Impairment of HERG K⁺ Channel Function by Tumor Necrosis Factor-α

ROLE OF REACTIVE OXYGEN SPECIES AS A MEDIATOR

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Congestive heart failure (CHF) is associated with susceptibility to lethal arrhythmias and typically increases levels of tumor necrosis factor-α (TNF-α) and its receptor, TNFR1. CHF down-regulates rapid delayed-rectifier K⁺ current (IₓKr) and delays cardiac repolarization. We studied the effects of TNF-α on cloned HERG K⁺ channel (human ether-a-go-go-related gene) in HEK293 cells and native IₓKr in canine cardiomyocytes with whole-cell patch clamp techniques. TNF-α consistently and reversibly decreased HERG current (IₓHERG). Effects of TNF-α were concentration-dependent, increased with longer incubation period, and occurred at clinically relevant concentrations. TNF-α had similar inhibitory effects on IₓKr and markedly prolonged action potential duration (APD) in canine cardiomyocytes. Immunoblotting analysis demonstrated that HERG protein level was slightly higher in canine hearts with tachypacing-induced CHF than in healthy hearts, and TNF-α slightly increased HERG protein level in CHF but not in healthy hearts. In cells pretreated with the inhibitory anti-TNFRI1 antibody, TNF-α lost its ability to suppress IₓHERG, indicating a requirement of TNFRI1 activation for HERG suppression. Vitamin E or MnTBAP (Mn(III) tetraakis(4-benzoic acid) porphyrin chloride), a superoxide dismutase mimetic, prevented, whereas the superoxide anion generated by xanthine/xanthine oxidase mimicked, TNF-α-induced IₓHERG depression. TNF-α caused robust increases in intracellular reactive oxygen species, and vitamin E and MnTBAP abolished the increases, in both HEK293 cells and canine ventricular myocytes. We conclude that the TNF-α/TNFRI1 system impairs HERG/IₓKr function mainly by stimulating reactive oxygen species, particularly superoxide anion, but not by altering HERG expression; the effect may contribute to APD prolongation by TNF-α and may be a novel mechanism for electrophysiological abnormalities and sudden death in CHF.

** Experimental Procedures

Cell Disposition—HEK293 cells stably expressing HERG were a kind gift from Drs. Zhou and January. Cell culture and handling procedures have been described previously (10). Cardiomyocytes were isolated from healthy adult mongrel dogs as described in detail previously (11, 12). The procedures for animal use were in accordance with institutional guidelines.

Whole-cell Patch Clamp Recording—Patch clamp techniques have been described in detail elsewhere (13–16). Experiments were conducted at 36 ± 1 °C. For current recordings in canine myocyte studies, the following were included in the bath to block contaminating currents: CdCl₂ (200 μmol/liter, L-type Ca²⁺ current), 4-aminopyridine (1 mmol/liter, transient outward K⁺ currents), glyburide (10 μmol/liter, ATP-sensitive K⁺ current), and 293B (10 μmol/liter, slow delayed-rectifier K⁺ current). Action potentials were recorded in the current clamp mode with Tyrode solution free of ion channel blockers. TNF-α was either added to the extracellular solution 10 min after formation of

1 The abbreviations used are: TNF-α, tumor necrosis factor-α; CHF, congestive heart failure; APD, action potential duration; EAD, early afterdepolarization; ROS, reactive oxygen species; CM-H2DFDA, 5-(and-6)-chloromethyl-2′,7′-dihydrodihydrofluorescein diacetate; VitE, vitamin E; MnTBAP, Mn(III) tetraakis(4-benzoic acid) porphyrin chloride; X/O, xanthine/xanthine oxidase.
whole-cell configuration (acute studies), or cells were incubated with TNF-α in the medium for 10 h before patch clamp recording (long term exposure).

Western Blot—The procedures were similar to those described previously (15). Polyclonal anti-HERG raised in rabbit against highly purified peptide (CY/EEL PAGAPELPQD GPT, corresponding to residues 1118–1133 of human HERG was purchased from Alomone Laboratories (Jerusalem, Israel).

Intracellular Reactive Oxygen Species (ROS) Measurement—5- and (6)-Chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) from Molecular Probes was used to detect oxidative activity in living cells as detailed previously (16).

RESULTS

$I_{\text{HERG}}$ was elicited by 2-s depolarizations followed by 2-s repolarizing steps (Fig. 1, *inset*). Currents were recorded immediately after formation of whole-cell configuration and series resistance compensation. Comparisons were made between control cells (without TNF-α) and cells incubated for 10 h with various TNF-α concentrations from 0.01 to 10 ng/ml, which are within the pathophysiological range of TNF-α levels (0.1 ng/ml) (17–19). $I_{\text{HERG}}$ density was reduced by TNF-α, with effects that were concentration- and voltage-dependent, being larger at more negative potentials (Fig. 1A). $I_{\text{HERG}}$ kinetics were unaltered by TNF-α.

Exposure to TNF-α for 15 min concentration-dependently decreased $I_{\text{HERG}}$. $I_{\text{HERG}}$ amplitude was decreased by 9, 16, and 35% by TNF-α at 1, 10, and 100 ng/ml, respectively. Results at 100 ng/ml are shown in Fig. 1B. Depression of $I_{K_0}$ by TNF-α was reproduced in both dog atrial and ventricular myocytes (Fig. 1C). APD_{30} and APD_{90}, duration at 50 and 90% repolarization, respectively, were both significantly longer in single ventricular cells preincubated with TNF-α at 10 ng/ml in Tyrode solution for 10 h relative to control cells (Fig. 1D).

Western blot analysis of HERG protein levels in the membrane preparations extracted from HERG-expressing HEK293 cells and from the ventricular myocytes of healthy dogs or dogs with tachypacing induced CHF was performed. A band of around 135 kDa was identified by anti-HERG antibody, and the band was abolished after the antibody had been neutralized by its antigenic peptide. TNF-α treatment neither significantly alter HERG protein level in HERG293 cells nor in healthy dogs. HERG protein level was slightly higher in CHF than in healthy dogs and was slightly increased by TNF-α in CHF dogs (Fig. 2A).

To clarify whether TNF-α acts on $I_{\text{HERG}}$ via activation of TNF receptor I (TNFR1), we incubated HEK293 cells with H389 (an inhibitory anti-TNFR1 antibody) for 1 h before patch clamp recording upon acute exposure to 100 ng/ml TNF-α or beginning 1 h before prolonged (10 h) exposure to 1 ng/ml TNF-α. H389 prevented suppression of $I_{\text{HERG}}$ by subsequent acute or prolonged application of TNF-α. Data from prolonged exposure experiments are shown in Fig. 2B.

Activation of TNFR1 can stimulate overproduction of intracellular ROS (20). To investigate whether ROS mediates TNF-α-induced HERG depression, we assessed the effects of TNF-α on $I_{\text{HERG}}$ in cells pretreated with the antioxidant vitamin E (VitE). Pretreatment with VitE for 2 h prevented $I_{\text{HERG}}$ reduction by TNF-α (Fig. 2C). Another antioxidant MnTBAP (Mn(III) tetrakis(4-benzoic acid) porphyrin chloride), a superoxide dismutase mimetic, produced similar preventative effects on TNF-α-induced HERG impairment (Fig. 2D). By contrast, preincubation of cells with superoxide anion generating system xanthine/xanthine oxidase (X/XO) mimicked the inhibitory effect of TNF-α on $I_{\text{HERG}}$ (Fig. 2E).

To confirm that intracellular ROS production was indeed stimulated by TNF-α, we detected ROS level using CM-H2DCFDA fluorescence dye to stain the cells. The cells stained with fluorescence intensity ≥5 times the background were defined as positive staining, and the number of cells with positive staining was pooled from five fields. The intensity of staining was analyzed by densitometric scanning using the LSM pro-
Fig. 2. Mechanisms for HERG depression by TNF-α. A, Western blot analysis of HERG protein levels. Membrane protein samples were extracted from left ventricular myocytes isolated from healthy dogs and dogs with ventricular tachypacing-induced CHF and from HEK293 cells. HEK293 cells were treated with TNF-α (10 ng/ml) for 10 h in culture medium, and isolated myocytes were treated with TNF-α in Tyrode solution for 10 h. Mean data were calculated from a total of four independent samples for each group. MK, protein marker. *, p < 0.05 versus control (Ctl), B, inhibitory anti-TNFRI antibody H398 prevents HERG depression by TNF-α. Cells were incubated with H398 (10 μg/ml) for 1 h before prolonged (10 h) exposure to TNF-α (10 ng/ml, n = 7). C and D, antioxidants VitE (100 μM, n = 14) or superoxide dismutase mimic MnTBAP (5 μM, n = 12) prevents HERG depression by TNF-α. *, p < 0.05 versus TNF-α alone. E, superoxide anion generating system X/XO (400 μM/units/ml, n = 16) mimicked the effects of TNF-α on I_{HERG}. F, alterations of the intracellular level of ROS by TNF-α (0.1 or 10 ng/ml) and antioxidants VitE (100 μM) and MnTBAP (5 μM), respectively, determined by CM-H2DCFDA fluorescence dye staining (green) in HERG-expressing HEK293 cells. The upper panels show examples of confocal microscopic images, and the lower panels show the percentage of cells with positive staining (% Cell) and the intensity of positive staining (n = 4 batches of cells for each group). *, p < 0.05 versus Ctl; +, p < 0.05 versus TNF-α alone. G, increase in ROS by TNF-α (0.1 ng/ml) and reversal by VitE (100 μM) or MnTBAP (5 μM) in canine ventricular myocytes. The upper panels show examples of confocal microscopic images, and the lower panels show the intensity of positive staining (n = 3). *, p < 0.05 versus Ctl; +, p < 0.05 versus TNF-α alone.

**DISCUSSION**

Heart failure is associated with APD and QT interval prolongation, believed to contribute to the occurrence of sudden cardiac death (6, 7). We show here that TNF-α suppresses I_{HERG} in HEK293 and I_{Kr} in dog cardiomyocytes and prolonged APD. Depression of I_{HERG}/I_{Kr}, as produced by TNF-α in this study, may contribute to delayed repolarization and associated malignant ventricular tachyarrhythmias with increased TNF-α level in patients with CHF.

Ionic remodeling in CHF has been studied (21). L-type Ca^{2+} current density appears to be unaltered (20). The inward-rectifier K⁺ current is consistently reduced (5). The transient outward K⁺ current (I_{to}) is also reduced, potentially causing APD prolongation (5, 22). However, inhibition of I_{to} reduces APD in human atrial cells (23), canine atrial cells (12), and dog Purkinje fibers (24). The effect of I_{to} on the AP depends largely on the magnitude of I_{Kr} (25). Tsuji et al. (26) showed I_{Kr}, measured as E-4031-sensitive tail current, to be ~36% smaller in rabbits with ventricular tachypacing-induced CHF than in healthy rabbits. Lodge and Normandin (27) demonstrated earlier that I_{Kr}, measured as dofetilide-sensitive tail current, reduced by ~45% in the BIO TO-2 strain of cardiomyopathic
hamster of 10 months old, derived from the BIO 53.58 animals and providing a model of dilated low output heart failure, compared with the 10-month-old control (BIO F1B) hamsters. A recent study by London et al. (9) showed significant APD prolongation in transgenic mice which overexpressed TNF-α and developed heart failure. Our study suggests that TNF-α may be an important mediator of CHF-induced J

We further demonstrated that pretreatment with VitE or MnTBAP prevented, whereas X/XO mimicked, TNF-α-induced I}_{HERG} depression. The effects of VitE and MnTBAP are likely due to their antioxidant actions because TNF-α increased the intracellular ROS level in a concentration-dependent manner in both HEK293 cells and canine ventricular myocytes, more specifically O$_2^-$ level because VitE or MnTBAP effectively prevented the increase. In line with our finding, a recent study published during the course of this study clearly demonstrated the ability of TNF-α to stimulate mitochondrial production of ROS in cardiomyocytes (20). It has also been shown that ROS impairment of HERG channels by TNF-α occurs at the functional level, but not at the expression levels (TNF-α did not alter HERG protein content), and the functional impairment of HERG channels by TNF-α is mediated by ROS, particularly O$_2^-$. Circulating TNF-α levels predict mortality in CHF, and therapies directed against TNF-α may limit the pathophysiological consequences (1). In healthy human subjects, the TNF-α level is below 0.01 ng/ml, but in patients with heart failure, it can increase to over 0.1 ng/ml (17–19). TNF-α significantly inhibited I}_{HERG} over this concentration range (e.g. by ~35% at plateau voltages from -10 to +10 mV in cells exposed to 0.1 ng/ml TNF-α for 10 h). Our study might have underestimated the effects of TNF-α on APD because the myocytes were incubated with TNF-α at 4 °C to maintain good quality of the cells. Our observations provide new insights into the potential molecular mechanisms underlying electrophysiological abnormalities and sudden arrhythmic death in patients with CHF.

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REFERENCES

1. McTiernan, C. F., and Feldman, A. M. (2000) Curr. Cardiol. Rep. 2, 189–197
2. Akhurst, P., Ueland, T., Lien, E., Bendtzen, K., Muller, F., Andreassen, A. K., Nordoy, I., Aasen, H., Espevik, T., Simonsen, S., Froland, S. S., and Gullesstad, I. (1999) Am. J. Cardio. 83, 376–382
3. Irwin, M. W., Mak, S., Mann, D. L., Qu, R., Penninger, J. M., Yan, A., Dawood, F., Wen, W. H., Zhou, Z., and Liu, P. (1999) Circulation 99, 1492–1498
4. Deswal, A., Petersen, N. J., Feldman, A. M., Young, J. B., White, B. G., and Mann, D. L. (2001) Circulation 103, 2055–2059
5. Beuckelmann, D. J., Nahauer, M., and Erdmann, E. (2002) Circ. Res. 73, 379–385
6. Marban, E. (2002) Nature 415, 213–218
7. Nuss, H. B., Kaab, S., Kass, D. A., Tomasselli, G. F., and Marban, E. (1999) Am. J. Physiol. 277, H580–H591
8. Priebe, L., and Beuckelmann, D. J. (1998) Circ. Res. 82, 1206–1223
9. London, B., Baker, L. C., Lee, J. S., Shusterman, V., Choi, B-B., Kubota, T., McTiernan, C. F., Feldman, A. M., and Slama, G. (2003) Am. J. Physiol. 284, H1431–H1441
10. Zhou, Z., Gong, Q., Ye, B., Fan, Z., Makielski, J. C., Robertson, G. A., and January, C. T. (1998) Biophys. J. 74, 230–241
11. Shi, H., Wang, H., and Wang, Z. (1999) Mol. Pharmacol. 55, 487–507
12. Yue, L., Feng, J., Li, G. R., and Nattel, S. (1996) Am. J. Physiol. 270, H2157–H2168
13. Wang, J., Wang, H., Han, H., Zhang, Y., Yang, B., Nattel, S., and Wang, Z. (1999) Circulation 100, 2649–2654
14. Zhang, Y., Wang, H., Wang, J., Han, H., Nattel, S., and Wang, Z. (2003) FEBS Lett. 534, 125–132
15. Wang, H., Zhang, Y., Cao, L., Han, H., Wang, J., Yang, B., Nattel, S., and Wang, Z. (2003) Cancer Res. 63, 4843–4848
16. Zhang, Y., Han, H., Wang, J., Wang, H., Yang, B., and Wang, Z. (2003) J. Biol. Chem. 278, 10417–10426
17. Anker, S. D., Volterrani, M., Egerer, K. R., Felton, C. V., Kox, W. J., Poole-Wilson, P. A., and Coats, A. J. (1998) Q. J. Med. 91, 199–203
18. Ferrari, R., Bachiotti, T., Confortini, R., Opaich, C., Febo, O., Curti, A., Cassani, G., and Visioli, O. (1995) Circulation 92, 1479–1486
19. Liu, L., and Zhao, S. P. (1999) Int. J. Cardiol. 69, 77–82
20. Suematsu, N., Tsutsui, H., Wen, J., Kang, D., Ikeuchi, M., Ide, T., Hayashidani, S., Shiomori, T., Kubota, T., Hamaaki, N., and Takeshita, A. (2003) Circulation 107, 1418–1425
21. Tomasselli, G. F., and Marban, E. (1999) Cardiovasc. Res. 42, 270–283
22. Nahauer, M., Beuckelmann, D. J., and Erdmann, E. (1999) Circ. Res. 73, 386–394
23. Escande, D., Coubolle, A., Faivre, J. F., Deroubaix, E., and Coraboeuf, E. (1997) Am. J. Physiol. 272, H1423–H1448
24. Lee, J. H., and Rosen, M. R. (1994) J. Cardiovasc. Electrophysiol. 5, 232–240
25. Nygren, A., Fiset, C., Firek, L., Clark, J. W., Lindblad, D. S., Clark, R. B., and Lodge, N. J. (1997) J. Mol. Cell. Cardiol. 29, 3211–3221
26. Lodge, N. J., and Normandin, D. E. (1997) Circ. Res. 80, 309–309
27. Lodge, N. J., and Normandin, D. E. (1997) J. Mol. Cell. Cardio. 29, 3211–3221
28. Choudhary, G., and Dudler, S. C., Jr. (2002) Congest. Heart Fail. 8, 145–155
29. Byrne, J. A., Grieve, D. J., Cave, A. C., Shah, A. M. (2003) Arch. Mal. Coeur. Vaiss. 96, 214–223
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