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Protective effects of benidipine on hydrogen peroxide-induced injury in rat isolated hearts

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Abstract

We investigated the effects of benidipine (hydrochloride), a calcium antagonist, on hydrogen peroxide (H_2O_2)-induced injury in Langendorff-perfused rat hearts. The hearts were aerobically perfused at a constant flow and exposed to H_2O_2 (600 μmol L^{-1}) for 4 min, resulting in the oxidative stress-induced myocardial dysfunction (e.g., decrease in the left ventricular developed pressure) and myocardial cell injury (e.g., increase in the release of lactate dehydrogenase). Pretreatment of the hearts with benidipine or nifedipine was performed for 20 min until the start of H_2O_2 exposure. Benidipine at 1 nmol L^{-1} and nifedipine at 10 nmol L^{-1} decreased the myocardial contractility and perfusion pressure to a similar degree in the hearts under normal conditions. Benidipine (1 nmol L^{-1}) significantly reduced the H_2O_2-induced myocardial damage. Nifedipine (10 nmol L^{-1}) also tended to exhibit similar effects. Benidipine inhibited the increase in tissue lipid peroxidation induced by H_2O_2. The results suggest that, in addition to the calcium antagonism, benidipine possesses other actions responsible for the cardioprotective effects, to which the antioxidant activity of benidipine may partly contribute.

Introduction

Benidipine (hydrochloride) is a 1,4-dihydropyridine calcium antagonist with a slow onset of action and long-lasting properties (Karasawa & Kubo 1988a; Karasawa et al 1988a), which is clinically useful in the treatment of hypertension and angina pectoris (Fuji et al 1988; Yamada et al 1990). In experimental models, benidipine exhibited beneficial effects against ischaemic and reperfusion injury of the heart (Karasawa & Kubo 1988b; Karasawa et al 1988b). Myocardial ischaemia and reperfusion induces intracellular calcium overload, resulting in myocardial injury (Kloner & Braunwald 1987). The inhibition of calcium currents is probably the main mechanism for the cardioprotective action of benidipine.

During reperfusion after ischaemia, reactive oxygen species (known as potent oxidants), such as superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2), are generated and cause lipid peroxidation of the cell membrane and intracellular Ca^{2+} accumulation, which induces mechanical and metabolic derangements or damage (Kaneko et al 1994). Antioxidants, as well as oxygen radical scavengers, protect the myocardium against ischaemia–reperfusion damage (Hess & Manson 1984; Lucchesi 1990; Loesser et al 1991). Dihydropyridine calcium antagonists, including benidipine, have an antioxidant property (Mason & Trumbore 1996; Sugawara et al 1996; Yao et al 2000). In fact, benidipine has been shown to preserve the vascular endothelial function in rat arteries subjected to ischaemia and reperfusion (Karasawa et al 1991; Yao & Karasawa 1994), which was independent of the calcium-channel blocking action (Sato et al 1992). Thus, the antioxidant action is expected to contribute to the cardioprotective effect of benidipine. We reported that benidipine prevented myocardial injury following ischaemia and reperfusion in rat isolated perfused hearts (Yao & Karasawa 1994). In this study, to evaluate the contribution of the antioxidant action of

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benidipine to its cardioprotection in the therapeutic range of concentrations, we examined the effects of benidipine on the H_2O_2-induced myocardial injury in rat isolated hearts.

Materials and Methods

Drugs and chemicals

Benidipine hydrochloride was synthesized at Kyowa Hakko Kogyo. Nifedipine was purchased from the Sigma Chemical Co. (St Louis, MO). Benidipine and nifedipine were initially dissolved in polyethylene glycol 400 (Kanto Chemical, Tokyo, Japan) to make a 1 mmol L^{-1} solution, and the solution was further diluted with distilled water before use in the experiments. All other chemicals were of reagent grade and were from commercial sources.

Animals

Male Wistar rats, 300–390 g (Japan SLC Inc., Hamamatsu, Japan), were used. The rats were kept at 19–25°C under a 12-h light–dark cycle, and they had free access to tap water and commercial chow (FR-2, Funabashi Farm, Funabashi, Japan). All rats received humane care in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, formulated by the Japanese Pharmacological Society, and the protocol was approved by the Bioethical Committee of Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co. Ltd.

Heart perfusion

Rats were anaesthetized with sodium pentobarbital (Nembutal 50 mg kg^{-1}, i.p.; Dainippon Pharmaceutical, Osaka, Japan) 30 min after an injection with heparin (500 IU/rat, i.p.). Under anaesthesia, the chest was opened and the heart was excised. The aorta was cannulated, and the heart was perfused at a constant flow of 10 mL min^{-1} with oxygenated (95% O_2+5% CO_2) Krebs–Henseleit bicarbonate (KHB) buffer (25°C) with the following composition (mmol L^{-1}): NaCl, 112; $NaHCO_3$, 25; KCl, 5; $MgSO_4$, 1.2; KH_2PO_4, 1; $CaCl_2$, 1.25; dextrose, 11.5; pyruvate, 2. A balloon-tipped catheter filled with water was then inserted into the left ventricle via the left atrium. The heart was mounted on a Langendorff apparatus and perfused with warmed KHB buffer (37°C). Perfusion pressure (PP) and left ventricular pressure (LVP) were measured with the aortic cannula and the balloon-tipped catheter, which were connected to pressure transducers (SCK-590, Nihon Kohden, Tokyo, Japan), respectively. The maximum rate of rise in LVP (LV dP/dt max) was derived using an electric differentiator (ED-600G, Nihon Kohden). All parameters were continuously recorded on a thermal array chart recorder (WS-681, Nihon Kohden). The left ventricular end-diastolic pressure (LVEDP) was measured from the recorded LVP signal.

The heart was allowed to equilibrate for 15 min, and then LVEDP was adjusted to about 5 mmHg by changing the volume of the balloon. The left ventricular developed pressure (LVDP) was calculated from the difference between left ventricular peak systolic pressure and LVEDP.

Experimental protocol

Once the baseline values were measured, the hearts were treated with benidipine (hydrochloride, 0.1–1 nmol L^{-1}), nifedipine (1–10 nmol L^{-1}) or vehicle (0.001% polyethylene glycol 400) 20 min before H_2O_2 treatment. The concentrations of benidipine were within its therapeutic range. The concentrations of nifedipine were selected to cause a similar degree of negative inotropic effects under normal conditions. The drug solution or the vehicle was infused into the perfusion line leading to the aortic cannula using a constant flow infusion pump (Pump 22; Harvard Apparatus Inc., South Natick, MA) at a rate of 0.1 mL min^{-1} for 20 min. After the post-drug cardiac functions were measured and the perfusate was collected for 1 min, H_2O_2 solution (Kokusan Chemical Works, Tokyo, Japan) was infused into the aortic cannula at a rate of 0.1 mL min^{-1} for 4 min. The final concentration of H_2O_2 in the perfusate was 600 μmol L^{-1}. The heart was then perfused with control buffer for 30 min. At 0, 10, 20 and 30 min after stoppage of H_2O_2 infusion, the perfusate was sampled for 1 min. The activity of lactate dehydrogenase (LDH) in the perfusate samples was measured with an autoanalyser (AU600; Olympus, Tokyo, Japan).

Measurement of lipid peroxidation

To measure the tissue levels of lipid peroxidation in normal, H_2O_2-treated and benidipine+H_2O_2-treated hearts, the hearts were frozen at the end of the experiment with freezing clamps previously chilled in liquid nitrogen. The frozen myocardial samples were stored at −80°C until the analysis was performed.

The samples were pulverized in a mortar cooled with liquid nitrogen. A part of the pulverized tissue powder (about 100 mg) was weighed and homogenized 1:10 (w/v) in 10% KCl. Levels of thiobarbituric acid reactive substances (TBARS) were determined by the method of Ohkawa et al (1979) as an index of the extent of lipid peroxidation. TBARS formed in the solution were determined after heating at 100°C for 60 min in the presence of 0.2% thiobarbituric acid in a total volume of 4.0 mL. The TBARS were extracted into n-butanol and pyridine (15:1, v/v). The fluorescence was measured at an excitation wavelength of 515 nm and an emission wavelength of 553 nm (F-3000, Hitachi, Tokyo).

Statistical analysis

All values are expressed as means±s.e.m. The drug-treatment values of the parameters measured were compared with the vehicle-treatment values. Inter-group comparisons were made by the nested variance analysis, and then significant differences were tested by one-way analysis of variance followed by Dunnett's test or the Kruskal–Wallis test followed by Steel's test for multiple comparison, or the

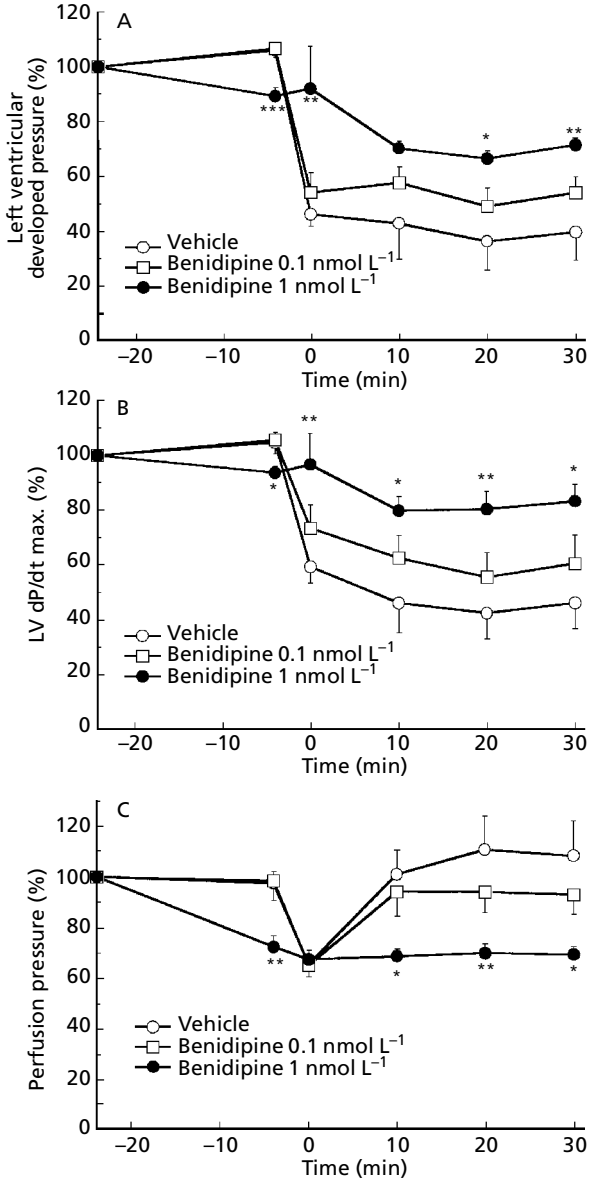

Figure 1 Effects of benidipine on left ventricular developed pressure (LVDP, Panel A), maximal rate of rise in left ventricular pressure (LV dP/dt max, Panel B) and perfusion pressure (PP, Panel C) in rat isolated perfused hearts subjected to H_2O_2. The hearts were treated with benidipine (0.1 or 1 nmol L^{-1}) for 20 min until the start of H_2O_2 infusion. The ordinate indicates percentage of the pre-drug value. Values are means±s.e.m. of 6 or 7 hearts. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs vehicle. Basal values of LVDP, LV dP/dt max and PP in each group were as follows. LVDP (mmHg): control, 107.2±3.4; benidipine 0.1 nmol L^{-1}, 104.0±6.5; and benidipine 1 nmol L^{-1}, 99.1±3.4. LV dP/dt max (mmHg s^{-1}): control, 3329±174; benidipine 0.1 nmol L^{-1}, 2983±193; and benidipine 1 nmol L^{-1}, 2980±144. PP (mmHg): control, 68.4±6.9; benidipine 0.1 nmol L^{-1}, 61.9±5.6; and benidipine 1 nmol L^{-1}, 67.3±6.5. No significant difference was observed among all the parameters in each group.

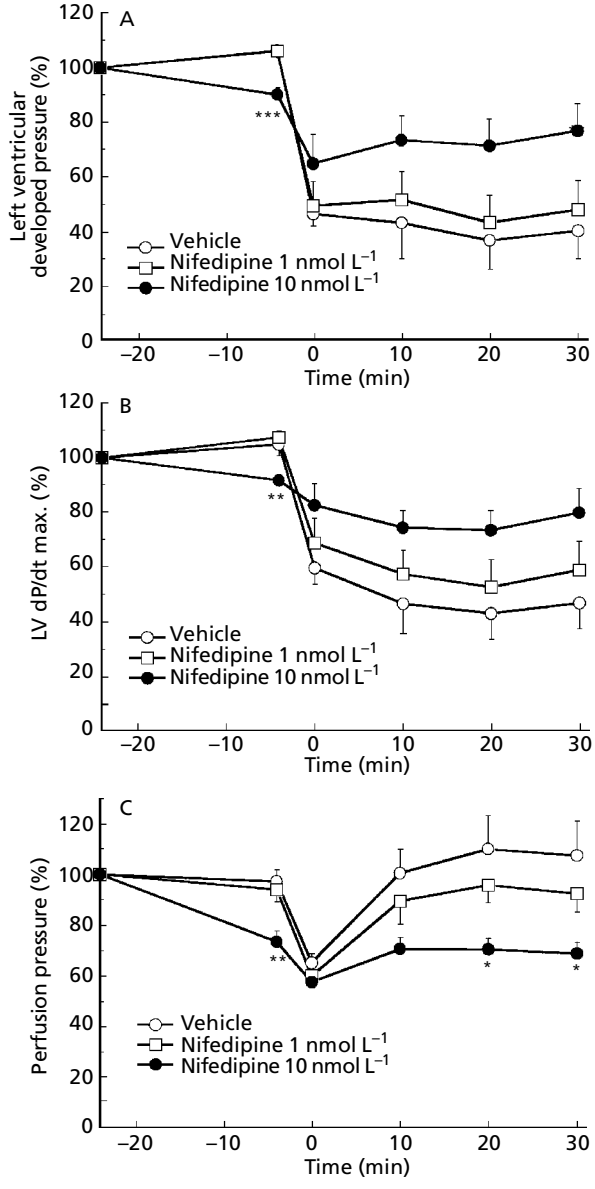

Figure 2 Effects of nifedipine on left ventricular developed pressure (LVDP, Panel A), maximal rate of rise of left ventricular pressure (LV dP/dt max, Panel B) and perfusion pressure (PP, Panel C) in rat isolated perfused hearts subjected to H_2O_2. The hearts were treated with nifedipine (1 or 10 nmol L^{-1}) for 20 min until the start of H_2O_2 infusion. The ordinate indicates the percentage of the pre-drug value. Values are means±s.e.m. of 6 or 7 hearts. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs vehicle. Basal values of LVDP, LV dP/dt max and PP in each group were as follows, LVDP (mmHg): control, 107.2±3.4; nifedipine 1 nmol L^{-1}, 97.8±5.1; and nifedipine 10 nmol L^{-1}, 95.2±3.2. LV dP/dt max (mmHg s^{-1}) control, 3329±174; nifedipine 1 nmol L^{-1}, 2991±100; and nifedipine 10 nmol L^{-1}, 2996±209. PP (mmHg): control, 68.4±6.9; nifedipine 1 nmol L^{-1}, 67.9±6.8; and nifedipine 10 nmol L^{-1}, 71.1±5.2. No significant difference was observed among all the parameters in each group.

F test followed by Student's *t*-test for non-matched pair comparison. A difference was considered as statistically significant at $P < 0.05$. All statistics were calculated with the SAS statistical software (version 6.12; SAS Institute Inc., Cary, NC).

Results

Figures 1 and 2, respectively, show the effects of benidipine and nifedipine on the H_2O_2-induced changes in LVDP, LV dP/dt max and PP. There were no significant differences in the baseline values among the groups. Benidipine at 1 nmol L^{-1} and nifedipine at 10 nmol L^{-1} decreased the LV dP/dt max as an index of myocardial contractility. No significant differences in the effects on any cardiac parameters were detected between benidipine at 1 nmol L^{-1} and nifedipine at 10 nmol L^{-1} 20 min after the beginning of infusion of each drug. H_2O_2 produced contractile failure as demonstrated by the decreases in LVDP and LV dP/dt max. H_2O_2 produced the decrease in PP, indicating vasodilatation in the hearts perfused at a constant flow, at 0 min after the stoppage of H_2O_2 perfusion, and then gradual increase in PP. At 30 min after H_2O_2 perfusion, the mean LVDP value of the hearts in the vehicle group was 45% of the pre-H_2O_2 value. Treatment with benidipine at 1 nmol L^{-1} significantly inhibited the decreases in LVDP and LV dP/dt max, and the increase in PP. Nifedipine at 10 nmol L^{-1} reduced the H_2O_2-induced changes in LVDP, LV dP/dt max and PP, but the effects were not significant. Benidipine at 0.1 nmol L^{-1} and nifedipine at 1 nmol L^{-1} did not affect these parameters.

Figure 3 shows the time course of changes in the LDH activity. Perfusion with vehicle alone caused an increase in the LDH release that peaked 20 min after the start of the treatment with H_2O_2. Benidipine and nifedipine reduced the release of LDH. At 1 nmol L^{-1}, the effect of benidipine on the release of LDH was significant.

Hearts treated with H_2O_2 had significantly elevated levels of TBARS (16.3 nmol mL^{-1} 10% homogenates, $P < 0.001$) compared with normal hearts (10.4 nmol mL^{-1}). Benidipine at 1 nmol L^{-1} significantly reduced the rise in TBARS (12.6±1.3 nmol mL^{-1}, $P < 0.05$).

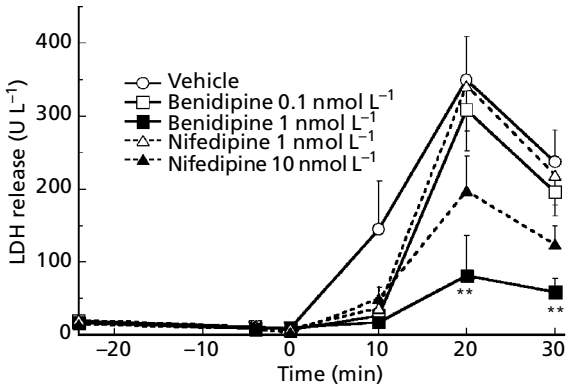

Figure 3 Effects of benidipine and nifedipine on the release of lactate dehydrogenase (LDH) in rat isolated perfused hearts subjected to H_2O_2. The hearts were treated with benidipine (0.1 or 1 nmol L^{-1}) or nifedipine (1 or 10 nmol L^{-1}) for 20 min until the start of H_2O_2 infusion. Values are means±s.e.m. of 6 or 7 hearts. $^{**}P < 0.01$ vs vehicle.

Discussion

In this study, H_2O_2 produced myocardial membrane damage and subsequent mechanical dysfunction in isolated perfused hearts from rats. Several studies indicated that H_2O_2 decreased the tissue levels of high-energy phosphates in the heart, leading to cardiac dysfunction (Hara et al 1993; Hara & Abiko 1996; Kokita & Hara 1996; Xiao et al 1999). According to recent physiological studies, H_2O_2 activated the Na^+/Ca^{2+} exchanger through increases in intracellular Na^+ in ventricular myocytes (Yanagida et al 1995; Goldhaber 1996; Ward & Giles 1997). In addition, H_2O_2 was reported to induce the enhancement of the L-type calcium currents in the myocytes (Thomas et al 1998). These effects probably cause the calcium overload, which is responsible for irreversible myocardial derangements in the cardiac myocytes (Goldhaber 1996).

We examined the effects of benidipine and nifedipine on the H_2O_2-induced myocardial injury in rat isolated perfused hearts. These calcium antagonists protected the hearts against the H_2O_2-induced injury. In particular, benidipine exhibited a protective effect even at a concentration of 1 nmol L^{-1}, which corresponds to the therapeutic plasma concentrations. One nmol L^{-1} of benidipine decreased LVDP, LV dP/dt max, and PP. L-type Ca^{2+}-channel-blocking action, leading to increasing coronary flow and decreasing cardiac mechanical function, may partly contribute to the cardioprotective effects of benidipine. One nmol L^{-1} of benidipine, but not 10 nmol L^{-1} of nifedipine, significantly prevented the H_2O_2-induced myocardial injury, even though both decreased the myocardial contractility and perfusion pressure to a similar degree in the hearts under normal conditions. The protective effects of benidipine could not be totally attributed to its inhibition of L-type calcium current. Just after the treatment with H_2O_2 (0 min), LVDP and the LV dP/dt max were decreased without increases in LDH release, indicating that the contractile dysfunction was not secondary to the myocardial cell injury. Benidipine at 1 nmol L^{-1} inhibited these decreases at 0 min, suggesting that benidipine may directly prevent the H_2O_2-induced myocardial dysfunction.

Benidipine has been shown to increase cardiac NO levels and exhibit protective effects against ischaemic damage in canine hearts via bradykinin- and NO-dependent mechanisms (Kitakaze et al 1999b; Asanuma et al 2001). NO donors had beneficial effects on the LVDP in the ischaemia–reperfusion injury of Langendorff hearts (Hotta et al 1999). NO had inhibitory effects on left ventricular dysfunction after H_2O_2-mediated oxidative stress in rat isolated hearts (Valen et al 1996). NO may contribute to the cardioprotective effects of benidipine in our study.

Ischaemia–reperfusion-induced increases in the level of free radicals are paralleled by elevated rates of lipid peroxidation (Ambrosio et al 1991; Blasig et al 1995; Cordis et al 1995). Some radical scavengers and antioxidants have been demonstrated to protect the myocardium against oxidative stress (Nakaya et al 1987). Several studies indicate that some calcium antagonists possess lipid antioxidant properties (Mason & Trumbore 1996; Sugawara et al 1996; Yao et al 2000). We have also reported that eight dihydro-

pyridine (DHP) calcium antagonists, including benidipine and nifedipine, had concentration-dependent antioxidant effects in a rat brain homogenate (Yao et al 2000). A hydroxyl radical that is generated from H_2O_2 by the Fenton reaction easily removes a hydrogen atom from a methylene carbon of an unsaturated fatty acid of the membrane phospholipids, and hence produces peroxidation of lipids (Hess & Manson 1984). In fact, H_2O_2 increased the tissue level of TBARS, products of lipid peroxidation. It has been demonstrated that myocardial changes induced by reactive oxygen are accompanied by lipid peroxidation of the cell membrane of the heart (Hess & Manson 1984). Antioxidant substances have been shown to attenuate both myocardial dysfunction and lipid peroxidation induced by H_2O_2 (Onodera et al 1992; Nagy et al 1996). In this study, benidipine inhibited the H_2O_2-induced lipid peroxidation, suggesting that the protective effects of benidipine on the H_2O_2-induced injury may be due, in part, to its antioxidant effect. However, the exact mechanism remains unknown, and further investigations are necessary.

In general, DHP calcium antagonists appear to be distributed in cell membranes according to their lipophilicity (Mason & Trumbore 1996). Nosaka & Ishii (1991) reported that, in the octanol–buffer system and lipid membranes, benidipine had the highest partition coefficient of the DHP calcium antagonists, including nifedipine. In addition, the membrane approach theory is considered as a putative mechanism of long-lasting antihypertensive effects of benidipine, and explains the lack of correlation between the effects and plasma levels of benidipine (Kitakaze et al 1999a). These results suggest that benidipine is highly lipophilic and effectively accumulates in membranes. In fact, the vasorelaxant effect of benidipine in isolated coronary arteries was sustained, even after washout of the drug (Karasawa & Kubo 1988a). In this study, benidipine may effectively and continuously accumulate in the myocardial membranes, contributing to the cardioprotective efficacy of benidipine.

Conclusions

Benidipine has protective effects on the H_2O_2-induced injury in rat isolated hearts. These results suggest that, in addition to calcium antagonism, benidipine possesses other actions responsible for the cardioprotective effects, to which the antioxidant activity of benidipine may partly contribute.

References

Ambrosio, G., Flaherty, J. T., Duilio, C., Tritto, I., Santoro, G., Elia, P. P., Condorelli, M., Chiariello, M. (1991) Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfused hearts. *J. Clin. Invest.* **87**: 2056–2066

Blasig, I. E., Grune, T., Schonheit, K., Rohde, E., Jakstadt, M., Haseloff, R. F., Siems, W. G. (1995) 4-Hydroxynonenal, a novel indicator of lipid peroxidation for reperfusion injury of the myocardium. *Am. J. Physiol.* **269**: H14–H22

Cordis, G. A., Maulik, N., Das, D. K. (1995) Detection of oxidative stress in heart by estimating the dinitrophenylhydrazine derivative of malonaldehyde. *J. Mol. Cell. Cardiol.* **27**: 1645–1653

Fuji, Y., Suzuki, H., Katsumata, H., Nakajima, S., Saruta, T. (1988) Hormonal and renal responses to oral once-daily calcium entry blocker in normotensive and hypertensive persons. *J. Cardiovasc. Pharmacol.* **11**: 438–443

Goldhaber, J. I. (1996) Free radicals enhance Na^+/Ca^{2+} exchange in ventricular myocytes. *Am. J. Physiol.* **271**: H823–H833

Hara, A., Abiko, Y. (1996) Protective effects of dilazep and its novel derivative, K-7259, on mechanical and metabolic derangements induced by hydrogen peroxide in the isolated perfused rat hearts. *J. Pharmacol. Exp. Ther.* **277**: 565–571

Hara, A., Matsumura, H., Abiko, Y. (1993) Lidocaine attenuates both mechanical and metabolic changes induced by hydrogen peroxide in the rat heart. *Am. J. Physiol.* **265**: H1478–H1485

Hess, M. L., Manson, N. H. (1984) Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. *J. Mol. Cell Cardiol.* **16**: 969–985

Hotta, Y., Otsuka-Murakami, H., Fujita, M., Nakagawa, J., Yajima, M., Liu, W., Ishikawa, N., Kawai, N., Masumizu, T., Kohno, M. (1999) Protective role of nitric oxide synthase against ischemia-reperfusion injury in guinea pig myocardial mitochondria. *Eur. J. Pharmacol.* **380**: 37–48

Kaneko, M., Matsumoto, Y., Hayashi, H., Kobayashi, A., Yamazaki, N. (1994) Oxygen free radicals and calcium homeostasis in the heart. *Mol. Cell. Biochem.* **139**: 91–100

Karasawa, A., Kubo, K. (1988a) Calcium antagonistic effects and the in vitro duration of actions of KW-3049, a new 1, 4-dihydropyridine derivative, in isolated canine coronary arteries. *Jpn. J. Pharmacol.* **47**: 35–44

Karasawa, A., Kubo, K. (1988b) Inhibitory effect of KW-3049, a new 1,4-dihydropyridine calcium antagonist, on the reduction of myocardial creatine kinase activity and high energy phosphate content in rats subjected to coronary artery ligation. *J. Pharm. Dyn.* **11**: 722–729

Karasawa, A., Kubo, K., Shuto, K., Oka, T., Nakamizo, N. (1988a) Vasodilating effects of the new calcium antagonist benidipine hydrochloride in anesthetized dogs and cats. *Arzneimittelforsch.* **38**: 1707–1712

Karasawa, A., Kubo, K., Shuto, K., Oka, T., Nakamizo, N. (1988b) Beneficial effects of the new calcium antagonist benidipine hydrochloride on myocardial dysfunction following coronary occlusion and reperfusion in anesthetized dogs. *Arzneimittelforsch.* **38**: 1717–1721

Karasawa, A., Rochester, J. A., Ma, X.-L., Lefer, A. M. (1991) Protection of endothelial damage and systemic shock by benidipine, a calcium antagonist, in rats subjected to splanchnic ischemia and reperfusion. *Circ. Shock* **33**: 135–141

Kitakaze, M., Karasawa, A., Kobayasi, H., Tanaka, B. S., Kuzuya, T., Hori, M. (1999a) Benidipine: a new Ca^{2+} channel blocker with a cardioprotective effect. *Cardiovasc. Drug Reviews* **17**: 1–15

Kitakaze, M., Node, K., Minamino, T., Asanuma, H., Kuzuya, T., Hori, M. (1999b) A Ca channel blocker, benidipine, increases coronary blood flow and attenuates the severity of myocardial ischemia via NO-dependent mechanisms in dogs. *J. Am. Coll. Cardiol.* **33**: 242–249

Kloner, R. A., Braunwald, E. (1987) Effects of calcium antagonists on infarcting myocardium. *Am. J. Cardiol.* **59**: 84B–94B

Kokita, N., Hara, A. (1996) Propofol attenuates hydrogen peroxide-induced mechanical and metabolic derangements in the isolated rat heart. *Anesthesiology* **84**: 117–127

Loesser, K. E., Kukreja, R. C., Kazziha, S. Y., Jesse, R. L., Hess, M. L. (1991) Oxidative damage to the myocardium: a fundamental mechanism of myocardial injury. *Cardioscience* **2**: 199–216

Lucchesi, B. R. (1990) Myocardial ischemia, reperfusion and free radical injury. *Am. J. Cardiol.* **65**: 14I–23I

Mason, R. P., Trumbore, M. W. (1996) Differential membrane interactions of calcium channel blockers. Implications for antioxidant activity. *Biochem. Pharmacol.* **51**: 653–660

Nagy, A., Sellei, P., Valen, G., Sjoquist, P. O., Vaage, J. (1996) Effects of a novel low-molecular weight antioxidant on cardiac injury induced by hydrogen peroxide. *Free Radic. Biol. Med.* **20**: 567–572

Nakaya, H., Tohse, N., Kanno, M. (1987) Electrophysiological derangements induced by lipid peroxidation in cardiac tissue. *Am. J. Physiol.* **253**: H1089–H1097

Nosaka, C., Ishii, A. (1991) Partition of [^{3}H] benidipine hydrochloride into the lipid membrane. *Clin. Rep.* **25**: 3887–3893

Ohkawa, H., Ohishi, N., Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351–358

Onodera, T., Takemura, G., Oguro, T., Ashraf, M. (1992) Effect of exogenous hydrogen peroxide on myocardial function and structure in isolated rat heart. *Can. J. Cardiol.* **8**: 989–997

Sato, H., Karasawa, A., Kubo, K. (1992) Protective effects of benidipine on rat endothelial injury induced by sodium citrate. *Clin. Rep.* **26**: 4397–4402 (Abstract in English)

Sugawara, H., Tobise, K., Kikuchi, K. (1996) Antioxidant effects of calcium antagonists on rat myocardial membrane lipid peroxidation. *Hypertens. Res.* **19**: 223–228

Thomas, G. P., Sims, S. M., Cook, M. A., Karmazyn, M. (1998) Hydrogen peroxide-induced stimulation of L-type calcium current in guinea pig ventricular myocytes and its inhibition by adenosine A1 receptor activation. *J. Pharmacol. Exp. Ther.* **286**: 1208–1214

Valen, G., Skjelbakken, T., Vaage, J. (1996) The role of nitric oxide in the cardiac effects of hydrogen peroxide. *Mol. Cell. Biochem.* **159**: 7–14

Yamada, K., Yasui, S., Kishida, H., Hiejima, K., Murakami, E., Takekoshi, N. (1990) Clinical evaluation of benidipine hydrochloride (KW-3049) on angina pectoris. A double-blind comparative study with nifedipine. *Ther. Res.* **11**: 1637–1656 (Abstract in English)

Yanagida, S., Luo, C. S., Doyle, M., Pohost, G. M., Pike, M. M. (1995) Nuclear magnetic resonance studies of cationic and energetic alterations with oxidant stress in the perfused heart. Modulation with pyruvate and lactate. *Circ. Res.* **77**: 773–783

Yao, K., Karasawa, A. (1994) Protective effects of benidipine against myocardial damage following ischemia and reperfusion in the isolated perfused rat heart. *Biol. Pharm. Bull.* **17**: 517–521

Yao, K., Ina, Y., Nagashima, K., Ohmori, K., Ohno, T. (2000) Antioxidant effects of calcium antagonists in the rat brain homogenates. *Biol. Pharm. Bull.* **23**: 766–769

Ward, C. A., Giles, W. R. (1997) Ionic mechanism of the effects of hydrogen in rat ventricular myocytes. *J. Physiol.* **500**: 631–642

Xiao, C. Y., Hara, A., Hashizume, H., Tanaka, K., Abiko, Y. (1999) Both D-cis- and L-cis-diltiazem attenuate hydrogen peroxide-induced derangements in rat hearts. *Eur. J. Pharmacol.* **374**: 387–398