0026-895X/93/050655-05$03.00/0

MOLECULAR PHARMACOLOGY, 43:655–659

ACCELERATED COMMUNICATION

Novel Potent Inhibitor of Receptor-Activated Nonselective Cation Currents in HL-60 Cells

D. KRAUTWURST, J. HESCHELER, D. ARNDTS, W. LÖSEL, R. HAMMER, and G. SCHULTZ

Institut für Pharmakologie, Freie Universität Berlin, D-1000 Berlin 33, Germany (D.K., J.H., G.S.), and Boehringer Ingelheim KG, D-6507 Ingelheim am Rhein, Germany (D.A., W.L., R.H.)

Received January 13, 1993; Accepted February 9, 1993

SUMMARY

A pharmacological classification of receptor-activated nonselective cation channels has not been possible because of the lack of specific and potent pharmacological blockers. In dibutyryl-cAMP-differentiated HL-60 cells, we recently identified ATP- and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-stimulated cation currents that were blocked by an organic inhibitor of receptor-mediated Ca^{2+} entry, 1-β-[3-(4-methoxyphenyl)-propoxy]-4-methoxyphenethyl-1*H*-imidazole hydrochloride (SK&F 96365), with an IC_{50} of about 3 μM. Here, we describe a new compound, (*RS*)-(3,4-dihydro-6,7-dimethoxyisoquinoline-1-γ1)-2-phenyl-*N*,*N*-di-[2-(2,3,4-trimethoxyphenyl)ethyl]acetamide (LOE 908), that fully blocked these currents at 3 μM. Half-maximal inhibition of agonist-activated nonselective inward currents was seen at 40 nM LOE 908, whereas voltage-dependent K^+ currents in undifferentiated HL-60 cells were blocked with an IC_{50} of 620 nM. fMLP-induced single-channel currents of 4–5-pS conductance were abolished when the excised inside-out patch was exposed to 3 μM LOE 908. The rank order of potency of cations blocking ATP- and fMLP-induced inward currents was $Gd^{3+} > Ni^{2+} > Cd^{2+}$.

The activation of neutrophil leukocytes during inflammatory events results in chemotaxis, exocytosis, and superoxide anion production. These reactions depend strongly on extracellular Ca^{2+} but are not influenced by activators or blockers of voltage-dependent Ca^{2+} channels (1, 2). These observations point to the existence of another class of ion channels that are characterized by a lack of voltage dependence and cation selectivity, because Ca^{2+} influx is parallelled by a substantial Na^+ influx. There are a variety of nonexcitable cells, including platelets, lymphocytes, and neutrophils, in which transmembranous Ca^{2+} and Na^+ influx involves NSC channels (1–11). These channels lack a voltage-dependent gating mechanism but are opened by receptor agonists (for reviews, see Refs. 6 and 7). It has been suggested that these NSC channels play a prominent role in prolonged cell activation and in the refilling of internal Ca^{2+} stores (12, 13). Under pathophysiological conditions, NSC channels may be involved in cell degradation and necrosis (14). In contrast to voltage-operated Ca^{2+} channels, which are classified on the basis of their different sensitivities to Ca^{2+} channel blockers (15), a pharmacological classification of NSC channels has not yet been possible because of the lack of specific and potent blockers.

Me_2SO- or Bt_2cAMP-differentiated HL-60 cells are widely used as a model system for neutrophils (16–21). Like neutrophils (4, 22), HL-60 cells possess NSC channels that can be activated by the chemotactic peptide fMLP or ATP (3, 23–25). In the present study, we characterized agonist-induced NSC channels in undifferentiated and Bt_2cAMP-differentiated HL-60 cells, using the whole-cell and single-channel patch-clamp techniques (26). We compared the efficacy of inorganic and organic blockers, and we introduce a new potent blocker (LOE 908) of receptor-activated NSC channels.

Experimental Procedures

Materials. SK&F 96365 was kindly provided by Dr. J. E. Merritt, Smith Kline Beecham (Welwyn, UK). LOE 908 [(*RS*)-(3,4-dihydro-6,7-dimethoxyisoquinoline-1-γ1)-2-phenyl-*N*,*N*-di-[2-(2,3,4-trimethoxyphenyl)ethyl]acetamide] was prepared by Bischler-Napieralsky cyclization ($CH_3CN/POCl_3$) from (*RS*)-phenyl-*N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N'*-[2-(2,3,4-trimethoxyphenyl)ethyl]malonyldiamide and was transformed to its monooxalate. Stock solutions of LOE 908 (30 mM) and SK&F 96365 (30 mM) were prepared in Me_2SO and were

This work was supported by grants from the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie. D.K. was supported by a predoctoral fellowship from the Freie Universität Berlin. This work contains data from the doctoral thesis of D.K.

ABBREVIATIONS: NSC, nonselective cation; Me_2SO, dimethylsulfoxide; Bt_2cAMP, dibutyryl-cAMP; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; fMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; GTPγS, guanosine-5'-*O*-(3-thio)triphosphate; G protein, guanine nucleotide-binding protein; TEA, tetraethanolamine.

stored at −20°. Dilutions of LOE 908 and SK&F 96365 were made immediately before experiments. Isradipine was from Sandoz (Basel, Switzerland). Sources of other materials have been described elsewhere (3).

Cell culture. HL-60 cells were grown in suspension culture in RPMI 1640 medium supplemented with 10% (v/v) horse serum as described (17). To induce differentiation, cells were seeded at 1×10^6/ml and were cultured for 48 hr in the presence of 0.2 mM Bt_2cAMP (17).

Electrophysiology. HL-60 cells (8×10^6 cells) were centrifuged at $250 \times g$ for 5 min at 20° and were resuspended in standard extracellular Tyrode solution (E1) containing (in mM) 140 NaCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5.4 KCl, 10 glucose, and 10 HEPES/NaOH, pH 7.4 at 37°. Cells were transferred into a perfusion chamber (0.2 ml) mounted on an inverted microscope. Cells settled to the bottom of the chamber within 3 min. Patch pipettes were prepared from glass capillaries (Jencons, Leight Buzzard, UK) according to the method of Hamill *et al.* (26). With an open diameter of 1–2 μm, the average resistance of the electrodes was 4–6 MΩ.

Whole-cell recordings. Pipettes were filled with a solution (I1) containing (in mM) 90 potassium aspartate, 50 KCl, 1.0 $MgCl_2$, 3.0 Na_2-ATP, 1 EGTA, and 10 HEPES/KOH, pH 7.4 at 37°. Electrical approach to HL-60 cells was gained by suction. Membrane potentials and whole-cell membrane currents were measured according to the method of Hamill *et al.* (26), using a List LM/EPC7 patch-clamp amplifier (List Electronics Darmstadt, Germany). Resting potential in HL-60 cells ranged between −60 and −30 mV, and cell capacitance was between 4 and 8 pF. At the resting potential, we determined the input conductance to be 45 ± 10 pS/pF ($n = 23$). Currents and conductances were referred to membrane capacitance. HL-60 cells were superfused at 2 ml/min with solution E1.

Single-channel recordings. Excised inside-out patches with gigaohm seal resistance were obtained from the cell-attached configuration. Single-channel currents were recorded at 25°. The pipette solution (I2) contained (in mM) 116.5 NaCl, 13.5 KCl, 18 TEA-HCl, and 2 HEPES/NaOH, pH 7.4 at 25°. TEA was added to block K^+ currents. Inside-out patches faced the bath solution (E2), containing (in mM) 126 potassium aspartate, 14 KCl, 1.0 EGTA, and 25 HEPES/KOH, pH 7.4 at 25°. The Nernst potentials for K^+ and Cl^- under these conditions were −60 mV. The unfiltered currents were continuously recorded on a Sony DTR 1200 digital audiotape recorder (Science Products, Frankfurt, Germany). For off-line analysis, data were filtered at a frequency of 400 Hz using an eight-pole Bessel filter (Frequency Devices, Haverhill, MA) and were sampled at 3 kHz using an Axon AD/DA converter (Axon, Foster City, CA).

Results

Whole-cell NSC inward currents in HL-60 cells were activated by the receptor agonists ATP (30 μM) and bacterial chemotactic peptide fMLP (100 nM). The magnitude of receptor-activated NSC currents depended on the differentiation status of the HL-60 cells. In undifferentiated cells ATP induced about half the current observed in Bt_2cAMP-differentiated cells (Fig. 1, A and C; Fig. 2, A and B; Table 1). The chemotactic peptide fMLP had no effect in undifferentiated HL-60 cells but activated NSC currents in differentiated cells (Figs. 1E and 2C). Both ATP and fMLP stimulated NSC currents with comparable biophysical properties (Fig. 1; for conductances, see Table 1). The current-voltage relations measured during voltage-ramp pulses from −100 to −20 mV were linear, with an apparent reversal at 0 mV. When Na^+ was removed from the bath solution, the reversal potential shifted by about −20 mV (values for fMLP-stimulated currents: −19 ± 2 mV, $n = 6$), and the amplitude was diminished by about 90%, suggesting that Na^+ is the main charge carrier permeating NSC channels (3). The hormonal activation of whole-cell NSC currents co-

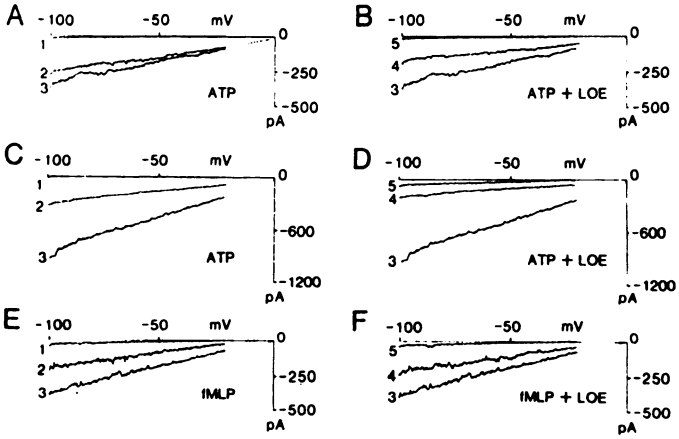

Fig. 1. Blockade of ATP- and fMLP-induced NSC currents by LOE 908 in undifferentiated and Bt_2cAMP-differentiated HL-60 cells. Current-voltage relations were measured during linear voltage-ramp pulses from −100 to −20 mV (0.4 V/sec). A and B, undifferentiated HL-60 cells; C, D, E, and F, Bt_2cAMP-differentiated HL-60 cells. NSC currents were evoked by either ATP (A-D) or fMLP (E and F). Each experiment is represented by current-voltage relations. *Curves 1*, *2*, and *3*, control currents and currents after 6- and 12-sec superfusion with the agonist, respectively. *Curves 4* and *5*, currents after 6- and 12-sec superfusion of agonist-stimulated cells with LOE 908 (1 μM). *Dotted lines*, extrapolations of the currents to the reversal potential. For each condition, one of eight similar experiments is shown.

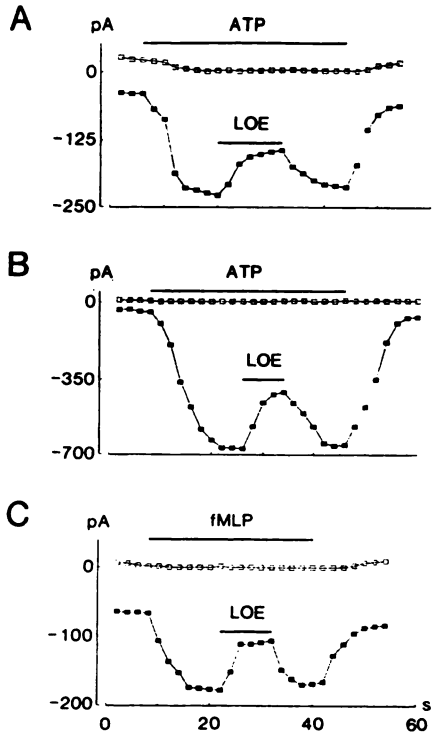

Fig. 2. Time courses of the effects of ATP, fMLP, and LOE 908 on NSC currents. Currents were measured at −60 mV (■) or 0 mV (□). In A and B, ATP (30 μM) was superfused to undifferentiated and Bt_2cAMP-differentiated HL-60 cells, respectively; C represents the fMLP (100 nM) effect on a differentiated HL-60 cell. In all three cases, LOE 908 (40 nM, corresponding to the IC_{50}; see Fig. 3) inhibited the current at −60 mV but not that at 0 mV. *Horizontal bars*, times during which the agonists or LOE 908 were applied. Each *panel* represents one of four similar experiments.

TABLE 1

Agonist-induced NSC conductances in undifferentiated and Bt_2cAMP-differentiated HL-60 cells

Conductances were related to the membrane capacity and expressed as pS/pF (mean ± standard deviation; numbers of observations are given in parentheses). In 40 cells, we determined the membrane capacity to be 6 ± 2 pF.

Stimulus	Conductance
	pS/pF
Undifferentiated HL-60 cells	
None	45 ± 10 (23)
ATP (30 μM)	825 ± 90 (23)
Bt_2cAMP-differentiated HL-60 cells	
None	45 ± 9 (25)
ATP (30 μM)	1560 ± 370 (9)
fMLP (100 nM)	785 ± 72 (16)

incided with an increase in the current noise that could be due to single-channel fluctuations. The effects of ATP and fMLP on NSC currents occurred with a delay of several seconds and were fully reversible when the agonists were washed out (Fig. 2). With sustained stimulation, NSC currents showed a slight desensitization, with about 10% decay per minute. The stimulation of NSC current corresponded to a depolarization of the membrane potential seen under current-clamp conditions. The receptor-activated NSC current was insensitive to (+)-isradipine (300 nM), an organic Ca^{2+} channel blocker of the 1,4-dihydropyridine type (data not shown). In HL-60 cells, SK&F 96365 blocked NSC currents with an IC_{50} of about 2 μM (Fig. 3B).

Similarly to the existence of different types of K^+ channels in human neutrophils (27), HL-60 cells are known to express lineage-specific K^+ currents (28). SK&F 96365 is not specific for NSC currents (29); in undifferentiated HL-60 cells SK&F 96365 blocked voltage-dependent K^+ outward currents with an IC_{50} of 1.9 ± 0.6 μM (Fig. 3C). In pituitary GH_3 cells (data not shown) and arterial smooth muscle cells (2), 1 μM SK&F 96365 reduced L-type voltage-dependent Ca^{2+} currents by about 50%.

A more specific and more potent blocker of NSC channels is LOE 908. As demonstrated in the current-voltage relations of Fig. 1, extracellular application of 1 μM LOE 908 almost completely suppressed the ATP- and fMLP-activated NSC currents, without affecting the linear voltage dependence. The block occurred independently of the holding potential, developed within seconds, and was fully reversible when LOE 908 was washed out (see Fig. 2). The LOE 908 concentration-inhibition curves were identical for the ATP- and fMLP-stimulated NSC currents in undifferentiated and Bt_2cAMP-differentiated HL-60 cells (see Fig. 3B). Half-maximal inhibition and maximal inhibition were observed at about 40 nM and 3 μM, respectively, whereby LOE 908 was almost 2 orders of magnitude more potent than SK&F 96365.

LOE 908 blocked outwardly rectifying, voltage-operated, K^+ currents in undifferentiated HL-60 cells with an IC_{50} of 620 ± 90 nM (see Fig. 3C). In PC-12 cells, LOE 908 blocked voltage-operated Ca^{2+} channels with an IC_{50} of about 5 μM.[1]

In neutrophils and Me_2SO-differentiated HL-60 cells, fMLP-induced Ca^{2+} or Mn^{2+} entry has been demonstrated to be sensitive to La^{3+}, Ni^{2+}, and Cd^{2+} (2, 30, 31). In our hands, ATP- and fMLP-induced NSC currents in Bt_2cAMP-differentiated HL-60 cells were blocked by Gd^{3+}, Ni^{2+}, and Cd^{2+} with IC_{50}

[1] D. Krautwurst, unpublished observations.

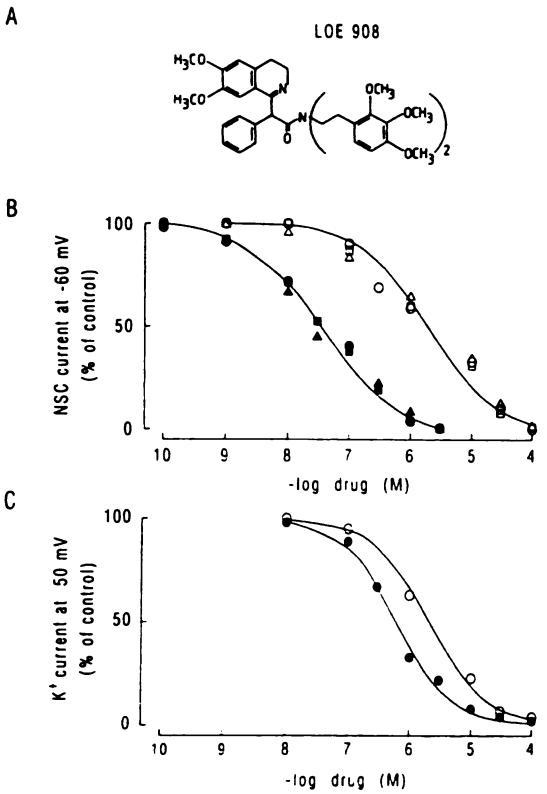

Fig. 3. Concentration-inhibition curves for LOE 908 and SK&F 96365 on NSC currents and voltage-dependent K^+ currents in HL-60 cells. A, Structure of LOE 908. B, Concentration-response curves for LOE 908 (*closed symbols*) and SK&F 96365 (*open symbols*) on agonist-induced NSC currents. Maximal NSC current was induced by ATP (30 μM) in undifferentiated (*squares*) and differentiated (*triangles*) HL-60 cells, as well as by fMLP (100 nM) in differentiated cells (*circles*). Currents were measured at −60 mV. Each *point* represents the mean percentage of NSC current amplitude at the respective concentration of SK&F 96365 (n = 3–6, standard deviation < 15%) or LOE 908 (n = 3–8, standard deviation < 15%). Nonlinear regression analysis revealed for LOE 908 an IC_{50} value of 40 ± 10 nM and a Hill coefficient of 0.7 ± 0.1. For SK&F 96365, the IC_{50} value was 2.4 ± 0.9 μM and the Hill coefficient was 0.8 ± 0.1. C, Concentration-response curves for LOE 908 (●) and SK&F 96365 (○) on voltage-dependent outward K^+ currents in undifferentiated HL-60 cells. Outwardly rectifying K^+ whole-cell currents (data not shown) were activated during linear voltage-ramp pulses from −100 to 50 mV (0.4 V/sec). K^+ current amplitudes were measured at 50 mV. Each *point* represents the mean percentage of K^+ current amplitude at the respective concentration of SK&F 96365 (n = 4–6, standard deviation < 15%) or LOE 908 (n = 4–6, standard deviation < 15%). *Curves* represent best fits according to the logistic equation (37).

values of 0.19 ± 0.03 mM, 0.51 ± 0.07 mM, and 1.9 ± 0.3 mM, respectively (Fig. 4). The Hill coefficients were 0.9–1.0.

In Bt_2cAMP-differentiated HL-60 cells, we measured single-channel NSC currents activated by fMLP (10 nM) in the cell-attached configuration under voltage-clamp conditions (Fig. 5). After being changed to the inside-out configuration, channels in the excised patch were still active over a period of at least 3 min. At −60 mV, inward currents with an amplitude of 0.23 ± 0.03 pA (mean ± standard deviation, n = 10) and a linear conductance of 4–5 pS were observed. Currents reversed at about 0 mV (data not shown; see also Ref. 3). The open probability ($n \times p_o$, where n is the number of channels being active and p_o is the open probability) was 0.03 ± 0.01 (mean ± standard deviation, n = 10), as calculated from open-time analysis of at least 3–4-min recordings. Single NSC channel

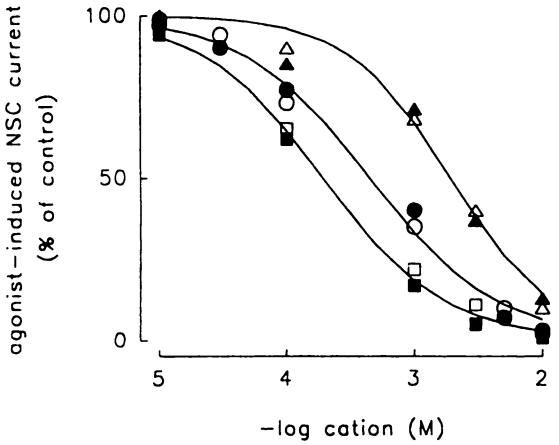

Fig. 4. Concentration-inhibition curves for various cations on ATP- and fMLP-induced whole-cell NSC currents in Bt_2cAMP-differentiated HL-60 cells. Currents were stimulated with ATP (30 μM) (*closed symbols*) or fMLP (100 nM) (*open symbols*) in the presence of various concentrations of Gd^{3+} (*squares*), Ni^{2+} (*circles*), or Cd^{2+} (*triangles*). Currents were measured at a holding potential of −60 mV. Each *point* represents the mean of three to eight experiments; the standard deviation generally amounted to <15% of the mean. *Curves* represent best fits according to the logistic equation (37).

openings were blocked when the excised patch was exposed to 3 μM LOE 908 in the bath solution. The block was reversible when LOE 908 was washed out.

Discussion

The results indicate that LOE 908 is a potent and reversible blocker of NSC currents activated by ATP or by fMLP in HL-60 cells. The lipophilic characteristics of LOE 908 may explain its blocking effects on whole-cell and single-channel currents when it is applied to the external and cytoplasmic sides of the membrane, respectively. Because the concentration dependence of the LOE 908-induced block was identical for NSC currents evoked by either agonist, it is plausible that LOE 908 affects the channel rather than the receptors.

In HL-60 cells, LOE 908 displayed higher potency in blocking NSC channels, compared with the blockade of voltage-dependent K^+ channels. Moreover, LOE 908 was about 2 orders of magnitude more potent in blocking NSC currents in HL-60 cells than in blocking inward currents through voltage-dependent Ca^{2+} channels in PC-12 cells.[1] This points to a selectivity of LOE 908 for NSC channels in HL-60 cells over voltage-operated channels.

In our hands, Gd^{3+}, Ni^{2+}, and Cd^{2+} blocked ATP- and fMLP-induced NSC whole-cell currents with similar potencies (Gd^{3+} > Ni^{2+} > Cd^{2+}), indicating that the activation of different receptors may activate the same NSC channels. A similar rank order of potency for La^{3+}, Ni^{2+}, and Cd^{2+} in blocking fMLP-induced Ca^{2+} entry in Me_2SO-differentiated HL-60 cells has been reported (31). By their insensitivity to typical blockers of voltage-dependent Ca^{2+} channels and a different pattern of sensitivity to divalent cations, NSC channels in HL-60 cells are pharmacologically distinct from voltage-dependent Ca^{2+} channels. Whereas Cd^{2+} is a weak blocker of agonist-induced NSC inward currents in HL-60 cells, Cd^{2+} is at least 1 order of magnitude more potent in blocking voltage-operated L-type

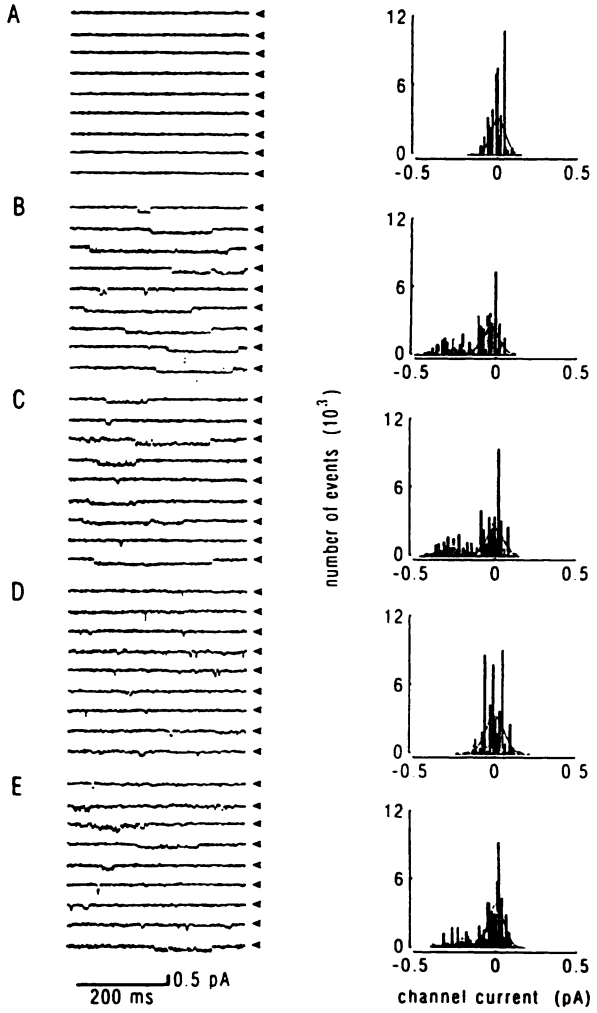

Fig. 5. fMLP-induced single-channel currents in Bt_2cAMP-differentiated HL-60 cells. *Left*, selected original current recordings from one cell. *Arrows*, zero current levels. During the entire experiment, the patch was clamped to −60 mV. A, Control currents recorded in I2/E2 in the cell-attached configuration; B, single-channel openings in the cell-attached configuration in the presence of 10 nM fMLP; C, current recordings from the same patch in the inside-out configuration; D, current recordings from the same excised patch after exposure to 3 μM LOE 908; E, current recordings 1 min after washout of LOE 908. *Right*, amplitude histograms from the selected current traces. The same single-channel amplitudes (−0.23 ± 0.02 pA) were derived from direct measurements of channel openings in the filtered current traces and from fitting two Gaussian functions to the histograms in B, C, and E. Similar results were obtained with nine different patches.

Ca^{2+} channels, and Ni^{2+} blocks T-type Ca^{2+} channels at low submillimolar concentrations in a variety of cells (15).

We recently described the existence of a NSC channel with a conductance of 4–5 pS in membranes of Bt_2cAMP-differentiated HL-60 cells, which current was not observed in the presence of 10 μM SK&F 96365 (3). In the present study, we demonstrate a 4–5-pS NSC channel with Na^+ as charge carrier, in Bt_2cAMP-differentiated HL-60 cells, that could be activated by fMLP at low concentrations. Although of similar conductance, NSC channels in HL-60 cells may be different from Ca^{2+}-stimulated NSC channels occurring in neutrophils (4) with respect to their Ca^{2+} dependence (3, 23, 32). Once activated in the intact cell, HL-60 channels remained active for several minutes even if the cytoplasmic side of the patch was exposed

to Ca^{2+}-free bath solution. Therefore, an inhibitory effect of LOE 908 on the opening of Ca^{2+}-stimulated cation channels (4) by suppression of inositol 1,4,5-trisphosphate-induced Ca^{2+} release from intracellular stores is unlikely. Similarly to the fMLP-induced whole-cell NSC currents, single-channel currents in the excised patch were fully and reversibly blocked by 3 μM LOE 908. This supports the idea that this compound may directly block the channel.

A possible involvement of phosphoinositide breakdown (PI response) and subsequent reactions in the receptor-mediated opening of NSC currents in neutrophils and HL-60 cells has been suggested (4, 18, 23, 31, 33), because activation of phospholipase C and depletion of intracellular Ca^{2+} stores (23, 31) coincide with the activation of NSC channels. In preliminary experiments with HL-60 cells, we observed that treatment with thapsigargin, which is known to release Ca^{2+} from stores by blocking a Ca^{2+}-ATPase (34), activated a LOE 908-sensitive NSC current. We recently demonstrated that G proteins are involved in the activation by receptor agonists of NSC currents in Bt_2cAMP-differentiated HL-60 cells (3). In preliminary experiments we found that whole-cell NSC currents stimulated by intracellularly applied GTPγS (see also Ref. 35) were also sensitive to LOE 908 (data not shown). Single-channel patch-clamp studies on human carcinoma A431 cells have revealed two types of Ca^{2+}-permeable channels with unitary conductances between 1 and 3 pS (36). These channels could be activated by application of epidermal growth factor to the extracellular side and GTPγS to the intracellular side of an excised membrane patch. Whereas an effect of inositol 1,4,5-trisphosphate-induced Ca^{2+} release on NSC current is obvious, an additional, possibly more direct, G protein-mediated activation of the NSC current cannot be excluded. In the elucidation of the exact mechanism of receptor- and G protein-mediated stimulation of NSC currents, as well as their physiology and pathophysiology, LOE 908 is likely to become an important tool.

Acknowledgments

We are indebted to Inge Reinsch and Monika Bigalke for valuable technical assistance.

References

1. Seifert, R., and G. Schultz. The superoxide-forming NADPH oxidase of phagocytes: an enzyme system regulated by multiple mechanisms. *Rev. Physiol. Biochem. Pharmacol.* **117**:1–332 (1991).
2. Merritt, J. E., R. Jacob, and T. J. Hallam. Use of manganese to discriminate between calcium influx and mobilization from internal stores in stimulated human neutrophils. *J. Biol. Chem.* **264**:1522–1527 (1989).
3. Krautwurst, D., R. Seifert, J. Hescheler, and G. Schultz. Formyl peptides and ATP stimulate Ca^{2+} and Na^+ inward currents through non-selective cation channels via G-proteins in dibutyryl cyclic AMP-differentiated HL-60 cells: involvement of Ca^{2+} and Na^+ in the activation of β-glucuronidase release and superoxide production. *Biochem. J.* **288**:1025–1035 (1992).
4. Von Tscharner, V., B. Prod'hom, M. Baggiolini, and H. Reuter. Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration. *Nature (Lond.)* **324**:369–372 (1986).
5. Penner, R., G. Matthews, and E. Neher. Regulation of calcium influx by second messengers in rat mast cells. *Nature (Lond.)* **334**:499–504 (1988).
6. Meldolesi, J., and T. Pozzan. Pathways of Ca^{2+} influx at the plasma membrane: voltage-, receptor-, and second messenger-operated channels. *Exp. Cell Res.* **171**:271–283 (1987).
7. Putney, J. W. Calcium-mobilizing receptors. *Trends Pharmacol. Sci.* **8**:481–486 (1987).
8. Schultz, G., W. Rosenthal, J. Hescheler, and W. Trautwein. Role of G-proteins in calcium channel modulation. *Annu. Rev. Physiol.* **52**:275–292 (1990).
9. Hallam, T. J., and T. J. Rink. Receptor-mediated Ca^{2+} entry: diversity of function and mechanism. *Trends Pharmacol. Sci.* **10**:8–10 (1989).
10. Meldolesi, J., E. Clementi, C. Fasolato, D. Zacchetti, and T. Pozzan. Calcium influx following receptor activation. *Trends Pharmacol. Sci.* **12**:289–292 (1991).
11. Barritt, G. J., and B. P. Hughes. The nature and mechanism of activation of the hepatocyte receptor-activated Ca^{2+}-inflow system. *Cell. Signalling* **3**:283–292 (1991).
12. Irvine, R. F., R. M. Moor, W. K. Pollock, P. M. Smith, and K. A. Wregget. Inositol phosphates: proliferation, metabolism and function. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **320**:281–298 (1988).
13. Berridge, M. J. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu. Rev. Biochem.* **56**:159–193 (1987).
14. Choi, D. W. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci.* **11**:465–468 (1988).
15. Bean, B. P. Classes of calcium channels in vertebrate cells. *Annu. Rev. Physiol.* **51**:367–384 (1989).
16. Chaplinski, T. J., and J. E. Niedel. Cyclic nucleotide-induced maturation of human promyelocytic leukemia cells. *J. Clin. Invest.* **70**:953–964 (1982).
17. Seifert, R., R. Burde, and G. Schultz. Activation of NADPH oxidase by purine and pyrimidine nucleotides involves G proteins and is potentiated by chemotactic peptides. *Biochem. J.* **259**:813–819 (1989).
18. Cowen, D. S., B. Baker, and G. R. Dubyak. Pertussis toxin produces differential inhibitory effects on basal, P_2-purinergic, and chemotactic peptide-stimulated inositol phospholipid breakdown in HL-60 cells and HL-60 cell membranes. *J. Biol. Chem.* **265**:16181–16189 (1990).
19. Thokin, M. N., N. Morishima, T. Iiri, K. Takahashi, M. Ui, and T. Katada. Interaction of guanine-nucleotide-binding regulatory proteins with chemotactic peptide receptors in differentiated human leukemic HL-60 cells. *Eur. J. Biochem.* **195**:527–533 (1991).
20. Seifert, R., A. Höer, S. Offermanns, A. Buschauer, and W. Schunack. Histamine increases cytosolic Ca^{2+} in dibutyryl-cAMP-differentiated HL-60 cells via H_1 receptors and is an incomplete secretagoque. *Mol. Pharmacol.* **42**:227–234 (1992).
21. Seifert, R., A. Höer, I. Schwaner, and A. Buschauer. Histamine increases cytosolic Ca^{2+} in HL-60 promyelocytes predominantly via H_2 receptors with an unique agonist/antagonist profile and induces functional differentiation. *Mol. Pharmacol.* **42**:235–241 (1992).
22. Schumann, M. A., T. Tanigaki, D. N. Heller, and T. A. Raffin. Ca^{2+}-dependent and Ca^{2+}-independent mechanisms modulate whole-cell cationic currents in human neutrophils. *Biochem. Biophys. Res. Commun.* **185**:531–538 (1992).
23. Demaurex, N., W. Schlegel, P. Varnai, G. Mayr, D. P. Lew, and K.-H. Krause. Regulation of Ca^{2+} influx in myeloid cells. *J. Clin. Invest.* **90**:830–839 (1992).
24. Aviram, A., and M. Shaklai. The role of increased calcium influx rate in receptor-mediated function of differentiating HL-60 cells. *Cell Calcium* **11**:269–274 (1990).
25. Gusovsky, F., J. A. Bitran, T. Yasumoto, and J. W. Daly. Mechanism of maitotoxin-stimulated phosphoinositide breakdown in HL-60 cells. *J. Pharmacol. Exp. Ther.* **252**:466–473 (1990).
26. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391**:85–100 (1981).
27. Krause, K.-H., and M. J. Welsh. Voltage-dependent and Ca^{2+}-activated ion channels in human neutrophils. *J. Clin. Invest.* **85**:491–498 (1990).
28. Wieland, S. J., Q. Gong, R. H. Chou, and L. H. Brent. A lineage-specific Ca^{2+}-activated K^+-conductance in HL-60 cells. *J. Biol. Chem.* **267**:15426–15431 (1992).
29. Merritt, J. E., W. P. Armstrong, C. D. Benham, T. J. Hallam, R. Jacob, A. Jaxa-Chamiec, B. K. Leigh, S. A. McCarthy, E. K. Moores, and T. Rink. SK&F 96365, a novel inhibitor of receptor-mediated calcium entry. *Biochem. J.* **271**:515–522 (1990).
30. Andersson, T., C. Dahlgren, T. Pozzan, O. Stendahl, and D. Lew. Characterization of fMet-Leu-Phe receptor-mediated Ca^{2+} influx across the plasma membrane of human neutrophils. *Mol. Pharmacol.* **30**:437–443 (1986).
31. Demaurex, N., D. P. Lew, and K.-H. Krause. Cyclopiazonic acid depletes intracellular Ca^{2+} stores and activates an influx pathway for divalent cations in HL-60 cells. *J. Biol. Chem.* **267**:2318–2324 (1992).
32. Nasmith, P. E., and S. Grinstein. Are Ca^{2+} channels in neutrophils activated by a rise in cytosolic free Ca^{2+}? *FEBS Lett.* **221**:95–100 (1987).
33. Pittet, D., D. P. Lew, G. W. Mayr, A. Monod, and W. Schlegel. Chemoattractant receptor promotion of Ca^{2+} influx across the plasma membrane of HL-60 cells. *J. Biol. Chem.* **264**:7251–7261 (1989).
34. Thastrup, O. Role of Ca^{2+}-ATPases in regulation of cellular Ca^{2+} signalling, as studied with the selective microsomal Ca^{2+}-ATPase inhibitor thapsigargin. *Agents Actions* **29**:8–23 (1990).
35. Nüsse, O., and M. Lindau. GTPγS-induced calcium transients and exocytosis in human neutrophils. *Biosci. Rep.* **10**:93–103 (1990).
36. Mozhayeva, G. N., A. P. Naumov, and Y. A. Kuryshev. Calcium-permeable channels activated via guanine nucleotide-dependent mechanism in human carcinoma cells. *FEBS Lett.* **277**:227–229 (1990).
37. DeLean, A., P. J. Munson, and D. Rodbard. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.* **235**:E97-E102 (1978).

Send reprint requests to: G. Schultz, Institut für Pharmakologie, Freie Universität Berlin, Thielallee 69–73, D-1000 Berlin 33, Germany.