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Differential effect of serum from bipolar versus schizophrenic patients on spontaneous acetylcholine release at mammalian neuromuscular junction

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Abstract

Objective: The diagnosis of bipolar disease frequently requires a long time since the age of onset, especially because the disease is misdiagnosed with schizophrenia. The aim of the present work was to investigate whether sera from bipolar patients have an active substance that allows making a fast identification of the disease.
Methods: Sera from healthy volunteers, euthymic and non-stabilized bipolar patients, and schizophrenic patients were passively transferred into CF1 mice and after 2 day injections, MEPP frequency from diaphragm muscles was recorded. The same procedure was performed with sera fraction of high and low MW (cut-off 3000).
Results: Sera from non-stabilized bipolar patients induced a decreased MEPP frequency and occluded the presynaptic inhibitory effect of the specific adenosine A_1 receptor agonist 2-chloro-N^6-cyclopentyl-adenosine (CCPA) in the recipient mice, while in the euthymic bipolar group spontaneous secretion reached control values although the action of CCPA was still prevented. Similar results were obtained with low MW sera fraction from euthymic and non-stabilized bipolar patients. The addition of adenosine deaminase to the sera fraction prevented the modification of spontaneous ACh release. In mice injected with sera from schizophrenic patients, MEPP frequency was within control values and CCPA induced its typical inhibitory action.
Conclusions: These results indicate that bipolar patients contain in their blood an active substance compatible with adenosine, which was able to modify spontaneous ACh release in the recipient mice. This effect was not observed with sera from healthy volunteers and schizophrenic patients. The increase of adenosine concentration may result from synaptic hyperactivity that presumably plays a role in the symptoms of bipolar disorder and/or may derive from peripheral cells through a more general mechanism.
Significance: The different results obtained with bipolar and schizophrenic sera raise the possibility that the passive transfer model could be used as a diagnostic test in the future.

Keywords: Bipolar disorder; Schizophrenia; Adenosine; Differential diagnosis; Mammalian neuromuscular junction

1. Introduction

Bipolar disorder (BD) is a severe chronic psychiatric disease that affects 2–6% of the population (Akiskal et al., 2000; Angst et al., 2003; Judd and Akiskal, 2003). Despite being recognized for almost 2000 years, BD is frequently underdiagnosed. Many factors may interfere with making an accurate diagnosis. These include: the broad range of clinical presentation and co-morbidity and the age of onset.

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Moreover, symptoms frequently overlap with those observed in other psychiatric disorders such as schizophrenia, depression and personality disorders (Thomas, 2004). Onset of BD usually occurs before the age of 25 years. However, recent studies have shown that in some cases treatment for the illness is not started until as much as 10 years after onset (Hantouche et al., 2003; Lish et al., 1994), reflecting the poor recognition of the disorder. Early and accurate identification and diagnosis of BD can lead to specific treatment choices that may improve prognosis.

BD has a complex genetic, biological and psychosocial etiology. There is increasing evidence that the immune system, in close interaction with the CNS and the endocrine system, plays a role in BD (Breunis et al., 2003; Maes et al., 1995; Rapaport et al., 1999; Tsai et al., 2001). It has been demonstrated that this illness is associated with organ-specific autoimmunity to the antigens thyroperoxidase, H^+/K^+ adenosine triphosphatase and glutamic acid decarboxylase-65 (Padmos et al., 2004).

On the other hand, researchers hypothesized that symptoms of affective disorders are caused by an overactivity of certain neural circuits (Post, 1992; van Calker et al., 2000) that may be associated with increased neurotransmitter secretion.

Synaptic transmission is subject to a variety of modulatory influences that can modify the probability of neurotransmitter secretion. Adenosine (AD) is an essential nucleoside which regulates neurotransmitter release by binding presynaptic adenosine receptors. AD is a physiological constituent of all body fluids, including the extracellular space. At the synaptic cleft, AD concentration is closely matched to the intracellular space through AD transporters (see review by Fredholm, 1995). In addition, AD can be formed extracellularly after ATP (which is released together with the neurotransmitter) is hydrolyzed to the nucleoside via the ectonucleotidase cascade (Meriney and Grinnell, 1991; Redman and Silinsky, 1994; Ribeiro and Sebastião, 1987). In the brain, activation of inhibitory A_1 receptors probably has an important adaptive function in situations of pathologically exaggerated neuronal activity, as it appears to occur in BD. Recently, we have characterized the effect of AD on neuromuscular synapses. AD and the specific A_1 receptor agonist 2-chloro-N^6-cyclopentyl-adenosine (CCPA) induce presynaptic inhibition of spontaneous ACh release due to modulation of L-type voltage-dependent calcium channels (VDCC) (De Lorenzo et al., 2004). On the other hand, we also demonstrated that when secretion is increased by exposing nerve terminals to high K^+ concentration, endogenous AD induces presynaptic inhibition by modulating the P/Q-type VDCC, as a means of preventing excessive neurotransmitter secretion. These results with potassium depolarization are highly relevant to the effects of adenosine on synchronous, neurally evoked neurotransmitter release as adenosine inhibits physiologically relevant electrically evoked ACh release by inhibiting Ca currents through P/Q type VDCCs (Silinsky, 2004).

The aim of the present work was to investigate whether sera from bipolar patients contain an active substance (i.e. immunoglobulins, neuromodulators) that may influence neurotransmitter release at the neuromuscular junction, a synapse which is easily accessible and extensively studied. We analyzed the miniature end plate potential (MEPP) frequency at mice neuromuscular junctions, after the passive transfer of serum from non-stabilized bipolar patients (last manic or depressive episode) and euthymic bipolar patients. A second approach was to compare the obtained results with those from schizophrenic patients in order to establish new elements for the differential diagnosis.

2. Patients and methods

2.1. Sera collection

Following approval from the Ethics Committee of the Instituto de Investigaciones Médicas *Alfredo Lanari*, sera samples were obtained from bipolar and schizophrenic patients and healthy volunteers. Informed consent was obtained from all participants and in order to preserve the confidentiality of the data they were identified by a code. In all cases, sera were frozen to −20 °C and stored until used. All psychiatric diagnosis of the subjects from whom serum was collected was made by using DSM-IV criteria (American Psychiatric Association, 1994) by a senior psychiatrist after an extensive case history review. The bipolar patients consisted of 19 males and 22 females, in the age range from 21 to 66 years with a mean of 51.33 ± 3.30 years for euthymic bipolar patients and 42.87 ± 3.41 years for non-stabilized bipolar patients; the schizophrenic patients were 13 males and 15 females, in the age range from 18 to 68 years with a mean of 41.85 ± 2.87 years. Sera were obtained during the course of routine therapy (LiCl or typical antipsychotics) or from patients virgin of treatment. Because Li^+ was reported to induce changes on spontaneous ACh release, mainly at high concentrations or when used replacing Na^+ (Kouniniotou-Krontiri, 1985; Muchnik and Venosa, 1969; Sebastião and Ribeiro, 1990; Waud et al., 1982), diaphragm muscles were exposed to a Ringer solution containing 2 mM LiCl, a concentration that was similar to the therapeutic serum level. Lithium chloride neither modified MEPP frequency when compared to control solution, nor altered the modulatory action of CCPA (normal Ringer 1.05 ± 0.02 MEPP/s, normal Ringer + CCPA: 0.54 ± 0.01/s, Li^+ 1.06 ± 0.01 MEPP/s, Li^+ + CCPA: 0.53 ± 0.01 MEPP/s, $n = 4$; ANOVA: $F = 500.3$; d.f. = 3, 12; $P < 0.05$; Tukey: normal Ringer vs normal Ringer + CCPA, $P < 0.05$; normal Ringer vs Li^+, $P > 0.05$; Li^+ vs Li^+ + CCPA, $P < 0.05$; normal Ringer + CCPA vs Li^+ + CCPA, $P > 0.05$). On the other hand, administration of an antipsychotic drug seems not to be related to any abnormality on neuromuscular junction since passive transfer of sera from schizophrenic patients, with or with-

out this type of medication, resulted in MEPP frequencies similar to the control group. Control samples were collected from 4 males and 7 females volunteers (age range from 29 to 50 years with a mean of 35.91 ± 1.97 years) from staff members of the Instituto de Investigaciones Médicas with no previous history of psychiatric illness.

2.2. Sera passive transfer

CF1 mice weighting 30 ± 5 g were divided into different groups: control group (CG) mice uninjected; healthy volunteer group (HVG) mice receiving sera from healthy volunteers; euthymic bipolar group (EBG) mice receiving sera from treated euthymic bipolar patients; non-stabilized bipolar group (NSBG) mice receiving sera from bipolar depressive or manic patients; schizophrenic group (SG): mice receiving sera from schizophrenic patients (in course or in remission). Animals in HVG, EBG, NSBG and SG received daily intraperitoneal (i.p.) injection of 1 ml of serum for 2 days, and 24 h later, they were anesthetized with sodium thiopental (50 mg/kg, i.p.) and the left hemidiaphragm was excised for electrophysiological recording. Each mouse received serum from individual subjects.

2.3. Electrophysiological studies

The intracellular recording techniques have been described elsewhere (Losavio and Muchnik, 1997). Briefly, muscles were transferred to a 5-ml chamber superfused (3 ml/min) with Ringer–Krebs solution (mM: NaCl 135, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, D-glucose 11, HEPES 5, pH 7.3–7.4, bubbled with O_2). All recordings were made at room temperature (20–23 °C). MEPPs were recorded at the end-plate region of the muscle fibers using borosilicate glass microelectrodes (WP Instruments) with a resistance of 5–10 MΩ when filled with 3 M KCl. Muscle fibers with a resting membrane potential less negative than −60 mV or MEPPs with a rise time greater than 1 ms were rejected. In each experimental group, the muscles were allowed to equilibrate in the respective solution for at least 20 min, after observing that the MEPPs represented a period of stable spontaneous neurotransmitter release. MEPP frequency was recorded for 100 s from at least 10 different neuromuscular junctions and the values were averaged.

After MEPP recording in Ringer Krebs solution, we investigated the action of the A_1 receptor agonist CCPA (500 nM, Sigma). MEPP frequency was analyzed after 20 min of incubation with CCPA and in the presence of it.

2.4. Filtering procedure

Sera from euthymic and non-stabilized bipolar patients were filtered through a Centrifugal Filter Devices Centricon YM-3, 3000 MW cut-off. Then the high MW fraction (>3000), and the low MW fraction (<3000) were injected into two different groups of mice and MEPP frequency was evaluated. Then, the low MW fraction sera were pretreated (before i.p. injection to the mice) with adenosine deaminase type VI (ADA, Sigma, 0.5 U/ml), an enzyme that degrades adenosine into the inactive metabolite inosine. Injection of 0.5 U/ml ADA during 2 days to control mice did not modify MEPP frequency compared to control values (1.17 ± 0.03/s, $n = 3$).

2.5. Statistical analysis

The data given in Section 3 represent mean values ± SEM and n represents the number of animals (only the left hemidiaphragm was used from each mouse for a given experiment). The frequencies were measured by direct observation from the oscilloscope screen or as MEPP amplitudes, acquired through an A/D converter controlled by computer and analyzed using WCP software (Dagan Corp.). The recorded MEPPs were digitized at 25 kHz. Experiments were done under double-blinded procedure. Statistical significance of differences between means was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test, Dunnett's test or by Student's test when required. Differences were considered to be significant when $P < 0.05$.

3. Results

3.1. Effect of passive transfer of serum from patients suffering from bipolar disorder and schizophrenia on MEPP frequency

Fig. 1 shows the mean MEPP frequency values obtained from individual diaphragm muscles belonging to non-injected mice and to animals transferred with sera from healthy volunteers, euthymic bipolar patients, non-stabilized bipolar patients, and schizophrenic patients. MEPP frequency in the CG ranged from 0.90 to 1.12/s ($n = 8$) and that of the HVG ranged from 0.98 to 1.18 /s ($n = 11$). ANOVA between data obtained from CG and data obtained from HVG did not show any difference in spontaneous release ($F = 0.4130$, d.f. = 11, 111, $P > 0.05$). MEPP frequency recorded from mice treated with sera from euthymic bipolar patients behaved similar to CG (EBG ranged from 0.96 to 1.35/s, $n = 21$). When MEPP frequency values from each patient were compared to data obtained from CG, there was no significant difference (ANOVA: $F = 1.266$; d.f. = 21, 196; $P > 0.05$).

Conversely, passive transfer of serum from non-stabilized bipolar patients markedly reduced spontaneous release of ACh in the recipient mice (range from 0.44 to 0.76 MEPP/s, $n = 20$). MEPP frequency values from each patient and the CG differed significantly in all cases, regardless of whether the patient was in a manic or depressive episode (ANOVA: $F = 3.076$; d.f. = 20, 187; $P < 0.05$; see Fig. 1).

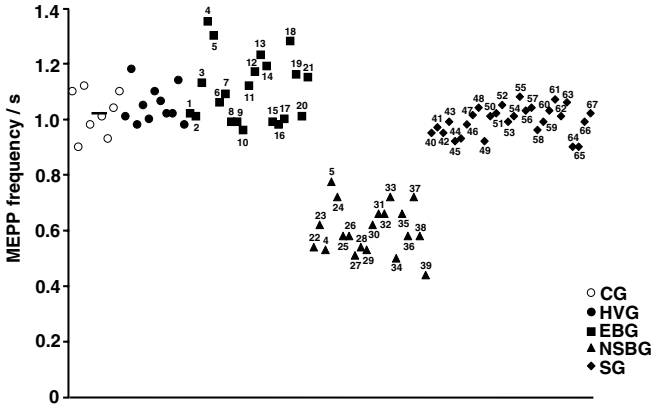

Fig. 1. MEPP frequency recorded in normal Ringer solution from diaphragm muscles of non-treated mice (CG, $n = 8$) and from those treated with sera from healthy volunteers (HVG, $n = 11$), euthymic bipolar patients (EBG, $n = 21$), non-stabilized bipolar patients coursing last depressive or manic episode (NSBG, $n = 20$), and schizophrenic patients (SG, $n = 28$). The heavy black line indicates the mean MEPP frequency value from the CG ($n = 70$ fibers). Numbers adjacent to the symbols correspond to different mice treated with sera from individual patients. Numbers 1–21 correspond to euthymic bipolar patients, numbers 22–30 to non-stabilized bipolar patients in manic state, numbers 31–39 to non-stabilized bipolar patients in depressive state, numbers 40–47 to schizophrenic patients in remission and numbers 48–67 to schizophrenic patients in active state. Note that sera from bipolar patients 4 and 5 were obtained when they were in both euthymic and manic states.

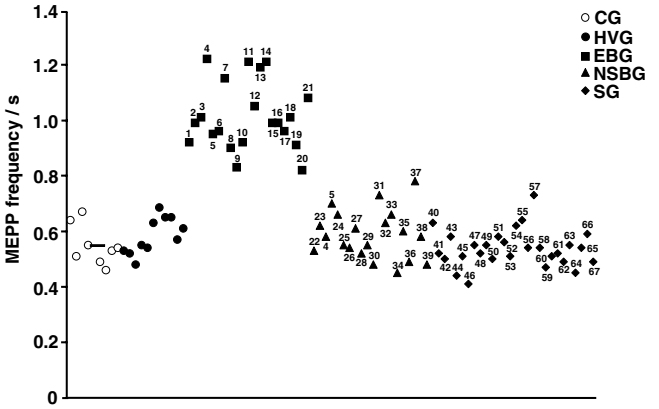

Fig. 2. MEPP frequency recorded in the presence of CCPA (500 nM) from diaphragm muscles of non-injected mice (CG, $n = 8$) and from those treated with sera from healthy volunteers (HVG, $n = 11$), euthymic bipolar patients (EBG, $n = 21$), non-stabilized bipolar patients coursing last depressive or manic episode (NSBG, $n = 20$), and schizophrenic patients (SG, $n = 28$). The heavy black line indicates the mean MEPP frequency value from CG. Numbers close to the symbols correspond to the same mice injected with sera from the patients included in Fig. 1.

When values of MEPP frequency were analyzed in mice treated with sera from schizophrenic patients, we found similar results to those observed in the CG, regardless of the phase of the disease (range from 0.90 to 1.08 MEPP/s; $n = 28$, ANOVA: $F = 0.3751$; d.f. $= 28$, 266; $P > 0.05$, Fig. 1).

On the other hand, passive transfer of serum from euthymic and non-stabilized bipolar patients and from schizophrenic patients did not affect MEPP amplitudes in the recipient mice, suggesting that their sera did not have a postsynaptic action (ANOVA: $F = 0.3287$; d.f. $= 4$, 15; $P > 0.05$; See Table 1).

3.2. Effect of CCPA on MEPP frequency in the bipolar and schizophrenic groups

Values of MEPP frequency obtained in normal Ringer in mice treated with sera from non-stabilized bipolar patients were similar to those observed in our previous paper when AD (100 μM) or the specific AD A_1 receptor agonist CCPA (500 nM) was added to the normal saline (De Lorenzo et al., 2004). One possibility is that non-stabilized bipolar patients have an increase of AD concentration in their sera; therefore, we considered it of interest to study the effect of this nucleoside on spontaneous neurotransmitter secretion in the injected mice.

As shown in Fig. 2, all animals belonging to CG exhibited a significant decrease of MEPP frequency when the preparations were bathed with 500 nM CCPA (range from 0.46 to 0.67/s). Similar results were obtained when the HVG was treated with the nucleoside (range from 0.48 to 0.69 MEPP/s). Conversely, in mice treated with serum from both euthymic and non-stabilized bipolar patients, the typical inhibitory effect of CCPA on spontaneous ACh release could not be observed; MEPP frequency values obtained in CCPA solution were not significantly different from those obtained in normal Ringer solution for each animal (EBG range from 0.82 to 1.22 MEPP/s; NSBG range from 0.45 to 0.78 MEPP/s). The action of CCPA in the SG was similar to the CG and HVG (range from 0.41 to 0.73 MEPP/s). Fig. 3 summarizes MEPP frequency values obtained in all groups (means ± SEM) in normal Ringer and in the presence of CCPA. When the values were analyzed using the paired t test, it is clear that CCPA induced a significant decrease of MEPP frequency in CG (normal Ringer: 1.03 ± 0.03/s; CCPA: 0.56 ± 0.03/s, $n = 8$; $P < 0.05$), HVG (normal Ringer: 1.05 ± 0.02/s;

Table 1
MEPP amplitude obtained in normal Ringer solution from non-treated mice (CG) and from those treated with sera from healthy volunteers (HVG), euthymic bipolar patients (EBG), non-stabilized bipolar patients (NSBG), and schizophrenic patients (SG)

	CG	EBG	NSBG		SG
			Manic	Depressive	
mV	1.02 ± 0.12 ($n = 4$)	0.99 ± 0.14 ($n = 4$)	1.01 ± 0.09 ($n = 4$)	0.93 ± 0.10 ($n = 4$)	1.11 ± 0.11 ($n = 4$)

Values are means ± SEM.

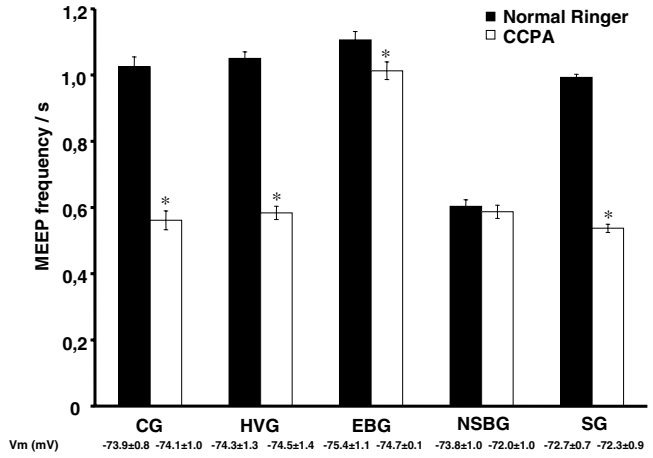

Fig. 3. Summarized bar graph showing mean MEPP frequency obtained in normal Ringer solution and in the presence of CCPA from non-treated mice (CG, $n = 8$) and from those treated with sera from healthy volunteers (HVG, $n = 11$), euthymic bipolar patients (EBG, $n = 21$), non-stabilized bipolar patients (NSBG, $n = 20$), and schizophrenic patients (SG, $n = 28$). $^{*}P < 0.05$ (obtained by paired Student's t test) corresponds to comparisons between mean MEPP frequency recorded in normal Ringer and in the presence of CCPA.

CCPA: 0.58 ± 0.02/s, $n = 11$; $P < 0.05$) and SG (normal Ringer: 0.99 ± 0.01/s; CCPA: 0.54 ± 0.01/s, $n = 28$; $P < 0.05$). In EBG, although individual comparisons of MEPP frequency recorded in normal Ringer solution and CCPA were not significantly different, averaged values depicted a significant difference (normal Ringer: 1.11 ± 0.03/s; CCPA: 1.01 ± 0.03/s, $n = 21$; $P < 0.05$). On the other hand, in NSBG, CCPA failed to exert any modulatory effect on spontaneous ACh release (normal Ringer: 0.60 ± 0.02/s; CCPA: 0.59 ± 0.02/s, $n = 20$ $P > 0.05$). A possible explanation for the lack of action of CCPA may be due to the fact that the A_1 receptors were occupied by AD present in the serum of bipolar patients.

3.3. Effect of passive transfer of high and low molecular weight sera fraction from bipolar patients on MEPP frequency

In order to know the nature of the substance involved in the biological findings observed with the EBG and NSBG on MEPP frequency, sera from bipolar patients

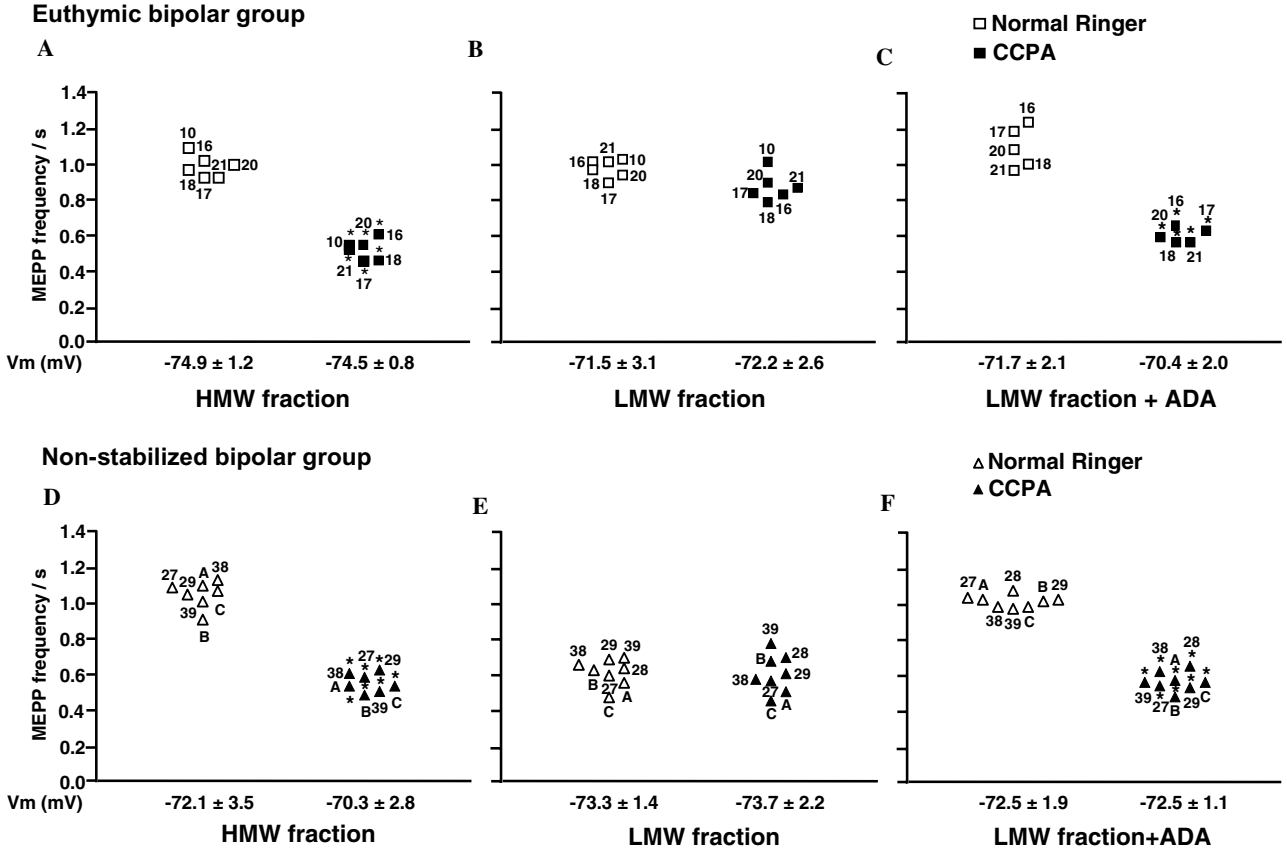

Fig. 4. (A–F) MEPP frequency recorded in normal Ringer and in the presence of CCPA from diaphragm muscles of mice treated with high molecular weight (HMW) sera fraction, and low molecular weight (LMW) sera fraction from euthymic and non-stabilized bipolar patients in the absence and in the presence of ADA. Numbers close to the symbols correspond to the same patients included in Figs. 1 and 2. A, B and C correspond to non-stabilized bipolar patients not included in Figs. 1 and 2. $^{*}P < 0.05$ (obtained by paired Student's t test) corresponds to comparisons between MEPP frequency recorded in normal Ringer and in the presence of CCPA.

were filtered through a Centrifugal Filter with a 3000 MW cut-off resulting in a high MW fraction (>3000) and a low MW fraction (<3000). Then, each fraction from each individual patient was transferred into different mice and MEPP frequency was recorded. As shown in Fig. 4A and D, MEPP frequency recorded from diaphragms of mice treated with high MW sera fraction from euthymic and non-stabilized bipolar patients was within control range and diminished when the muscles were treated with CCPA. Therefore, it is unlikely that immunoglobulins were involved in the results observed with sera from bipolar patients. On the contrary, MEPP frequency recorded from mice treated with low MW sera fraction from euthymic bipolar patients has control values and was not modified by CCPA (Fig. 4B). Besides, low MW sera fraction from non-stabilized bipolar patients decreased MEPP frequency compared to values obtained with CG; low MW sera fraction from these patients also occluded the action of CCPA (Fig. 4E). The results observed with the injection of low MW sera fraction from euthymic and non-stabilized bipolar patients were similar to those observed with the entire sera. These findings have relevance since, in most of the cases, sera fraction and entire sera, came from the same patients.

3.4. ADA prevented the effect of low MW sera fraction on MEPP frequency

We then focused our analysis on the substance of the low MW fraction obtained from sera of euthymic and non-stabilized bipolar patients that presented biological effects on MEPP frequency. To examine the possibility that the substance was AD, we pretreated the low MW fraction with ADA, the enzyme that inactivates endogenous AD by converting it into inosine (Redman and Silinsky, 1994). Previously, to evaluate the effect of ADA on AD in our experimental system, we studied its effect in mice treated with 100 μM AD (1 ml for 2 days). MEPP frequency recorded from diaphragms of animals treated only with AD was diminished to 0.73 ± 0.05/s ($n = 7$) when compared to CG (1.03 ± 0.03/s, $n = 8$), while mice injected with AD + ADA (0.5 U/ml) showed values similar to CG (1.11 ± 0.09/s, $n = 6$), demonstrating that the ADA concentration was adequate (ANOVA: $F = 12.91$; d.f. = 2, 18; $P < 0.05$; Tukey's test: CG vs AD, $P < 0.05$; CG vs AD + ADA, $P > 0.05$; AD vs AD + ADA, $P < 0.05$). Afterwards, we studied the effect of ADA on low MW sera fraction from euthymic and non-stabilized bipolar patients. As can be seen in Fig. 4C, the injection of low MW sera fraction from euthymic bipolar patients + ADA into mice allowed the action of CCPA. In the same way, the injection of low MW sera fraction from non-stabilized bipolar patients + ADA prevented the decrease of MEPP frequency in normal Ringer solution and the occlusion of CCPA action (Fig. 4f).

4. Discussion

In the present investigation, we have demonstrated that bipolar patients contain in their blood an active substance which when passively transferred into mice modifies spontaneous neurotransmitter release at the neuromuscular junction. As a matter of fact, sera belonging to non-stabilized bipolar patients induced a decreased MEPP frequency and occluded the presynaptic inhibitory effect of CCPA.

Hyperactivity of certain neural pathways presumably provokes some of the symptoms of bipolar disorder, and a progressive sensitisation of certain neural pathways is thought to be related to the increase in the frequency and severity of episodes, which occurs during the course of the disease (Post, 1992; van Calker et al., 2000). Moreover, the use of lithium and anticonvulsants such as mood-stabilizers for bipolar affective disorders seems to be related to a reduction of excessive transmitter release in brain, thereby regulating aberrant intracellular and extracellular signalling in critical regions of the limbic forebrain (Xie and Hagan, 1998). For example, it is known that carbamazepine behaved as an A_1 receptor antagonist and the chronic treatment induced "up-regulation" of A_1 receptors (Biber et al., 1999; Daval et al., 1989; van Calker et al., 1991). We obtained similar results at the mouse neuromuscular junction (data not shown), suggesting that the therapeutic effect of carbamazepine may influence neuronal excitability. One possible explanation for the results obtained with sera from non-stabilized bipolar patients would be that they contain a high concentration of AD as a result of synaptic hyperactivity, since the magnitude of MEPP frequency decrease was equivalent to that observed when AD is added to the saline in *in vitro* experiments. In a previous paper, we demonstrated that, when asynchronous secretion is increased by exposing motor nerve terminals to high K^+ concentrations, endogenous AD induces presynaptic inhibition by occupying the A_1 receptors, occluding the effect of the specific A_1 agonist CCPA (De Lorenzo et al., 2004). In the same way, the lack of action of CCPA observed in the diaphragm of mice treated with sera from non-stabilized bipolar patients may be due to the occupation of A_1 receptors by the AD present in the serum of bipolar patients.

The possibility that the source of AD was the hyperactivity of central neural pathways is challenged by the fact that the rate of AD efflux across blood–brain barrier, under physiological conditions, is small compared to the rate of uptake into neurons and glia. However, under conditions of elevated brain interstitial fluid AD such as hypoxia/ischemia, blood–brain barrier efflux transport becomes an important mechanism (Isakovic et al., 2004). On the other hand, we cannot exclude an increase of AD concentration occurring through a more general mechanism; it may be possible that AD in the serum samples derives from peripheral cells (platelets, erythrocytes, leucocytes). Alterations of second messenger pathways were observed in analyses of postmortem brain (Fields et al., 1999; Friedman and

Wang, 1996; Wang and Friedman, 1996, 2001; Young et al., 1993; see review by Ackenheil, 2001; Gould and Manji, 2002) as well as in platelets and leukocytes from bipolar patients (Perez et al., 2000; Tardito et al., 2003; van Calker et al., 1993; Wang et al., 1999).

The behavior obtained with sera from non-stabilized bipolar patients was observed in all animals treated, irrespective of whether the patient was in a manic or depressive episode, suggesting that the mechanism(s) underlying the alterations in neurosecretion is independent of the clinical states. MEPP frequency recorded in normal Ringer solution and in the presence of CCPA was not significantly different in either states of bipolar disease. Moreover, we did not find statistical difference when we analyzed the results by age and gender (data not shown). In the EBG, even though spontaneous neurotransmitter secretion reached control values, the action of CCPA was still prevented in the individual cases. However, if we consider the results as a group, the effect of CCPA was statistically different from the control solution, suggesting that the patients are improving when compared with the non-stabilized bipolar patients. This is consistent with the results observed with patients 4 and 5 (see Figs. 1 and 2): during their manic state, sera from both patients induced a fall in MEPP frequency in normal Ringer and an occlusion of the action of CCPA in the recipient mice. After treatment, these patients not only improve clinically, but MEPP frequency became normal and CCPA induced an inhibition of ≅26% and 27%, respectively.

In order to know more accurately the nature of the active substance, sera from bipolar patients were filtered (cut-off 3000) obtaining two fractions which were transferred into mice. MEPP frequency recorded from diaphragms of mice injected with high MW sera fraction from euthymic and non-stabilized bipolar patients was within control range, while the injection of low MW sera fraction from both groups induced changes in mice neurosecretion similar to those produced by the bipolar entire sera. These results make it unlikely that an immunoglobulin is responsible for the observed biological effects since their MW are over 3000 (high MW fraction). On the other hand, AD has a MW below 3000 (267.2).

Consistent with the notion that AD is the active substance, we found that treatment of the low MW sera fraction with ADA, which completely inactivates AD, caused a return of spontaneous neurotransmitter release to control values and allowed the inhibitory action of CCPA to be observed.

Symptoms of bipolar disease frequently overlap with those of other psychiatric disorders, mainly schizophrenia. Our results are relevant, since sera from all schizophrenic patients neither modified spontaneous release nor interfered with the presynaptic action of CCPA in the recipient mice. So, this raises the possibility that the passive transfer model could be used as a diagnostic test in the future (after trying the test out in a blinded trial). Early diagnosis may lead to an adequate treatment improving the outcome of patients, especially with respect to the course and suicidal mortality.

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