Tyrosine kinase-dependent modulation by interferon-α of the ATP-sensitive K^+ current in rabbit ventricular myocytes

Manabu Nishio[a], Yoshizumi Habuchi[b,*], Hideo Tanaka[b], Junichiro Morikawa[a], Takeshi Okanoue[a], Kei Kashima[a]

[a] *Department of Internal Medicine III, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-0841, Japan*
[b] *Department of Laboratory Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-0841, Japan*

Received 28 December 1998

Abstract We examined the effects of interferon-α on the ATP-sensitive K^+ current ($I_{K,ATP}$) in rabbit ventricular cells using the patch-clamp technique. $I_{K,ATP}$ was induced by NaCN. Whole-cell experiments indicated that interferon-α (5×10^2–2.4×10^4 U/ml) inhibited $I_{K,ATP}$ in a concentration-dependent manner (60.7 ± 7.5% with 2.4×10^4 U/ml). In cell-attached configuration, interferon-α (2.4×10^4 U/ml) applied to the external solution also inhibited the activity of the single ATP-sensitive K^+ (K_{ATP}) channel by 56.0 ± 5.8% without affecting the single channel conductance. The inhibitory effect of $I_{K,ATP}$ by interferon-α was blocked by genistein and herbimycin A, tyrosine kinase inhibitors, but was not affected by *N*-(2-metylpiperazyl)-5-isoquinolinesulfoamide (H-7), an inhibitor of protein kinase C and cAMP-dependent protein kinase. These findings suggest that interferon-α inhibits the cardiac K_{ATP} channel through the activation of tyrosine kinase. The tyrosine kinase-mediated inhibition of $I_{K,ATP}$ by cytokines may aggravate cell damage during myocardial ischemia.

Key words: Interferon-α; Adenosine triphosphate; Potassium channel; Rabbit; Ventricular myocyte; Tyrosine kinase

1. Introduction

Interferon is a cytokine, which plays important roles in the host defense mechanisms against viral infection and cancer. It binds to specific receptors on target cells including infected cells, cancer cells and immune cells, resulting in the inhibition of virus proliferation, the activation of immune cells and the regulation of oncogenes [1]. The heart may be a target site for interferon, as the production of various cytokines including interferons by leukocytes increases during cardiovascular disease such as myocarditis, cardiomyopathy and ischemic heart disease [2,3], and because these cytokines are known to aggravate heart failure [2].

Recent studies have indicated that interferon activates tyrosine kinase via the specific receptor-mediated mechanism [4]. This kinase was recently found to be an important modulator in ion channels [5], including L-type Ca^{2+} [6], Cl^- [7] and K^+ channels [8–11]. In the heart, the delayed rectifier K^+ [8] and the K_{ATP} channels [11] were reported to be modulated by tyrosine kinase.

The K_{ATP} channel plays important protective roles in the heart. Activation of K_{ATP} channel shortens the action potential duration to reduce energy consumption, and protects the cardiac cells against ischemia [12–14]. Since the secretion of interferon is increased under cardiac pathological conditions, where the K_{ATP} channel is likely to be activated [3], it is important to examine the effects of this cytokine on cardiac $I_{K,ATP}$. The aim of this study was to elucidate the regulatory role of interferon in the K_{ATP} channel in single cardiac myocytes.

2. Materials and methods

2.1. Cell isolation

Isolation of single ventricular cells and voltage-clamp experiments were conducted as described [15]. Rabbits weighing 2–2.5 kg were anesthetized by intravenous injection of 40 mg/kg pentobarbital and 500 IU heparin, then were killed by a blow to the neck. The excised heart was retrogradely perfused with Ca^{2+}-free, phosphate-buffered solution containing (in mM): NaCl, 142; KCl, 5.4; $MgCl_2$, 1.0; NaH_2PO_4, 0.33; Na_2HPO_4, 2.24; and glucose, 10 (pH = 7.4) for 10 min. The perfusates were then switched to the Ca^{2+}-free solution containing 0.02 mg/ml collagenase (Yakult, Tokyo, Japan) and 0.01 mg/ml protease (Type 14, Sigma, St. Louis, MO, USA) for 15 min. The perfusates were continuously bubbled with 100% O_2 at 37°C. After a 10 min perfusion of the first enzyme treatment, the right ventricular free wall was cut into small pieces. They were then stirred in the second enzyme solution containing 1 mg/ml collagenase (Wako, Osaka, Japan). Isolated cells were placed in the stock solution at 4°C with 0.1% bovine serum albumin (Sigma, Fraction V). The stock solution contained (in mM): K-glutamate, 90; oxalate, 10; KCl, 25; KH_2PO_4, 10; $MgSO_4$, 1; taurine, 10; ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.5; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 5; and glucose, 10 (pH = 7.2 adjusted with KOH).

2.2. Electrical measurements

For electrical measurements an Axopatch-1D amplifier was used (Axon Instruments, Foster City, CA, USA). For whole-cell current measurements, the cells were voltage-clamped under superfusion with glucose-free Tyrode solution containing (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.0; $MgCl_2$, 1.0 and HEPES, 5 (pH = 7.4 adjusted with HCl). The pipette solution contained (in mM): K-aspartate, 110; KCl, 20; $CaCl_2$, 1.0; $MgCl_2$, 1.0; EGTA, 10; K_2-ATP, 1; Na_3-GTP, 0.2; and HEPES, 5 (pH = 7.2 adjusted with KOH). The pipette used had a tip resistance of 2–2.5 MΩ. The cell capacity was 84 ± 18 pF (n = 135, mean ± S.D.). For induction of $I_{K,ATP}$, the cells in the recording chamber were pretreated with 1-mM NaCN-containing glucose-free Tyrode solution for 10–20 min at 37°C. During the voltage-clamp experiments the concentration of NaCN in the superfusate was increased to 10 mM to effectively activate $I_{K,ATP}$ (Fig. 1A).

*Corresponding author. Fax: (81) (75) 251-5678.
E-mail: yhabuchi@koto.kpu-m.ac.jp

Abbreviations: $I_{K,ATP}$, ATP-sensitive K^+ current; K_{ATP} channel, ATP-sensitive K^+ channel; PKC, protein kinase C; PKA, cAMP-dependent protein kinase; H-7, *N*-(2-metylpiperazyl)-5-isoquinolinesulfoamide; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid

PII: S0014-5793(99)00083-6

Single-channel recordings were conducted in both the cell-attached and inside-out configurations [16]. The pipette's resistance was around 10 MΩ. The pipette was heat-polished immediately before use and the shank coated with Sylgard (Dow Corning Co., Midland, MI, USA). The pipette solution contained (in mM): NaCl, 140; KCl, 5.4; and HEPES, 5 (pH = 7.2 adjusted with KOH). For the cell-attached configuration, NaCN (1 mM) was added to the stock solution (4°C) 4–6 h before the use of the cells. The NaCN-loaded cells were perfused with the bathing solution containing (in mM): KCl, 135; $MgCl_2$, 2.0; EGTA, 5; NaCN, 10; and HEPES, 5 (pH 7.4 adjusted with KOH). Cromakalim (10 μM) was added to the perfusate to maintain the single channel activity. In the inside-out mode, the excised patch from the non-loaded cell was exposed to a bathing solution containing (in mM): KCl, 135; $MgCl_2$, 1.0; EGTA, 5; NaCN, 10; HEPES, 5; ATP, 0.02; and ADP, 0.1 (pH 7.2 adjusted with KOH). All the experiments were carried out at 37°C.

2.3. Data acquisition and analysis

The frequencies of the low-pass filter (E-3201B, NF Electrics, Osaka, Japan) were 0.5 kHz for the whole-cell recording and 2 kHz for the single-channel recording, respectively. In the single-channel experiments, the data were analyzed using pClamp software (Axon Instruments, Foster City, CA, USA). The mean open probability was measured every 15 s.

The data were presented as means ± S.E.M., unless otherwise specified. Student's *t*-test was used for statistical analysis, and *P* values smaller than 0.05 were considered to be significant.

2.4. Drugs and chemicals

We used interferon-α, provided by Sumitomo Pharmaceutical Co. (Osaka, Japan). Interferon-α was stored at 4°C as a 3.47×10^6 U/ml stock solution in distilled water. Glibenclamide, Na_2-ATP, K_2-ATP, ADP, Na_2-phosphocreatine and Na_3-GTP were purchased from Sigma. All other chemicals were from Wako Pure Chemicals (Osaka). Glibenclamide was dissolved in dimethylsulfoxide as a 10 or 100 mM stock solution.

3. Results

3.1. Interferon-α inhibition of $I_{K,ATP}$

Fig. 1A shows a representative effect of interferon-α on $I_{K,ATP}$ in whole-cell configuration at a membrane potential of −10 mV. After pretreatment with 1 mM NaCN, switching of the solution to that containing 10 mM NaCN produced the immediate activation of glibenclamide-sensitive $I_{K,ATP}$ [15]. During the plateau following the initial run-down of $I_{K,ATP}$, the bath application of interferon-α at 2.4×10^4 U/ml inhibited the $I_{K,ATP}$ by 60.7 ± 7.5% (n = 16). In Fig. 1B, inhibitory effects on $I_{K,ATP}$ at −10 mV are plotted against the concentration of interferon-α. Interferon-α inhibited $I_{K,ATP}$ in a concentration-dependent manner; it began to inhibit $I_{K,ATP}$ at 0.05×10^4 U/ml (10.1 ± 11%, n = 4).

Fig. 2A shows the effect of interferon-α (2.4×10^4 U/ml) on the single-channel current using the cell-attached configuration. Bath application of interferon-α significantly reduced the channel openings. The mean NP_o decreased by 56.0 ± 5.8% during superfusion with interferon-α (n = 10, Fig. 2A,C). However, interferon-α at 2.4×10^4 U/ml did not alter NP_o when it was applied to the bath solution in the inside-out configuration (n = 4, Fig. 2C). These results indicate that the site for interferon-α to modify the K_{ATP} channel is on the outer surface of the membrane. The *I-V* relationships for single K_{ATP} channel (Fig. 2B) clearly show that interferon-α did not change the conductance of the single K_{ATP} channel (25.2 ± 0.3 pS during control and 25.4 ± 0.3 pS in the presence of interferon-α, n = 6). Therefore, the inhibition of $I_{K,ATP}$ by interferon-α is attributed to a decrease in the open probability of the channels.

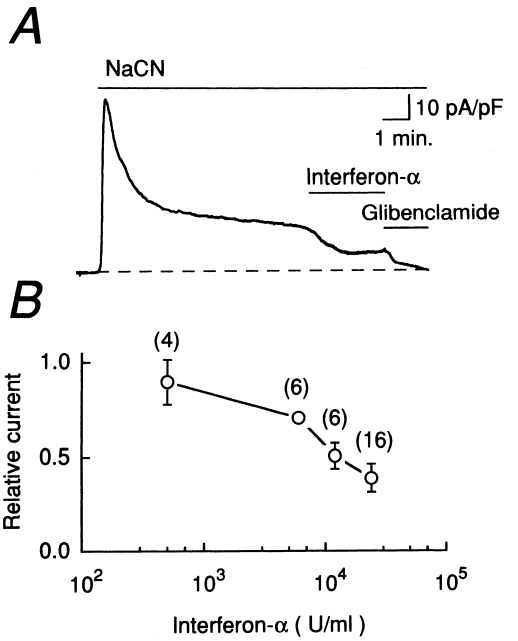

Fig. 1. Effects of interferon-α on $I_{K,ATP}$. A: Representative record of the holding current at −10 mV. The pipette solution contained 1 mM ATP. Horizontal bars indicate the period of extracellular applications of the drugs. The concentrations of interferon-α and glibenclamide were 2.4×10^4 U/ml and 1 μM, respectively. NaCN was first applied at a concentration of 1 mM. After the whole-cell configuration was established, the concentration of NaCN was increased to 10 mM. The dotted line indicates the baseline current level obtained by the application of glibenclamide. B: Concentration-dependent inhibitions of $I_{K,ATP}$ by interferon-α. The $I_{K,ATP}$ in the presence of interferon-α relative to that immediately before the application of interferon-α is plotted against the interferon-α concentration. $I_{K,ATP}$ was measured as the glibenclamide-sensitive current. Parentheses indicate the number of cells. A single concentration of the drug was tested on one cell.

3.2. Effect of protein kinase inhibitors on inhibition of $I_{K,ATP}$ by interferon-α

Interferon-α induces tyrosine phosphorylation, which is a key mediator of the receptor-effect couplings of interferon in various cells [4,17]. To assess the involvement of tyrosine kinase in the $I_{K,ATP}$ inhibition by interferon-α, we examined the effects of interferon in the presence of tyrosine kinase inhibitors, genistein and herbimycin A. Either genistein at 100 μM or herbimycin A at 1.5 μM was applied to the cell milieu through the pipette. Fig. 3A shows the effect of genistein. Both genistein (n = 6) and herbimycin A (n = 6, data not shown) completely blocked the inhibitory effect of interferon-α on $I_{K,ATP}$. The effect of genistein was also observed in non-dialyzed cells. Fig. 3C shows the effect of genistein on K_{ATP} channels in the cell-attached configuration. The cells were incubated with 10 μM genistein in the stock solution for 4–6 h before use, and superfused with the bath solution containing genistein (100 μM). Interferon-α (2.4×10^4 U/ml) did not significantly affect the single K_{ATP} channel activity in the presence of genistein.

The aforementioned results indicate that the interferon-α inhibition of $I_{K,ATP}$ is mediated by tyrosine kinase. K_{ATP} channels are known to be regulated by other protein kinases including protein kinase C (PKC) [18] and protein kinase A (PKA) [19]. The possibility that PKA and PKC is involved in

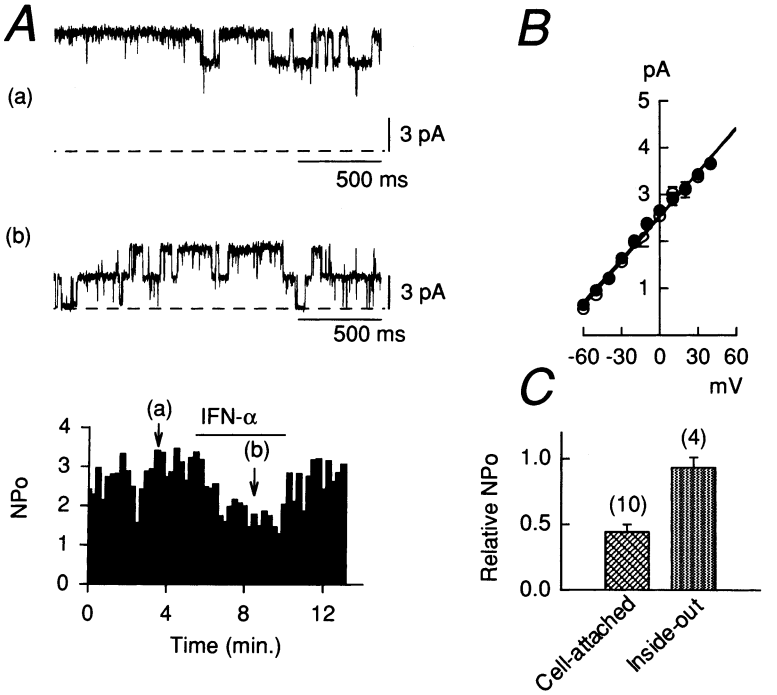

Fig. 2. Interferon-α inhibition of single K_{ATP} channels in the cell-attached configuration. A: Upper trace, single-channel recording in the absence (a) and presence (b) of interferon-α (2.4×10^4 U/ml). The membrane potential was maintained at −10 mV. Interferon-α was added to the bath solution. The dotted line indicates 0-current level, which was determined by applying 1 μM glibenclamide at the end of the experiment. Lower panel, temporal change in the open probability of the K_{ATP} channels following the application of interferon-α. The NP_o measured every 15 s is plotted as a function of time. The horizontal bar indicates the period of interferon-α (2.4×10^4 U/ml) application. The channel recordings indicated by (a) and (b) in this panel are shown as the upper traces. B: Conductance of the single K_{ATP} channel current. ○ and ● denote the single-channel amplitude at various holding potentials before and after the application of interferon-α (2.4×10^4 U/ml), respectively ($n=6$). C: The effects of interferon-α on NP_o in cell-attached and inside-out configurations. The NP_o was averaged for a period of 1 min immediately before and after the application of interferon-α (2.4×10^4 U/ml). The relative NP_o denotes the ratio of the averaged NP_o during the interferon-α perfusion to that during control. Parentheses indicate the number of cells.

the interferon-induced inhibition of $I_{K,ATP}$ was examined using 100 μM H-7, an inhibitor for PKC and PKA, in the pipette solution. H-7 did not affect the inhibition of $I_{K,ATP}$ by interferon-α (59.4 ± 6.3%; $n=6$; Fig. 3B).

4. Discussion

Our results showed that interferon-α inhibited $I_{K,ATP}$ in rabbit ventricular myocytes. The inhibitory effect of interferon-α occurred when the outer membrane surface was exposed to interferon-α. The attenuation of $I_{K,ATP}$ by interferon-α was blocked by the application of genistein and herbimycin A, tyrosine kinase inhibitors. It has been generally regarded that interferons exert their cellular effects by binding to the specific receptors of target cells [23,26]. Interferon-bound receptors activate a family of tyrosine kinases, known as Janus kinases [4,23,27]. These tyrosine kinases are key mediators in the interferon receptor-response couplings. Thus, our finding that the extracellular application of interferon-α inhibited $I_{K,ATP}$ via activation of tyrosine kinase indicates that interferon-α induced the suppression of $I_{K,ATP}$ through the physiological receptor-response couplings.

Since tyrosine kinases phosphorylate tyrosine residues of various proteins [28], interferon-α may inhibit $I_{K,ATP}$ by phosphorylating the channel protein. Indeed, Kwak et al. [11] demonstrated that tyrosine phosphorylation of the K_{ATP} channel prompted the run-down of the channel activity in the inside-out configuration in rat cardiac myocytes. Using a cloned K_{ATP} channel, Ashford et al. [29] and Inagaki et al. [30] found that the K_{ATP} channel has sites sensitive to tyrosine phosphorylation. The tyrosine phosphorylation was reported also in other K^+ channels, such as the delayed-rectifier K^+ (RAK) channel [8], cloned Kv3.1 and Kv2.1 channels [31] and the voltage-gated K^+ channel in *Aplysia* bag-cell neurons [10]. Huang et al. [8] found that the RAK channel inhibition by muscarinic stimulation was absent in the mutant RAK channel lacking tyrosine phosphorylation sites. Based on these findings, it seems that tyrosine kinases participate in the functional regulation of various types of membrane K^+ channels, and that interferon modulates $I_{K,ATP}$ through tyrosine phosphorylation of the channel protein.

The phosphorylation of various channel proteins plays a key role in the regulation of the channel function [32]. PKA and PKC, rather than tyrosine kinase, are known to cause large and rapid changes in various ionic currents. $I_{K,ATP}$ is also regulated by the phosphorylation of PKA and PKC [18,19,33]. In addition, PKC regulates the tyrosine kinase [34], and recent electrophysiological studies have also shown that PKC-mediated tyrosine kinase activation is involved in the muscarinic suppression of K_{ATP} and RAK channels [8,35]. PKA may also modify tyrosine phosphorylation of channel proteins [10]. However, our finding that H-7 did not alter the effects of interferon-α in this study indicates that PKA and PKC played a small, if any, role in the inhibition of $I_{K,ATP}$ by interferon-α.

Tyrosine phosphorylation is closely related with gene tran-

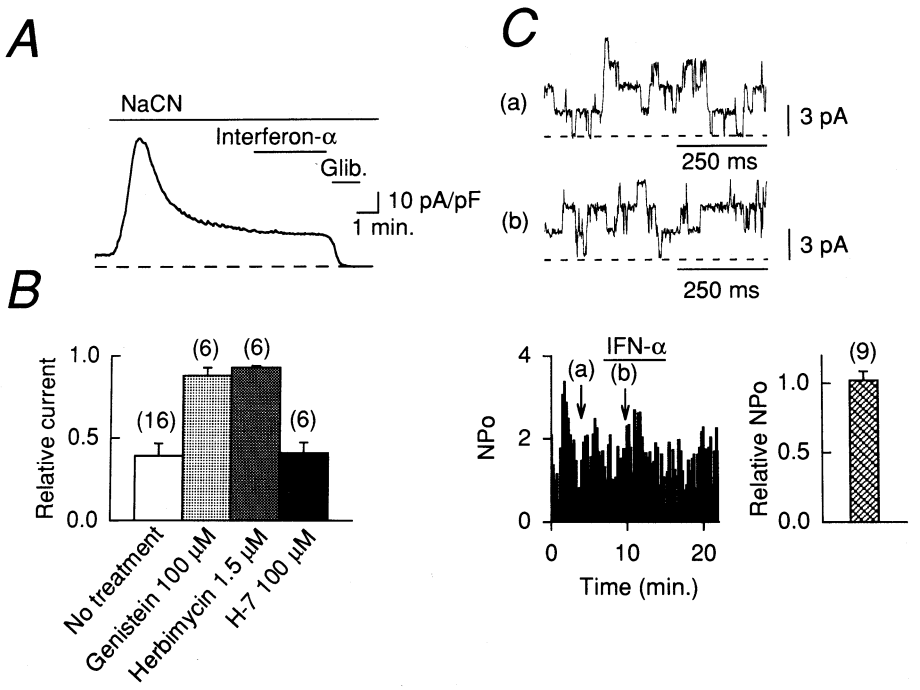

Fig. 3. Effects of protein kinase inhibitors on the inhibition of $I_{K,ATP}$ by interferon-α. A: Representative record of the holding current at −10 mV in the presence of genistein. The pipette solution contained genistein (100 μM). The bath solution and protocol were the same as in Fig. 1. B: The effects of genistein (100 μM), herbimycin A (1.5 μM) and H-7 (100 μM). These protein kinase inhibitors were applied to the pipette solution. The relative $I_{K,ATP}$ value in the presence of interferon-α (2.4×10^4 U/ml) is shown. Parentheses indicate the number of cells. C: The absent effect of interferon-α (2.4×10^4 U/ml) on single K_{ATP} channels in the presence of genistein. The cell was loaded with genistein (10 μM) for 4–6 h. Genistein (100 μM) was also added to the test solution, and the records were made in the cell-attached configuration. The temporal plot of the NP_o and the relative NP_o are shown in the lower diagrams.

scription [4,27]. The interferon-activated Janus kinases phosphorylate some protein complexes (interferon-stimulated gene factors). The phosphorylated gene factors induce gene transcription and cause the effects. Interferon-α is reported to induce gene transcription within minutes in human fibroblast cells [21]. Therefore, it is possible that some effectors following interferon-induced gene transcription inhibit $I_{K,ATP}$.

Our experiments demonstrated that bath-applied interferon-α inhibited the K_{ATP} channel activity at a threshold concentration of 500 U/ml. In previous reports, experimental concentrations between 200–1000 U/ml [21–24] were used for human interferon to stimulate human cells; i.e. a high dose of interferon seems to be necessary for the inhibition of $I_{K,ATP}$. The tissue concentration of interferon has not yet been determined. The cytokine secretion, including interferons, exhibits an autocrine property [20]. Therefore, the concentration of interferon may be high in tissues, in sufficient amounts to modulate $I_{K,ATP}$. In addition, in our experiments we used human interferon-α and rabbit cardiac cells. The interferon-receptor reaction is highly species-dependent and shows only a weak cross reaction [25]. Thus, the use of the cardiac cells isolated from different species may have diminished the activity of human interferon in this study.

In summary, we showed that cardiomyocytes are a target of interferon-α, which regulates $I_{K,ATP}$ via tyrosine kinase-mediated mechanisms. Cytokines such as tumor necrosis factor, interleukins, and interferons are expressed during heart failure and aggravate the condition [2,3,20,36]. Therefore, the inhibition of $I_{K,ATP}$ by interferons may diminish the cardioprotective mechanisms of the K_{ATP} channel and may aggravate cell damage during ischemia. This is the first report showing that a biological cytokine modulates cardiac $I_{K,ATP}$ through tyrosine kinase.

References

[1] Baron, S., Tyring, S.K., Fleischmann Jr., W.R., Coppenhaver, D.H., Niesel, D.W., Klimpel, G.R., Stanton, G.J. and Hughes, T.K. (1991) J. Am. Med. Assoc. 266, 1375–1383.
[2] Matsumori, A., Shioi, T., Yamada, T., Matsui, S. and Sasayama, S. (1994) Circulation 89, 955–958.
[3] Vaddi, K.D.V.M., Nicolini, F.A., Mehta, P. and Mehta, J.L. (1994) Circulation 90, 694–699.
[4] Larner, A. and Reich, N.C. (1996) Biotherapy 8, 175–181.
[5] Siegelbaum, S.A. (1994) Curr. Biol. 4, 242–245.
[6] Katsube, Y., Yokoshiki, H., Nguyen, L., Yamamoto, M. and Sperelakis, N. (1998) Eur. J. Pharmacol. 345, 309–314.
[7] Sorota, S. (1995) Pflügers Arch. 431, 178–185.
[8] Huang, X.-Y., Morielli, A.D. and Peralta, E.G. (1993) Cell 75, 1145–1156.
[9] Xiong, Z., Burnette, E. and Cheung, D.W. (1995) Eur. J. Pharmacol. 290, 117–123.
[10] Wilson, G.F. and Kaczmarek, L.K. (1993) Nature 366, 433–438.
[11] Kwak, Y.G., Park, S.K., Cho, K.P. and Chae, S.W. (1996) Life Sci. 58, 897–904.
[12] Noma, A. (1983) Nature 305, 147–148.
[13] Nichols, C.G. and Lederer, W.J. (1991) Am. J. Physiol. 261, H1675–H1686.
[14] Parret, J.R. and Kane, K.A. (1994) Cardiovasc. Res. 28, 783–787.
[15] Nishio, M., Habuchi, Y., Tanaka, H., Morikawa, J., Yamamoto, T. and Kashima, K. (1998) J. Pharmacol. Exp. Ther. 287, 293–300.
[16] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
[17] Lev, S., Moreno, H., Martinez, R., Canoll, P., Peles, E., Musac-

chio, J.M., Plowman, G.D., Rudy, B. and Schlessinger, J. (1995) Nature 376, 737–745.
[18] Light, P.E., Allen, B.G., Walsh, M.P. and French, R.J. (1995) Biochemistry 34, 7252–7257.
[19] Schackow, T.E. and TenEick, R.E. (1994) J. Physiol. (Lond.) 474, 131–145.
[20] Wang, D., McMillin, J.B., Bick, R. and Buja, L.M. (1996) Lab. Invest. 75, 809–818.
[21] Dale, T.C., Ali-Iman, A.M., Kerr, I.M. and Stark, G.R. (1989) Proc. Natl. Acad. Sci. USA 86, 1203–1207.
[22] Schindler, C., Shuai, K., Prezioso, V.R. and Darnell Jr., J.E. (1992) Science 257, 809–813.
[23] Novick, D., Cohen, B. and Rubinstein, M. (1994) Cell 77, 391–400.
[24] Korutla, L. and Kumar, R. (1996) Anticancer Res. 16, 2789–2796.
[25] Rehberg, E., Kelder, B., Hoal, E.G. and Pestka, S. (1982) J. Biol. Chem. 257, 11497–11502.
[26] Uzé, G., Lutfalla, G. and Gresser, I. (1990) Cell 60, 225–234.
[27] Darnell Jr., J.E., Kerr, I.M. and Stark, G.R. (1994) Science 264, 1415–1421.
[28] Darnell Jr., J.E. (1997) Science 277, 1630–1635.
[29] Ashford, M.L.J., Bond, C.T., Blair, T.A. and Adelman, J.P. (1994) Nature 370, 456–459.
[30] Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M., Seino, Y., Mizuta, M. and Seino, S. (1995) J. Biol. Chem. 270, 5691–5694.
[31] Solomon, D.H., Critz, S.M., Wible, B.A., Brown, A.M. and Schwartz, J.H. (1993) Soc. Neurosci. Abstr. 19, 711.
[32] Levitan, I.B. (1994) Annu. Rev. Physiol. 56, 193–212.
[33] Liang, B.T. (1997) Am. J. Physiol. 273, H847–H853.
[34] Kribben, A., Wieder, E.D., Li, X., Putten, V.V., Granot, Y., Schrier, R.W. and Nemenoff, R.A. (1993) Am. J. Physiol. 265, C939–C945.
[35] Hatakeyama, N., Wang, Q., Goyal, R.K. and Akbarali, H.I. (1995) Am. J. Physiol. 268, C877–C885.
[36] Yue, P., Massie, B.M., Simpson, P.C. and Long, C.S. (1998) Am. J. Physiol. 275, H250–H258.