ELSEVIER

European Journal of Pharmacology 447 (2002) 31–36

ejp

www.elsevier.com/locate/ejphar

Effects of Ca^{2+} influx through nonselective cation channel on noradrenaline-induced mitogenic responses

Yoshihumi Kawanabe [a,b,*], Nobuo Hashimoto [b], Soichi Miwa [a], Tomoh Masaki [a]

[a] *Department of Pharmacology, Kyoto University Graduate School of Medicine, Kyoto, Japan*
[b] *Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan*

Received 20 December 2001; received in revised form 24 April 2002; accepted 22 May 2002

Abstract

We have recently shown that noradrenaline induces extracellular Ca^{2+} influx through nonselective cation channel (NSCC) in Chinese hamster ovary cells expressing α_{1A}-adrenoceptors (CHO-α_{1A}). Moreover, this NSCC is sensitive to (*R*,*S*)-(3,4-dihydro-6,7-dimethoxy-isoquinoline-1-yl)-2-phenyl-*N*,*N*-di-[2-(2,3,4-trimethoxyphenyl)ethyl]-acetamide (LOE 908) and resistant to 1-[*b*-(3-[4-Methoxyphenyl]propoxy)-4-methoxyphenethyl]-1*H*-imidazole hydrochloride (SK&F 96365). In the present study, we characterized the effects of extracellular Ca^{2+} influx through NSCC on noradrenaline-induced mitogenic responses and activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) of CHO-α_{1A} using LOE 908 and SK&F 96365. Noradrenaline induced a mitogenic response in CHO-α_{1A}. LOE 908 completely inhibited the noradrenaline-induced mitogenesis, whereas SK&F 96365 did not inhibit it. The IC_{50} value of LOE 908 for noradrenaline-induced mitogenesis was similar to that for the noradrenaline-induced increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). Noradrenaline stimulated ERK1/2 activity. The magnitude of noradrenaline-induced ERK1/2 activity in the absence of extracellular Ca^{2+} was 40% of that in the presence of extracellular Ca^{2+}. LOE 908 partially (60%) inhibited the noradrenaline-induced ERK1/2 activity, whereas SK&F 96365 did not inhibit it. The IC_{50} value of LOE 908 for noradrenaline-induced ERK1/2 activity was similar to that for the noradrenaline-induced increase in $[Ca^{2+}]_i$. Collectively, these results demonstrate that extracellular Ca^{2+} influx through LOE 908-sensitive and SK&F 96365-resistant NSCC may be essential for noradrenaline-induced mitogenesis in CHO-α_{1A}. Moreover, the noradrenaline-induced ERK1/2 activity involves two distinct pathways, one dependent on extracellular Ca^{2+} influx through NSCC, whereas the other is independent of the influx.

Keywords: Noradrenaline; Nonselective cation channel; Cell proliferation

1. Introduction

α_1-Adrenoceptors are members of the G-protein-coupled receptor family and mediate many of the important physiological effects of catecholamines such as noradrenaline. Vascular smooth muscle cells and cardiac myocytes express at least three subtypes of α_1-adrenoceptors, namely α_{1A}, α_{1B} and α_{1D} receptors (Price et al., 1994; Hieble et al., 1995). α_1-Adrenoceptors play a particularly important role in controlling cardiovascular responses such as regulation of blood pressure via activation of smooth muscle contraction (Graham et al., 1996). Activation of α_1-adrenoceptors also stimulates cardiac and vascular smooth muscle growth and hypertrophy (Jackson and Schwartz, 1992; Milano et al., 1994). Moreover, sustained increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) due to extracellular Ca^{2+} influx plays a critical role in noradrenaline-induced mitogenic response in rat-1 fibroblasts expressing α_{1A}-, α_{1B}- or α_{1D}-adrenoceptors (Chen et al., 1999). However, which type of Ca^{2+} channels may be involved in noradrenaline-induced mitogenic responses is presently unknown. This is mainly due to a lack of available specific Ca^{2+} channel blockers. Recently, we have constructed Chinese hamster ovary cells stably expressing α_{1A}-, α_{1B}- or α_{1D}-adrenoceptors (CHO-α_{1A}, -α_{1B} or -α_{1D}, respectively), and compared the Ca^{2+} channels activated by noradrenaline using whole-cell recordings and monitoring of $[Ca^{2+}]_i$ along with the receptor-operated Ca^{2+} channel blockers, (*R*,*S*)-(3,4-dihydro-6,7-dimethoxy-isoquinoline-1-yl)-2-phenyl-*N*,*N*-di-[2-(2,3,4-trimethoxyphenyl)ethyl]-acetamide (LOE 908)

* Corresponding author. Membrane Biology Program, Brigham and Women's Hospital, Harvard Institute of Medicine, Room 520, 77 Avenue Louis Pasteur, Boston, MA 02115, USA. Tel.: +1-617-525-5827; fax: +1-617-525-5821.

E-mail address: ykawanabe@rics.bwh.harvard.edu (Y. Kawanabe).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01832-0

(Encabo et al., 1996) and 1-[*b*-(3-[4-Methoxyphenyl]propoxy)-4-methoxyphenethyl]-1*H*-imidazole hydrochloride (SK&F 96365) (Meritt et al., 1990). A noradrenaline-induced sustained increase in $[Ca^{2+}]_i$ due to extracellular Ca^{2+} influx involved a Ca^{2+}-permeable nonselective cation channel (NSCC), which was sensitive to LOE 908 and resistant to SK&F 96365 in all transfectants (Kawanabe et al., 2001a). The transfection and functional expression of the cDNA clone for α_{1A}-, α_{1B}- or α_{1D}-adrenoceptors into the same cell type provides a model system to study and compare the precise signal transduction pathways of a single receptor subtype without any ambiguity resulting from the presence of multiple receptor subtypes. Thus, the first objective of the present study was to clarify the effects of Ca^{2+} influx through NSCC on the noradrenaline-induced mitogenic response in CHO-α_{1A}.

Extracellular signal-regulated kinase 1 and 2 (ERK1/2), members of the mitogen-activated protein kinase (MAPK) family, are considered to represent a major signaling pathway mediating noradrenaline-induced mitogenic response (Xin et al., 1997; Hu et al., 1999). It is generally accepted that extracellular Ca^{2+} influx plays a critical role in α_1-adrenoceptor-induced MAPK activation (Muthalif et al., 1996; Romanelli and van de Werve, 1997; Hu et al., 1999). In rabbit vascular smooth muscle cells, noradrenaline, by promoting extracellular Ca^{2+} influx, increases CaM kinase II activity, leading to activation of MAPK (Muthalif et al., 1996). On the other hand, ERK1/2 activation is Ca^{2+}-independent in CHO cells expressing muscarinic acetylcholine receptors (Wylie et al., 1999). Thus, it is unknown whether ERK1/2 activation by noradrenaline also involves extracellular Ca^{2+} influx-independent process. The second objective of the present study was therefore to examine the role of Ca^{2+} influx through NSCC on the noradrenaline-induced ERK1/2 activation in CHO-α_{1A}.

2. Materials and methods

2.1. Cell culture

Stable expression of α_{1A}-adrenoceptors in CHO cells was accomplished as described previously (Kawanabe et al., 2001a). CHO-α_{1A} was routinely maintained in F-12 medium supplemented with 10% fetal calf serum under a humidified atmosphere of 5% CO_2/95% air.

2.2. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) assay and [^{3}H]thymidine incorporation

Cells were seeded into 96-well plates at 5×10^3 cells/well for the assay using MTT and into 24-well plates at 4×10^4 cells/well for [^{3}H]thymidine incorporation. They were incubated overnight in F-12 supplemented with 10% fetal calf serum at 37 °C. The cells were deprived of serum for 24 h, washed with phosphate-buffered saline (PBS), and incubated with noradrenaline for a further 48 h in serum-free F-12 with or without Ca^{2+} channel blockers.

The MTT assay as a measure of the number of viable cells was performed as described previously (Sugawara et al., 1996). Briefly, the cells were exposed to 1 mg/ml MTT during the last 4 h of culture with noradrenaline, and then lysed with acidic lysis buffer (20% (w/v) sodium dodecyl sulfate (SDS) in 50% *N*,*N*-dimethyl formamide solution at pH 4.7). The absorbance of the lysate at 562 and 630 nm was measured with an EL340 Microtiter Plate Reader (Bio-Tek Instruments, Winooski, VT). The difference between the values at 562 and 630 nm was used as an index of the number of viable cells.

Measurement of [^{3}H]thymidine incorporation was performed as described previously (Kawanabe et al., 2001b). Briefly, for measurement of [^{3}H]thymidine incorporation, [^{3}H]thymidine (1 μCi/ml) was added during the last half of the 48-h incubation period with noradrenaline. To stop the reaction, the cells were washed three times with ice-cold PBS, incubated with 10% (w/v) trichloroacetic acid at 4 °C for 30 min, and subsequently washed three times with ice-cold PBS to remove the trichloroacetic acid-soluble material. The radioactivity incorporated into the trichloroacetic acid-insoluble fraction was recovered in 0.1 N NaOH and counted using a liquid scintillation counter (Aloka, Tokyo, Japan) with the solid scintillator Luma-Cap (Packard, Groningen, The Netherlands).

2.3. Measurement of ERK1/2 activity

ERK1/2 activity was measured as described previously (Kawanabe et al., 2001b). Briefly, cells at 80% confluency in 10-cm dishes were starved for 24 h before being stimulated by noradrenaline for various periods in serum-free F-12 in the presence or absence of Ca^{2+} channel blockers. The reaction was terminated by washing once with PBS and twice with 20 mM Tris–HCl (pH 7.4). After the addition of 1 ml of ice-cold extraction buffer (10 mM Tris–HCl, 0.5 mM EDTA, 0.5 mM EGTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 5 mg/ml aprotinin, 0.05 mM NaF, 0.5 mM Na_3PO_4, 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride, and 5 mM β-glycerophosphate, at pH 7.4), the cells were removed with a scraper. For partial purification of ERK, the cell suspension was transferred to a 15-ml conical tube, sonicated for 10 s×3 at 10-s intervals, and centrifuged at 25,000×*g* for 20 min. The supernatant was applied to a DEAE-Sephadex column (bed volume, 0.5 ml) pre-equilibrated with equilibration buffer (extraction buffer containing 100 mM NaCl). The enzyme was eluted with the elution buffer (extraction buffer containing 500 mM NaCl) and concentrated using Centricon YM-30 (Millipore, Bedford, MA, USA). The protein concentration of the partially purified enzyme in each sample was determined with a bovine serum albumin Microprotein Assay Kit (Pierce, Rockford, IL, USA), and 5 μg of the enzyme was used for each assay. ERK1/2

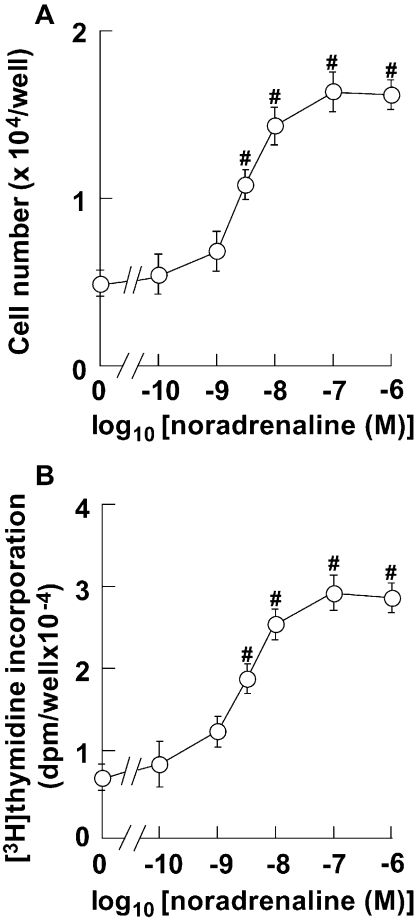

Fig. 1. Effects of various concentrations of noradrenaline on the number of viable cells (A) and DNA synthesis (B) in CHO-α_{1A}. After cells had been deprived of serum for 24 h, they were stimulated with increasing concentrations of noradrenaline for a further 48 h. The numbers of viable cells (MTT assay) (A) and [^{3}H]thymidine incorporation (B) were determined as described in Materials and methods. Data are presented as mean±S.E.M. of three determinations, each done in triplicate. #P<0.05; significantly different from the control values in each experiment.

activity was determined using a MAP Kinase Assay Kit (Amersham, Buckinghamshire, UK) according to the manufacturer's instructions.

2.4. Statistical analysis

All results were expressed as mean±S.E.M. The data were subjected to a two-way analysis of variance, and when a significant F value was encountered, the Newman–Keuls' multiple range test was used to test for significant differences between treatment groups. A probability level of P<0.05 was considered statistically significant.

2.5. Drugs

Boehringer Ingelheim (Ingelheim, Germany) kindly provided LOE 908. Other reagents were obtained commercially from the following sources: noradrenaline from Wako (Osaka, Japan); MTT from Sigma (St. Louis, MO, USA); [^{3}H]thymidine from NEN (Boston, MA, USA); SK&F 96365 from Biomol (Plymouth Meeting, PA, USA).

3. Results

3.1. Effects of LOE 908 on the noradrenaline-induced mitogenic response

After stimulation with 100 nM noradrenaline, both the number of viable cells as estimated by the MTT assay and mitogenic activity as estimated by [^{3}H]thymidine incorporation increased with time up to 48 h (data not shown). Therefore, in subsequent experiments the stimulation time was set at 48 h.

Noradrenaline stimulated a mitogenic response in CHO-α_{1A} in a concentration-dependent manner, with an EC_{50}

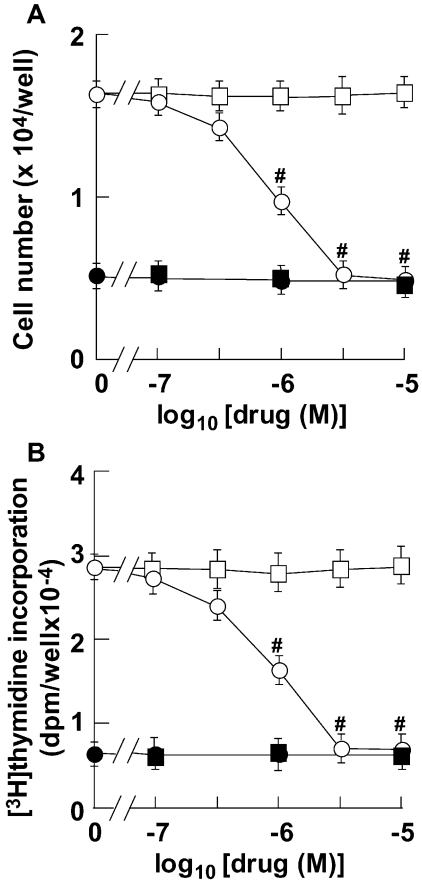

Fig. 2. Effects of various concentrations of LOE 908 or SK&F 96365 on noradrenaline-induced increases in the number of viable cells (A) and DNA synthesis (B) in CHO-α_{1A}. Starved cells were incubated for 15 min with increasing concentrations of LOE 908 (circles) or SK&F 96365 (squares), and then they were stimulated with (open symbols) or without (closed symbols) 100 nM noradrenaline. The numbers of viable cells (MTT assay) and [^{3}H]thymidine incorporation were determined as described in Materials and methods. Data are presented as mean±S.E.M. of three determinations, each done in triplicate. #P<0.05; significantly different from the control values in each experiment.

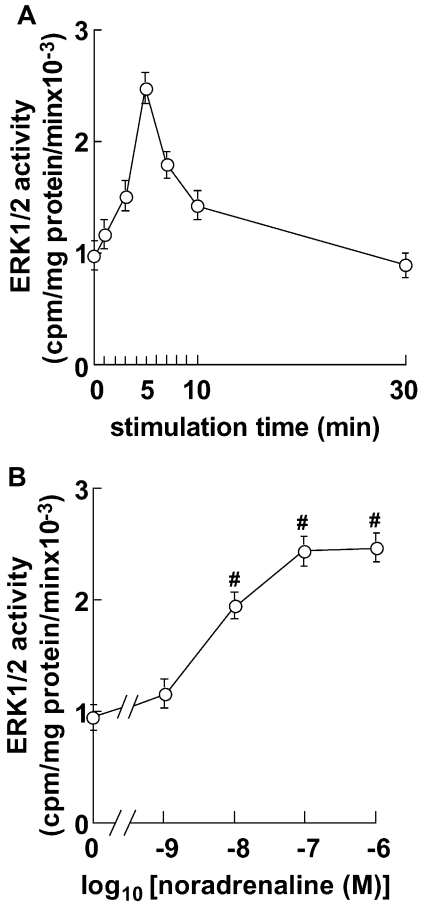

Fig. 3. (A) Time course of ERK1/2 activity following stimulation with noradrenaline in CHO-α_{1A}. After cells had been cultured in serum-free medium for 24 h, they were stimulated with 100 nM noradrenaline for the indicated time periods. (B) Effects of various concentrations of noradrenaline on ERK1/2 activity in CHO-α_{1A}. After cells had been deprived of serum for 24 h, they were stimulated with increasing concentrations of noradrenaline for 5 min. ERK1/2 activity was determined as described in Materials and methods. Data presented are the mean±S.E.M. of three determinations, each done in triplicate. #P<0.05; significantly different from the control values in each experiment.

value of around 3 nM for both the MTT assay and [^{3}H]thymidine incorporation; the maximal effects, a 3.2-fold increase in the MTT assay and 3.8-fold for [^{3}H]thymidine incorporation, were obtained at concentrations ≥100 nM (Fig. 1). The noradrenaline-induced mitogenic response was abolished by pretreatment of the cells with phentolamine or prazosin (data not shown).

Next, we examined the effects of Ca^{2+} influx through NSCC on the noradrenaline-induced mitogenic response using LOE 908 and SK&F 96365. LOE 908 inhibited noradrenaline-induced mitogenesis in a concentration-dependent manner with an IC_{50} value of around 1 μM both for the MTT assay and the [^{3}H]thymidine incorporation assay (Fig. 2). Complete inhibition was observed at concentrations ≥3 μM (Fig. 2). In contrast, SK&F 96365 up to 10 μM did not affect noradrenaline-induced mitogenic response (Fig. 2).

3.2. Time course and concentration-dependency of ERK1/2 activation by noradrenaline

After stimulation with 100 nM noradrenaline, ERK1/2 activity in the cytosolic fraction increased with time and at 5 min reached a peak of 2.5-fold greater than prestimulation values (Fig. 3A). Thereafter, activity rapidly decreased and by 30 min had reverted to the control level (Fig. 3A). Therefore, in subsequent experiments the stimulation time was set at 5 min.

Noradrenaline stimulated ERK1/2 activity in a concentration-dependent manner with an EC_{50} value of around 3 nM; the maximal effect (2.5-fold increase) was observed at concentrations ≥100 nM (Fig. 3B).

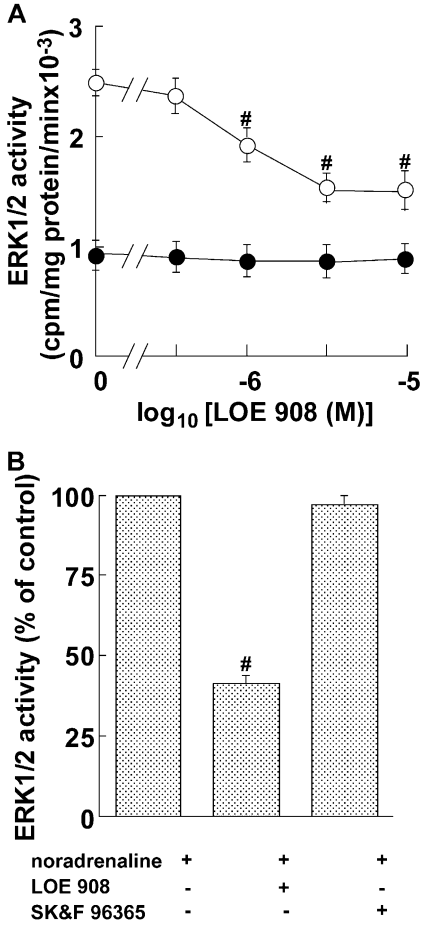

Fig. 4. (A) Effects of LOE 908 on noradrenaline-induced ERK1/2 activity in CHO-α_{1A}. Starved cells were incubated for 15 min with LOE 908 and then stimulated with (open circles) or without (closed circles) 100 nM noradrenaline. (B) Effects of extracellular Ca^{2+} and a maximal effective concentration of LOE 908 and SK&F 96365 on noradrenaline-induced ERK1/2 activity in CHO-α_{1A}. Starved cells were incubated in medium containing 3 mM EGTA, 10 μM LOE 908 or 10 μM SK&F 96365, and then stimulated with 100 nM noradrenaline. The ERK1/2 activity following stimulation with 100 nM noradrenaline was set at 100%, and the ERK1/2 before stimulation with noradrenaline was set at 0%. ERK1/2 activity was determined as described in Materials and methods. Data presented are the mean±S.E.M. of three determinations, each done in triplicate. #P<0.05; significantly different from the control values in each experiment.

3.3. Effects of LOE 908 on ERK1/2 activation

LOE 908 inhibited the noradrenaline-induced increase in ERK1/2 activity in a concentration-dependent manner with an IC_{50} value of around 1 μM (Fig. 4A); the maximal inhibitory effect (60%) was observed at concentrations ≥3 μM (Fig. 4). The magnitude of noradrenaline-induced ERK1/2 activity in the presence of maximal effective concentration of LOE 908 was similar to that in the absence of extracellular Ca^{2+} (Fig. 4B). In contrast, SK&F 96365 up to 10 μM did not affect noradrenaline-induced ERK1/2 activity (Fig. 4B).

4. Discussion

As reported for a variety of cells (Xin et al., 1997; Hu et al., 1999; Chen et al., 1999), noradrenaline was also found to act as a mitogen on CHO-α_{1A} based on the data from the MTT and [^{3}H]thymidine incorporation assays (Fig. 1).

The inhibitory action of LOE 908 on the noradrenaline-induced mitogenic response is mediated by blockade of Ca^{2+} entry through NSCC, as shown by the following data: (1) In our recent work using patch-clamp and $[Ca^{2+}]_i$ monitoring, noradrenaline was found to activate LOE 908-sensitive and SK&F 96365 resistant NSCC in CHO-α_{1A} (Kawanabe et al., 2001a). (2) In the present study, the IC_{50} value of LOE 908 for the noradrenaline-induced mitogenic response (Fig. 2) correlated well with that for the noradrenaline-induced $[Ca^{2+}]_i$ response (Kawanabe et al., 2001a). (3) LOE 908 is considered to exert no cytotoxic effects on quiescent cells, judging from the data of the MTT and [^{3}H]thymidine incorporation assays (Fig. 2). (4) SK&F 96365, Ca^{2+} channel blockers (Meritt et al., 1990), did not affect either noradrenaline-induced sustained increase in $[Ca^{2+}]_i$ (Kawanabe et al., 2001a) or mitogenic responses (Fig. 2). With LOE 908 at concentrations ≥3 μM, the noradrenaline-induced mitogenic response was completely blocked (Fig. 2). Thus, it can be concluded that the noradrenaline-induced mitogenic response is absolutely dependent on Ca^{2+} influx through LOE 908-sensitive and SK&F 96365-resistant NSCC in CHO-α_{1A}.

As described previously (Hu et al., 1999; Chen et al., 1999; Zhong and Minneman, 1999), noradrenaline stimulated ERK1/2 in CHO-α_{1A} (Fig. 3). Involvement of the ERK1/2-dependent cascade in the noradrenaline-induced mitogenic response is consistent with previous reports (Hu et al., 1999; Chen et al., 1999). Because previous reports indicated that stimulation of ERK1/2 by noradrenaline was dependent on extracellular Ca^{2+} influx (Romanelli and van de Werve, 1997; Hu et al., 1999), we examined the effects of Ca^{2+} influx through NSCC on stimulation of ERK1/2 by noradrenaline in CHO-α_{1A}. The IC_{50} value of LOE 908 for the noradrenaline-induced ERK1/2 activation (Fig. 4A) correlated well with that for the noradrenaline-induced $[Ca^{2+}]_i$ response (Kawanabe et al., 2001a). Moreover, the magnitude of noradrenaline-induced ERK1/2 activity in the presence of 10 μM LOE 908 was similar to that in the absence of extracellular Ca^{2+} (Fig. 4B). These results show that the inhibitory action of LOE 908 on noradrenaline-induced ERK1/2 activation is mediated by blockade of Ca^{2+} entry through NSCC. LOE 908 only partially inhibited ERK1/2 activation (Fig. 4). Thus, noradrenaline activates ERK1/2 via both extracellular Ca^{2+} influx-dependent and -independent cascades. Because LOE 908 completely blocked the noradrenaline-induced mitogenic response (Fig. 2), the noradrenaline-induced mitogenic response via extracellular Ca^{2+} influx-independent ERK1/2 cascades is also dependent on extracellular Ca^{2+} influx. These results suggest that a noradrenaline-induced mitogenic cascade downstream of ERK1/2 may be dependent on Ca^{2+} influx through NSCC (Fig. 5). Indeed, Ca^{2+} influx regulates the activation of p34cdc2 kinase and subsequent phosphorylation of pRB (the dephosphorylated form of retinoblastoma protein), leading to DNA synthesis (Takuwa et al., 1993). Phosphorylation of pRB occurs in the mid- to late-G_1 phase and is required for entry into S phase (Weinberg, 1995). Thus, it is currently thought that a Ca^{2+}-dependent process acting relatively far downstream in the intracellular signaling pathway plays a pivotal role in the regulation of cell cycle progression. However, it is unknown whether the same signaling pathways are operating in CHO-α_{1A}. It remains to be determined which signaling cascades downstream of ERK1/2 are involved in the noradrenaline-induced mitogenic response and at which step(s) extracellular Ca^{2+} influx is required.

In summary, we demonstrate in the present study that (1) extracellular Ca^{2+} influx through LOE 908-sensitive and SK&F 96365-resistant NSCC may be essential for noradrenaline-induced mitogenesis in CHO-α_{1A}, and (2) the noradrenaline-induced ERK1/2 activity involves two distinct pathways, one dependent on extracellular Ca^{2+} influx through NSCC, whereas the other is independent of the influx.

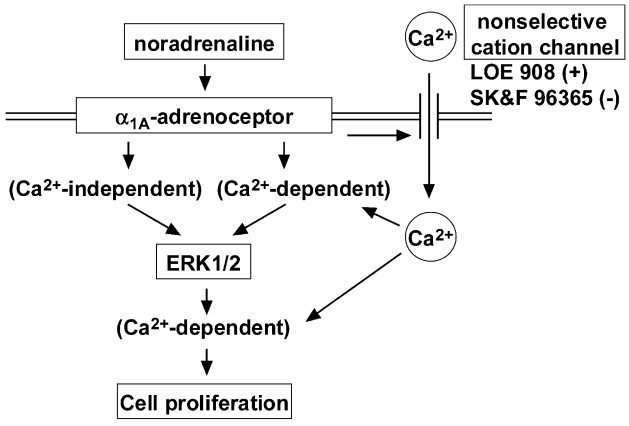

Fig. 5. Schematic representation of the involvement of extracellular Ca^{2+} influx through NSCC in noradrenaline-induced cell proliferation via ERK1/2-dependent cascade in CHO-α_{1A}. See text for details.

Acknowledgements

We thank Boehringer Ingelheim for the kind donation of LOE 908.

This work was supported by a grant-in-aid from the Ministry of Education, Science, Sports and Culture of Japan, by Special Coordination Funds for Science and Technology from the Science and Technology Agency (STA), by a Research Grant for Cardiovascular Disease (11C-1) from the Ministry of Health and Welfare, and by a grant from the Smoking Research Foundation, Japan.

References

Chen, J., Lin, R., Hu, Z.W., Hoffman, B.B., 1999. Alpha1-adrenergic receptor activation of c-fos expression in transfected rat-1 fibroblasts: role of Ca^{2+}. J. Pharmacol. Exp. Ther. 289, 1376–1384.

Encabo, A., Romanin, C., Birke, F.W., Kukovetz, W.R., Groschner, K., 1996. Inhibition of a store-operated Ca^{2+} entry pathway in human endothelial cells by the isoquinoline derivative LOE 908. Br. J. Pharmacol. 119, 702–706.

Graham, R.M., Perez, D.M., Hwa, J., Piascik, M.T., 1996. Alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ. Res. 78, 737–749.

Hieble, J.P., Bylund, D.B., Clarke, D.E., Eikenburg, D.C., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Ruffolo, R.R., 1995. International Union of Pharmacology: X. Recommendation for nomenclature of alpha 1-adrenoceptors: consensus update. Pharmacol. Rev. 47, 267–270.

Hu, Z.W., Shi, X.Y., Lin, R.Z., Chen, J., Hoffman, B.B., 1999. α_1-Adrenergic receptor stimulation of mitogenesis in human vascular smooth muscle cells: role of tyrosine protein kinases and calcium in activation of mitogen-activated protein kinase. J. Pharmacol. Exp. Ther. 290, 28–37.

Jackson, C.L., Schwartz, S.M., 1992. Pharmacology of smooth muscle cell replication. Hypertension 20, 713–736.

Kawanabe, Y., Hashimoto, N., Masaki, T., Miwa, S., 2001a. Ca^{2+} influx through nonselective cation channels plays an essential role in noradrenaline-induced arachidonic acid release in Chinese hamster ovary cells expressing α_{1A}-, α_{1B}- or α_{1D}-adrenergic receptors. J. Pharmacol. Exp. Ther. 299, 901–907.

Kawanabe, Y., Hashimoto, N., Masaki, T., 2001b. B103 neuroblastoma cells predominantly express endothelin type B receptor; effects of extracellular Ca^{2+} influx on endothelin-1-induced mitogenesis. Eur. J. Pharmacol. 425, 173–179.

Meritt, J.E., Airmstrong, W.P., Benham, C.D., Hallam, T.J., Jacob, R., Jaxa-Chamiec, A., Leigh, B.K., Mccarthy, S.A., Moores, K.E., Rink, T.J., 1990. SK&F 96365, a novel inhibitor of receptor-mediated calcium entry. Biochem. J. 271, 515–522.

Milano, C.A., Dolber, P.C., Rockman, H.A., Bond, R.A., Venable, M.E., Allen, L.F., Lefkowitz, R.J., 1994. Myocardial expression of a constitutively active alpha 1B-adrenergic receptor in transgenic mice induces cardiac hypertrophy. Proc. Natl. Acad. Sci. U. S. A. 91, 10109–10113.

Muthalif, M.M., Benter, I.F., Uddin, M.R., Malik, K.U., 1996. Calcium/calmodulin-dependent protein kinase IIalpha mediates activation of mitogen-activated protein kinase and cytosolic phospholipase A2 in norepinephrine-induced arachidonic acid release in rabbit aortic smooth muscle cells. J. Biol. Chem. 271, 30149–30157.

Price, D.T., Lefkowitz, R.J., Caron, M.G., Berkowitz, D., Schwinn, D.A., 1994. Localization of mRNA for three distinct alpha 1-adrenergic receptor subtypes in human tissues: implications for human alpha-adrenergic physiology. Mol. Pharmacol. 45, 171–175.

Romanelli, A., van de Werve, G., 1997. Activation of mitogen-activated protein kinase in freshly isolated rat hepatocytes by both a calcium- and a protein kinase C-dependent pathway. Metabolism 46, 548–555.

Sugawara, F., Ninomiya, H., Okamoto, Y., Miwa, S., Mazda, O., Katsura, Y., Masaki, T., 1996. Endothelin-1-induced mitogenic responses of Chinese hamster ovary cells expressing human endothelinA: the role of a wortmannin-sensitive signaling pathway. Mol. Pharmacol. 49, 445–447.

Takuwa, N., Zhou, W., Kumada, M., Takuwa, Y., 1993. Ca^{2+}-dependent stimulation of retinoblastoma gene product phosphorylation and p34cdc2 kinase activation in serum-stimulated human fibroblasts. J. Biol. Chem. 268, 138–145.

Weinberg, R.A., 1995. The retinoblastoma protein and cell cycle control. Cell 81, 323–330.

Wylie, P.G., Challiss, R.A., Blank, J.L., 1999. Regulation of extracellular-signal regulated kinase and c-Jun N-terminal kinase by G-protein-linked muscarinic acetylcholine receptors. Biochem. J. 338, 619–628.

Xin, X., Yang, N., Eckhart, A.D., Faber, J.E., 1997. α_{1D}-Adrenergic receptors and mitogen-activated protein kinase mediate increased protein synthesis by arterial smooth muscle. Mol. Pharmacol. 51, 764–775.

Zhong, H., Minneman, K.P., 1999. Differential activation of mitogen-activated protein kinase pathways in PC12 cells by closely related alpha1-adrenergic receptor subtypes. J. Neurochem. 72, 2388–2396.