Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 344 (2006) 189–193
BBRC
www.elsevier.com/locate/ybbrc

Tumor necrosis factor-α inhibits the cardiac delayed rectifier K current via the asphingomyelin pathway

Katsuharu Hatada [a], Takashi Washizuka [a], Minoru Horie [b], Hiroshi Watanabe [a], Fumio Yamashita [a], Masaomi Chinushi [a], Yoshifusa Aizawa [a,*]

[a] *Division of Cardiology, Niigata University Graduate School of Medical and Dental Science, Niigata, Japan*
[b] *The Second Department of Internal Medicine, Shiga Medical College, Ohtsu, Japan*

Received 15 March 2006
Available online 29 March 2006

Abstract

Tumor necrosis factor-α (TNF-α) affects contractility and ionic currents in the heart. However, the electrophysiological effects, especially on delayed rectifier K currents (IK), have not yet been fully elucidated. We examined the effects of TNF-α on IK. Using a voltage-clamp method, IK was measured in guinea pig ventricular myocytes in the basal state and after pharmacological intervention. To specify the site of the action of TNF-α, the myocytes were incubated with pertussis toxin or *N*-oleoylethanolamine, a ceramidase inhibitor, and IK was measured. TNF-α suppressed IK when it was enhanced by isoproterenol, histamine or forskolin but not in the basal state or when IK was augmented by an internal application of cyclic AMP. Both pre-incubation with pertussis toxin and *N*-oleoylethanolamine abolished the inhibitory action of TNF-α on isoproterenol-augmented IK. TNF-α inhibits IK, mainly IKs, when it is augmented by PKA as a result of the generation of sphingosine.

Keywords: TNF-α; Delayed rectifier K current; c-AMP; PKA pathway; Sphingosine; Ceramide

Genes of tumor necrosis factor-α (TNF-α) are up-regulated in many myocardial diseases: ischemia [1], experimental and human myocarditis [2–4], dilated cardiomyopathy [5,6], and congestive heart failure [7–10], and can exert mechanical and electrophysiological effects on myocardial cells [11–14].

In the diseased heart, the PKA pathway is activated via enhanced adrenergic activity leading to the augmentation of the delayed rectifier potassium current (IK) as well as calcium currents [11,15]. However, the action of TNF-α on IK is unknown, so we studied the effect of TNF-α on IK in rat ventricular myocytes. We found that TNF-α inhibits IK but only when it was augmented by PKA activation and the effect was a result of the generation of sphingosine by TNF-α.

Materials and methods

Cell preparation. Single ventricular myocytes were isolated from the left ventricle of adult guinea pigs weighing 250–400 g using an enzymatic dissociation procedure as reported previously [16]. Briefly, after deep anesthesia with pentobarbital sodium given intraperitoneally at 50 mg/kg, the chest was opened under artificial respiration, the aorta was cannulated with Langendorff's apparatus, and the heart was quickly excised. Using the retrograde perfusion, normal Tyrode's solution (36 °C) was applied for 5 min, followed by nominally Ca^{2+}-free Tyrode's solution until contraction ceased. Then, with Tyrode's solution supplemented with 0.4 mg/ml of collagenase type 1 (Sigma Co., St. Louis, MO), the heart was retrogradely perfused for 15–20 min. The composition of normal Tyrode's solution was NaCl 145, KCl 5.4, $CaCl_2$ 1.8, NaH_2PO_4 0.3, $MgCl_2$ 0.5, glucose 5.5, and Hepes 5 (pH adjusted to 7.4 with NaOH) (mmol/L).

Finally, the heart was perfused with KB medium at room temperature to rinse the collagenase off [17]. The composition of KB medium was L-glutamic acid 70, KCl 25, taurine 20, KH_2PO_4 10, $MgCl_2$ 3, EGTA 0.5, glucose 11, and Hepes 10 (pH adjusted to 7.3 with KOH) (mmol/L). The partially digested heart was gently minced with scissors in the KB solution and after filtration through 105-μm mesh, cells were stored at room temperature and used within 8 h of isolation.

* Corresponding author. Fax: +81 25 228 5611.
E-mail address: aizaways@med.niigata-u.ac.jp (Y. Aizawa).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.03.115

Measurement of IK. The pipette solution contained potassium aspartate 110, KCl 20, $MgCl_2$ 7.0, $CaCl_2$ 0.69, K_2-ATP 5, Na_2-GTP 0.1, creatine phosphate-K_2 5, EGTA 5, and Hepes 5 (pH 7.4 with KOH) (mmol/L). According to the stabilizing constants proposed by Fabiato and Fabiato [18], with the correction of Tsien and Rink [19], the *p*Ca of the internal solution was calculated to be 8.0.

A few drops of cell suspension were dispersed into a small chamber superfused with Tyrode's medium on the stage of an inverted microscope (Olympus, Tokyo, Japan). A gigaohm seal was obtained in the center of the cells by applying negative pressure to the interior of the pipettes by gentle suction and the whole-cell currents were measured with low-resistance pipettes (2 MΩ) using an Axopatch 200B amplifier with a CV-203BU headstage and pClmap software (Axon Instruments, Foster City, CA).

In all experiments, the L-type calcium current (ICaL) was inhibited by 2 μmol/L of nisoldipine (Bayer Pharmaceutical Co., Osaka, Japan). The liquid junction potential was corrected by a voltage offset on the patch-clamp amplifier. Cell membrane capacitance was measured using the internal circuit for capacitance-current compensation. Series resistance was compensated for to minimize the duration of the capacitive surge.

After a depolarization pulse of 2 s, the voltage was clamped back to the holding potential (−40 mV) and IK steady-state currents were defined as the difference between the peak point of time-dependently activated component and the holding currents. All data were filtered at 1 kHz, digitized at 4 kHz using a Digidata 1200 (Axon Instruments, Foster City, CA), and stored on a custom-made computer (Intermedical, Nagoya, Japan). IK was measured in the basal state and after the addition of isoproterenol at 20 nmol/L and the pharmacological interventions were achieved as follows.

Measurements of IK in the altered PKA pathway. Rat TNF-α was dissolved in distilled water until use and the effect of TNF-α (Sigma Co.) was tested at 20 ng/ml before and after the addition of isoproterenol to the external medium. To examine the role of the intracellular signal transudation, we employed several pharmacological interventions.

Histamine is known to increase c-AMP and increase IK via the stimulatory G protein (Gs)/adenylate cyclase pathway [20]. Histamine (Sigma Co.) freshly made immediately before use was prepared to result in a 250 nmol/L and the effect of TNF-α was studied. Similarly, forskolin (Sigma Co.), a diterpene plant alkaloid known to increase intracellular c-AMP by directly stimulating adenylate cyclase [21], was dissolved in ethanol (10 mmol/L stock solution) and prepared at 500 nmol/L to augment IK and the effect of TNF-α was measured. Histamine and forskolin were administered to activate IK in a comparable magnitude as that of 20 nmol/L of isoproterenol. Then, c-AMP was administered intracellularly via direct dialysis of intrapipette c-AMP (1 μmol/L).

Since the intracellular c-AMP concentration is modulated by adenylate cyclase coupled with Gs and pertussis toxin (PTX)-sensitive G-protein (Gi), we examined the action of TNF-α on IK after the incubation of myocytes with PTX (Sigma Co.) at 5 μg/ml and at 36 °C for 120 min. After PTX treatments, we determined the effect of carbacol on IK [22].

Finally, we tested whether the ceramide–sphingosine pathway is involved concerning the action of TNF-α on IK. Sphingosine 1-phosphate, a product of the sphingomyelin pathway, has already been shown to modulate IKAch via PTX-sensitive G-proteins [23], therefore we incubated the myocytes with *N*-oleoylethanolamine (NOE) (Sigma Co.), a ceramidase inhibitor, and examined the effect of TNF-α on the

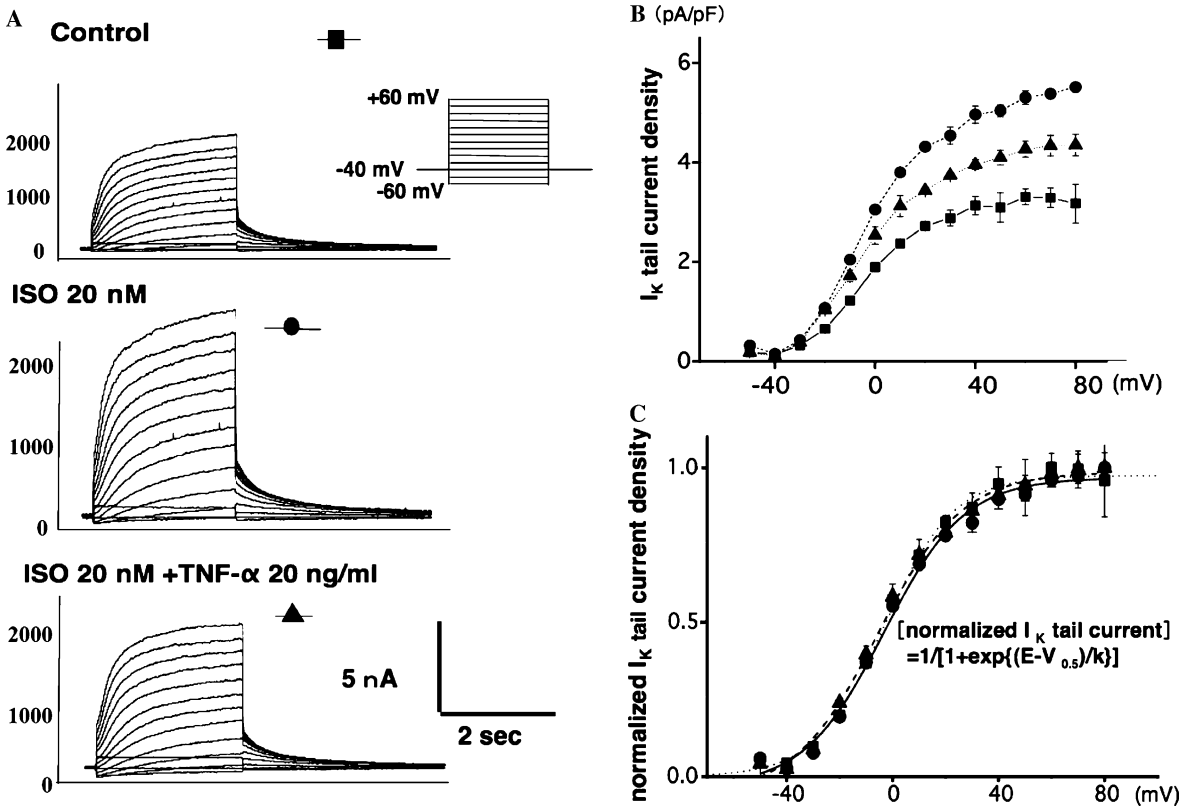

Fig. 1. The effects of TNF-α on current–voltage relationships for IK enhanced by isoproterenol. (A) Three sets of current traces at test potentials between −60 and +60 mV in 10-mV increments. (B) Tail current amplitudes plotted against test potentials. (C) Activation curves obtained by normalizing the IK tail current amplitudes. The solid curves are expressed as a function of membrane potential (E) as follows: normalized IK tail current $= 1/\{1 + 1/\exp[(E - V_{0.5})/k]\}$, where k is the slope factor. Solid square indicates the control condition; solid circle, exposure to 20 nmol/L ISO; and solid triangle, after addition of 20 ng/ml TNF-α. Symbols and bars represent mean ± SE.

isoproterenol augmented IK. During the measurement of IK, the chamber perfusate was continuously drained by suction, and the complete exchange of the perfusate could be achieved in 2 min. The effect on membrane currents of solvents, DMSO (0.1%) and ethanol (<0.1%), was shown to be negligible.

Numerical data were presented as means ± SEM and they were compared by Student's *t* test. A *P* value of less than 0.05 was considered to be significant.

Results and discussion

Effect of TNF-α on IK

TNF-α caused no substantial effect on basal IK ($n = 4$) but when IK was increased to 179 ± 34% by isoproterenol at 20 nmol/L ($n = 20$), TNF-α reduced IK to 62 ± 10% (Figs. 1A and B). IK tail currents normalized by those measured after the application of a +80 mV potential test well fitted with the Boltzmann equation (Fig. 1C). Membrane potentials for $V_{0.5}$ were 8.4 ± 1.3 mV in the control condition, 9.6 ± 2.1 mV in the presence of isoproterenol, and 9.8 ± 1.2 mV after the addition of TNF-α. The threshold potential for IK activation was not altered by TNF-α. The dose–response curve of TNF-α on IK in the presence of 20 nmol/L isoproterenol ($n = 22$) was similar to the Hill equation (Fig. 2). TNF-α cancelled IK augmented by isoproterenol in a dose-dependent manner with IC_{50} at 11.6 ± 0.7 ng/ml and a Hill coefficient of 1.1 ± 0.1 and the inhibitory effect of TNF-α was not reversed after washout.

Histamine (250 nmol/L) enhanced IK tail current to 170 ± 69% as shown in Fig. 3A which was reversed by TNF-α (20 ng/ml) to 37 ± 13% ($n = 4$). Similarly, forskolin at 500 nmol/L augmented IK (175 ± 4.7%, $n = 5$) to a comparable degree as that induced by isoproterenol and TNF-α (20 ng/ml) again reversed IK to 58 ± 13% (Fig. 3B). However, direct dialysis of myocytes with intrapipette cAMP (1 μmol/L) caused an increase of IK but its reduction by TNF-α was negligible 8 ± 1% ($n = 5$) (Fig. 3C). These results suggested that TNF-α inhibits IK by reducing intracellular c-AMP.

IK is composed of two components: IKr and IKs [24]. Though IKr is partly sensitive to isoproterenol sensitive [25], we confirmed the same inhibitory property on IK after

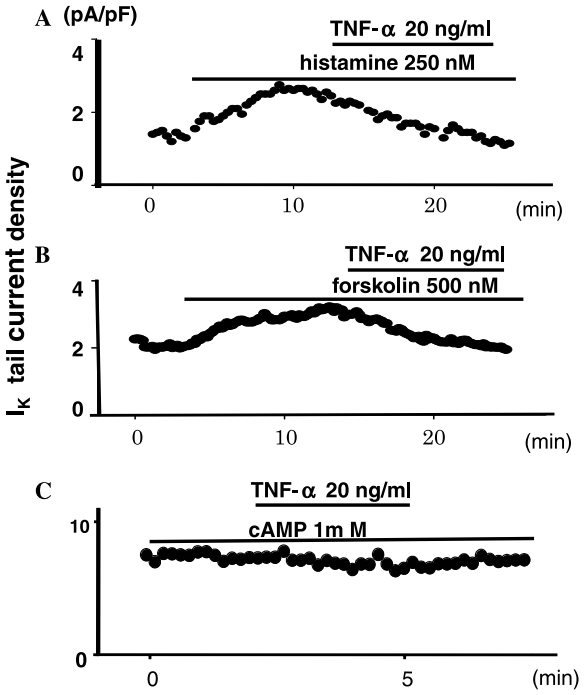

Fig. 3. The inhibitory effect of TNF-α on other types of PKA-enhanced IK. (A–C) Three time courses showing TNF-α (20 ng/ml) actions on the tail current of IK potentiated by histamine (250 nM/L), forskolin (500 nM/L), and intrapipette cAMP (1 mM/L).

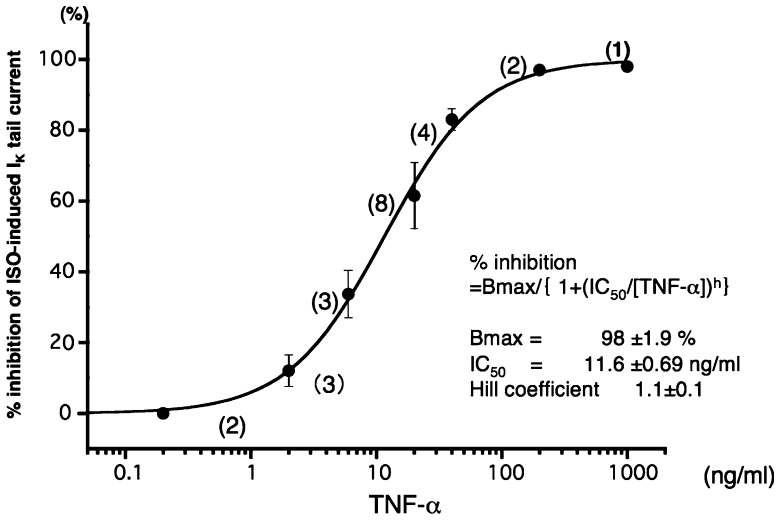

Fig. 2. The effects of TNF-α concentration–inhibition relationships for IK enhanced by isoproterenol. TNF-α dependent inhibitions accessed by normalizing amplitudes of IK tails after +40-mV test potential in the presence of various concentrations of TNF-α by that measured in the absence of the peptide and plotted as a function of the TNF-α concentration. Symbols represent the mean percent inhibition; the smooth line, the best fit to the Hill equation: % inhibition = $B_{max}/\{1 + (IC_{50}/[TNF\text{-}\alpha])^h\}$, where B_{max} indicates the maximal inhibition, and *h*, the Hill coefficient. Vertical bars represent SE. Numbers in parentheses indicate the number of experiments.

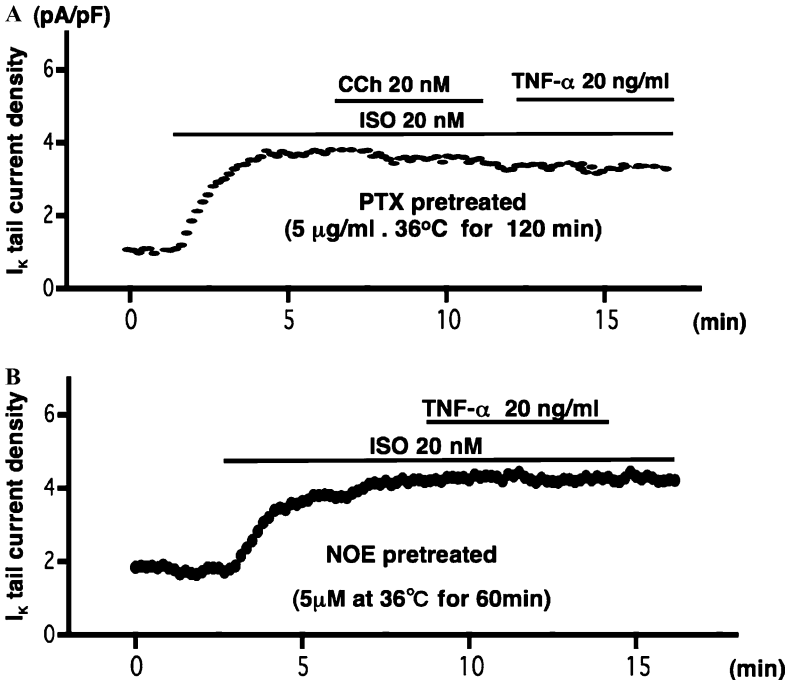

Fig. 4. The inhibitory effect of TNF-α is mediated by PTX-sensitive G-proteins and *N*-oleoylethanolamine. (A) Preincubation with PTX (5 μg/ml at 36 °C > 60 min) abolished the inhibitory effect of both CCh (20 nM) and TNF-α (20 ng/ml). (B) Preincubation with ceramidase inhibitor, *N*-oleoylethanolamine, also abolished the inhibitory effect of TNF-α (20 ng/ml).

the use of E-4031: an IKr inhibitor (up to 2 μmol/L) prior to the present study and the inhibitory effect of TNF-α on the isoproterenol activated IK would represent mainly that of IKs. TNF-α has no effect on basal IKs but reduced it when it is augmented by isoproterenol, histamine, or forskolin.

Effects of PTX and the ceramide–sphingosine phosphate pathway

Carbachol reduced intracellular c-AMP by stimulating the PTX-sensitive G-protein (Gi) and inhibited the isoproterenol-augmented IK but when the myocytes were preincubated with PTX, such action of carbachol was abolished and the addition of TNF-α showed no effect (Fig. 4A). Furthermore, when the myocytes were pre-incubated with *N*-oleoylethanolamine (at 5 μM), a ceramidase inhibitor, the inhibitory effect on IK by TNF-α was also abolished to 10 ± 4% (Fig. 4B).

Sphingosine-1-phosphate is considered to be generated from the ceramide-sphingomyelin by TNF-α and to activate Gi leading to a fall of c-AMP [26,27]. The fall of c-AMP must result in an immediate effect on contractile dysfunction [26,28–30] as well as on ionic currents [31–33] including IKs as shown in the present study.

In summary, TNF-α was shown to reverse IK when it is augmented by the PKA pathway. A fall of c-AMP would be involved as the mechanism of the reversal of IK by TNF-α since TNF-α induces a generation of sphingosine-1-phosphate from ceramide-sphingomyelin leading to the activation of Gi. However, because of diverse actions of TNF-α on several ionic currents, the net electrophysiological effects of TNF-α and their roles need to be further studied.

References

[1] J. Gurevitch, I. Frolkis, Y. Yuhas, Y. Paz, M. Matsa, R. Mohr, V. Yakirevich, Tumor necrosis factor-alpha is released from the isolated heart undergoing ischemia and reperfusion, J. Am. Coll. Cardiol. 28 (1996) 247–252.

[2] S. Hirono, M.O. Islam, M. Nakazawa, Y. Yoshida, M. Kodama, A. Shibata, T. Izumi, S. Imai, Expression of inducible nitric oxide synthase in rat experimental autoimmune myocarditis with special reference to changes in cardiac hemodynamics, Circ. Res. 80 (1997) 11–20.

[3] Y. Okura, T. Yamamoto, S. Goto, T. Inomata, S. Hirono, H. Hanawa, L. Feng, C.B. Wilson, I. Kihara, T. Izumi, A. Shibata, Y. Aizawa, S. Seki, T. Abo, Characterization of cytokine and iNOS mRNA expression in situ during the course of experimental autoimmune myocarditis in rats, J. Mol. Cell Cardiol. 29 (1997) 491–502.

[4] A. Henke, M. Nain, A. Stelzner, D. Gemsa, Induction of cytokine release from human monocytes by coxsackievirus infection, Eur. Heart J. 12 (Suppl. D) (1996) 134–136.

[5] A. Matsumori, T. Yamada, H. Suzuki, Y. Matoba, S. Sasayama, Increased circulating cytokines in patients with myocarditis and cardiomyopathy, Br. Heart J. 72 (1994) 561–566.

[6] M. Satoh, M. Nakamura, H. Saitoh, H. Satoh, C. Maesawa, I. Segawa, A. Tashiro, K. Hiramori, Tumor necrosis factor-alpha-converting enzyme and tumor necrosis factor-alpha in human dilated cardiomyopathy, Circulation 99 (1999) 3260–3265.

[7] T. Bachetti, L. Comini, L. Agnoletti, G. Gaia, B. Milanesi, S. Curello, A. Corti, R. Ferrari, O. Visioli, Activation and role of the tumor necrosis factor-alpha in congestive heart failure, Cardiologia 41 (1996) 343–347.

[8] T. Kubota, M. Miyagishima, R.J. Alvarez, R. Kormos, W.D. Rosenblum, A.J. Demetris, M.J. Semigran, G.W. Dec, R. Holubkov,

C.F. McTiernan, D.L. Mann, A.M. Feldman, D.M. McNamara, Expression of proinflammatory cytokines in the failing human heart: comparison of recent-onset and end-stage congestive heart failure, J. Heart Lung Transplant. 19 (2000) 819–824.
[9] G. Torre-Amione, S. Kapadia, C. Benedict, H. Oral, J.B. Young, D.L. Mann, Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD), J. Am. Coll. Cardiol. 27 (1996) 1201–1206.
[10] S.P. Zhao, T.D. Xu, Elevated tumor necrosis factor alpha of blood mononuclear cells in patients with congestive heart failure, Int. J. Cardiol. 71 (1999) 257–261.
[11] H.C. Hartzell, Regulation of cardiac ion channels by catecholamines, acetylcholine and second messenger systems, Prog. Biophys. Mol. Biol. 52 (1998) 165–247.
[12] J.I. Goldhaber, K.H. Kim, P.D. Natterson, T. Lawrence, P. Yang, J.N. Weiss, Effects of TNF-alpha on [Ca2+]i and contractility in isolated adult rabbit ventricular myocytes, Am. J. Physiol. 271 (1996) H1449–H1455.
[13] K.A. Krown, K. Yasui, M.J. Brooker, A.E. Dubin, C. Nguyen, G.L. Harris, P.M. McDonough, C.C. Glembotski, P.T. Palade, R.A. Sabbadini, TNF alpha receptor expression in rat cardiac myocytes: TNF alpha inhibition of L-type Ca2+ current and Ca2+ transients, FEBS Lett. 376 (1995) 24–30.
[14] K. Iino, H. Watanabe, T. Saito, S. Kibira, T. Iijima, M. Miura, TNF-alpha rapidly antagonizes the beta-adrenergic responses of the chloride current in guinea-pig ventricular myocytes, Circ. J. 67 (2003) 347–353.
[15] K.B. Walsh, R.S. Kass, Regulation of a heart potassium channel by protein kinase A and C, Science 242 (1988) 67–69.
[16] T.C. Hwang, M. Horie, A.C. Nairn, D.C. Gadsby, Role of GTP-binding proteins in the regulation of mammalian cardiac chloride conductance, J. Gen. Physiol. 99 (1992) 465–489.
[17] G. Isenberg, U. Klockner, Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium", Pflugers Arch. 395 (1982) 6–18.
[18] A. Fabiato, F. Fabiato, Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells, J. Physiol. (Paris) 75 (1979) 463–505.
[19] R.Y. Tsien, T.J. Rink, Neutral carrier ion-selective microelectrodes for measurement of intracellular free calcium, Biochim. Biophys. Acta 599 (1980) 623–638.
[20] H. Tanaka, T. Furukawa, M. Hayafuji, Y. Habuchi, Modulation of the delayed K+ current by histamine in guinea pig ventricular myocytes, Naunyn Schmiedebergs Arch. Pharmacol. 344 (1991) 582–588.
[21] K.B. Seamon, J.W. Daly, Forskolin: its biological and chemical properties, Adv. Cyclic Nucleotide Protein Phosphorylation Res. 20 (1986) 1–150.
[22] D.P. Rardon, A.J. Pappano, Carbachol inhibits electrophysiological effects of cyclic AMP in ventricular myocytes, Am. J. Physiol. 251 (1986) H601–H611.
[23] A.F. James, L.H. Xie, Y. Fujitani, S. Hayashi, M. Horie, ETa receptors mediate inhibition of the cardiac PKA-dependent Cl current via pertussis toxin sensitive mechanism, Nature 370 (1994) 297–300.
[24] M.C. Sanguinetti, N.K. Jurkiewicz, Two components of cardiac delayed rectifier K+ current: differential sensitivity of block by class III antiarrhythmic agents, J. Gen. Physiol. 96 (1990) 195–215.
[25] J. Cui, Y. Melman, E. Palma, G.I. Fishman, T.V. McDonald, Cyclic AMP regulates the HERG K(+) channel by dual pathways, Curr. Biol. 10 (2000) 671–674.
[26] H. Oral, G.W. Dorn 2nd, D.L. Mann, Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian cardiac myocyte, J. Biol. Chem. 272 (1997) 4836–4842.
[27] J. Kwiatkowska, Sphingomyelin pathway in signal transduction, Postepy Biochem. 40 (1994) 130–134.
[28] K.L. MacDonell, D.L. Severson, W.R. Giles, Depression of excitability by sphingosine 1-phosphate in rat ventricular myocytes, Am. J. Physiol. 275 (1998) H2291–H2299.
[29] C. Sharma, T. Smith, S. Li, G.J. Schroepfer Jr., D.H. Needleman, Inhibition of Ca2+ release channel (ryanodine receptor) activity by sphingolipid bases: mechanism of action, Chem. Phys. Lipids 104 (2000) 1–11.
[30] T. Yokoyama, L. Vaca, R.D. Rossen, W. Durante, P. Hazarika, D.L. Mann, Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart, J. Clin. Invest. 92 (1993) 2303–2312.
[31] M. Bunemann, B. Brandts, D.M. zu Heringdorf, C.J. van Koppen, K.H. Jakobs, L. Pott, Activation of muscarinic K+ current in guinea-pig atrial myocytes by sphingosine-1-phosphate, J. Physiol. 489 (1995) 701–777.
[32] X.Q. Li, M.G. Zhao, Q.B. Mei, Y.F. Zhang, W. Guo, H.F. Wang, D. Chen, Y. Cui, Effects of tumor necrosis factor-alpha on calcium movement in rat ventricular myocytes, Acta Pharmacol. Sin. 24 (2003) 1224–1230.
[33] K. Yasui, P. Palade, Sphingolipid actions on sodium and calcium currents of rat ventricular myocytes, Am. J. Physiol. 270 (1996) C645–C649.