; Young et al 1993), although their results are often contradictory (see Introduction). These data could support the existence of an altered regulation of the receptor-G protein-AC cycle in major depressive disorders. The modifications in the production of cAMP reported in our study could be a direct (or compensatory) consequence of this alteration.

As previously mentioned, chronic antidepressant treatment has been shown to reduce both the density of β-adrenoceptors and β-adrenergic-induced stimulation of AC in rat brain (Hudson et al 1993), as well as to modify the levels of G proteins (Dowlatshahi et al 1999) and the activation of AC (Chen and Rasenick 1995). Thus, it could be suggested that the modifications in AC response found in brain tissue from depressed patients could be due to the effects of the medication; however, our data do not support the influence of antidepressants in the decreased response of AC to xamoterol; this effect was fully maintained when the analysis was restricted to the samples from those patients with negative laboratory records for antidepressants, showing that the functional desensitization of β-adrenergic-induced cAMP production is intrinsic to the mood disorder. On the other hand, the lack of significance for this response in the antidepressant-treated group could simply be due to small sample size ($n = 3$).

The decrease in the production of cAMP in brain tissue from patients with major depressive disorders following β-adrenergic stimulation suggests the existence of a disturbance of the cerebral transignaling mechanisms in the depressive illness, particularly with respect to noradrenergic neurotransmission. Taking into account the growing evidence suggesting the involvement of downstream targets of cAMP signaling in mood disorders (Dowlatshahi et al 1999), this deficit in AC functionality might be of special relevance for the etiopathogenesis of depression.

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