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Involvement of mitogen-activated protein kinases and reactive oxygen species in the inotropic action of ouabain on cardiac myocytes. A potential role for mitochondrial K_{ATP} channels

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Abstract

Binding of ouabain to Na^+/K^+-ATPase activated multiple signal transduction pathways including stimulation of Src, Ras, p42/44 MAPKs and production of reactive oxygen species (ROS) in rat cardiac myocytes. Inhibition of either Src or Ras ablated ouabain-induced increase in both $[Ca^{2+}]_i$ and contractility. While PD98059 abolished the effects of ouabain on $[Ca^{2+}]_i$, it only caused a partial inhibition of ouabain-induced increases in contractility. On the other hand, pre-incubation of myocytes with N-acetyl cysteine (NAC) reduced the effects of ouabain on contractility, but not $[Ca^{2+}]_i$. Furthermore, 5-hydroxydecanoate (5-HD) blocked ouabain-induced ROS production and partially inhibited ouabain-induced increases in contractility in cardiac myocytes. Pre-incubation of myocytes with both 5-HD and PD98059 completely blocked ouabain's effect on contractility. Finally, we found that opening of mitochondrial K_{ATP} channel by diazoxide increased intracellular ROS and significantly raised contractility in cardiac myocytes. These new findings indicate that ouabain regulates cardiac contractility via both $[Ca^{2+}]_i$ and ROS. While activation of MAPKs leads to increases in $[Ca^{2+}]_i$, opening of mitochondrial K_{ATP} channel relays the ouabain signal to increased ROS production in cardiac myocytes. (Mol Cell Biochem **242**: 181–187, 2003)

Key words: Na^+/K^+-ATPase, ouabain, contractility, $[Ca^{2+}]_i$, Ras/MAPK, mitochondrial K_{ATP} channel, reactive oxygen species

Introduction

Na^+/K^+-ATPase is an energy-transducing ion pump in most mammalian cells [1, 2]. It carries out the active transport of Na^+ and K^+ across the plasma membrane using the energy generated from hydrolysis of ATP. In the heart, this enzyme also serves as a functional receptor for digitalis compounds such as digoxin and ouabain [3–6]. Binding of ouabain to cardiac Na^+/K^+-ATPase inhibits the ion pumping function of the enzyme and increases myocyte contractility in the heart. This effect on cardiac contractility serves as the basis for the therapeutic use of digitalis drugs in the management of congestive heart failure [3–6].

In recent years we have demonstrated that binding of ouabain to the Na^+/K^+-ATPase can also convert the enzyme into a signal transducer [7–14]. It appears that ouabain promotes the interaction of the Na^+/K^+-ATPase with Src, resulting in activation of the kinase. The activated Src in turn binds to and transactivates the epidermal growth factor receptor (EGFR), leading to recruitment of adaptor protein Shc and subsequent stimulation of Ras [12, 13]. Downstream from Ras ouabain stimulates p42/44 mitogen-activated protein kinases (MAPKs) and increases mitochondrial production of reactive oxygen species (ROS) [10, 11]. Interestingly, activation of some of these signal transduction pathways by ouabain is independent of ouabain-induced changes in intracellular ion concentrations

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as well as in contractility of cardiac myocytes [13]. Significantly, we have recently shown that the classic effects of ouabain on intracellular calcium ($[Ca^{2+}]_i$) also depend on the signal transducing function of the Na^+/K^+-ATPase [14]. Inhibition of either protein tyrosine kinases or Ras or p42/44 MAPKs, but not ROS production diminishes ouabain-induced increases in $[Ca^{2+}]_i$. These findings led us to extend the above investigation and test the role of the signal transducing function of the Na^+/K^+-ATPase in ouabain-induced regulation of contractility in cardiac myocytes. We report here that the effects of ouabain on cardiac contraction not only depend on activation of MAPKs and the subsequent increase in $[Ca^{2+}]_i$, but also require opening of mitochondrial K_{ATP} channels ($mitoK_{ATP}$), which causes an increase in intracellular ROS.

Materials and methods

Materials

Collagenase Type II was from Worthington (Freehold, NJ, USA). Indo-1-AM and CM-DCFH diacetate were obtained from Molecular Probes (Eugene, OR, USA). Diazoxide and 5-HD were from Sigma (Saint Louis, MO, USA). PP2 was purchased from Calbiochem (San Diego, CA, USA).

Cell preparation and culture

The same protocols were used to prepare Ca^{2+}-tolerant adult rat ventricular myocytes as described in our previous work [14, 15]. In brief, Sprague–Dawley rats weighing between 250–300 g were anesthetized with sodium pentobarbital (60 mg/kg i.p.). The hearts were rapidly removed, attached to an aortic cannula, and retrograde perfused for 15 min with Joklik medium to wash out the blood, followed by 5 min perfusion with a nominally Ca^{2+}-free Joklik medium supplemented with 20 mM creatine and 60 mM taurine. The heart was then perfused with collagenase type II until the heart became soft and flaccid. Myocytes were dissociated from the left ventricle, harvested, and plated onto laminin-coated coverslips as previously described [14]. Medium was changed 2 h post plating. Over 95% of myocytes were quiescent, and they were used for the experiments after an overnight culture.

Fluorescence microscopic measurements of $[Ca^{2+}]_i$, contractility and ROS

Myocytes cultured on coverslips were perfused and paced at 0.5 Hz. $[Ca^{2+}]_i$ was measured by indo-1 as previously described [14]. Myocytes were loaded with 10 μM indo-1-AM for 30 min. Indo-1 fluorescence was recorded using a microscope-based fluorescence system (Photon Technology International, Monmouth Junction, NJ, USA). The probe was excited at 365 nm, and fluorescence emitted at 405 and 485 nm was recorded at 60 Hz in real time. $[Ca^{2+}]_i$ was calculated based on the fluorescence ratio and the Ca^{2+}calibration curve [14]. Myocyte contractility was measured as cell shortening using an edge detector as previously described [16]. Under each experimental condition signals were obtained from about 12 single cells from 3–5 different preparations. Intracellular ROS concentration was measured in cells loaded with 10 μM CM-DCFH diacetate as previously described [11]. Under each experimental condition about 15 single myocytes were imaged with an Attofluor imaging system, and CM-DCF fluorescence was measured at an excitation wavelength of 480 nm and an emission wavelength of 520 nm.

Preparation of replication-defective adenoviruses and adenovirus infection of cardiac myocytes

Replication-defective adenoviruses expressing a dominant negative Asn^{17} Ras were generated, amplified, purified, and used for the infection of myocytes as described before [10]. An identical virus containing the β-galactosidase gene (β-Gal), instead of the Asn^{17} Ras, was used as the control [10].

Analysis of data

Data are given as the mean ± S.E. Statistical analysis was performed using the Student's *t*-test, and significance was accepted at $p < 0.05$. Each presented immunoblot is representative of the similar results from at least three separate experiments.

Results

Ouabain regulation of cardiac contractility requires activation of Src, Ras, and MAPKs

We showed previously that binding of ouabain to the Na^+/K^+-ATPase activated Src, resulting in transactivation of the EGFR and subsequent stimulation of the Ras/MAPK cascade in cardiac myocytes [12, 14]. Inhibition of either Src or Ras or MAPKs blocked ouabain-induced increases in $[Ca^{2+}]_i$ [14]. Because increases in $[Ca^{2+}]_i$ are essential for ouabain-induced rise in contractility [17, 18], we postulated that the effects of ouabain on myocyte contractility must also be mediated via the above pathways. As depicted in Fig. 1, ouabain, at non-

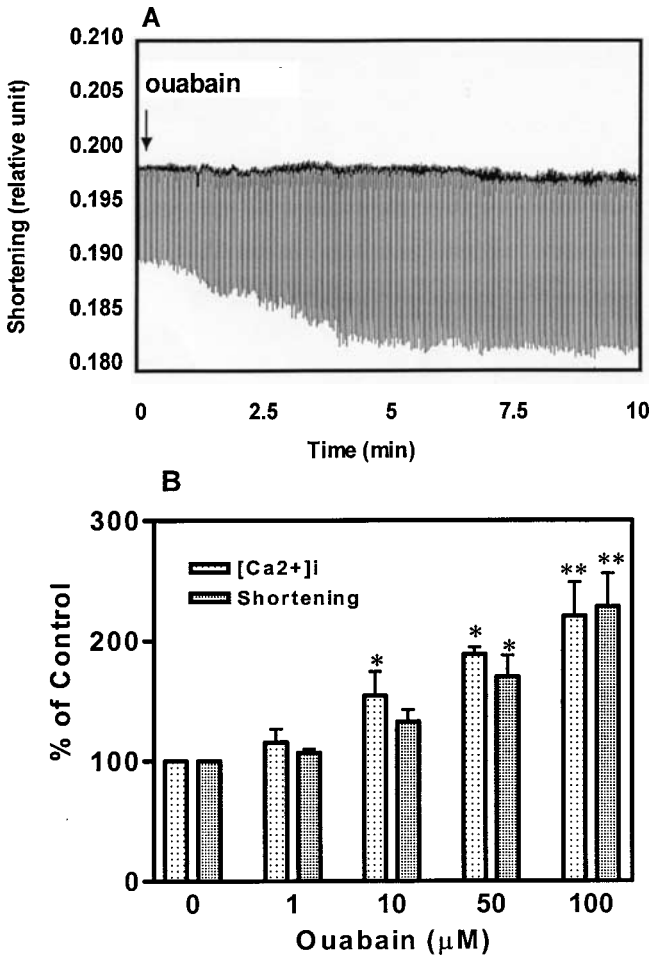

Fig. 1. Effects of ouabain on contractility and $[Ca^{2+}]_i$ in adult rat cardiac myocytes. To measure myocyte contractility and $[Ca^{2+}]_i$, cells were perfused and paced at 0.5 Hz as described under Materials and methods. Cell contractility was measured as cell shortening using an edge detector, and $[Ca^{2+}]_i$ was determined based on the indo-1 fluorescence ratio of 405 and 485 nm. Panel A shows a representative trace of contractility in a single cell. Ouabain (100 μM) was added to the medium at the time as indicated by the arrow. Panel B shows that ouabain increases both contractility and $[Ca^{2+}]_i$ in a dose dependent manner. Values are presented as mean ± S.E. of 15 single cells from 4 different experiments. *p < 0.05 and **p < 0.01 vs. control.

toxic concentrations, increased contractility in cultured adult rat cardiac myocytes in a time- and dose-dependent manner. The effects of ouabain on contractility correlated well with the rise in $[Ca^{2+}]_i$. As expected, inhibition of Src by PP2 completely blocked the effects of ouabain on contractility (Fig. 2). In addition, in cells expressing dominant negative mutant of Asn^{17} Ras, ouabain also failed to stimulate myocyte contraction (Fig. 2). These data are consistent with the findings that inhibition of either Src or Ras blocks ouabain-induced increases in $[Ca^{2+}]_i$ [14]. Surprisingly, while pre-incubation of myocytes with PD 98059 to inhibit MAPK completely

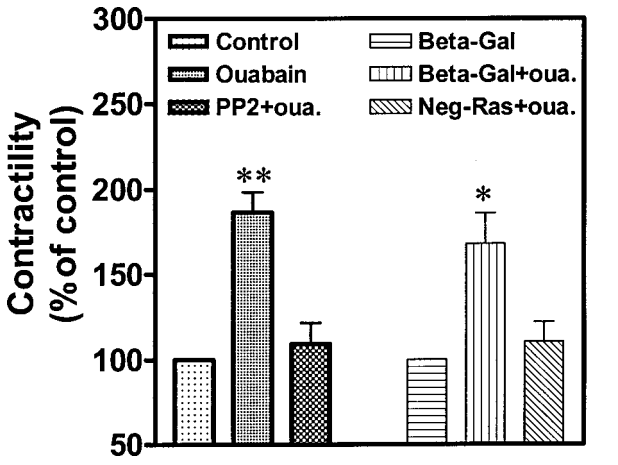

Fig. 2. Effects of PP2 and dominant negative Ras on ouabain-induced increase in contractility. Myocytes were pre-incubated with 1 μM PP2 for 20 min or transduced with adenoviruses expressing a dominant negative Ras for 12 h. (Cells transduced with the same amount of β-gal viruses were used as viral control.) Both treated and control myocytes were then exposed to 100 μM ouabain for 10 min and contractility was measured as in Fig. 1. Values are presented as mean ± S.E. of 10 single cells. *p < 0.05 and **p < 0.01 vs. control.

abolished ouabain-induced increases in $[Ca^{2+}]_i$, it only caused a partial inhibition of ouabain-induced rise in contractility (Fig. 3). These findings indicate that factors other than increases in $[Ca^{2+}]_i$ also contribute to ouabain regulation of cardiac contractility.

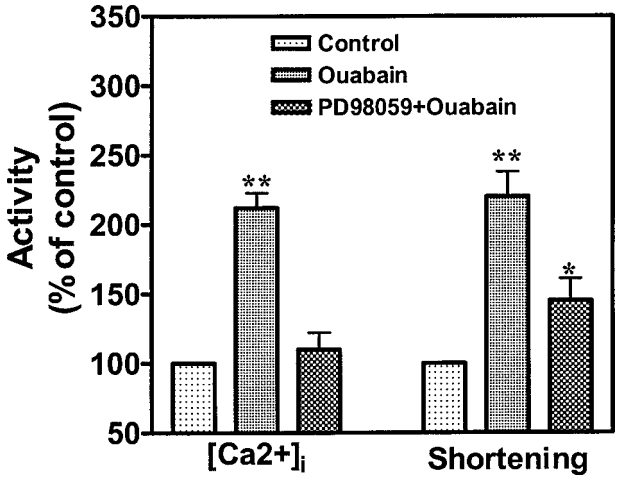

Fig. 3. Effects of PD 98059 on ouabain-induced increases in contractility and $[Ca^{2+}]_i$. Myocytes were pre-incubated with 30 μM PD98059 for 30 min, and then exposed to 100 μM ouabain for 10 min. Contractility and $[Ca^{2+}]_i$ were measured as in Fig. 1. Values are presented as mean ± S.E. of 15 single cells from 4 different experiments. *p < 0.05 and **p < 0.01 vs. control.

Involvement of ROS in ouabain-induced increases in contractility

Since activation of Ras by ouabain also increased mitochondrial production of ROS [13, 14], the above findings led us to examine whether ROS are involved in ouabain-induced regulation of cardiac contractility. As depicted in Fig. 4, ouabain increased ROS production in cardiac myocytes. Pre-incubation of myocytes with 10 mM NAC abolished ouabain-induced rise in intracellular ROS (Fig. 4) as previously noted in neonatal cardiac myocytes. Interestingly, NAC also caused a significant inhibition of ouabain-induced increases in contractility.

Involvement of mitoK$_{ATP}$ in the effects of ouabain on contractility

MitoK$_{ATP}$ opening plays a pivotal role in cardioprotection by K$_{ATP}$ channel openers and ischemic preconditioning [19–22]. In particular, mitoK$_{ATP}$ opening is required to trigger cardioprotective signaling pathways [23], and we have proposed that this function is mediated by inducing mitochondrial ROS production [24]. Accordingly, we assessed the role of inhibition of mitoK$_{ATP}$ in ouabain-induced ROS production and rise in contractility. As depicted in Fig. 5, pre-incubation of myocytes with 200 μM 5-HD inhibited the effects of ouabain on intracellular ROS. Interestingly, 5-HD also suppressed ouabain-induced increases in contractility under the same experimental conditions (Fig. 5). These findings support a proposal that opening of mitoK$_{ATP}$ and subsequent rise in ROS production are involved in ouabain-induced regulation of cardiac contractility. To gain additional support that mitoK$_{ATP}$ is involved in regulation of cardiac contraction, myocytes were exposed to diazoxide, a specific mitoK$_{ATP}$ agonist [22], and monitored for changes in intracellular ROS and contractility. As depicted in Fig. 6, diazoxide increased both intracellular ROS and contractility in cardiac myocytes. Interestingly, pre-incubation of myocytes with either 10 mM NAC or 200 μM 5-HD caused a complete inhibition of diazoxide-induced increases in both ROS and myocyte con-

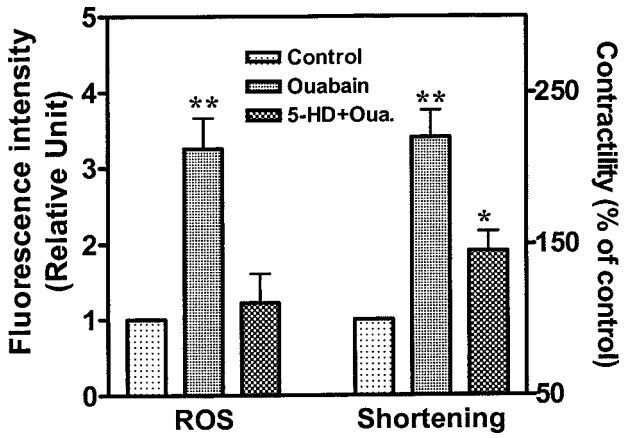

Fig. 5. Effects of 5-HD on oubain-induced increases in intracellular ROS and myocyte contractility. Myocytes were preincubated with 200 μM 5-HD for 30 min, and then exposed to 100 μM ouabain. Intracellular ROS and contractility was measured as in Fig. 4. Values are presented as mean ± S.E. of 10–15 single cells. *p < 0.05, **p < 0.01 vs. control

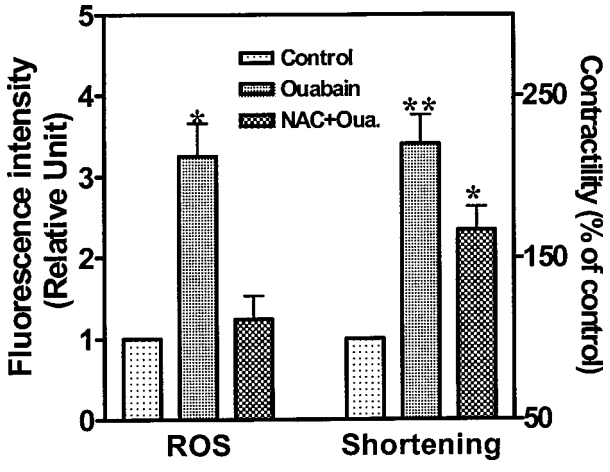

Fig. 4. Effects of NAC on ouabain-induced increases in intracellular ROS and contractility in adult rat cardiac myocytes. Myocytes were loaded with 5- (and 6-)chloromethyl-2′,7′-dichlorofulorescin diacetate, and treated with 100 μM ouabain for 10 min in the presence or absence of 10 mM NAC. Both ROS and myocytes contractility were measured as described under Materials and methods. Values are mean ± S.E. of 12 cells from 4 independent experiments. *p < 0.05, **p < 0.01 vs. control.

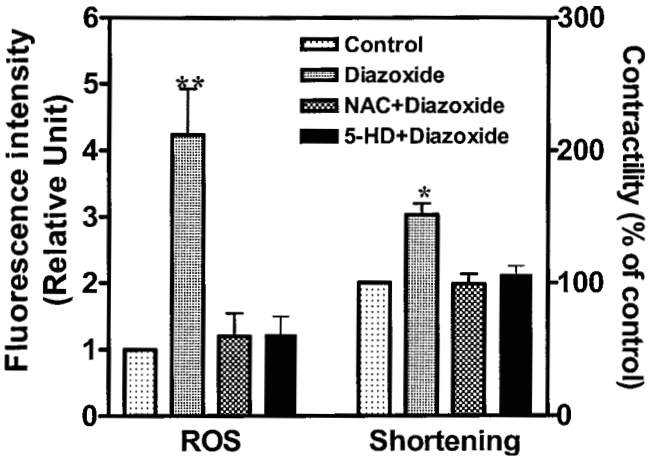

Fig. 6. Effects of diazoxide on intracellular ROS and myocytes contractility. Myocytes were treated with 10 μM diazoxide in the presence or absence of either 10 mM NAC or 200 μM 5-HD. Intracellular ROS and contractility were then measured as in Fig. 4. Values are presented as mean ± S.E. *p < 0.05, **p < 0.01 vs. control.

traction (Fig. 6). Since activation of MAPKs and opening of mitoK$_{ATP}$ are required for ouabain-induced increases in $[Ca^{2+}]_i$ and ROS, respectively, we reasoned that inhibition of these two pathways should completely abolish the effects of ouabain on myocyte contraction. Indeed, as shown in Fig. 7, pre-incubation of myocytes with both PD98059 and 5-HD blocked the ouabain-induced increases in contractility.

Discussion

It has been known for long time that cardiac glycosides including ouabain increase $[Ca^{2+}]_i$ and contractility in cardiac myocytes by binding to the Na^+/K^+-ATPase [3–6]. In rat cardiac myocytes we showed that 10–100 μM ouabain caused about 20–50% inhibition of Na^+/K^+-ATPase in a dose-dependent manner [8, 15]. It is important to note that under our experimental conditions, ouabain at concentrations up to 100 μM did not cause arrhythmic contraction and Ca^{2+} overload in 15 min. It also had no effect on diastolic cell length. On the other hand, these non-toxic concentrations of ouabain caused a rapid stimulation of multiple signal transduction pathways including activation of Ras, p42/44 MAPKs and mitochondrial production of ROS (Fig. 4 and [14]). Concomitantly, it also raised $[Ca^{2+}]_i$ and contractility in a time- and dose-dependent manner in these cells. When the relation between the signal transducing function of the enzyme and the pharmacological effects of ouabain on $[Ca^{2+}]_i$ and contractility

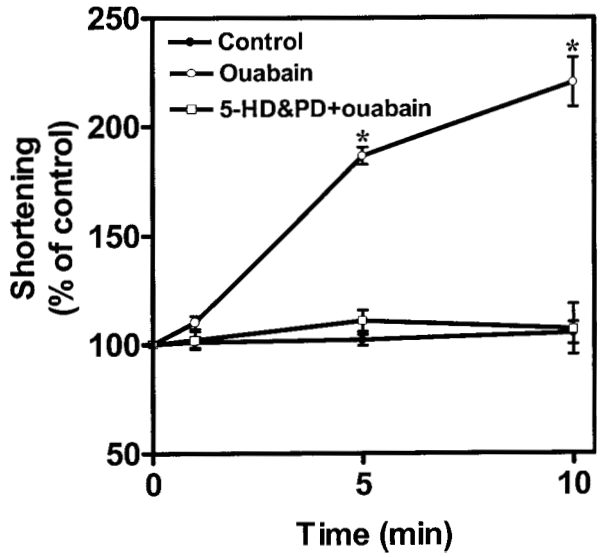

Fig. 7. Combination of PD98059 and 5-HD abolished ouabain-induced increases in contractility. Myocytes were simultaneously pre-incubated with 200 μM 5-HD and 30 μM PD98059 for 30 min, then exposed to 100 μM ouabain for 5 or 10 min and assayed for contractility as in Fig. 1. Values are mean ± S.E. of 10 measurements from 3 different preparations. *p < 0.05, **p < 0.01 vs. control.

were determined (Figs 1–5), we showed that inhibition of either Src by PP2 or Ras by expression of Asn^{17} Ras ablated ouabain-induced increases in both $[Ca^{2+}]_i$ and contractility. Clearly, the factors that relay extracellular ouabain to increases in $[Ca^{2+}]_i$ and contractility must be the effectors of Ras.

It is well established that $[Ca^{2+}]_i$ is the central regulator of cardiac contractility [17, 18]. Since ouabain raised $[Ca^{2+}]_i$ via a MAPK-dependent pathway, we postulated that inhibition of MAPKs by PD98059 should abolish the effects of ouabain on both $[Ca^{2+}]_i$ and contractility in cardiac myocytes. Surprisingly, while pre-incubation of myocytes with PD 98059 completely blocked ouabain-induced rise in $[Ca^{2+}]_i$, it only caused a partial inhibition of the effects of ouabain on contractility. These findings led us to propose that factors other than MAPKs and $[Ca^{2+}]_i$ are also involved in ouabain-induced increases in contractility. Because inhibition of Ras completely blocked the effects of ouabain on contractility, we reasoned that the additional regulatory element(s) must be the other effector(s) of Ras. We showed previously that downstream from Ras ouabain also increased mitochondrial production of ROS in cardiac myocytes [13, 14]. Early studies of others also indicated that antioxidant α-tocopherol reduced the positive inotropic action of digitalis on atria muscle [25]. Therefore, we postulated that ROS might work in concert with $[Ca^{2+}]_i$ in regulation of contractility in response to ouabain. This notion was supported by the experiments shown in Fig. 4, in which pre-incubation of myocytes with NAC caused a significant inhibition of the effects of ouabain on contractility. Since NAC showed no effect on ouabain-induced changes in Na/K-ATPase activity (data not shown), $[Ca^{2+}]_i$ [14], and c-fos expression [11], its effect on ouabain-induced increases in contractility strongly suggest an involvement of ROS in ouabain regulation of cardiac contraction.

We showed previously that ouabain stimulated mitochondrial production of ROS via a Ras-dependent pathway [13]. We noted that the ouabain signaling pathway [7–14] and the cardioprotective signaling pathway [26] contain many elements in common, including mitochondrial ROS production. Moreover, cardioprotection by diazoxide [19] has been proposed to begin with mitoK$_{ATP}$-dependent stimulation of mitochondrial ROS production [23, 24]. Therefore, we reasoned that opening of mitoK$_{ATP}$ might relay the signal from Ras to ROS production. This hypothesis is supported by the findings shown in Fig. 5 in which inhibition of mitoK$_{ATP}$ by 5-HD blocked ouabain-induced increases in intracellular ROS. Furthermore, 5-HD also caused a significant inhibition of ouabain-induced rise in contractility, and pre-incubation of myocytes with both PD98059 and 5-HD completely abolished the effects of ouabain on contractility in cardiac myocytes. Interestingly, opening mitoK$_{ATP}$ by incubation of myocytes with diazoxide was sufficient to raise intracellular ROS concentration and increase contractility in myocytes. These effects were

blocked by either 5-HD or NAC (Fig. 6). Clearly, a modest increase in intracellular ROS via opening of mitoK$_{ATP}$ by either ouabain or diazoxide can cause a significant rise in contractility in cardiac myocytes.

Although the mechanism by which ROS regulate cardiac contractility remains to be resolved, it is appropriate to consider the following two alternatives. First, the effects of ROS may be mediated through sensitizing myofilament to $[Ca^{2+}]_i$ by its direct action on contractile proteins or regulation of protein phosphorylation via activation of protein kinases such as PKC [27, 28]. Interestingly, activation of PKC was also found to be responsible for endothelin-1-induced increases in myofilament Ca^{2+} sensitivity [29]. In addition, early studies had suggested a role of PKC in ouabain-induced inotropy in the heart [30, 31], and we have recently demonstrated that ouabain activates multiple isozymes of PKC in cardiac myocytes under our experimental conditions [32]. Because there is evidence that increases in ROS are sufficient to activate PKC [33], it is quite possible that ROS may exert its effect on myocyte contraction by increasing myofilament Ca^{2+} sensitivity through activation of PKC. Alternatively, since mitoK$_{ATP}$ plays an important role in regulation of mitochondrial energy metabolism [21], it is also possible that opening of mitoK$_{ATP}$ and the subsequent rise in ROS production may prime mitochondria into a higher working state so that ATP can be delivered more efficiently to the myofibrils for contraction. Interestingly, early studies did suggest that digitalis affect mitochondrial energy metabolism [34, 35].

In summary, we demonstrated here that ouabain regulates cardiac contractility via activation of at least two pathways. Activation of p42/44 MAPKs and inhibition of the ion pumping function of the Na^+/K^+-ATPase by ouabain increased $[Ca^{2+}]_i$ whereas opening of mitoK$_{ATP}$ stimulated the production of ROS. Both $[Ca^{2+}]_i$ and ROS, in turn, worked in concert, resulting in increases in contractility in cardiac myocytes.

Acknowledgements

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References

1. Skou JC: The Na,K-pump. Meth Enzymol 156: 1–29, 1988
2. Lingrel JB, Kuntzweiler T: Na^+,K^+-ATPase. J Biol Chem 269: 19659–19662, 1994
3. Akera T, Ng YC: Digitalis sensitivity of Na^+,K^+-ATPase, myocytes and the heart. Life Sci 48: 97–106, 1991
4. Schwartz A, Grupp G, Wallick E, Grupp IL, Ball WJ Jr: Role of the Na^+K^+-ATPase in the cardiotonic action of cardiac glycosides. Prog Clin Biol Res 268B: 321–338, 1988
5. Eisner DA, Smith TW: The Heart and Cardiovascular System. In: H.A. Fozzard, E. Haber, R.B. Jennigs, A.M. Katz, H.E. Morgan (eds). Raven Press, New York, 1992, pp 863–902
6. Barry WH, Hasin Y, Smith TW: Sodium pump inhibition, enhanced calcium influx via sodium–calcium exchange, and positive inotropic response in cultured heart cells. Circ Res 56: 231–241, 1985
7. Xie Z: Ouabain interaction with cardiac Na/K-ATPase reveals that the enzyme can act as a pump and a signal transducer. Cell Mol Biol 47: 383–390, 2001
8. Peng M, Huang L, Xie Z, Huang W-H, Askari A: Partial inhibition of Na^+/K^+-ATPase by ouabain induces the Ca^{2+}-dependent expressions of early-response genes in cardiac myocytes. J Biol Chem 271: 10372–10378, 1996
9. Huang L, Li H, Xie Z: Ouabain-induced hypertrophy in cultured cardiac myocytes is accompanied by changes in expressions of several late response genes. J Mol Cell Cardiol 29: 429–437, 1997
10. Kometiani P, Li J, Gnudi L, Kahn BB, Askari A, Xie Z: Multiple signal transduction pathways link Na^+/K^+-ATPase to growth-related genes in cardiac myocytes: The roles of ras and mitogen-activated protein kinases. J Biol Chem 273: 15249–15256, 1998
11. Xie Z, Kometiani P, Liu J, Li J, Shapiro JI, Askari A: Intracellular reactive oxygen species mediate the linkage of Na^+/K^+-ATPase to hypertrophy and its marker genes in cardiac myocytes. J Biol Chem 274: 19323–19328, 1999
12. Haas M, Askari A, Xie Z: Involvement of Src and epidermal growth factor receptor in the signal transducing function of Na^+/K^+-ATPase. J Biol Chem 275: 27832–27837, 2000
13. Liu J, Tian J, Haas M, Shapiro JI, Askari A, Xie Z: Ouabain interaction with cardiac Na^+/K^+-ATPase initiates signal cascades independent of changes in intracellular Na^+ and Ca^{2+} concentrations. J Biol Chem 275: 27838–27844, 2000
14. Tian J, Gong X, Xie Z: Signal-transducing function of Na^+/K^+-ATPase is essential for ouabain's effect on $[Ca^{2+}]_i$ in rat cardiac myocytes. Am J Physiol 281: H1899–H1907, 2001
15. Xie Z, Wang Y, Askari A, Huang W-H, Klaunig JE, Askari A: Studies on the specificity of the oxygen free radical effects on cardiac sodium pump. J Cell Mol Cardiol 22: 911–920, 1990
16. Bassani RA, Bassani JWM, Bers DM: Mitochondrial and sarcolemmal Ca^{2+} transport reduce $[Ca^{2+}]_i$ during caffeine contractures in rabbit cardiac myocytes. J Physiol (Lond) 453: 591–608, 1992
17. Bers DM: Calcium fluxes involved in control of cardiac myocyte contraction. Circ Res 87: 275–281, 2000
18. Satoh H, Ginsburg KS, Qing K, Terada H, Hayashi H, Bers DM: KB-R7943 block of Ca(2+) influx via Na(+)/Ca(2+) exchange does not alter twitches or glycoside inotropy but prevents Ca(2+) overload in rat ventricular myocytes. Circulation 101: 1441–1446, 2000
19. Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, Grover GJ: Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels: Possible mechanism of cardioprotection. Circ Res 81: 1072–1082, 1997
20. Grover GJ, Garlid KD: ATP-sensitive potassium channels: A review of their pharmacology. J Mol Cell Cardiol 32: 677–695, 2000
21. Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD: Bioenergetic consequences of opening the ATP-sensitive K(+) channel of heart mitochondria. Am J Physiol Heart Circ Physiol 280: H649–657, 2001
22. Garlid KD, Paucek P, Yarov-Yarovoy V, Sun X, Schindler PA: The mitochondrial K_{ATP} channel as a receptor for potassium channel openers. J Biol Chem 271: 8796–8799, 1996
23. Pain T, Yang XM, Critz SD, Yue Y, Nakano A, Liu GS, Heusch G, Cohen MV, Downey JM: Opening of mitochondrial K(ATP) channels

triggers the preconditioned state by generating free radicals. Circ Res 87: 460–466, 2000
24. Garlid KD: Opening mitochondrial K(ATP) in the heart – what happens, and what does not happen. Basic Res Cardiol 95: 275–279, 2000
25. Tawfik H, Schlieper P: The influence of a-tocopherol-nicotinate (Renascin) on cardiac glycoside effects. In: E. Erdmann, K. Greff, J.C. Skou (eds). Cardiac Glycosides 1785–1985. 1986, pp 109–117
26. Baines CP, Cohen MV, Downey JM: Signal transduction in ischemic preconditioning: The role of kinases and mitochondrial K(ATP) channels. J Cardiovasc Electrophysiol 10: 741–754, 1999
27. Steinberg SF, Goldberg M, Rybin VO: Protein kinase C isoform diversity in the heart. J Mol Cell Cardiol 27: 141–153, 1995
28. Baudet S, Weisser J, Janssen AP, Beulich K, Bieligk U, Pieske B, Noireaud J, Janssen PML, Hasenfuss G, Prestle J: Increased basal contractility of cardiomyocytes overexpressing protein kinase C and blunted positive inotropic response to endothelin-1. Cardiovasc Res 50: 486–494, 2001
29. Wang H; Endoh M: Chelerythrine and genistein inhibit the endothelin-1-induced increase in myofilament Ca^{2+} sensitivity in rabbit ventricular myocytes. Eur J Pharmacol 424: 91–96, 2001
30. Gotoh H, Kamiyama A, Shibayama R, Sawada M, Kashimoto T: Involvement of phosphoinositide turnover in ouabain inotropism. Biochem Biophys Res Commun 194: 72–78, 1993
31. Otani B, Otani H, Morita M, Das DK: Mol Cell Biochem 90: 111–120, 1989
32. Mohammadi K, Kometiani P, Xie Z, Askari A: Role of protein kinase C in the signal pathways that link Na^+/K^+-ATPase to ERK1/2. J Biol Chem 276: 42050–42056, 2001
33. Konishi H, Tanaka M, Takemura Y, Matsuzaki H, Ono Y, Kikkawa U, Nishizuka Y: Activation of protein kinase C by tyrosine phosphorylation in response to H_2O_2. Proc Natl Acad Sci USA 94: 11233–11237, 1997
34. Lee KS; Klaus W: The subcellular basis for the mechanism of inotropic action of cardiac glycosides. Pharmacol Rev 23: 193–229, 1971
35. Wollenberger A: The energy metabolism of the failing heart and the metabolic action of the cardiac glycosides. Pharmacol Rev 1: 311–344, 1949