Associated Disturbances in Calcium Homeostasis and G Protein–Mediated cAMP Signaling in Bipolar I Disorder

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Background: Evidence of extensive cross-talk between calcium (Ca\(^{2+}\))- and cAMP-mediated signaling systems suggests that previously reported abnormalities in Ca\(^{2+}\) homeostasis in bipolar I (BP-I) patients may be linked to disturbances in the function of G proteins that mediate cAMP signaling.

Methods: To test this hypothesis, the \( \beta \)-adrenergic agonist, isoproterenol, and the G protein activator, sodium fluoride (NaF), were used to stimulate cAMP production in B lymphoblasts from healthy and BP-I subjects phenotyped on basal intracellular calcium concentration ([Ca\(^{2+}\)]\(_B\)). cAMP was measured by radioimmunoassay and [Ca\(^{2+}\)]\(_B\) by ratiometric fluorometry with fura-2.

Results: Isoproterenol- (10 \( \mu \)M) stimulated cAMP formation was lower in intact B lymphoblasts from BP-I patients with high [Ca\(^{2+}\)]\(_B\) (> 2 SD above the mean concentration of healthy subjects) compared with patients having normal B lymphoblast [Ca\(^{2+}\)]\(_B\) and with healthy subjects. Although basal and NaF-stimulated cAMP production was greater in B lymphoblast membranes from male BP-I patients with high versus normal [Ca\(^{2+}\)]\(_B\), there were no differences in the percent stimulation. This suggests the differences in NaF response resulted from higher basal adenylyl cyclase activity.

Conclusions: These findings suggest that trait-dependent disturbances in processes regulating \( \beta \)-adrenergic receptor sensitivity and G protein–mediated cAMP signaling occur in conjunction with altered Ca\(^{2+}\) homeostasis in those BP-I patients with high B lymphoblast [Ca\(^{2+}\)]\(_B\).

Key Words: Bipolar disorder, G proteins, signal transduction, cAMP, calcium homeostasis, B lymphoblasts

Introduction

Evidence from studies of postmortem brain and peripheral blood cells implicate disturbances in G protein–mediated cAMP signaling and Ca\(^{2+}\) homeostasis in the pathophysiology of bipolar affective disorder (BD; reviewed in Li et al 2000; Manji 1992; Warsh and Li 1996). For instance, increased stimulatory G protein \( \alpha \) subunit (Go\(_s\)) levels (Manji et al 1995; Young et al 1994), blunted receptor- and G protein–stimulated cAMP accumulation (Ebstein et al 1988; Mann et al 1985; Pandey et al 1979) and increased basal intracellular calcium levels ([Ca\(^{2+}\)]\(_B\); Dubovsky et al 1992b; Emamghoreishi et al 1997) have all been observed in mononuclear leukocytes from patients with BD. Moreover, some of these signaling disturbances appear to be trait dependent, possibly reflecting underlying molecular “vulnerability” factors in BD (Emamghoreishi et al 1997; Warsh et al 1999). Although it has been speculated that these signal transduction abnormalities are linked through cross-talk mechanisms (Dubovsky et al 1992a; Warsh and Li 1996), to our knowledge there is no empirical evidence for such a link.

Extensive cell-type specific cross-talk occurs between G protein–coupled cAMP- and phospholipase C (PLC)–linked second-messenger systems (Hill and Kendall 1989; Liu and Simon 1996). Multiple adenylyl cyclase (AC) subtypes provide the potential for Ca\(^{2+}\) either to synergise or attenuate cAMP signaling (Sunahara et al 1996). Thus, Ca\(^{2+}\)/CaM directly activates AC isotypes I and VIII, whereas Ca\(^{2+}\) inhibits isotypes V and VI (Sunahara et al 1996). Ca\(^{2+}\)-activated protein kinase C (PKC) may also increase or decrease cAMP levels by phosphorylating \( \beta \)-adrenergic receptors (\( \beta \)-ARs; Bell and Brunton 1987), Gso, Gix (Katada et al 1985; Sugden and Klein 1988; Yoshimasa et al 1987) or some AC subtypes (Cooper et al 1995). Recently, it has been shown that Ca\(^{2+}\)-activated...
PKC suppresses protein kinase A (PKA)-mediated signaling in fibroblast cell lines (Dobbing and Berchtold 1996), providing another site of interaction between these signaling pathways.

cAMP-mediated processes also affect Ca²⁺ signaling by inhibiting Ca²⁺ influx (Rasmussen 1986), desensitizing inositol trisphosphate receptors (Supattapone et al 1988), stimulating Ca²⁺ removal from the cytosol by Ca²⁺ ATPase (Helman et al 1986), and inhibiting PLCβ₂ (Liu and Simon 1996). In other instances, G protein–mediated cAMP signaling enhances that of Ca²⁺, including PKA-mediated phosphorylation of voltage-dependent Ca²⁺ channels in excitable cells (Chad et al 1987), or the ion gate that closes K⁺ channels in cells in general, thus prolonging depolarization and enhancing Ca²⁺ influx (Kandel et al 1987). There is also evidence that in non-excitable cells, receptor-activated Ca²⁺ channels are opened by an increase in the concentration of cytoplasmic cAMP (Barritt 1999). Such mechanisms as noted above would account for the types of interaction (i.e., stimulation/suppression) that occur between cAMP and phosphoinositide signaling pathways.

As disturbances in both G protein–mediated cAMP and Ca²⁺ signaling have been previously reported in BD, cross-talk regulatory mechanisms are potentially important candidates to be considered in explaining abnormalities in these two signal transduction pathways. Thus, the increased lymphocyte and platelet intracellular Ca²⁺ ([Ca²⁺]ₜ) reported in BD might result from the altered G protein function previously identified in these patients, or vice versa. In effect, defects in one signaling system may decompensate or, alternatively, induce adaptive changes in other signaling systems in an attempt to maintain homeostasis.

To test this hypothesis, we used Epstein–Barr virus (EBV)–immortalized B lymphoblasts derived from BD patients, a cellular model that demonstrates trait-dependent abnormalities in Ca²⁺ homeostasis (Emamghoreishi et al 1997) and in β-AR sensitivity (Kay et al 1993) in this disorder. These cell lines can be grown in continuous culture removed from in vivo neurohormonal influences and medications, which circumvents the potential of such variables to hamper detection of signal transduction abnormalities and interactions between signaling cascades. To screen for possible relationships between disturbed G protein function and altered basal intracellular [Ca²⁺]ₜ ([Ca²⁺]ₜ), isoproterenol and sodium fluoride (NaF)-stimulated cAMP formation were determined in B lymphoblasts from bipolar I (BP-I) patients stratified based on [Ca²⁺]ₜ compared with healthy subjects. We report here the coexistence of altered receptor- and G protein–mediated cAMP signaling and calcium homeostasis, supporting the view that these abnormalities are both interrelated and trait-dependent.

### Methods and Materials

**Subjects**

Cell lines were selected from a subset of BP-I patients and healthy subjects recruited and assessed as previously described (Emamghoreishi et al 1997). Briefly, subjects were recruited from the Mood Disorders inpatient and outpatient units of the Clarke Institute of Psychiatry. After a clinical diagnosis of BP-I disorder was made by a consulting psychiatrist, the diagnosis was confirmed for research purposes using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P, version 2.0; First et al 1995b) administered by a research psychiatrist or trained psychiatric research assistant, complemented by review of medical records. All interview and historical material was further reviewed by a research psychiatrist to ensure the reliability of the diagnoses. Patients had no history of recent (>3 months) substance abuse. Healthy comparison subjects were recruited through posted advertisements in the University of Toronto and were assessed by a trained research assistant and a research psychiatrist using the SCID-I/NP, version 2.0 (First et al 1995a). Healthy subjects had no past or family (first-degree relatives) history of psychiatric disorder. All subjects were physically healthy, had no past history of serious medical illness, and had no personal or family history of diabetes or hypertension. The study was approved by the Human Subjects Review Committee of the University of Toronto and, after providing a complete description of the study, all subjects provided written informed consent. Table 1 shows the demographics and [Ca²⁺]ₜ

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Healthy</th>
<th>BP-I high [Ca²⁺]ₜ</th>
<th>BP-I normal [Ca²⁺]ₜ</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>38.6 ± 3.60</td>
<td>37.7 ± 4.00</td>
<td>39.8 ± 4.27</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>7/4</td>
<td>6/5</td>
<td>3/8</td>
</tr>
<tr>
<td>Lifetime psychiatric comorbidity</td>
<td>NA</td>
<td>—Panic disorder, 2—alcohol dependence</td>
<td>5—Alcohol abuse or dependence</td>
</tr>
<tr>
<td>B lymphoblast [Ca²⁺]ₜ</td>
<td>53.2 ± 1.75</td>
<td>74.8 ± 1.70</td>
<td>57.5 ± 1.03</td>
</tr>
</tbody>
</table>

*Mean ± SEM.

*BP patients with a lifetime history of alcohol abuse or dependence had no recent history (>3 months) of substance abuse at the time of study. One BP-I subject with normal [Ca²⁺]ₜ also had a past, but not recent, history of polysubstance abuse.

*Tukey p < .001, compared with healthy subjects and BP-I patients with normal [Ca²⁺]ₜ.
of the BP-I patients and healthy subjects from whom cell lines were selected for study.

**B Lymphoblast Transformation and Intracellular Calcium Measurements**

Mononuclear leukocyte preparations were isolated by density gradient separation through Ficoll-Hypaque and B lymphocytes in these isolates were transformed by infection with EBV as previously described (Emamghoreishi et al 1997). The transformed, immortalized B lymphoblasts were grown in RPMI-1640 medium containing L-glutamine (2 mmol/L), pyruvate (1 mmol/L), 20% fetal bovine serum (FBS), 100 μg/mL streptomycin, 100 units/mL penicillin in an incubator (95% air/5% CO2 at 37°C) with passages every 3–4 days. At 12–15 passages, aliquots of cells were removed and [Ca2+]B determined as described above. The remaining cells were frozen in aliquots (107 cells/mL) and stored over the vapour phase of liquid nitrogen until below. The remaining cells were thawed quickly (<5 min) at 37°C and transferred gently into 25-cm2 culture flasks containing 5 mL pre-warmed (37°C) B lymphoblast medium and regrown as previously described (Emamghoreishi et al 1997). BP-I subjects were stratified based on [Ca2+]B greater than (high) or less than (normal) two standard deviations above the mean [Ca2+]B determined in cells from healthy subjects, to classify patients into these two phenotypes.

**Regeneration of B lymphoblast Cell Lines**

B lymphoblasts were removed from the liquid nitrogen freezer and immediately placed on dry ice. Cells were then thawed quickly (<5 min) at 37°C and transferred gently into 25-cm2 culture flasks containing 5 mL pre-warmed (37°C) B lymphoblast medium and regrown as previously described (Emamghoreishi et al 1997). After nine passages from the time of regeneration (total passages 21–24), cells were transferred to B lymphoblast medium containing 10% FBS and incubated for three additional passages. Finally, cells were transferred to B lymphoblast medium without FBS and incubated for 24 hours, then harvested and divided in two aliquots. One aliquot (8–10 × 107 cells) was used immediately to assay isoproterenol-stimulated cAMP formation, and the other was stored at −70°C until used to assay G protein-stimulated AC activity.

**Determination of Basal and Isoproterenol-Stimulated cAMP Production in Intact B Lymphoblasts**

Isoproterenol was used to assess β-adrenergic receptor-stimulated cAMP formation in intact B lymphoblasts using a method modified from Kay et al (1993). Cells were washed once in buffer containing 130 mmol/L NaCl, 0.54 mmol/L KCl, 0.005 mmol/L CaCl2, 0.098 mmol/L MgCl2, 0.1% glucose and 15 mmol/L Tris.HCl pH 7.6, resuspended at 3 × 106 cells/mL, and incubated in 0.9 mL aliquots (30 min, 37°C, 95% air/5% CO2). The reactions were begun by adding 50 μL isobutyl-methylxanthine (IBMX, final concentration 0.5 mmol/L), 50 μL ascorbic acid (final concentration 0.1 mmol/L) with or without isoproterenol, and incubating at 37°C for 10 min. Reactions were stopped by boiling for 10 min, then centrifuging at 12,000 g for 10 min at 4°C. The protein concentration in each pellet was determined by the Lowry method (Lowry et al 1951). The supernatants, which contained the cAMP, were evaporated to dryness under vacuum, stored at −70°C until needed, then reconstituted with 100 μL assay buffer containing 50 mmol/L sodium acetate at pH 6.2, and 0.01% sodium azide. The cAMP content was measured using an 125I-radioimmunoassay kit following the supplier’s instructions (Mandel, Guelph, Canada). Maximally stimulating and saturating concentrations of isoproterenol (1 and 10 μmol/L, respectively) were determined in preliminary dose-response studies, and subsequently used to assess B lymphoblast receptor-stimulated cAMP production in the comparison groups.

**Determination of Basal and NaF-Stimulated AC Activity in B Lymphoblast Membranes**

**MEMBRANE PREPARATION.** B lymphoblasts were thawed on ice, resuspended in 20 volumes ice-cold buffer (10 mmol/L Tris, pH 7.4, 1 mmol/L EGTA) and homogenized by hand using a glass-teflon homogenizer (15 strokes). The homogenates were centrifuged at 20,000 g (20 min, 4°C), then the pellets were re-suspended and centrifuged twice more. Each pellet was resuspended in 500 μL ice-cold TEMS buffer (50 mmol/L Tris, pH 7.4, 1 mmol/L EGTA, 5 mmol/L MgCl2, 12% sucrose), and protein concentrations were determined by the method of Bradford (Bradford 1976), using bovine serum albumin as a standard.

**ASSAY OF NAf-STIMULATED AC ACTIVITY.** Membrane homogenates were diluted with TEMS buffer to give 1 μg membrane protein/μL and the samples kept on ice until use. Reaction mixtures (200 μL) contained 50 mmol/L Tris, pH 7.4, 1 mmol/L EGTA, 3.5 mmol/L MgCl2, 0.1 mmol/L IBMX, 1.5 mg/mL BSA, 0.5 mmol/L adenosine triphosphate (ATP), 25 mmol/L phosphocreatine, and 250 units/mL creatine phosphokinase. Membrane homogenates (50 μL) were incubated at 37°C for 15 min in 100 μL reaction mixture. The reactions were initiated by adding 50 μL ATP (2 mmol/L) in the absence or presence of NaF (2.5, 5, and 10 mmol/L), followed by gently vortexing and incubation at 37°C for 10 min with gentle shaking. The reaction was terminated by boiling the tubes for 10 min, followed by cooling on ice and centrifuging at 12,000 g for 10 min at 4°C. The supernatants were then removed and stored at −70°C until used for cAMP measurements as described above.

**Statistical Analysis**

Differences in dependent variables (basal and stimulated cAMP formation) were assessed by repeated-measures multivariate analysis of variance (MANOVA). Concentration (basal and various concentrations of isoproterenol or NaF) was the repeated “within” factor and diagnosis was the “between” subject factor. NaF-stimulated cAMP formation values were logarithmically transformed to normalize the distribution of the data. Based on previous studies, we tested directional hypotheses (i.e., of reduced responsivity to isoproterenol stimulation) [Extein et al
levels was 17% lower than that achieved with 1 μmol/L isoproterenol respectively; Table 2). The increase in absolute cAMP stimulation above basal was also observed with 1 μmol/L isoproterenol-stimulation of B lymphoblasts from healthy subjects, although this difference did not reach statistical significance (37%, t = 1.58, p = .065).

Additionally, the reduced isoproterenol-stimulated cAMP production in BP-I patients with high [Ca\(^{2+}\)]\(_{\text{in}}\) was more marked at 10 μmol/L and was significantly different from that determined at 1 μmol/L isoproterenol (t = 2.0, p = .025). In preliminary experiments using membrane preparations, isoproterenol-stimulation was very low and variable, as we have also observed for other tissues (Young et al 1993). Thus, there was no further attempt to measure isoproterenol-stimulated cAMP formation in membranes from the subject samples studied.

### Table 2. Basal and Isoproterenol-Stimulated cAMP Production in B Lymphoblasts from Healthy Subjects and Bipolar I Patients with High (>69.4 nmol/L) and Normal (<69.4 nmol/L) [Ca\(^{2+}\)]\(_{\text{in}}\)

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>High [Ca(^{2+})](_{\text{in}})</th>
<th>Normal [Ca(^{2+})](_{\text{in}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>9.24 ± 1.28</td>
<td>10.7 ± 1.71</td>
<td>12.0 ± 1.62</td>
</tr>
<tr>
<td>Isoproterenol (1 μmol/L)</td>
<td>16.9 ± 2.55</td>
<td>16.8 ± 4.24</td>
<td>20.5 ± 3.08</td>
</tr>
<tr>
<td>% stimulation</td>
<td>82.9 ± 12.5</td>
<td>52.3 ± 15.7</td>
<td>69.0 ± 12.6</td>
</tr>
<tr>
<td>Isoproterenol (10 μmol/L)</td>
<td>16.6 ± 3.00</td>
<td>14.7 ± 3.83</td>
<td>21.6 ± 3.42</td>
</tr>
<tr>
<td>% stimulation</td>
<td>79.4 ± 19.4</td>
<td>33.5 ± 15.2(^{a,b})</td>
<td>81.8 ± 17.6</td>
</tr>
</tbody>
</table>

cAMP production (see Methods) is expressed as pmol/mg protein/10 min (mean ± SEM, 11 subjects in each group). The % stimulation over the basal level is shown.

\(^{a}\)Significantly different (t = 2.0, p = .025) from stimulation by 1 μmol/L isoproterenol in the same patient group.

\(^{b}\)Significantly different from BP-I subjects with normal [Ca\(^{2+}\)]\(_{\text{in}}\) (t = 1.95, p = .03) and from healthy subjects (t = 1.85, p = .035).

Results

Basal and Isoproterenol-Stimulated cAMP Production in Intact B Lymphoblasts

Repeated-measures analysis of variance (ANOVA) performed on the absolute values for cAMP formation showed a significant effect of isoproterenol [F(2,60) = 26.1, p < .001], but no significant interaction between diagnosis and isoproterenol [F(4,60) = 1.0, p = .2] or main effect of diagnosis [F(2,30) = 0.71, p = .25]. When the isoproterenol-stimulated AC activity was expressed as the percent stimulation above basal, there was a significant interaction between diagnosis and the percent stimulation [F(4,60) = 2.04, p = .05] and a trend toward a significant main effect of diagnosis [F(2,30) = 1.92, p = .08]. Post hoc comparison of cell means by contrasts showed a significant reduction in the percent stimulation above basal at 10 μmol/L isoproterenol in BP-I patients with high [Ca\(^{2+}\)]\(_{\text{in}}\) compared with normal [Ca\(^{2+}\)]\(_{\text{in}}\), and compared with healthy subjects (59%, t = 1.95, p = .03 and 58%, t = 1.85, p = .035, respectively; Table 2). The increase in absolute cAMP levels was 17% lower than that achieved with 1 μmol/L isoproterenol stimulation in B lymphoblasts from BP-I patients with high [Ca\(^{2+}\)]\(_{\text{in}}\), but was not statistically significant compared with the cAMP levels reached following stimulation of B lymphoblasts from healthy subjects with 10 μmol/L isoproterenol. Reduced percent stimulation above basal was also observed with 1 μmol/L isoproterenol in BP-I patients with high [Ca\(^{2+}\)]\(_{\text{in}}\) compared with healthy subjects, although this difference did not reach statistical significance (37%, t = 1.58, p = .065).

Basal and NaF-Stimulated AC Activity in B Lymphoblast Membranes

NaF increased cAMP production in a concentration-dependent manner, with maximal stimulation at 5 mmol/L and a reduced response at 10 mmol/L NaF (Figure 1A). A repeated-measures MANOVA test showed significant main effects of NaF concentration [F(3,72) = 23.1, p < .001] and gender [F(1,24) = 4.5, p = .02], but not of diagnosis [F(2,24) = 1.5, p = .12]. Moreover, statistically significant interactions were found between sex and NaF concentration [F(3,72) = 6.5, p = .001], and between diagnosis and NaF [F(6,72) = 1.95, p = .04]. Simple effects were performed to examine the interactions. Contrasts of cell means revealed that male subjects were significantly different from female subjects in the magnitude of the response to 2.5 mmol/L NaF [F(1,24) = 21, p < .001] and in the reduced response to 10 mmol/L versus 5 mmol/L NaF [F(1,24) = 4.96, p = .02] (Figure 1, C and D).
the extent of reduction in response to 10 mmol/L compared to 5 mmol/L NaF \[ F(1.23) = 6.2, \ p = .01 \] (Figure 1 E and F).

Figure 1 (C–F) shows NaF-stimulated cAMP production expressed in absolute values and as a percent above basal cAMP production, in the comparison groups stratified by gender. Because the number of female BP-I subjects with normal [Ca\(^{2+}\)]\(_{i}\) was small, further statistical analysis of data was performed only on male bipolar subjects. Repeated-measures MANOVA showed significant effects of NaF concentration \[ F(3,42) = 48.8, \ p < .001 \], an interaction between NaF concentration and diagnosis \[ F(6.42) = 2.4, \ p = .02 \], and a significant main effect of diagnosis \[ F(2,14) = 3.5, \ p = .028 \] (Figure 1C). Male BP-I patients with high [Ca\(^{2+}\)]\(_{i}\) had significantly higher absolute values of basal cAMP (142% greater, \( p < .05 \)) and NaF-stimulated cAMP production (86% greater at 2.5 mmol/L NaF, \( p = .05 \); 94% at 5 mmol/L, \( p < .05 \); 81% at 10 mmol/L, \( p < .05 \)) compared with male BP-I patients with normal [Ca\(^{2+}\)]\(_{i}\). Their cAMP values were also higher than healthy male subjects, but the differences were not statistically significant (84% at 2.5 mmol/L, \( p = .09 \); 73% at 5 mmol/L, \( p = .08 \)). In contrast, analysis of the NaF-stimulated cAMP levels in B
lymphoblast membranes from male subjects expressed as a percent increase above their respective basal activities (Figure 1E) showed only a significant effect of NaF concentration \[F(2,28) = 35.4, \ p < .001\].

**Discussion**

We have used B lymphoblasts from BP-I patients that display high \(\left[Ca^{2+}\right]_B\) to test whether alterations in calcium homeostasis are related to disturbances in receptor-G-protein coupled cAMP formation in BP-I patients manifesting this cellular endophenotype. The principal findings are reduced \(\beta\)-AR-stimulated cAMP formation in intact B lymphoblasts, and elevated basal G protein/effector-dependent cAMP production in membranes of B lymphoblasts from BP-I patients with high \(\left[Ca^{2+}\right]_B\) compared with healthy subjects. Specifically, the \(\beta\)-AR response to 10 \(\mu\)mol/L isoproterenol was significantly lower in BP-I patients with high \(\left[Ca^{2+}\right]_B\) than in healthy subjects, and although the response to 1 \(\mu\)mol/L isoproterenol was also lower, it was not statistically significant. Male BP-I patients with elevated B lymphoblast \(\left[Ca^{2+}\right]_B\) also had significantly higher basal and NaF-stimulated AC activity compared with male BP-I subjects with normal \(\left[Ca^{2+}\right]_B\) and a trend towards higher NaF-stimulated AC activity compared with healthy subjects. These findings suggest that the alterations in intracellular calcium homeostasis in B lymphoblasts from BP-I patients are indeed associated with disturbances in receptor- and G protein–coupled AC activity. Moreover, the co-expression of these disturbances in cells from BP-I subjects implies that this spectrum of signal transduction abnormalities is trait dependent.

Maximally stimulating and saturating concentrations of isoproterenol were used to assess \(\beta\)-AR-stimulated cAMP production in B lymphoblasts because of the limited signal-to-noise ratio of the response and to ensure a maximal level of cAMP stimulation was achieved in all cases in the absence of full dose response studies on each cell line. \(\beta\)-ARs in transformed B lymphoblasts show qualitatively similar ligand binding characteristics and AC activation to those in B lymphocytes, but lower density and maximal enzyme activity (Ehstein et al 1985; Elliot 1987). Furthermore, because the functional interaction between receptors and G proteins is strongly influenced by their relative expression levels (Kenakin 1996), the small responses observed here likely reflect the low density of \(\beta\)-ARs in these cell lines. Dose-response studies were not performed, and inferences about the efficacy of receptor-G protein coupling (reflected in the EC_{50} and exact nature of the altered responsivity will require future concentration-response characterization.

The small magnitude and inter-sample variability in the isoproterenol-stimulated cAMP production at 1 \(\mu\)mol/L may have contributed to the lack of statistically significant differences in B lymphoblast \(\beta\)-AR responses, between BP-I patients with high \(\left[Ca^{2+}\right]_B\) and either patients with normal \(\left[Ca^{2+}\right]_B\) or healthy subjects. Nevertheless, our results highlight the possibility that factors regulating the responsivity of the \(\beta\)-AR-G protein-AC complex are differentially altered in BP-I patients who manifest the endophenotype of high B lymphoblast \(\left[Ca^{2+}\right]_B\). Although \(\beta\)-AR density was not determined in this study, previous studies have shown reduced \(\beta\)-AR-stimulated AC activity in lymphocytes (Mann et al 1985) and B lymphoblasts (Kay et al 1993) from BD patients in the absence of changes in \(\beta\)-AR density or affinity. Furthermore, we have not found differences in \(\alpha\)s and \(\alpha\)i levels in B lymphoblasts from BP-I patients compared with their matched healthy subjects (unpublished data). These observations argue against the possibility that altered levels of \(\beta\)-AR and of these G protein \(\alpha\) subunits account for the reduced \(\beta\)-AR-stimulated response in B lymphoblasts from BP-I patients observed here. Other downstream processes that regulate the response of the receptor-G protein-AC transduction apparatus should be considered, and it is notable that Kay et al (1993) found less \(\beta\)-AR down-regulation in response to isoproterenol in B lymphoblasts from BD patients than in cells from control subjects. These observations suggested the process(es) regulating \(\beta\)-AR desensitization and/or down-regulation may be compromised in BD. \(\beta\)-AR down-regulation and/or desensitization is mediated by receptor phosphorylation through PKA and/or \(\beta\)-AR kinase (Sibley et al 1987). Therefore, the lower \(\beta\)-AR responsiveness in BP-I patients compared with healthy subjects and BP-I patients with normal \(\left[Ca^{2+}\right]_B\), could similarly be related to alterations in \(\beta\)-AR kinase or PKA activity.

Disturbances in cross-talk mechanisms could also be involved. For example, PKC induces \(\beta\)-AR sequestration and desensitization (Kassis et al 1985, Pitcher et al 1992; Sibley et al 1987) and decreases \(\beta\)-AR affinity and coupling to G protein in S49 lymphoma cells (Bell and Brunton 1987). \(Ca^{2+}\) may also affect PKA activity, either directly or indirectly (Blumenthal et al 1986; Dobbeling and Berchtold 1996). Thus, disturbances in \(Ca^{2+}\) homeostasis in BD could readily affect \(\beta\)-AR-mediated cAMP signaling at the receptor level through the intricate framework of cross talk that exists between these signaling systems.

NaF stimulation of cAMP formation was used to probe the integrity of G protein function, as in preliminary experiments NaF stimulated cAMP formation in B lymphoblasts more robustly than GTP\(_\gamma\)S. The latter GTP analogue also acts directly on G protein \(\alpha\) subunits stimulating their coupling to effector enzymes (Yamanaka...
et al 1986). Our NaF stimulation studies, although not definitive, show some potentially important trends. Although there was no main effect of diagnosis in the NaF-induced responses, there was a significant but unexpected interaction with gender, as well as an interaction between increasing concentrations of NaF and the diagnosis. Male subjects differed from female subjects in their concentration-response curves for NaF-stimulated AC activity. For this reason, and because of the small number of female BP-I patients with normal \([Ca^{2+}]_{iB}\) \((n = 3)\), only male subjects were considered in further statistical analyses. Male BP-I patients with high \([Ca^{2+}]_{iB}\) showed higher basal (142%) and NaF-stimulated AC activity (81%–94%) compared with BP-I patients with normal \([Ca^{2+}]_{iB}\). NaF-stimulated AC activity was also higher (73%–84%) in male BP-I patients with high \([Ca^{2+}]_{iB}\) compared with healthy subjects, although these differences did not reach statistical significance, possibly because of high variability and small sample size; however, the greater NaF-stimulated AC activity in BP-I patients with high \([Ca^{2+}]_{iB}\) likely reflects higher basal AC activity in this group, as there were no significant differences in percent stimulation over basal levels among the comparison groups.

In contrast to the significantly higher basal cAMP formation in the B lymphoblast membrane preparations from male BP-I patients with high \([Ca^{2+}]_{iB}\) compared to other comparison groups, no difference was observed in basal cAMP formation in intact B lymphoblasts. This discrepancy is most readily explained by the different cellular matrices and assay conditions used: the NaF-stimulation employed a membrane preparation, whereas intact cells were used in the isoproterenol studies. Basal and stimulated responses in intact cells were assayed in the presence of \(Ca^{2+}\) (i.e., at physiological extracellular \(Ca^{2+}\) concentrations), whereas the membrane assay used EGTA-washed membranes, and was therefore \(Ca^{2+}\) free. The differences in basal cAMP formation in membranes unmasked by removing \(Ca^{2+}\) suggest altered regulation of one or more \(Ca^{2+}\)-dependent processes that influence cAMP formation. In effect, basal cAMP formation appears to be “locked” at a higher baseline in membranes from BD compared with healthy male subjects and is not further upregulated in the presence of \(Ca^{2+}\), as may occur in B lymphoblast membranes from healthy subjects. Because cAMP formation was measured following phosphodiesterase inhibition with IBMX, it is most likely that the postulated dysregulation of cAMP formation in B lymphoblast membranes from BP-I patients with high \([Ca^{2+}]_{iB}\) involves the AC subtypes expressed in these cells and/or factors modulating their activity. Multiple AC isotypes exist that differ in their activation and inhibition by \(G\) protein \(\alpha\) and \(\beta\gamma\) subunits, \(Ca^{2+}\), PKC, and PKA (Sunahara et al 1996). Therefore, higher basal AC activity in B lymphoblasts from BP-I patients with high \([Ca^{2+}]_{iB}\) than in the other comparison groups might reflect differences in catalytic activity or levels of AC isotype (Pieroni et al 1995), particularly because \(G\) protein \(\alpha\) subunit levels did not differ; however, the specific AC isotypes expressed in human B lymphoblasts have not been characterized.

The finding of higher basal AC activity in male BP-I patients with high \([Ca^{2+}]_{iB}\) may be important pathophysiologically. In addition to the factors discussed above, the levels and activity of phosphodiesterase isozymes (Houslay and Milligan 1997) and the extent and type of cross-talk (Cooper et al 1995) also contribute to determining basal cAMP levels. If basal cAMP is near the threshold for activating PKA, a small increase in cAMP either from increased AC activity or reduced phosphodiesterase activity will have a large and rapid effect (Houslay and Milligan 1997). In contrast, when basal AC activity is low, increased AC activity may be the predominant means of triggering PKA responses. Thus, the predicted physiological consequences of modulating PDE and/or AC activity can differ.

In summary, the reduced isoproterenol stimulation and elevated basal AC activity in B lymphoblasts from BP-I patients with high \([Ca^{2+}]_{iB}\) suggests a link between disturbances in calcium homeostasis and processes regulating \(\beta\)-AR sensitivity and \(G\) protein-coupled AC functionality in a subgroup of patients with BD. Further replications are warranted, however, to ensure the replicability of these preliminary findings given the small sample size. Which signaling system (\(Ca^{2+}\) or cAMP) is first affected, and to what extent the changes in the linked transduction cascade are secondary or adaptive responses, remain to be elucidated. Regardless, these disturbances in signal transduction systems, which are expressed in transformed B lymphoblasts from BP-I patients, appear to represent trait-dependent abnormalities that distinguish a pathophysio logically distinct subgroup of BP-I disorder.

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