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ORIGINAL ARTICLE

Abnormal bradykinin signalling in fibroblasts deficient in the PIP_2 5-phosphatase, ocrl1

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Summary The oculocerebrorenal syndrome of Lowe (Lowe syndrome) is an X-linked disorder of phosphatidylinositol metabolism characterized by congenital cataracts, renal proximal tubulopathy and neurological deficits. The disorder is due to the deficiency of the phosphatidylinositol 4,5-bisphosphate (PIP_2) 5-phosphatase, ocrl1. PIP_2 is critical for numerous cellular processes, including cell signalling, actin reorganization and protein trafficking, and is chronically elevated in patients with Lowe syndrome. The elevation of PIP_2 cells of patients with Lowe syndrome provides the unique opportunity to investigate the roles of this phospholipid in fundamental cellular processes. We previously demonstrated that ocrl1 deficiency causes alterations in the actin cytoskeleton. Since actin remodelling is strongly activated by $[Ca^{+2}]$, which increases in response to IP_3 production, we hypothesized that altered calcium signalling might contribute to the observed abnormalities in actin organization. Here we report a specific increase in bradykinin-induced Ca^{+2} mobilization in Lowe fibroblasts. We show that the abnormal bradykinin signalling occurs in spite of normal total cellular receptor content. These data point to a novel role for ocrl1 in agonist-induced calcium release.

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Competing interests: None declared

References to electronic databases: Oculocerebrorenal syndrome of Lowe: OMIM 309000. *OCRL1*: OMIM 300535. Phosphatidylinositol 4,5-bisphosphate 5-phosphatase: EC 3.1.3.36.

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Abbreviations

2-APB	2-aminoethoxydiphenylborate
EGF	epidermal growth factor
GPCR	G-protein-coupled receptor
PDGF	platelet-derived growth factor
PIP_2	phosphatidylinositol 4,5-bisphosphate
TGN	*trans*-Golgi network

Introduction

The oculocerebrorenal syndrome of Lowe (Lowe syndrome) is a rare X-linked disorder characterized by bilateral congenital cataracts, renal proximal tubulopathy including low-molecular-weight proteinuria, albuminuria, aminoaciduria, hypercalciuria, metabolic acidosis, phosphaturia and nephrocalcinosis (Bockenhauer et al. 2008) and neurological deficits. It is due to the deficiency of ocrl1, a type II phosphatidylinositol 4,5 bisphosphate (PIP_2) 5-phosphatase. This enzyme catalyzes the hydrolysis of PIP_2, which has a prominent role in a number of essential cellular processes including cell signalling, protein trafficking and actin polymerization

(Di Paolo and De Camilli 2006; Sheetz et al 2006). Lowe syndrome is the first known human disorder caused by the deficiency of a PIP_2 5-phosphatase. Thus the study of cells derived from patients with Lowe syndrome provides a unique opportunity to investigate the roles of PIP_2 metabolism in cells and tissues.

The ocrl1 protein is ubiquitously expressed, except for haematopoietic tissues; its localization to the *trans*-Golgi network (TGN), endosomes (Choudhury et al 2005; Dressman et al 2000; Ungewickell et al 2004) and the plasma membrane (Erdmann et al 2007; Faucherre et al 2005) suggest that ocrl1 plays a role in protein trafficking. The deficiency of ocrl1 leads to elevated cellular levels of PIP_2 (Wenk et al 2003; Zhang et al 1998). However, it is unknown at present why a deficiency of this widely expressed protein primarily affects the lens, kidney and brain in Lowe syndrome. PIP_2 plays an important role as a second messenger in regulating cell adhesion through the actin cytoskeleton (Raucher et al 2000). Since actin reorganization is required for the formation and maintenance of cell–cell contacts (Lee et al 2000; Shen and Turner 2005), the defects in lens epithelial cell differentiation and renal proximal tubule function in Lowe syndrome could result, in part, from abnormalities in the actin cytoskeleton. We have previously shown that actin remodelling is disrupted in Lowe fibroblasts (Suchy and Nussbaum 2002). Further evidence for a role for type II inositol polyphosphate 5-phosphatases in cell–cell contact formation, particularly in polarized cells, is suggested by work in the mouse. A mouse knockout of the closest paralogue to *Ocrl1*, *Inpp5b*, showed abnormal germ cell adhesion and abnormal Sertoli cell junctions (Hellsten et al 2002). However, the loss of *ocrl1* by itself did not result in a detectable phenotype in the mouse as it does in humans (Janne et al 1998).

Actin polymerization and the formation of actin-dependent structures such as tight junctions are calcium-dependent processes (Vasioukhin et al 2000). Intracellular calcium concentrations are strongly influenced by the hydrolysis of PIP_2, which results in the release of calcium from internal stores. We therefore hypothesized that a chronic PIP_2 5-phosphatase deficiency and elevation of PIP_2 in Lowe syndrome fibroblasts might also lead to increased calcium release from internal stores. This in turn might contribute to the observed abnormalities in actin remodelling, ultimately resulting in abnormal junction formation. Differences in calcium signalling between cell types might help explain the tissue specificity of the Lowe syndrome phenotype.

To test the hypothesis that calcium signalling is disrupted in Lowe cells, we measured calcium release from internal stores in Lowe fibroblasts and controls. We found that Lowe fibroblasts had an augmented response to bradykinin stimulation but, surprisingly, we did not observe a generalized increase in cell signalling with other agonists known to induce IP_3-mediated intracellular calcium release. These data point to a novel role for ocrl1 in altering only certain pathways in agonist-induced calcium release.

Materials and methods

Patients and cell lines

Normal human skin fibroblast cultures were obtained from American Type Culture Collection (Manassas, VA, USA), and from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ, USA). Fibroblast cultures from unrelated patients with Lowe syndrome, which had been obtained with the informed consent of a responsible parent or guardian, were used (NIH IRB protocol numbers 01-HG-0008, 01-HG-0095). Cells were grown in Dulbecco's modified essential medium (Gibco; Grand Island, NY, USA) with 15% fetal bovine serum and 2 mmol/L glutamine at 37°C with 5% CO_2.

Calcium imaging

Live cell imaging was performed with cell permeant, acetoxymethyl (AM) ester dyes, fluo 4 (0.5 μmol/L) and fura red (1.0 μmol/L), to measure calcium response to agonists. Upon binding calcium, fluo 4 fluorescence intensity increases and fura red intensity decreases (Molecular Probes; Carlsbad, CA, USA). Dyes were added to 20% pluronic solution (Molecular Probes and resuspended in nominally calcium free incubation buffer (1.06 mmol/L KH_2PO_4, 2.97 mmol/L $NaHPO_4$, 155 mmol/L NaCl, 20 mmol/L Hepes, 25 mmol/L glucose, 5.3 mmol/L KCl, 1 mmol/L sodium pyruvate, 0.8 mmol/L magnesium sulfate, 100 μmol/L EGTA, 1.5 mg/ml BSA, pH 7.3) (Holtzclaw et al 1995) with 0.08 mg/ml sulfinpyrazone to inhibit organic anion transport activity. To control for potential minor variations in buffer or dye prepared each day, a Lowe and control culture were run together as a pair. Seven different pairs of Lowe and control cultures were matched for passage number from four Lowe and four control cultures from different individuals. Each pair of cultures was plated two days prior to imaging, washed, and loaded into confocal chambers for a 20-min incubation with dyes at room temperature. Cultures were used at approximately 70% confluency.

Cells were rinsed three times, washed 20 min in incubation buffer, rinsed and loaded onto the confocal stage. A baseline calcium concentration was recorded for 5–10 frames, followed by stimulation with bradykinin (100 nmol/L), histamine (100 μmol/L) (Calbiochem, San Diego, CA, USA), or 50 ng/ml PDGF (platelet-derived growth factor; Sigma; St Louis, MO, USA). In other experiments, the cells were treated with the calcium ionophore A23187 (20 μmol/L) (Calbiochem) in order to measure stored calcium. Cells were imaged with a Zeiss Axiovert 100 M confocal microscope with LSM 510 software, using a Zeiss 20×/0.75 planapochromat objective. An excitation wavelength of 488 nm was used and images were collected with a 505–550 nm bandpass filter and a 650 nm long-pass filter, at a maximum pinhole setting, every 1–2 s for 3 min (for bradykinin, histamine and A23187) or 5 min (for PDGF and EGF). Data analysis was performed with the KaleidaGraph software (Synergy Software, Reading, PA, USA).

IP_3 receptor inhibition

Cells were incubated with the $[Ca^{2+}]$-sensitive dyes, as described above, washed and incubated for the final 5 min of the wash with 20–100 μmol/L 2-aminoethoxydiphenylborate (2-APB) (Calbiochem/ EMD Biosciences, La Jolla, CA, USA), and maintained in the presence of 2-APB during imaging. Cells were stimulated with 100 nmol/L bradykinin and imaged as described above.

Western blotting

Fibroblasts from four different Lowe patients and four controls were harvested and 20 μg of total cell protein was loaded on 10% polyacrylamide gels. Proteins were separated by electrophoresis and transferred to a PDVF membrane (Immobilon P, Millipore Corporation, Bedford, MA, USA). The membrane was blocked for 30 min at 37°C in 5% non-fat dry milk, TBST buffer (50 mM Tris, 150 mM NaCl, 0.1% Tween 20), before overnight incubation with monoclonal anti-B2 bradykinin receptor antibodies (1/1000) (BD Biosciences, Pharmingen, San Jose, CA, USA). The membranes were washed, incubated for 1 h with peroxidase-conjugated anti-mouse IgG antibody, washed again, and detected by chemiluminescence (ECL, Amersham Biosciences, Piscataway, NJ, USA). The membranes were stripped and reprobed with a monoclonal anti-β-tubulin antibody (1/200), AB3194 (Abcam, Inc., Cambridge, MA, USA), and the receptor protein was quantified by densitometry using β-tubulin as a control.

Results

Lowe fibroblasts show increased response to bradykinin

Agonist-induced intracellular calcium release was measured in primary cultures of Lowe and control fibroblasts. The studies were performed with the cells in calcium-free buffer in order to focus on the release of calcium from internal stores without having the results confounded by agonist-induced influx of extracellular calcium. Two cell-permeant calcium-sensitive fluorescent indicator dyes, fluo-4 and fura red, were used for ratiometric assessment of calcium release. Fluo-4 fluorescence increases upon binding of free calcium, whereas fura-red fluorescence decreases on free calcium binding. Following a baseline assessment in untreated cells, fibroblasts were stimulated with bradykinin, resulting in increased ratio of fluo 4/fura red that peaked approximately 15 s after stimulation (Table 1), then declined to baseline levels. Bradykinin was used to stimulate seven pairs of cultures of Lowe and control fibroblasts. The response was assessed by measurement of the peak calcium release in an average of 34 cells per genotype. In all seven pairs of cultures, Lowe cells had a higher mean calcium release than control (Table 1). This difference was statistically significant in six of seven pairs. Overall, Lowe cells showed a 26% increase in calcium release over controls. Lowe cells also tended to reach peak calcium concentrations faster than controls; the mean time to peak calcium in Lowe cells 12.4 seconds versus 18.3 seconds in control fibroblasts, although this was not statistically significant (paired t-test, $t = 2.10$, $p = 0.08$). The ratiometric method employed was not sensitive enough to detect baseline differences in intracellular free calcium between Lowe and control fibroblasts in the absence of agonist stimulation.

It is well established that bradykinin signalling occurs via the IP_3 receptor. We confirmed the calcium release we observed in response to bradykinin was IP_3 receptor-mediated by using 2-APB, a non-competitive inhibitor of the IP_3 receptor (Maruyama et al 1997; Missiaen et al 2001). Cells were preincubated with increasing concentrations of 2-APB (20–100 μmol/L) and then stimulated with 100 nmol/L bradykinin. We found that 2-APB inhibited agonist-stimulated calcium release in a dose-dependent manner (Fig. 1). At lower doses of 2-APB, there was a reduced peak response to bradykinin stimulation, as well as a reduction in the number of cells that responded to the agonist. Increasing concentrations of 2-APB diminished both the level of response and the number of cells that responded. At

Table 1 Release of calcium from internal stores in lowe and control fibroblasts in response to 100 nmol/L bradykinin

Experiment[a]	Number of cells analysed		Peak Ca^{2+} released (Fluo 4 / Fura red)				t[b]	p[c]
	Lowe	Control	Lowe		Control			
			Mean	SE[d]	Mean	SE		
1	29	24	2.37	0.09	1.69	0.08	5.76	<0.0001
2	28	22	2.15	0.07	1.87	0.05	3.35	0.002
3	48	48	1.44	0.04	0.92	0.04	8.57	<0.0001
4	39	49	1.49	0.03	1.40	0.05	1.73	0.087
5	26	15	2.84	0.16	2.26	0.15	2.67	<0.020
6	41	30	2.00	0.04	1.50	0.07	5.86	<0.0001
7	34	41	2.23	0.13	1.94	0.05	4.58	<0.0001

[a]Pairs of Lowe and control fibroblast cultures derived from different individuals.
[b]t, t-test.
[c]p, significance level.
[d]SE, standard error.

the lowest dose of 2-APB tested, the peak calcium release in response to bradykinin was reduced to 59.3% and 88.3% of that observed in untreated fibroblasts in Lowe and control cells, respectively. At higher doses of the compound very few cells responded to bradykinin, so we measured the dose-response of 2-APB inhibition by the number of cells that responded to bradykinin stimulation after treatment with the inhibitor. In the present study, concentrations of 2-APB ≤100 μmol/L were used to avoid additional effects beyond inhibition of the IP_3 receptor, which have previously been reported to occur at higher 2-APB concentrations (Missiaen et al 2001).

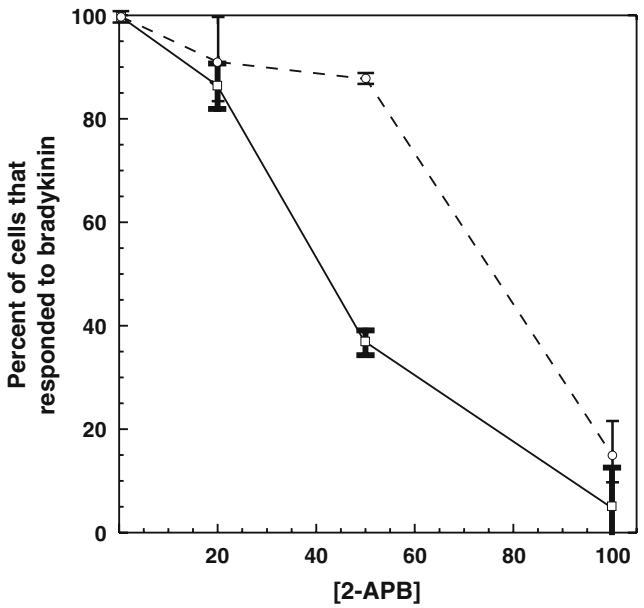

Fig. 1 Bradykinin stimulation caused an IP_3 receptor-dependent release of stored calcium. 2-Aminoethoxydiphenyl borate (2-APB), 20–100 μmol/L, a non-competitive inhibitor of the IP_3 receptor, reduced the response to 100 nmol/L bradykinin in both Lowe (solid line, squares) and control fibroblasts (broken line, circles) in a dose-dependent manner. The standard error of each data point is displayed as a vertical line, thick solid line for the standard error of measurement of the Lowe cells, thin line for the standard error of measurement of the control cells

Many cells have two major types of intracellular calcium release channels: those gated by the IP_3 receptor and those gated by the ryanodine receptor. Ryanodine receptor activation requires a higher baseline calcium level than that present in the calcium free media in experiments reported here. To confirm that we were measuring IP_3 receptor-mediated Ca^{2+} release, we tested whether we could observe ryanodine receptor activity in fibroblasts under the same conditions (nominally calcium-free medium) used here for measuring the IP_3-receptor-mediated responses. We stimulated cells with caffeine, an activator of the ryanodine receptor. We found no detectable calcium release in these fibroblasts even after stimulation with 100 mmol/L caffeine, confirming that the calcium release we were measuring in our experiments was IP_3-mediated.

Calcium stores are not abnormal in Lowe fibroblasts

To determine whether there was an increase in calcium stores in Lowe fibroblasts that might account for the increased response in Lowe cells, a calcium ionophore, A23187, was used to empty calcium stores. The relative total calcium released from the stores was measured as described above for agonist stimulation, and total stored calcium was assessed from the integrated area under the calcium release curve. Using

Table 2 Total Ca^{2+} released from internal stores in response to the calcium ionophore, A23187 (20 μmol/L)

Experiment[a]	Number of cells analysed		Total Ca^{2+} released				t[b]	p[c]
	Lowe	Control	Lowe		Control			
			Mean	SE[d]	Mean	SE		
1	38	34	52.82	1.66	59.13	2.62	2.03	<0.05
2	36	41	92.27	3.92	93.76	3.51	0.28	0.78
3	30	30	47.63	2.00	63.11	3.52	3.82	<0.001
4	32	37	79.52	4.30	96.21	3.56	3.02	<0.005

[a]Pairs of Lowe and control fibroblast cultures derived from different individuals.
[b]t, t-test.
[c]p, significance level.
[d]SE, standard error.

four pairs of cell cultures, we found no elevation in the total stored calcium in Lowe cells (Table 2). In fact, there was a trend for lower stored calcium in Lowe fibroblasts, with three of the four experiments showing a statistically significant reduction in stored calcium in Lowe fibroblasts. Therefore, higher calcium stores in Lowe cells is not the explanation for the increase in calcium release in response to bradykinin.

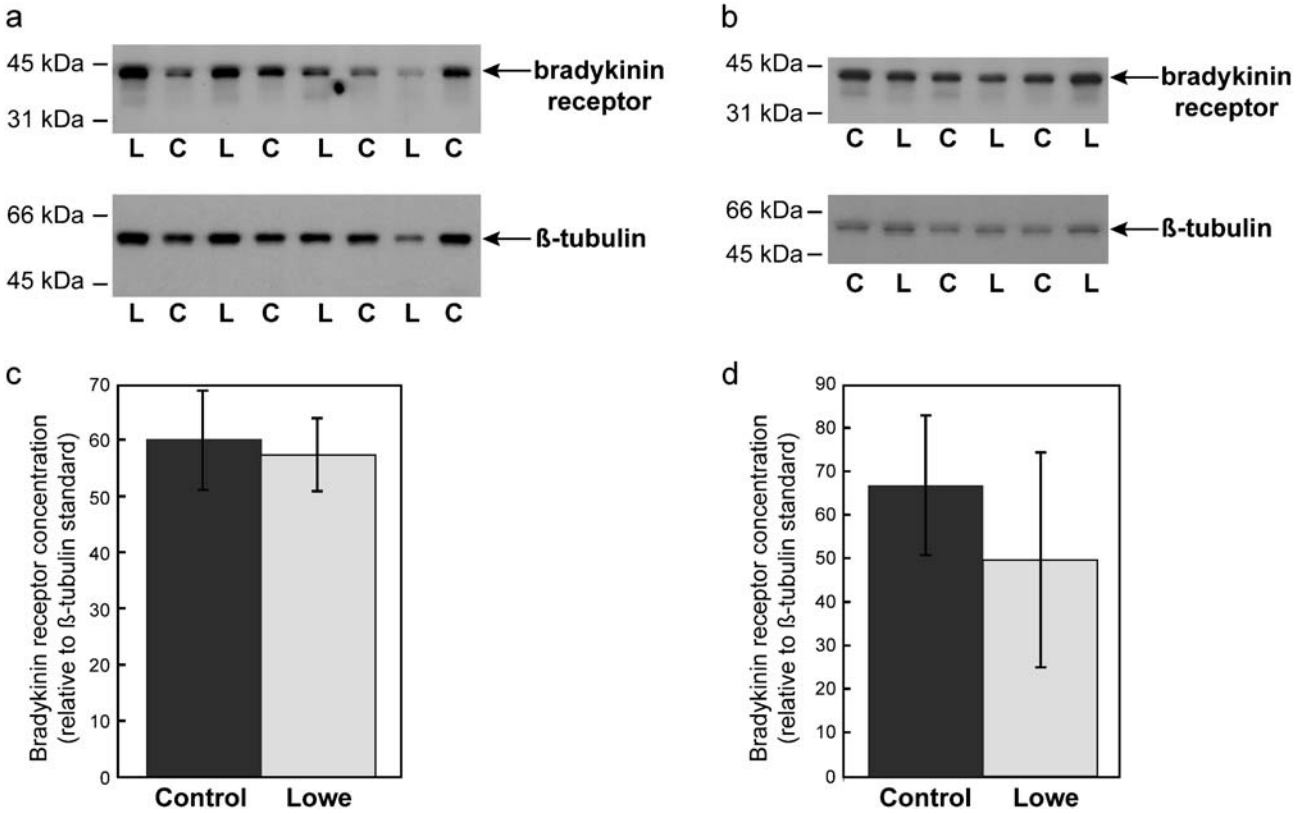

Fig. 2 The total cellular bradykinin receptor concentration was not increased in Lowe fibroblasts. Shown are the results of quantitative western analysis of two experiments, the first showing four different Lowe patient fibroblast cultures and four controls (experiment 1; panels **a**, **c**). Proteins were separated by electrophoresis and western blotting was performed using a monoclonal antibody to the bradykinin receptor and to β-tubulin protein, used as a loading control (panel **a**). The concentrations of receptor protein and β-tubulin protein were quantified by densitometry and the concentration of receptor protein was expressed relative to the β-tubulin protein. The mean and standard error of the relative bradykinin concentrations are shown in the histogram in panel **b**; the standard error is represented by the vertical bar. There was no significant difference in bradykinin receptor concentration observed between Lowe and control cells ($t = 0.249$, $p = 0.81$). These results were replicated in experiment 2 (panels **b**, **d**) using three different Lowe and control cell cultures, with similar results ($t = 1.04$, $p = 0.36$)

Table 3 Release of Ca^{2+} from internal stores upon stimulation with histamine (100 μmol/L)

Experiment[a]	Number of cells analysed		Peak Ca^{2+} released (Fluo 4 / Fura red)				t[b]	p[c]
	Lowe	Control	Lowe		Control			
			Mean	SE[d]	Mean	SE		
1	22	50	0.89	0.06	0.85	0.06	1.14	0.25
2	32	42	1.46	0.10	1.86	0.08	3.13	0.003
3	40	42	1.40	0.04	1.48	0.06	1.16	0.25
4	48	50	1.41	0.06	1.98	0.06	8.18	<0.0001

[a]Pairs of Lowe and control fibroblast cultures derived from different individuals.
[b]t, t-test.
[c]p, significance level.
[d]SE, standard error.

The concentration of bradykinin receptor is not increased in Lowe fibroblasts

We next investigated the possible cause for the abnormal bradykinin-induced calcium release in Lowe cells. The total bradykinin receptor expression was measured in four different Lowe patient fibroblast cultures and four control fibroblast cultures (Fig. 2a). Western analysis of total cell lysates, and quantification by densitometry relative to a β-tubulin control, revealed no difference in the total number of bradykinin receptors present in Lowe and control cells ($t = 0.249$, $p = 0.81$) (Fig. 2a and c). The results of a second set of experiments using three pairs of Lowe and control fibroblast cultures provided similar results (Fig. 2b and d).

Increased signalling in Lowe cells was not generalized

Our hypothesis was that the elevated calcium response to IP_3 was the result of elevated levels of PIP_2, the metabolic precursor of IP_3. This would imply that stimulation with other agonists that activate the phospholipase C-mediated hydrolysis of PIP_2 would also result in an increased response in Lowe cells. We therefore tested the response of Lowe and control cells to stimulation with a number of additional agonists. Histamine, like bradykinin, signals through a G-protein-coupled receptor (GPCR). PDGF signals through a tyrosine–kinase coupled receptor. We observed that, unlike the response to bradykinin, the response of Lowe fibroblasts to histamine was not increased over that of controls. Instead, Lowe fibroblasts tended to show a decreased response to histamine. In two experiments this decreased response to histamine was statistically significant (Table 3). The time to peak calcium concentration after histamine stimulation was 19 s in Lowe cells and 15 s in control cells, which was not significantly different ($t = 0.384$, $p = 0.72$). Thus, we found no evidence for an increase in response to histamine stimulation in Lowe cells. Furthermore, the response of Lowe fibroblasts to PDGF stimulation was not significantly different from controls in four experiments (Table 4). Nor did we

Table 4 Released of Ca^{2+} from internal stores upon stimulation with PDGF (50 ng/ml)

Experiment[a]	Number of cells analysed		Peak Ca^{2+} released (Fluo 4 / Fura red)				t[b]	p[c]
	Lowe	Control	Lowe		Control			
			Mean	SE[d]	Mean	SE		
1	29	29	1.08	0.07	1.12	0.04	0.58	0.56
2	17	32	0.93	0.08	0.80	0.05	1.39	0.17
3	32	32	0.83	0.07	0.81	0.05	0.20	0.85
4	35	41	0.79	0.09	0.80	0.05	0.06	0.95

[a]Pairs of Lowe and control fibroblast cultures derived from different individuals.
[b]t, t-test.
[c]p, significance level.
[d]SE, standard error.

observe a difference in time to peak calcium in PDGF stimulated cells; the mean time to peak calcium concentration was 111 s in Lowe cells and 112 s in controls ($t = 0.055$, $p = 0.96$). We also tested the response of Lowe and control fibroblasts to bombesin (signalling through a GPCR) and epidermal growth factor (signalling via a tyrosine-kinase coupled receptor) in several pairs of Lowe and control fibroblast cultures. Although only 50–70% of the fibroblasts responded to EGF or bombesin stimulation, compared with 92–100% with bradykinin or histamine and greater than 90% after PDGF stimulation, we observed no evidence for a consistent difference in the peak response of Lowe versus control cells (data not shown). These results indicate that the observed increased response to bradykinin stimulation in Lowe fibroblasts was not indicative of a generalized increase in the cell signalling response.

Discussion

We show here that Lowe patient fibroblasts have increased intracellular [Ca^{+2}] mobilization in response to bradykinin stimulation. This indicates that PIP_2 5-phosphatases can play a role in [Ca^{+2}] signalling and that abnormal [Ca^{+2}] signalling may contribute to the phenotype in Lowe syndrome. Bradykinin and the other agonists used in this study trigger calcium release from internal stores by the rapid phospholipase C-stimulated hydrolysis of PIP_2, producing IP_3 (Cruzblanca et al 1998). IP_3 binds to IP_3 receptors on several intracellular organelles, most notably the endoplasmic reticulum. A subsequent wave of calcium release that occurs by the parallel production of diacylglycerol, which activates protein kinase C and stimulates the influx of extracellular calcium, was not considered here, as the cells were incubated in nominally calcium free medium.

We predicted a generalized increase in calcium signalling in Lowe cells due to increased PIP_2 substrate availability for IP_3 production. However, the defect in signalling was observed only with bradykinin and not with any of the other G-protein-coupled receptors or the tyrosine kinase-coupled receptor agonists that we tested. Furthermore, we found no increase in stored calcium in Lowe cells. We conclude that the increased bradykinin-stimulated [Ca^{+2}] mobilization in Lowe syndrome fibroblasts must occur by a mechanism in addition to simply increasing the availability of PIP_2 substrate for IP_3 production and is specific to bradykinin signalling per se. We show here that the augmented response to bradykinin stimulation in Lowe cells was not due to a change in the total receptor content or to an increase in calcium stores. We hypothesize, therefore, that the observed response may be due to disrupted endocytosis/trafficking of the bradykinin receptors. Bradykinin receptors are trafficked by caveolae- or non-clathrin-mediated mechanisms (De Weerd and Leeb-Lundberg 1997; Haasemann et al 1998; Lamb et al 2002), whereas the other receptors tested here, PDGF and histamine, are not (Sato et al 2003; Self et al 2005; Newton et al 2005). The caveolin-1 binding motifs, $\Phi X\Phi(X)_4\Phi$ and $\Phi(X)_4\Phi X\Phi$, (where Φ is an aromatic amino acid (W,Y,F) and X is any amino acid) (Couet et al 1997) are present in ocrl1 at amino acids 223–230, 345–352, 555–562 of the ocrl1 protein sequence, U57627 (numbering from the putative *OCRL1* start site, corresponding to the second methionine in the reference sequence) (Suchy et al 1995). Overexpression of ocrl1 has been reported to block Shigatoxin b trafficking (Choudhury et al 2005), a process mediated by caveolae (Nichols and Lippincott-Schwartz 2001). Furthermore, the cellular localization of ocrl1 has implicated it in protein trafficking. Thus, an investigation of abnormalities in caveolar trafficking would be a logical next step in attempting to understand the cellular abnormalities caused by a deficiency in ocrl1.

How might defective bradykinin signalling contribute to the Lowe syndrome phenotype? The peptide hormone bradykinin is a common cellular agonist and the bradykinin receptor is expressed in most tissues, including the lens, kidney and brain (Vio et al 1996), the tissues primarily affected in Lowe syndrome. Disruptions in calcium signalling can affect cells and tissues in a way that can resemble the Lowe syndrome pathology. Abnormal calcium signalling has been shown to alter cell adhesion in polarized renal epithelial cells (De Blasio et al 2004; Stuart et al 1996) and appears to play a role in the development of lens cataracts including posterior subcapsular cataracts that occur in Lowe patients (Churchill and Louis 2002; Gupta et al 2004). Calcium is also critical in regulating the release of neurotransmitters and has been shown to play a role in initiating seizures, which occur in 50% of Lowe syndrome patients (Fletcher et al 1996; Pal et al 2001).

While abnormal bradykinin signalling may contribute to the phenotype in susceptible tissues, we suspect that the signalling defect may be one facet of a broader problem in actin reorganization and/or endocytosis in Lowe cells. PIP_2 is a second messenger affecting the reorganization of the actin cytoskeleton (Raucher et al 2000) and has a direct effect on endocytosis (Martin 2001; Cremona and De Camilli 2001). The phenotype of Lowe syndrome points strongly to a defect in epithelial tight junction formation that requires actin

polymerization. Interestingly, it has recently been reported that caveolae-mediated endocytosis is required for the formation of tight junctions and that the mechanism by which actin depolymerization disrupts tight junctions is by inhibiting caveolae-mediated endocytosis (Shen and Turner 2005). Caveolae are present in most tissues, including lens epithelial and fiber cells, and participate in signalling and endocytosis (Hnasko and Lisanti 2003; Lo et al 2004). Not all epithelial cells are affected in Lowe syndrome, indicating that some types of epithelial cells are more susceptible than others to the effects of ocrl1 deficiency. Ultimately, the explanation for how a deficiency of ocrl1 leads to the Lowe syndrome phenotype must elucidate both why the target tissues are affected and why other tissues are not. The explanation may lie in the differential concentration of PIP_2 and PIP_3 in the apical or basolateral membranes of epithelial cells (Gassama-Diagne et al 2006; Pilot et al 2006), in the differential organization of the actin cytoskeleton in different cell types (Lee et al 2000; Yonemura et al 2004), or in the selective expression and trafficking of endocytic proteins in different tissues (Lutcke et al 1994). Further studies on the role of ocrl1 in various epithelial cell types may lead to a better understanding of the complex phenotype of Lowe syndrome.

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