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Impaired Mast Cell Activation in Gene-Targeted Mice Lacking the Serum- and Glucocorticoid-Inducible Kinase SGK1

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The PI3K pathway plays a pivotal role in the stimulation of mast cells. PI3K-dependent kinases include the serum- and glucocorticoid-inducible kinase 1 (SGK1). The present study explored the role of SGK1 in mast cell function. Mast cells were isolated from bone marrow (BMMC) of SGK1 knockout mice (sgk1−/−) and their wild-type littermates (sgk1+/+). The BMMC number as well as CD117, CD34, and FcεRI expression in BMCCs were similar in both genotypes. Upon Ag stimulation of the FcεRI receptor, Ca2+ entry but not Ca2+ release from intracellular stores was markedly impaired in sgk1−/− BMMCs. The currents through Ca2+-activated K+ channels induced by Ag were significantly higher in sgk1+/+ BMMCs than in sgk1−/− BMMCs. Treatment with the Ca2+-activated K+ channels in both genotypes, indicating that the Ca2+-activated K+ channels are similarly expressed and sensitive to activation by Ca2+ in sgk1+/+ and sgk1−/− BMMCs, and that blunted stimulation of Ca2+-activated K+ channels was secondary to decreased Ca2+ entry. Ag-IgE-induced degranulation and early IL-6 secretion were also significantly blunted in sgk1−/− BMMCs. The decrease in body temperature following Ag treatment, which reflects an anaphylactic reaction, was substantially reduced in sgk1−/− mice, pointing to impaired mast cell function in vivo. Serum histamine levels measured 30 min after induction of an anaphylactic reaction were significantly lower in sgk1−/− than in sgk1+/+ mice. The observations reveal a critical role for SGK1 in ion channel regulation and the function of mast cells, and thus disclose a completely novel player in the regulation of allergic reaction. The Journal of Immunology, 2009, 183: 4395–4402.

Materials and Methods

Animals

All animal experiments were conducted according to the guidelines of the American Physiological Society as well as the German law for the welfare of animals and were approved by local authorities.

As described previously (36), a conditional targeting vector was generated from a 7-kb fragment encompassing the entire transcribed region on 12 exons. The neomycin resistance cassette was flanked by two loxP sites and inserted into intron 11. Exons 4–11, which code for the sgk1/ domain, were floxed by inserting a third loxP site into intron 3. Targeted R1 embryonic stem cells were transiently transfected with Cre recombinase. A clone with a recombination between the first and third loxP site (type 1 recombination) was injected into C57BL/6 blastocytes. Male chimeras were bred to 129/SvJ females. Heterozygous sgk1-deficient mice were

human; TNCB, trinitrochlorobenzene; TRPV2, transient receptor potential cation channel subfamily V, member 2; PDK1, phosphoinositide-dependent kinase.

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backcrossed to 129/Sv wild-type mice for two generations and then intercrossed to generate homozygous skg/+/ and skg−/− littermates. The animals were genotyped by PCR using standard methods. The study has been performed in 6- to 8-wk-old female and male skg+/+ and skg−/− mice.

Culture of bone marrow-derived mast cells (BMMC)
Femoral bone marrow cells from 6- to 8-wk-old skg+/+ and skg−/− naive mice were cultured for 4 wk in RPMI 1640 (Life Technologies) containing 10% FCS, 1% penicillin/streptomycin, 20 ng/ml IL-3 (R&D Systems), and 100 ng/ml concentrations of the c-kit ligand stem cell factor (Peprotech; Tebu-bio). BMMC maturation was confirmed by flow cytometry (FACSCalibur; BD Biosciences) using the following specific fluorochrome-labeled Abs: PE-labeled anti-FceRI (eBioscience); allophycocyanin-labeled anti-CD117 (BD Biosciences); and FITC-labeled anti-CD34 (BD Biosciences). For experiments, BMMCs were sensitized for 1 h with monoclonal mouse anti-dinitrophenyl IgE (anti-DNP IgE; 10 µg/ml per 1 × 106 cells, clone SPE-7; Sigma-Aldrich) in culture medium and then challenged acutely with dinitrophenyl-human serum albumin (DNP-HSA, 50 ng/ml; Sigma-Aldrich).

Determination of mast cell numbers in the ear
Anesthetized mice were euthanized by cervical dislocation, and the skin was cleansed with 70% ethanol. Ears were cut off at the base, fixed in 4% paraformaldehyde overnight, and finally embedded in paraffin. Tissue sections 3–4 µm thick taken from the middle of the ears were prepared, deparaffinized, and stained with toluidine blue. Mast cell numbers of at least three slices per ear were determined using a Zeiss Axiosvert 200 microscope with a LD Achromat ×40 lens in bright-field mode.

Peritoneal lavage
Anesthetized mice were euthanized by cervical dislocation, and the abdominal skin was cleansed with 70% ethanol. Sterile 0.9% NaCl (4 ml) was then instilled into the peritoneum. The abdomen was massaged gently for 1 min and then opened with sterile scissors. Recovered peritoneal lavage fluid was centrifuged at 1000 × g for 5 min, the cell pellets were resuspended in RBC lysis buffer for 1 min and recentrifuged, and then the cell pellet was resuspended in PBS. Slides were prepared as thick blood films and fixed with 4% paraformaldehyde. After staining with toluidine blue, at least 600 cells/slide were counted. Alternatively, peritoneal lavage fluid was analyzed by FACS analysis.

Measurement of intracellular Ca2+ concentration
Intracellular Ca2+ measurements were performed as described (46) using fura 2-AM. Fluorescence measurements were conducted with an inverted phase-contrast microscope (Axiovert 100; Zeiss). Cells were excited alternately at 340 and 380 nm, and the light was deflected by a dichroic mirror into the objective (Fluar 40/Ô/1.3 oil; Zeiss). Emitted fluorescence intensity was recorded at 505 nm; data were acquired using specialized computer software (Metafluor; Universal Imaging). As a measure of the increase in cytosolic Ca2+ activity, the slope and peak of the changes in the 340 nm:380 nm ratio were calculated for each experiment.

BMMCs were sensitized with IgE at 37°C either with 10 µg/ml for 1 h or with 100 ng/ml overnight and subsequently loaded with fura 2-AM (2 µM; Molecular Probes) for 20 min at 37°C. Intracellular Ca2+ was measured before and following addition of DNP-HSA to IgE-sensitized BMMCs in the absence or presence of extracellular Ca2+.

For intracellular calibration purposes, ionomycin (10 M) was applied at the end of each experiment. Experiments were performed with Ringer solution containing (in millimolar concentrations): 125 NaCl, 5 KCl, 1.2 MgSO4, 2 CaCl2, 2 NaHPO4, 32 HEPES, 5 glucose, pH 7.4. To reach nominally Ca2+-free conditions, experiments were performed using Ca2+-free Ringer solution containing (in millimolar concentrations): 125 NaCl, 5 KCl, 1.2 MgSO4, 2 NaHPO4, 32 HEPES, 0.5 EGTA, 5 glucose, pH 7.4.

Patch clamp
Patch clamp experiments have been performed at room temperature in voltage-clamp, fast-whole-cell mode (47). The cells were continuously perfused through a flow system inserted into the dish. The bath was grounded via a bridge filled with NaCl Ringer solution. Borosilicate glass pipets (2–4-MOhm tip resistance; GC 150 TF-10; Harvard Apparatus) manufactured by a micropipette-driven DMZ puller (Zeitz) were used in combination with a patch clamp headstage. Intracellular pipettes were filled with a solution containing (in millimolar concentrations): 125 NaCl, 5 KCl, 1.2 MgSO4, 2 NaHPO4, 32 HEPES, 0.5 EGTA, 5 glucose, pH 7.4.

Triton X-100 was used to induce delayed-type hypersensitivity reactions, which are strictly dependent on hapten-specific memory T cells and mast cells and are associated with a strong infiltrate of polymorphonuclear neutrophils (10, 49). These memory T cells lead to contact hypersensitivity reactions when the hapten is applied to the skin of sensitized mice (9). The mice were sensitized with 50 µl of a 1:9 mixture of acetone-olive oil at the shaved abdomen; size, 2 × 2 cm. One week later, mice were challenged with 1% TNCB (20 µl of a 1:9 mixture of acetone-olive oil) on both sides of the ear. In the 5% TNCB solution acetone is an irritant, whereas it is used holding potential to potentials between −115 mV and +65 mV. The currents were recorded with an acquisition frequency of 10 kHz and were 3 kHz low-pass filtered.

The pipet solution contained (in millimolar concentrations): 140 potassium gluconate, 5 KCl, 1.2 MgCl2, 2 EGTA, 1.26 CaCl2 (pCa 7), 2 NaATP, and 10 HEPES/KOH (pH 7.2), and was used in combination with NaCl Ringer bath solution (in millimolar concentrations): 145 NaCl, 5 KCl, 2 MgCl2, 1 CaCl2, 10 glucose, 10 HEPES/NaOH (pH 7.4). For some experiments, Ca2+-free Ringer solution was used that contained 1 mM EGTA. Where indicated, the Ag DNP-HSA (50 ng/ml; Sigma-Aldrich), the channel blockers clotrimazole (1.5 µM; Sigma-Aldrich), apamin (500 nM; Sigma-Aldrich), and iberiotoxin (100 nM; Sigma-Aldrich), as well as the Ca2+-ionophore ionomycin (1 µM; Sigma-Aldrich) were added to the bath solution. DNP-HSA, apamin, and iberiotoxin were prepared as stock solutions in water, clotrimazole in ethanol, and ionomycin in DMSO. The final concentrations of DMSO and ethanol were 0.01 and 0.005%, respectively.

The offset potentials between both electrodes were zeroed before sealing, and the potentials were corrected for liquid junction potentials as estimated according to the method of Barry and Lynch (48). The original whole-cell current traces are depicted without further filtering and currents of the individual voltage square pulses are superimposed. The applied voltages refer to the cytoplasmic face of the membrane with respect to the extracellular space. The inward currents, defined as flow of positive charge from the extracellular to the cytoplasmic membrane face, are negative currents and depicted as downward deflections of the original current traces.

B-Hexosaminidase release and IL-6 production
For the B-hexosaminidase release assay, mature BMMCs (0.3 × 106 per well) were seeded in a 96-well plate in fresh medium with 10 µg/ml anti-DNP IgE Ab for 1 h. Afterward, cells were washed in PBS, and the number of recovered peritoneal lavage cells was counted. Alternatively, peritoneal lavage fluid was analyzed by FACS analysis.

Data are shown as the percent of the total release (0.1% Triton X-100) corrected for spontaneous release.

The concentration of IL-6 released from mast cells, which were sensitized with 1 µg/ml IgE for 6 h at 3°C and then stimulated with DNP-HSA (50 ng/ml) for 30 min, 6 h, or 24 h, was measured by ELISA according to the instructions of the manufacturer (BD Biosciences).

Passive systemic anaphylaxis/Ag-induced anaphylaxis and histamine serum concentrations
Mice were sensitized with 2 µg/g weight (−50 µg per mouse) anti-DNP IgE by i.p. application. The next day, mice were challenged with either DNP-HSA (−120 µg/mouse or 4.8 µg/g weight) or PBS. Body temperature was monitored before and each 7 min after Ag challenge with a BAT-10 type T thermocouple thermometer and a RET-3 rectal probe for mice (Physitemp Instruments; distributed by World Precision Instruments) using a Duol18 data recording system (World Precision Instruments) during the midportion of the light phase of the light cycle. Mice were placed with the tail raised and the Vaseline petroleum jelly-covered probe was inserted a standardized distance of 2 cm until a stable temperature reading was obtained. Baseline temperature was measured after habituating mice to rectal probe insertion. Ambient room temperature was 23°C, and the animals were exposed to a 12-h light and 12-h dark cycle (7 a.m.–7 p.m.). Data are expressed as a change in body temperature following treatment (Δ°C). Thirty minutes after induction of an anaphylactic reaction, blood was taken for the analysis of histamine plasma concentration. Histamine was measured by ELISA according to the instructions of the manufacturer (IBL-Hamburg).

Triton X-100 was used to induce delayed-type hypersensitivity reactions, which are strictly dependent on hapten-specific memory T cells and mast cells and are associated with a strong infiltrate of polymorphonuclear neutrophils (10, 49). These memory T cells lead to contact hypersensitivity reactions when the hapten is applied to the skin of sensitized mice (9). The mice were sensitized with 5% TNCB (50 µl of a 1:9 mixture of acetone-olive oil) at the shaved abdomen; size, 2 × 2 cm. One week later, mice were challenged with 1% TNCB (20 µl of a 1:9 mixture of acetone-olive oil) on both sides of the ear. In the 5% TNCB solution acetone is an irritant, whereas it is used...
experiments. All data were tested for significance using Student’s unpaired

cultures) of sgk1

SE; cells in primary culture. Mean percent (±SE; n = 6 individual BMMC
cultures) of sgk1+/+ (■) and sgk1−/− (□) BMMCs acquired within the

mast cell gate. APC, Allophycocyanin.

FIGURE 1. Maturation of BMMCs from sgk1+/+ and sgk1−/− mice. A, Original

dot plots of CD117+, CD34- and FcεRI-positive BMMCs from sgk1+/+ and

sgk1−/− mice. Numbers depict the percent of cells in the respective

quadrant, acquired within the mast cell gate. B, Percent of mast
cells in primary culture. Mean percent (±SE; n = 6 individual BMMC
cultures) of sgk1+/+ (■) and sgk1−/− (□) BMMCs acquired within the

mast cell gate. APC, Allophycocyanin.

solely as a solvent in the 1% TNCB solution. Specific ear swelling was de-

termined by measuring ear thickness with a micrometer (Oditest; Kroepelin)

before and 4, 8, 12, and 24 h after TNCB challenge. Data are expressed as a

change in ear swelling comparing to thickness before treatment (Δμm), cor-

rected for irritation reaction in control mice (n = 4).

Statistics

Data are provided as means ± SE; n represents the number of independent

experiments. All data were tested for significance using Student’s unpaired
two-tailed t test or ANOVA (Dunnet’s test), where applicable. Results with

p < 0.05 were considered statistically significant.

Results

As illustrated in Fig. 1, BMMCs express CD117, CD34, and

FcεRI, i.e., receptors typically expressed by mast cells. No signifi-
cant difference in receptor abundance was observed between

BMMCs from SGK1 knockout mice (sgk1−/−) and their wild-type

littermates (sgk1+/+).

The number of mast cells in the peritoneum analyzed either by

FACS analysis (Fig. 2, A and B) or by toluidine blue staining (Fig. 2C)
as well as the number of mast cells in the skin, analyzed by

staining of ear sections with toluidine blue (Fig. 2D), was similar in

sgk1+/+ and sgk1−/− mice.

According to fura-2 fluorescence, IgE-dependent activation of

BMMCs was followed by an increase in cytosolic Ca2+

concentration. The increase was significantly steeper in sgk1−/− than in

sgk1+/+ BMMCs (Fig. 3, A and B). Accordingly, the maximal

fluorescence Δratio (peak) and the slope of the ratio (Δratio/time)

upon stimulation with Ag were significantly smaller in sgk1−/−

BMMCs than in sgk1+/+ BMMCs (Fig. 3B). Routinely, the cells

were sensitized with 10 μg/ml IgE for 1 h, but in one set of ex-

periments a lower concentration of IgE was used (100 ng/ml over-
night). In this case, stimulation with Ag increased cytosolic Ca2+

concentration to an extent similar to that of the higher IgE

concentration. At the lower IgE concentrations, the values were again

significantly different between sgk1−/− and sgk1+/+ BMMCs

(Fig. 3B, right). Stimulation of the BMMCs with Ag in Ca2+-free

solution allowed an estimate of Ca2+ release from intracellular

stores (Fig. 3C). This release was not significantly different be-

tween the genotypes (Fig. 3D).

In patch clamp experiments, Ag stimulation of BMMCs led to a

rapid increase of K+ selective conductance. Fig. 4A represents

original current traces obtained before (control) and after stimula-
tion of IgE-sensitized sgk1+/+ BMMC with Ag in a typical

experiment. Current-voltage relationships (I/V curve; Fig. 4C)

current conductances, calculated by linear regression (slope) of the

I/V curve (Fig. 4C) demonstrates that the current activated by Ag

was dependent on the presence of extracellular Ca2+. Removal of

Ca2+ from the bath solution significantly blunted the activation of

the current (Fig. 4, B and C). The current was inhibited by 1.5 μM

eclomizone (Fig. 4C) but was not sensitive to apamin (500 nM) or

iberitoxin (100 nM; data not shown). The same current could be

induced by the Ca2+ ionophore ionomycin (1 μM, Fig. 4F).

The current was thus carried by Ca2+-activated K+ channels, which

have recently been identified as KCa3.1 (46) and are known to be

activated following FcεRI cross-linking in human mast cells (18,

24). The current induced by Ag in sgk1−/− BMMCs was signifi-
cantly smaller than in wild-type cells (Fig. 4, D and E). Following

treatment of the cells with ionomycin, the K+ channels were

activated in both genotypes. The maximal current tended to be

slightly higher in sgk1+/+ as in sgk1−/− BMMCs (Fig. 4, F and

G), a difference, however, that was not statistically different. Thus,

Ca2+-activated K+ channels are similarly expressed and are

similarly sensitive to activation by Ca2+ in sgk1+/+ and sgk1−/−.
FIGURE 3. Ag-induced Ca\(^{2+}\) entry into BMMCs from sgk1\(^{+/+}\) and sgk1\(^{-/-}\) mice. A, Representative traces showing the ratio of 340:380 nm Fura-2 fluorescence in fura-2-AM-loaded BMMCs before and following acute addition of 50 ng/ml Ag. At the end of each experiment, ionomycin (10 \(\mu\)M) was added for calibration. For quantification of the Ca\(^{2+}\) entry into the BMMCs, the slope (Δ ratio/s, gray line) and peak (Δ ratio, dotted lines) were calculated following addition of Ag. B, Means ± SE of the peak (top) and slope (bottom) of the fluorescence ratio change for sgk1\(^{+/+}\) (n = 8; ■) and sgk1\(^{-/-}\) (n = 9; □) BMMCs sensitized with either 10 \(\mu\)g/ml IgE for 1 h (left) or 100 ng/ml IgE overnight (right) following stimulation with Ag (50 ng/ml). * (p < 0.05) and ** (p < 0.01) indicate significant difference between both groups (two-tailed unpaired t test). C, Representative traces showing the fura-2 fluorescence ratio before and after addition of Ag (50 ng/ml) in the absence of extracellular Ca\(^{2+}\) in fura-2-AM-loaded BMMCs. To create a Ca\(^{2+}\)-free environment, EGTA (0.5 mM) was added to the Ca\(^{2+}\)-free bath solution. D, Means ± SE of the peak value (left) and slope (right) of the fluorescence ratio change for sgk1\(^{+/+}\) (n = 6; ■) and sgk1\(^{-/-}\) (n = 4; □) BMMCs upon stimulation with Ag in Ca\(^{2+}\)-free solution.

BMMCs. Accordingly, the blunted activation by Ag results largely from decreased Ca\(^{2+}\) entry into sgk1\(^{-/-}\)BMMCs.

We also tested the ability of sgk1\(^{+/+}\) and sgk1\(^{-/-}\) BMMCs to undergo degranulation upon stimulation with Ag via the FceRI receptor. BMMCs release β-hexosaminidase, an enzyme stored in mast cell granules (50). Without stimulation, the release was 8.5 ± 1.5% (n = 5) in sgk1\(^{+/+}\) BMMCs and 7.9 ± 1.4% (n = 5) in sgk1\(^{-/-}\) BMMCs, values not significantly different between genotypes. Activation by Ag (50 ng/ml) or by ionomycin (1 \(\mu\)M) and PMA (100 ng/ml) stimulated degranulation in both genotypes. The effect was, however, significantly smaller in sgk1\(^{-/-}\) BMMCs than in sgk1\(^{+/+}\) BMMCs (Fig. 5A). The early Ag-induced release of IL-6 (30 min of stimulation with Ag) was also significantly blunted in sgk1\(^{-/-}\) cells (Fig. 5B). However, no difference between the two genotypes was observed when the cells were stimulated with Ag for a longer time (6 or 24 h; Fig. 5B). These data confirm that SGK1 is a critical component of the signaling cascade leading to release of mast cell mediators.

To evaluate whether the observed alterations in sgk1\(^{-/-}\) BMMCs were also relevant for mast cell function in vivo, we triggered passive systemic anaphylaxis. To this end, sgk1\(^{+/+}\) and sgk1\(^{-/-}\) mice were sensitized with anti-DNP IgE i.p. After overnight rest, mice received DNP-HSA or saline as a control by i.p. injection, and body temperature was monitored over time. As shown in Fig. 6, A and B, the decrease in body temperature following Ag treatment was substantially decreased in sgk1\(^{-/-}\) mice. Serum histamine levels measured 30 min after induction of the anaphylactic reaction were significantly lower in sgk1\(^{-/-}\) than in sgk1\(^{+/+}\) mice (Fig. 6C). Thus, these observations indeed point to severe impairment of in vivo mast cell function in SGK1-deficient mice.

Contact hypersensitivity responses to TNCB are prototypic T cell mediated delayed type hypersensitivity responses but involve several types of immune cells including mast cells (10). The ear thickness following TNCB challenge of sensitized mice shows an early immediate response, steadily increases, and displays a peak at ~24–48 h declining thereafter. We show here that there is no decrease in contact hypersensitivity responses in sgk1\(^{-/-}\) compared with sgk1\(^{+/+}\) mice at 24 h indicating unequivocal sensitization and T cell mediated delayed type hypersensitivity responses in both mouse strains (Fig. 7). The increase of ear thickness at 4 and 8 h was significantly reduced in sgk1\(^{-/-}\) mice, indicating that the early release of preformed mast cell mediators is also blunted in this type of immune response (Fig. 7 and Ref. 51).

Discussion

The present study reveals a role of SGK1 in the regulation of ion channel activity and function of mast cells. Distinct functional differences of mast cells are seen between gene-targeted mice lacking SGK1 (sgk1\(^{-/-}\)) and their wild-type littermates (sgk1\(^{+/+}\)). Deficiency of SGK1 blunts Ca\(^{2+}\) entry, the currents through Ca\(^{2+}\)-activated K\(^{+}\) channels and release of mast cell mediators. Most importantly, the anaphylactic reaction was strongly impaired in sgk1\(^{-/-}\) mice.

In theory, the blunted anaphylactic reaction may have been due to a decreased number of mast cells. We did not observe significant differences of mast cell numbers between sgk1\(^{+/+}\) and sgk1\(^{-/-}\) mice, but the data do not rule out a minor reduction of mast cell
number in sgk1−/− mice. However, the in vitro experiments demonstrate that the blunted anaphylactic reaction is at least partially due to defective function of sgk1−/− mast cells.

The activation of Ca2+ channels is critically important for the regulation of mast cell function, such as for the release of inflammatory mediators (15–22, 52). Stimulation of mast cells is paralleled by the activation of Ca2+-activated K+ channels (15, 23–25, 46). K+ channels maintain the cell membrane potential, which establishes the electrical driving force for Ca2+ entry (53).
and cytokine release (26). Moreover, PI3K was suggested to target adhesion, and migration, as well as Ag-IgE-induced degranulation (26). Though SGK1 is a stimulator of a wide variety of K⁺ channels, it is apparently not required for the activation of mast cell K⁺ channels by ionomycin. Along those lines, under control conditions capacitive Ca²⁺ entry is similar in sgk1⁺/⁺ lung fibroblasts and sgk1⁻/⁻ lung fibroblasts (66). However, the capacitive Ca²⁺ entry is down-regulated by serum deprivation and up-regulated by IGF-1.

In mast cells, PI3K has been shown to regulate cell proliferation, adhesion, and migration, as well as Ag-IgE-induced degranulation and cytokine release (26). Moreover, PI3K was suggested to target the transient receptor potential cation channel, subfamily V, member 2 (TRPV2) Ca²⁺ channel in these cells (54). SGK1 has previously been shown to increase the cell membrane abundance and activity of the Ca²⁺ channel TRPV5 (55, 56). It is conceivable that SGK1 has a similar stimulating effect on TRPV2.

SGK1-dependent Ca²⁺ entry further affects the activation of Ca²⁺-activated K⁺ channels following Ag exposure. K⁺ channel activation following ionomycin stimulation is, however, similar in sgk1⁺/⁺ and sgk1⁻/⁻ BMMCs. Thus, SGK1 blunts the Ag-induced activation of those channels largely by compromising Ca²⁺ entry with only little or no direct effect on the Ca²⁺-activated K⁺ channels. In other cells, SGK1 has been shown to activate a wide variety of different K⁺ channels (40, 55, 57–64). The present study does not rule out Ca²⁺-independent effects of SGK1 on K⁺ channels and other functions of mast cells.

Despite the broad role of SGK1 in channel regulation, the phenotype of the SGK1 knockout mouse is amazingly mild (32). PI3K activates phosphoinositide-dependent kinase (PDK1), which in turn activates the three SGK and the three protein kinase B/Akt isofoms (65). The ability of SGK1 to stimulate distinct carriers and channels is shared by several of those isofoms (32). Even though SGK1 is a stimulator of a wide variety of K⁺ channels, it is apparently not required for the activation of mast cell K⁺ channels by ionomycin. Along those lines, under control conditions capacitive Ca²⁺ entry is similar in sgk1⁺/⁺ lung fibroblasts and sgk1⁻/⁻ lung fibroblasts (66). However, the capacitive Ca²⁺ entry is down-regulated by serum deprivation and up-regulated by IGF-1.

**FIGURE 5.** β-Hexosaminidase and IL-6 release from sgk1⁺/⁺ and sgk1⁻/⁻ BMMCs. A, Mean β-hexosaminidase release (±SE; n = 5 individual experiments) from cultured sgk1⁻/⁻ BMMCs (□) and their wild-type sgk1⁺/⁺ littermates (■) stimulated for 15 min with 50 ng/ml Ag or 100 ng/ml PMA and 1 μM ionomycin. The stimulated β-hexosaminidase release in each experiment was corrected for the spontaneous release. **(p < 0.01) and * (p < 0.05), significant difference between genotypes, two-tailed unpaired t test. B, Mean IL-6 release (±SE; n = 3 individual experiments) from cultured sgk1⁻/⁻ BMMCs (□) and their wild-type sgk1⁺/⁺ littermates (■) sensitized for 6 h with IgE and then stimulated either for 30 min with Ag (50 ng/ml, left) or 6 h or 24 h (right). Each experiment was performed in duplicate; *** (p < 0.001), two-tailed unpaired t test.

**FIGURE 6.** Systemic anaphylactic reaction in sgk1⁺/⁺ and sgk1⁻/⁻ mice. A, Arithmetic means (±SE) of changes in body temperature (Δ°C) of sgk1⁻/⁻ mice (n = 8, □) and their wild-type sgk1⁺/⁺ littermates (n = 7, ■) following induction of anaphylaxis. Mice were given i.p. anti-DNP IgE (∼50 μg per mouse or 2 μg/l g weight) and challenged with DNP-HSA (∼120 μg per mouse or 4.8 μg/l g weight) after overnight rest. B, Arithmetic means (±SE) of maximal changes in body temperature (Δ°C) of sgk1⁻/⁻ mice (n = 8, □) and their wild-type littermates sgk1⁺/⁺ (n = 7, ■) following induction of anaphylaxis. **, Significant difference between genotypes (p = 0.002; two-tailed unpaired t test). C, Serum histamine levels 30 min after induction of anaphylaxis in sgk1⁻/⁻ mice (□) and their wild-type sgk1⁺/⁺ littermates (■). Data represent the results obtained from three mice, and from each mouse three individual probes were analyzed. *, Significant difference between genotypes; *, p < 0.05 two-tailed unpaired t test.

**FIGURE 7.** Contact hypersensitivity reaction to TNCB in sgk1⁺/⁺ and sgk1⁻/⁻ mice. Mean increase in ear thickness (Δμm, from 4 h-24 h) (±SE, n = 6) of sgk1⁻/⁻ mice (□) and their wild-type littermates sgk1⁺/⁺ (■) during TNCB-induced contact hypersensitivity reaction, corrected for irritation reaction in control mice. *, Significant difference from sgk1⁺/⁺ mice (p < 0.05; two-tailed unpaired t test).
and dexamethasone in sgk1−/− fibroblasts but not in sgk1−/− fibroblasts (66). As was expected, knockout of PDK1 is not compatible with survival of the animals (67). Reduced expression of PDK1 in PDK1 hypomorphic mice results in remarkably decreased size of the animals (67). The size of sgk1−/− mice is, however, normal, indicating that SGK1 could be replaced by the other five PDK1-dependent SGK and protein kinase B/Akt isoforms. One of those kinases is apparently SGK3, given that growth other five PDK1-dependent SGK and protein kinase B/Akt isoforms. Thus, at least in theory, pharmacological inhibition of SGK1 could be replaced by the other five PDK1-dependent SGK and protein kinase B/Akt isoforms. One of those kinases is apparently SGK3, given that growth

References


