Membrane potassium currents in human radial artery and their regulation by nitric oxide donor

Yongde Zhang\textsuperscript{a}, Tracy Tazzeo\textsuperscript{a}, Victor Chu\textsuperscript{b}, Luke J. Janssen\textsuperscript{a,*}

\textsuperscript{a} Department of Medicine, McMaster University, 50 Charlton Avenue East, Hamilton, Ontario, Canada L8N 4A6
\textsuperscript{b} Department of Surgery, McMaster University, Hamilton, Ontario, Canada L8N 4A6

Received 18 December 2005; received in revised form 27 March 2006; accepted 11 April 2006
Available online 21 April 2006
Time for primary review 19 days

Abstract

Objective: The human radial artery has demonstrated superior long-term results as a graft in coronary bypass surgery, but undesirable postsurgical spasm limits its clinical application. Few have examined its excitatory properties, especially the underlying ion channel mechanisms. In this study, we investigated the kinetic and pharmacological properties of the smooth muscle membrane potassium currents of this important artery.

Methods and results: Using whole cell patch-clamp techniques, we found the K\(^+\) current to be voltage-dependent and outwardly rectifying. Voltage-dependent inactivation was observed, being half-maximal at +28.0 mV but incomplete even at +40 mV. The K\(^+\) currents were predominantly sensitive to the KCa blocker tetraethylammonium (TEA; 63.9 \(\pm\) 12.1\% inhibition, \(p<0.05\)), less sensitive to the Kv blocker 4-aminopyridine (4-AP; 32.8 \(\pm\) 4.4\% inhibition, \(p<0.05\)), and the K\(_{ATP}\) blocker glibenclamide (28.7 \(\pm\) 8.5\% inhibition), at \(-20\) mV testing potential. Resting membrane potential was \(-52.0 \pm 6.8\) mV (\(n=5\)), and suppression of K\(^+\) currents by TEA and iberiotoxin (IbTx) caused membrane depolarization. Western blot analysis with channel-specific antibodies confirmed the presence of KCa and Kv channel proteins. TEA evoked 20.7 \(\pm\) 9.9\% of the contractile response to 60 mM KCl, whereas IbTx caused about 10\% of the above response at 10\(^{-7}\) M. The nitric oxide donor SNAP augmented membrane K\(^+\) currents in a concentration-dependent fashion; the augmentation was completely suppressed by TEA, but was relatively insensitive to the guanylate cyclase inhibitor ODQ.

Conclusions: The radial artery manifests mainly Ca\(^{2+}\)-dependent K\(^+\) currents at rest; this current is augmented by nitric oxide through a cGMP- and protein kinase G-independent action. The relatively depolarized membrane potential, as well as its muscular structure, predisposes the radial artery to spasm. Agents that activate the Ca\(^{2+}\)-dependent K\(^+\) current could be of therapeutic value in preventing postsurgical vasospasm.

\(\textcircled{D}2006\) European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: K\(^+\) current; Voltage-dependent; Ca\(^{2+}\)-activated; ATP-sensitive; Nitric oxide

1. Introduction

The radial artery was first proposed as an arterial graft in the early years of coronary artery by-pass grafting (CABG) surgery. However, it was soon found to exhibit a high incidence of near-term vasospasm (usually within 48 h postsurgery), which limited its application. Recently, favorable long-term follow-up studies \([1,2,18,24,25]\) have revived the clinical interests in this conduit graft.

The radial artery is intrinsically prone to vasospasm, partly due to its muscular structure. A number of endogenous vasoconstrictors are produced during the surgical crisis, including angiotensin II, endothelin, and thromboxin A\(_2\). Furthermore, adrenaline was routinely administered as an inotrope following the revascularization surgery to improve the myocardial and systemic perfusion. All these vasoconstrictors can cause graft spasm. Topical treatment
strategies have been advocated to improve the short-term outcome, among them are phenoxybenzamine, verapamil/nitroglycerin, and papaverine [5,11,16,27]. Postoperative calcium channel blocker therapy was also proposed to combat the spasm of radial artery graft [6,7]. However, none of these prophylactic treatment strategies demonstrated a universal protection against all vasoconstrictors during the surgery. A better understanding of the contractile properties and the underlying ion channel mechanisms of this tissue will help address the critical question of vasospasm and how to prevent it, thus increasing the success rate of this clinically important graft.

Vascular smooth muscle tone is directly dependent upon the intracellular calcium concentration, which in turn is largely determined by the regulation of Ca\(^{2+}\)-influx through voltage-gated Ca\(^{2+}\) channels. Membrane potassium ion efflux (outward K\(^+\) current) is the major ionic event determining the resting membrane potential. Therefore, membrane K\(^+\) and Ca\(^{2+}\) currents play important roles in regulating the vascular tone. Several factors including preoperative drug administration and surgical denervation during the harvesting could affect ion channel function and subsequently interfere with the vasomotor response. Despite this possibility, the basal membrane K\(^+\) and Ca\(^{2+}\) current properties of the radial arteries have never been reported. Meanwhile, it is known that K\(^+\) channel function is intricately regulated by endothelium-released autacoids, including prostaglandin I\(_2\), nitric oxide and endothelium-derived hyperpolarizing factor. A recent study demonstrated that histamine induced NO release through activation of H\(_2\) receptors, and suggested that a low expression of H\(_2\) receptors may be the causal factor in vasospasm in human radial artery [23]. Thus, the role of NO in the regulation of channel function warrants further investigation.

In the current study, we sought to examine the kinetic and pharmacological properties of the membrane K\(^+\) currents in the human radial artery, as well as their molecular composition and regulation by a nitric oxide donor.

2. Methods

2.1. Tissue preparation

Radial artery specimens were obtained with approval from local research ethics committee and upon informed consent from patients undergoing coronary arterial bypass grafting surgery. 55 patients 39 to 84 years of age (mean 63.4±10.3 years) participated in the study during February 2004 to October 2005. Among them were 47 males and 8 females. The radial artery was harvested with accompanying veins and adjacent connective tissue intact in the operating room, a surplus segment (1–2 cm in length) was cut and placed immediately in cold (4 °C) Krebs buffer solution. The segment was carefully dissected under the microscope, the fibrous adventitia and the endothelium were removed, and the remaining smooth muscle tissue was enzymatically dissociated at 37 °C for 40 min to liberate free smooth muscle cells for patch-clamp study. Cells were tested within 6 h after dissociation, and stored at 4 °C between experiments. For muscle bath experiments, the arteries were cut into vascular rings (with intact endothelium), each measuring approximately 2.5 mm in width.

2.2. Patch-clamping experiments

Electrophysiological responses were tested in cells that were phase dense and appeared relaxed. Membrane potassium currents were recorded at room temperature using the nystatin perforated patch configuration (0.3 mg/ml). Micropipettes (tip resistance 3 to 5 MΩ) were made using a programmable puller (model P-87, Sutter Instrument Co, Novato, CA) and then heat polished. Access resistances ranged from 11 to 40 MΩ; whole cell capacitance ranged from 9 to 35 pF. Membrane currents were filtered at 1 kHz and sampled at 2 KHz. Current signals were converted from analog to digital format (DigiData 1200, Axon Instruments, Foster City, CA) and stored on the hard-drive of a computer.

Vascular ring segments were mounted in 2.5-ml baths containing warm (37 °C) Krebs buffer, and constantly aerated with 95% O\(_2\)–5% CO\(_2\) to maintain a pH of 7.4. Preload tension was applied and kept at approximately 0.5 g. Experiments were conducted in the presence of 10\(^{-5}\) M indomethacin to inhibit endogenous prostanoid synthesis. Changes in isometric force were digitized (at 2 Hz) and recorded on-line using DigiMed System Integrator program (MicroMed, Louisville, KY). Tissues were equilibrated for 1–2 h, during which time they were challenged 2 or 3 times with 60 mM KCl, before the mechanical responses to K\(^+\)
channel blockers were assessed; the lattermost response to KCl was used to standardize all subsequent responses.

2.4. Western blot assay

The detailed methods of detecting smooth muscle cell membrane channel proteins were described in our previous paper [29]. Briefly, human radial artery specimens were homogenized in Tris buffer. After centrifugation, the supernatant was collected for immunoblot analysis. 10 μg of denatured protein was fractionated by electrophoresis. Proteins were then transferred onto nitrocellulose membranes (Hybond-ECL, Amersham). The membranes were probed with polyclonal rabbit anti-BKCa, anti-Kv1.2, and anti-Kv1.5 (1:300 dilution; Alomone Labs, Jerusalem, Israel), followed by secondary horseradish peroxidase-conjugated goat anti-rabbit IgG (1:15000 dilution; Sigma). Blots were detected with enhanced chemiluminescence (ECL, Amersham). β-Actin was used as loading control.

2.5. Chemicals and solutions

Dissociation solution contained the following components: collagenase (type F, from Clostridium histolyticum, 1 mg/ml), elastase (type IV, from porcine pancreas, 0.25 mg/ml), and bovine serum albumin (1 mg/ml), in Ca2+- and Mg2+-free Hanks solution. For K+ current recording, the electrode solution had the following components (in mM): 140 KCl, 1 MgCl2, 0.4 CaCl2, 20 HEPES, and 1 EGTA (pH = 7.2). The nystatin solution was prepared as 30 mg/ml stocks in dimethyl sulphoxide (DMSO), then diluted to a final concentration of 0.3 mg/ml in electrode solution. The pipette tip was filled with nystatin-free electrode solution, and the pipette back-filled with nystatin-containing solution. The cells were constantly perfused using standard Ringer.

Fig. 1. Voltage-dependent activation of K+ current was examined using simple-step depolarizing pulses (−60 mV to +50 mV, in 10-mV increments; 1.2 s duration) from a holding potential of −70 mV; representative recording is shown in upper left panel, current–voltage relationship is given in upper right panels. Tail currents were recorded using an additional step to −120 mV immediately following the activation protocol described above (lower left panel); mean amplitudes of tail current plotted against activation voltage (lower right panel) reveals a threshold voltage of approximately −30 mV and peak activation at +40 mV.
solution, which contained the following (in mM): 130 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 20 HEPES, and 10 d-glucose (pH = 7.4).

S-Nitroso-N-acetylpenicillamine (SNAP) was purchased from Calbiochem (Hornby, ON). DMSO was purchased from Caledon Laboratories Ltd. (Georgetown, ON). HEPES, MgCl₂ and KCl were purchased from BioShop Canada Inc. (Burlington, ON). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

2.6. Statistical analysis

All values are expressed as means ± S.E.M. unless otherwise stated. Paired sample data comparisons were performed by Student’s t-test, whereas group data analysis was performed by ANOVA when applicable. p < 0.05 was considered statistically different.

3. Results

3.1. Kinetics of membrane K⁺ currents

Depolarizing pulses (from −60 mV to +50 mV, 10-mV increments) were applied to radial arterial smooth muscle cells from a holding potential of −70 mV. K⁺ currents were activated at potentials greater than −40 mV, and exhibited voltage-dependent outward rectification, becoming relatively ‘noisy’ at positive voltages. A representative recording and its corresponding current–voltage relationship are given in Fig. 1. Tail currents were recorded at −120 mV immediately following the voltage protocol described above (left lower panel): threshold and peak activation occurred at −30 mV and +40 mV, respectively (right lower panel).

To examine the voltage dependence of inactivation of the K⁺ currents, cells were pre-conditioned by holding for 10 s at voltages ranging from −100 mV to +40 mV (10-mV increments) before delivering a brief test pulse to +40 mV. Representative experimental traces and an I/V plot (current vs. preconditioning voltage) are shown in Fig. 2. The K⁺ currents began to inactivate at −70 mV, but never fully inactivated: even at +40 mV, these were still approximately 20% of control. Boltzmann analysis (a finite-element analysis model) of the results from multiple cells is shown in Fig. 3: interpolation of those data shows inactivation to be half-maximal at −28.0 mV. Data were acquired from 12 cells from 9 patients.

3.2. Pharmacology of membrane K⁺ currents

Membrane K⁺ current composition was examined using an inhibitor of voltage-gated K⁺ currents (4-aminopyrididine, or 4-AP; 1 mM), an inhibitor of Ca²⁺-activated voltage-dependent K⁺ currents (tetrathylammonium, or
TEA; 1 mM), and an inhibitor of ATP-gated K⁺ currents (glibenclamide, or glib; 10 μM). Current–voltage relationships in the presence and absence of the blockers are presented in Fig. 4A; difference currents, reflecting the inhibitor-sensitive component of the current, are plotted as insets. The membrane currents were standardized by cell capacitance. In general, we found the K⁺ currents to be predominantly sensitive to TEA: currents were reduced to 34.5±2.7% (p<0.01, n=5) and 36.2±12.1% (p<0.05) of control at +40 mV and −20 mV, respectively. The large-conductance Ca²⁺-activated current blocker IbTx (0.1 μM) reduced the current to 61.3±10.3% and 55.3±14.5% (n=3) of control, while the small-conductance Ca²⁺-activated current blocker apamin (0.3 μM) reduced the current to 89.0±7.6% and 87.9±4.9% (n=4) at the above voltages. The K⁺ currents were less sensitive to 4-AP, being reduced to 88.7±7.5% of control at +40 mV, and 67.2±4.4% (p<0.05) at −20 mV (n=6). Glibenclamide inhibited the K⁺ currents to 73.8±8.0% and 71.3±8.5% of control at the above voltages (n=4). The mean inhibitory effects of these blockers on overall K⁺ current are shown in Fig. 4B.

Inward-rectifier K⁺ current was examined in cells voltage clamped at 0 mV and perfused with 140 mM K⁺ Ringers, then stepped to voltages ranging from −120 to +40 mV, in the presence vs. absence of Ba²⁺ (50 μM). No Ba²⁺-sensitive component was found in the resulting currents, suggesting the inward-rectifier K⁺ current is not present in this arterial smooth muscle.

Fig. 4. (A) Voltage-dependent activation was investigated in cells before and during introduction of the K⁺ channel blockers TEA (1 mM), IbTx (0.1 μM), apamin (0.3 μM), 4-AP (1 mM), or glibenclamide (10 μM), using voltage protocol described in Fig. 1. Currents were standardized by cell capacitance. Insets: Difference currents. (B) Mean magnitudes of inhibition of K⁺ current (at +40 mV and −20 mV testing pulses) caused by 4-AP (n=6), TEA (n=5), apamin (n=4), IbTx (n=3) and glibenclamide (n=4). Statistical results were given for the respective inhibitor effects vs. control, *p<0.05 and **p<0.01.
Mean resting membrane potential recorded in current-clamp mode was found to be $-50.0 \pm 7.8$ mV ($n = 4$); likewise, interpolation of current–voltage relationships to find the voltage at which current was equal to zero (which is by definition their resting membrane potential) yielded a mean value of $-52.0 \pm 6.8$ mV ($n = 5$). In addition to suppressing $K^+$ currents during voltage-clamp (Fig. 5, upper left panels), TEA (1 mM) also depolarized the membrane potential obtained during current-clamp mode (Fig. 5, upper right panel). On average, the membrane was depolarized by TEA from a resting value of $-52.0 \pm 6.8$ mV to a mean value of $-29.2 \pm 7.9$ mV ($n = 5$, $p < 0.05$). IbTx had a similar effect on the membrane potential, but 4-AP did not cause a significant depolarization of membrane (Fig. 5, lower panels). The above data were collected from 19 cells from 14 patients. These results highlight the inhibitory effect of TEA and IbTx on membrane $K^+$

![Fig. 5. Upper left panels: $K^+$ currents recorded before and during application of 1 mM TEA (by a micro puffer placed close to the cell). Upper right panel: Measurement of membrane potential (measured in current-clamp mode) in the same cell before and during application of 1 mM TEA. Lower panels: Membrane potential measurement before and during application of 1 mM 4-AP and 0.1 $\mu$M IbTx.](image)

![Fig. 6. Mean contractile responses to TEA, 4-AP, iberiotoxin and glybenclamide. Contractions are expressed as a percent of the response to 60 mM KCl evoked during the equilibration period. $n = 7$ for all.](image)

![Fig. 7. Relative abundances of BKCa, Kv1.2 and Kv1.5 channel proteins, assessed using Western blot approach and standardized by the amount of $\beta$-actin control present in the same gels.](image)
currents and the consequent depolarizing influence that ensues.

3.3. Vessel contractions evoked by $K^+$ channel blockers

In light of the membrane depolarizing effect of blocking $K^+$ channels, we also examined the effects of $K^+$ channel blockers on mechanical tone in vascular rings (73 rings from 12 patients) (Fig. 6). TEA evoked small and transient contractions at sub-millimolar concentrations: at millimolar concentrations, these were as large as $20.7 \pm 9.9\%$ ($n=7$) of the response to 60 mM KCl. Iberiotoxin, a highly selective blocker of large conductance $Ca^{2+}$-dependent $K^+$ channels, evoked a constrictor response of about 10% of the response to 60 mM KCl when applied at a concentration of $10^{-7}$ M. The Kv blocker 4-AP evoked contraction of $22.1 \pm 6.9\%$ ($n=7$) of the KCl response when applied at 1 mM. Glybenclamide

Fig. 8. (A) Nitric oxide donor SNAP augmented $K^+$ current–voltage relationship in a concentration-dependent and fully reversible manner. (B) Step depolarizing pulses (to +30 mV, from a holding potential of −70 mV; 850 ms duration) were delivered at 10-s intervals. Cells were pre-treated with 4-AP (1 mM). TEA and ODQ were delivered using micro-puffer placed near the test cells, whereas cumulative doses of SNAP were added directly to the bath chamber. SNAP (0.1–0.3 mM) augmented $K^+$ currents, which were completely suppressed by TEA (dotted lines indicate the amplitude of control currents). Addition of the soluble guanylate cyclase inhibitor ODQ (10 μM) had relatively little effect, while TEA again rapidly and reversibly reduced the overall $K^+$ current to the same level seen prior to addition of SNAP.
evoked little contraction in the experimental concentration range.

3.4. Plasmalemmal expression of K⁺ channel proteins

Excised human radial arteries (n = 8) were homogenized and subjected to Western blot analysis using antibodies directed against BKCa, Kv1.2 and Kv1.5 proteins. All three channel-proteins were found in these tissues (Fig. 7).

3.5. Regulation of K⁺ currents by the nitric oxide donor SNAP

The nitric oxide donor SNAP augmented membrane K⁺ currents in human radial arterial smooth muscle cells, with 55 ± 23% and 244 ± 71% (n = 5) augmentation for 8 × 10⁻⁵ M and 2.4 × 10⁻⁴ M SNAP, respectively. Representative experimental recordings and I/V relationships are given in Fig. 8A, showing the concentration dependence of the response. The salutary effect of SNAP on K⁺ currents was almost completely reversible upon wash out of SNAP. At a test voltage of +30 mV, SNAP (3 × 10⁻⁴ M) evoked dose-dependent augmentation of K⁺ currents in cells pretreated with 4-AP, which was completely suppressed by TEA (1 mM) (Fig. 8B, left panel). In separate experiments, the SNAP-augmented K⁺ currents were relatively insensitive to the soluble guanylate cyclase inhibitor 1H-[1,2,4]-oxadiazolo-[4,3]-quinoxalin-1-one (ODQ; 10 μM), in sharp contrast to the effect of TEA, suggesting that NO directly acts on the membrane K⁺ channel (Fig. 8B, right panel).

4. Discussion

Postoperative vasospasm is a continuing problem with radial arterial graft. Preexisting lesion (i.e., atherosclerosis) of the donor artery may further exacerbate the situation [8,22,30]. A better understanding of the contractile physiology of the human radial artery may lead to newer and better interventions to deal with this vasospasm. An important facet would include a characterization of the potassium channels present and their regulation by autacoids, a subject about which there is no previous literature.

The composition of potassium currents in human vascular smooth muscle has been reported in previous studies, which revealed an idiosyncrasy among different vascular beds. A voltage-dependent K⁺ current subtype has been identified and characterized in cultured human pulmonary arterial smooth muscle cells [13,17], while a Ca²⁺-activated voltage-dependent variety has been found in human and murine coronary arterioles [15,31] and human cerebral artery [26]. ATP-sensitive potassium channels have been found to mediate the regulatory mechanism of insulin on coronary vasculature [19] and cause vasodilation during acidosis in human internal mammary artery [20]. Little is known, however, about the properties (voltage dependence of activation/inactivation; kinetics; pharmacology) of the K⁺ current in human radial artery, a very important CABG graft material.

Our patch-clamp data indicate that the potassium currents in the human radial artery at rest consist of a mixture of voltage-dependent, Ca²⁺-dependent, and ATP-sensitive subtypes, with the TEA-sensitive (i.e., Ca²⁺-dependent) variety predominating. BKCa channels were the main subgroup among the Ca²⁺-dependent family, while SKCa showed relatively minor presence. Inwardly rectifier K⁺ current was not found in this arterial bed. Probing of the membrane channel proteins confirmed the presence of KCa and Kv varieties (the KATP channel protein was not tested). Also consistent with these findings, we found millimolar TEA and submicromolar iberiotoxin both evoked contractile responses in intact tissues, while glibenclamide did not. Interestingly, submillimolar 4-AP also evoked substantial contractions (comparable in magnitude to those evoked by TEA or iberiotoxin). This is consistent with our observation that a 4-AP-sensitive current is also active at physiological potentials (see Fig 4B). These currents were insensitive to the neuronal blocker tetrodotoxin (n = 3; data not shown), suggesting these contractions were likely not due to depolarization of nerve endings in the tissue.

The smooth muscle cell membrane potential of the radial artery was much more depolarized (−52.0 ± 6.8 mV) than that reported in the human internal mammary artery (−58.0 ± 0.84 mV) [12]. This difference could partially explain the greater tendency of the radial artery to undergo vasospasm following coronary bypass grafting, compared to the internal mammary artery. Application of TEA evoked further depolarization (to −29 ± 8 mV) and contraction (20% of the response to KCl, which nearly completely depolarizes the smooth muscle membrane). These data suggest that the KCa channels in the radial artery are active at rest: suppression of these basal currents following bypass surgery might account for the post-surgical vasospasm which plagues this clinical intervention. Voltage-dependent Ca²⁺ channels are responsible for the direct relationship between membrane depolarization and contraction; we have begun to characterize these and found them to exhibit “L-type” characteristics (data not shown). As such, agents which activate K⁺ currents may be useful in treating or even circumventing post-surgical vasospasm.

Consistent with its action as a vasodilator in other vascular beds [4,21], the nitric oxide donor SNAP elicited a marked increase in membrane K⁺ current in human radial artery cells. This augmentation was still seen in the presence of 4-aminopyridine, but was largely occluded by TEA, indicating that the current being activated was a Ca²⁺-dependent K⁺ current. Furthermore, this SNAP-evoked potassium current was relatively insensitive to the soluble guanylate cyclase inhibitor ODQ [9], suggesting that a substantial portion of the effect of nitric oxide might be a direct action on the channel per se. The effect of SNAP was
rapidly reversible, indicating that its mechanism of action did not involve nitrosylation of the channel.

The vasoconstrictor responses to the various potassium channel blockers tested here attest to the importance of voltage-dependent Ca\textsuperscript{2+}-influx in regulating tone in the human radial artery. As such, K\textsuperscript{+}-channel openers including the spasm. The Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current was augmented Ca\textsuperscript{2+}-current(s) present in the human radial artery have not prevent serotonin-evoked spasm\cite{9}. The voltage-dependent channel blocker verapamil was generally regarded as effective, a recent study found that postoperative oral calcium channel blocker therapy with diltiazem did not prevent serotonin-evoked spasm\cite{9}. The voltage-dependent Ca\textsuperscript{2+}-current(s) present in the human radial artery have not yet been characterized, and therefore provide a highly clinically relevant focus for future studies.

In conclusion, we found that the human radial artery manifests mainly Ca\textsuperscript{2+}-dependent K\textsuperscript{+} currents at rest. The activation of the plasmalemmal K\textsuperscript{+} channel leads to change in membrane potential of the smooth muscle cell: a relatively depolarized membrane may be partially responsible for the tendency of vasospasm. As such, agents activate Ca\textsuperscript{2+}-dependent K\textsuperscript{+} currents may prove useful in combating the spasm. The Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current was augmented by a nitric oxide donor through a cGMP- and PKG-independent action.

Acknowledgement

This work was supported by an operating grant from the Heart and Stroke Foundation of Ontario, Canada. The authors also wish to thank Mrs. Mary Helen Blackall for coordinating the transport of human radial artery specimens.

References

\begin{enumerate}
\item Harrison WE, Mellor AJ, Clark J, Singer DR. Vasodilator pre-
treatment of human radial arteries: comparison of effects of phenox-
\item Mussa S, Guzik TJ, Black E, Dipp MA, Channon KM, Taggart DP. Comparative efficacies and durations of action of phenoxbenzamine, varapamil/nitroglycerin solution, and papaverine as topical antispas-
\item Quast U, Stephan D, Bieger S, Russ U. The impact of ATP-sensitive K\textsuperscript{+} channel subtype selection of insulin secretagogues for the coronary vasculature and the myocardium. Diabetes 2004;Suppl. 3:156–64.
\item Stahli BE, Caduff RF, Greutert H, Kipfer B, Carrel TP, Tanner FC. Endothelial and smooth muscle cell dysfunction in human atheroscle-
\end{enumerate}
artery – implications for vasospasm of coronary artery bypass vessels.