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Cyclic AMP Second-Messenger Signal Amplification in Depression

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Beta-adrenergic-mediated cyclic AMP accumulation was reduced in lymphocytes obtained from depressed patients from that observed in an age- and sex-matched group of control subjects. Among the depressed patients, those not responding to treatment showed significantly lower pretreatment responses to isoproterenol compared with patients who exhibited significant clinical improvement during antidepressant treatment. Late-night (terminal) insomnia was significantly associated with the blunted response to beta-adrenergic stimulation. In depressed patients with the lowest isoproterenol response, the effect of forskolin (which acts distal to the receptor and directly stimulates the catalytic subunit) on cyclic AMP accumulation was also significantly decreased. This suggests that post-receptor modulations of signal amplification also play a role in the reduced response to beta-adrenergic stimulation in depression.

Peripheral blood elements have been widely used for the investigation of receptor-mediated signal-amplification processes in man (Ebstein *et al*, 1986*a*). Three published studies have used this approach to examine beta-adrenergic responsiveness in lymphocytes (Extein *et al*, 1979; Mann *et al*, 1985) and leucocytes (Pandey *et al*, 1979) from patients with endogenous depression. All reported reduced beta-adrenergic-mediated cyclic AMP synthesis in the depressed subjects.

The cyclic AMP signal-amplification system is now known to consist of several interacting enzyme and protein subunits located in the cell membrane (Codina *et al*, 1984). Hormone or neurotransmitter binding to the adenylate cyclase-linked receptor leads to activation of the N protein which in turn stimulates the catalytic subunit of the enzyme. Recent reports have suggested that antidepressant treatments, including electroconvulsive shock (ECS), may significantly alter the sensitivity of these post-receptor components (Menkes *et al*, 1983; Mooney *et al*, 1985; Newman *et al*, 1986; Ebstein *et al*, 1987*a*,*b*). Previous reports suggesting a reduction in beta-adrenergic receptor-mediated cyclic AMP amplification in depressed patients (Extein *et al*, 1979; Pandey *et al*, 1979; Mann *et al*, 1985) therefore merit re-examination using strategies which encompass activation of the adenylate cyclase complex at sites distal to the receptor.

In this study, we examined receptor- and post-receptor-stimulated cyclic AMP accumulation in lymphocytes obtained from patients with major depressive disorder. Isoproterenol was used to evaluate beta-adrenergic receptor-mediated responses, and forskolin, a unique diterpene that directly stimulates the catalytic subunit (Seamon & Daly, 1981), to assess cyclic AMP signal generation distal to the receptor. Prostaglandin E_1 (PGE_1) was used to evaluate the status of a receptor linked to the adenylate cyclase complex in lymphocytes, but was not thought to be implicated in depression.

Method

Subjects

Patients participating in this study were drawn from the Depression Unit of the Jerusalem Mental Health Center, Ezrath Nashim (JMHC). Normal control subjects (without any personal or family history of affective disorder or other psychiatric illness) were drawn from the staff of the hospital. Depressed and control subjects were all physically healthy and were free of psychotropic or other medication for at least 2 weeks prior to blood sampling. Informed consent was obtained according to the guide-lines laid down by the JMHC Internal Review Board.

The diagnosis of major depressive disorder (DSM-III criteria; American Psychiatric Association (1980)) was confirmed in the depressed patients by consensus of two psychiatrists (BL, BS, or ZS). Severity of depressive symptoms was assessed by the Hamilton Rating Scale for Depression (HRSD) (ECDEU version, first 16 items; Guy, 1976). Twenty-nine depressed subjects participated in the study. Their mean ± SEM age was 47.5 ± 2.6 years (range 20–74), and their mean HRSD score (27.5 ± 0.93). Twenty-two fulfilled DSM-III criteria for melancholia, eight had mood-congruent delusions and eight had a prior history of manic episodes and satisfied DSM-III criteria for bipolar disorder. None had any history of alcohol, or other substance, abuse, and social alcohol consumption was reported as minimal by all the subjects. The average age of the control subjects (mean 46.7 ± 3.7, range 22–76) was closely matched with the depressed group. Although previous studies have shown

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an age-associated decline in cyclic AMP synthesis in lymphocytes (Ebstein *et al*, 1984; 1986*a*,*b*), in the present study, no correlation was observed between cyclic AMP accumulation and age either for the control or depressed patients.

Blood samples

Blood samples were obtained by venipuncture in a 50-ml heparin-coated syringe between 0800–0930 h while the subject was in a sitting position. Lymphocytes were isolated from the sample using the method of Boyum (1964) modified in this laboratory as follows (Ebstein *et al*, 1984, 1985; Oppenheim *et al*, 1984; Stessman *et al*, 1985): to reduce platelet contamination to minimal levels, after the initial Ficoll purification step, lymphocytes are repeatedly centrifuged at low speed (250*g*) until microscopic examination shows greater than 95% lymphocyte purity. An additional centrifugation over Ficoll is often necessary to reduce platelet and red-blood-cell contamination.

Cyclic AMP determinations

One-millilitre aliquots of the purified lymphocyte suspension were pre-incubated for 30 min at 37°C in a standard balanced salt solution (pH 7.6) containing the following components: NaCl (0.13 M); glucose (0.01%); 1% bovine serum albumin; $CaCl_2$ (0.005 mM); $MgCl_2$ (0.098 mM); KCl (0.54 mM); Tris (0.015 M), and titrated with HCl to pH 7.6. The lymphocytes were then incubated for an additional 20 min in the presence and absence of drug. 3-isobutyl-1-methylxanthine (1mM), a potent phosphodiesterase inhibitor (Cheung, 1967), was present throughout. Cyclic AMP accumulation was linear under these conditions for at least 20 min. Lymphocytes were collected by centrifugation, suspended in absolute ethanol (1 ml) and disrupted with a Polytron homogeniser. Cyclic AMP was determined by protein-binding assay (Brown *et al*, 1971). All drugs (forskolin, 60 μM; isoproterenol, 10 μM; PGE_1, 1 μM) were used at concentrations previously shown to be maximally stimulating under the conditions employed in this assay (Ebstein *et al*, 1984, 1985; Oppenheim *et al*, 1984; Stessman *et al*, 1985).

Statistical analysis

Differences in group means were compared, using Student's *t*-test, (two-tailed), and correlations by the Pearson correlation coefficient. Two-way analysis of variance (ANOVA) was used to compare differences when the subjects were influenced by different levels of two factors.

Results

Significantly reduced basal and isoproterenol-stimulated cyclic AMP accumulation was observed in lymphocytes obtained from the depressed patients as compared with the control subjects (Fig. 1).

Forskolin-stimulated cyclic AMP accumulation was also lower in the depressed subjects, although the difference did not reach statistical significance ($t = 1.37$; $P = 0.18$). A correlation was, however, observed among the depressed patients between isoproterenol and forskolin-stimulated cyclic AMP accumulation ($r = 0.72$, $P < 0.0001$), suggesting a functional link between receptor and non-receptor mechanisms of activating adenylate cyclase. No correspondence between these two activities was observed for the control subjects ($r = 0.22$, NS). PGE_1-stimulated cyclic AMP accumulation was slightly, but not significantly, higher in the depressed patients (Fig. 1).

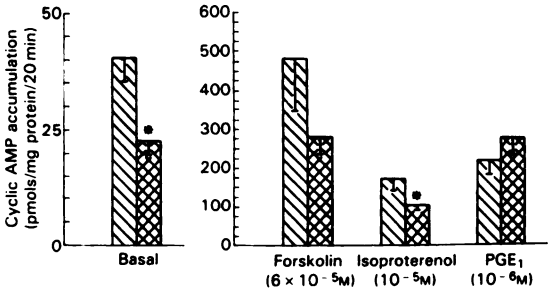


FIG. 1 Cyclic AMP accumulation in lymphocytes from all depressed and normal students. *$P = 0.024$ (isoproterenol); $P = 0.009$ (basal), Student's *t*-test, comparing depressed with control values. Basal cyclic AMP values were subtracted from the forskolin, isoproterenol, and PGE_1-stimulated values ▨, control ($n = 19$); ■, depressed ($n = 29$).

Each individual item in the HDRS was compared with the four biochemical measurements and a significant negative correlation was observed between late-night (terminal) insomnia and isoproterenol (Fig. 2), PGE_1 and basal cyclic AMP accumulation in lymphocytes. No other correlations were significant. No significant differences were observed between unipolar ($n = 21$) and bipolar ($n = 8$) patients, between patients with ($n = 8$) and without ($n = 21$) mood-congruent delusions, and between patients with ($n = 23$) and without ($n = 6$) melancholia.

Treatment-outcome data were available for 24 of the 29 depressed patients. Eight had been assigned to ECT, and 16 to at least 4 weeks of antidepressant medication with imipramine, doxepin, or maprotiline at clinically adequate

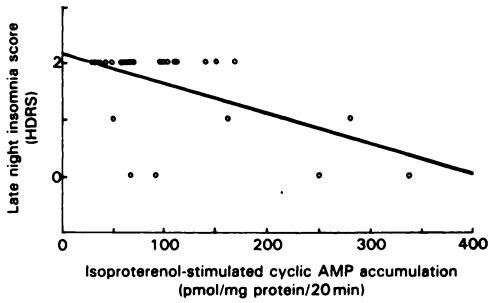


FIG. 2 Correlation of isoproterenol-stimulated cyclic AMP accumulation and HRSD late-night insomnia score. *r* = Pearson correlation coefficient = −0.55; $P = 0.0023$.

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dosages. Twenty of the 29 depressed patients had been referred to the depression unit because of a history of non-response to antidepressant treatment at other centres; twelve patients responded to treatment (HRSD improvement exceeding 50% of baseline, six ECT and six antidepressants), while 12 did not respond (2 ECT, 10 antidepressants). Two of the three patients who did not respond to antidepressants, who went on to receive ECT, did not respond to this treatment either. Pretreatment biochemical parameters for patients who responded to antidepressant therapy were compared with those for patients who did not. Significantly lower isoproterenol-stimulated cyclic AMP accumulation was observed in the group that did not respond, than in the group that did (Fig. 3). Patients not responding to treatment significantly differed from the control subjects, whereas patients who did respond did not. The former also showed a significantly reduced forskolin effect compared with the control subjects, while the latter were not significantly different. All subjects had been free of psychotropic medication for at least 2 weeks before the study. There was no difference in antidepressant dosage or length of treatment between the responding and non-responding groups prior to drug withdrawal, suggesting that the differences in cyclic AMP signal amplification between the two groups were intrinsic features and not a result of prior treatment.

Among the depressed patients, a significant difference between males and females was observed. Depressed males showed reduced isoproterenol-stimulated cyclic AMP accumulation compared with depressed females (129.6 ± 21.68 *v* 68.6 ± 10.2; $t = 2.45$; $P = 0.02$; d.f. = 27). No sex difference was observed among the control subjects. The effect of gender on beta-receptor-mediated cyclic AMP synthesis in the depressed subjects prompted us to examine whether the differences between depressed and control subjects, and between patients responding to treatment and those not, were influenced by the distribution of male and female subjects in these two groups. A two-way ANOVA was used to compare sex and isoproterenol response: (a) between all depressed and control subjects; and (b) between those depressed subjects who did and did not respond to treatment. Although a significant effect of diagnosis (all depressed *vs* control, $f = 6.51$, 1; $P = 0.014$) as well as treatment outcome (responding group *vs* non-responding group, $f = 5.52$, 1; $P = 0.025$) was observed on isoproterenol responsiveness, there was no significant interaction between gender and diagnosis ($f = 1.37$, 1; NS), or gender and treatment outcome ($f = -1.10$, 1; NS).

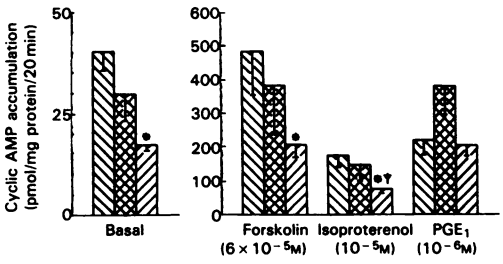


FIG. 3 Cyclic AMP accumulation in lymphocytes from patients who responded to treatment and those who did not. *$P = 0.0249$ (isoproterenol) comparing non-responding with responding patients; $P = 0.0017$ (isoproterenol) comparing non-responding patients with control subjects; *$P = 0.057$ (forskolin) comparing non-responding patients with control subjects; *$P = 0.0013$ (basal) comparing non-responding patients with control subjects ▨, control ($n = 19$); ▩, patients responding to treatment ($n = 12$); ▨, patients not responding to treatment, $n = 12$).

Discussion

The present findings are in agreement with those reported in three previous studies (Extein *et al*, 1979; Pandey *et al*, 1979; Mann *et al*, 1985) which examined beta-adrenergic-mediated cyclic AMP synthesis in peripheral-cell preparations from depressed patients. All showed a significant reduction in responsiveness to isoproterenol, underscoring the robust nature of this observation. In the present study, and two others (Extein *et al*, 1979; Mann *et al*, 1985), in which PGE_1-stimulated adenylate cyclase activity was measured, no decrease in this parameter was found. The normal PGE_1 response demonstrates that the blunted beta-adrenergic response is specific, and does not indicate a decline in all cyclic AMP second-messenger function in depression.

In the study of Mann *et al* (1985), the isoproterenol response in patients with psychomotor agitation was significantly lower than in control subjects, whereas patients without psychomotor agitation did not significantly differ from controls. In addition, patients with psychomotor agitation had significantly lower isoproterenol responses than depressed patients without psychomotor retardation. In the present study, we found no such relationship with agitation. On the other hand, we observed a significant inverse correlation between 'late-night insomnia' HRSD score, and receptor-mediated cyclic AMP synthesis. These results are noteworthy, since late-night or terminal insomnia has been classically regarded as pathognomonic for 'endogenous' depression. Insomnia-associated humoral fluctuations may explain the more strongly reduced isoproterenol responsiveness in these patients, since lymphocyte beta-adrenergic receptor function has been convincingly demonstrated to be sensitive to changes in plasma catecholamine concentrations (Fraser *et al*, 1981; Feldman *et al*, 1983).

The identification of patients not responding to treatment as a subgroup of depressed patients, with the most significant impairment in beta-adrenergic-mediated signal generation, is a potentially important finding. It is, however, limited by the fact that treatment assignment was not a progressive element of the study design, but nevertheless suggests that

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depressed patients with lower lymphocyte beta-adrenergic-receptor sensitivity may be more resistant to antidepressant treatment than those with higher values. Those who respond to treatment do not signficantly differ from control subjects, strengthening the proposed identification of a subgroup of depressed patients with the most significant impairment in adenylate cyclase function. Since all depressed patients had been previously exposed to periods of antidepressant therapy, the biochemical differences in the current study between patients who responded to treatment and those who did not are unlikely to have been influenced by previous histories of treatment.

In patients not responding to treatment, the group with the lowest reaction to isoproterenol, forskolin-stimulated adenylate cyclase activity was significantly lower than in the control subjects. A possible explanation for the reduced forskolin effect may be that second-messenger function 'downstream' to the receptor is influenced by chronic changes at the receptor/N protein level. This explanation is supported by the significant correlation between forskolin and isoproterenol-stimulated adenylate cyclase activity observed in depressed patients, but not in the control subjects. Other studies, including pre-clinical investigations that have examined the effect of chronic antidepressant treatment on cyclic AMP responsiveness to agents acting distal to the receptor, also support these conclusions (Menkes *et al*, 1983; Mooney *et al*, 1985; Newman *et al*, 1986; Ebstein *et al*, 1987*a*,*b*).

The reason for the blunted isoproterenol-stimulated cyclic AMP response in lymphocytes from depressed patients is still unclear. On the one hand, these results may reflect an inherited abnormality of the beta-adrenergic-stimulated adenylate cyclase complex as suggested by the investigations of Wright *et al* (1984), who observed reduced beta-receptor number in transformed lymphoblastoid cells from depressed patients. However, an alternative explanation that elevated plasma catecholamine levels in depression (Wyatt *et al*, 1971; Esler *et al*, 1982; Lake *et al*, 1982; Barnes *et al*, 1983) leads to down-regulation of the lymphocyte beta-adrenergic complex cannot be excluded.

Further studies are therefore needed to characterise more clearly the reduced beta-adrenergic responsiveness of depressed patients in terms of trait or state association. The role of post-receptor modifications of cyclic AMP second-messenger function also remains to be clarified. In the absence of direct access to brain tissue, important aspects of the pathophysiology of depression may be illuminated by these investigations.

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