INVOLVEMENT OF CALCIUM FLUX IN VASCULAR HYPOREACTIVITY INDUCED BY MECHANICAL INJURY

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The understanding and therapeutic control of abnormal vascular smooth muscle cells (VSMCs) growth is of considerable interest, since this abnormal growth, as consequence of an endothelial damage, is a common pathological feature in several vascular disease such as atherosclerosis, hypertension and restenosis after angioplastic balloon (**1**). After endothelial injury, VSMCs build up the neointima and, together with macrophages and T-lymphocytes, are the main cell type that take part in the formation of atherosclerotic and stenotic lesions. In the early stages of these processes, the VSMCs are modified, by differentiation, from a contractile phenotype to an immature phenotype with syntetic activity. This modification allows the VSMCs to migrate into the intima, to proliferate, and to secrete extracellular matrix components. The vascular reactivity to contracting agents is altered, and differences have been observed whether atherosclerotic lesion is produced by metabolic pathways (hypercholesterolemic diet) or mechanical injury (angioplasty). Indeed, it has been shown that in metabolic-induced atherosclerosis there is an increase in vascular reactivity to contracting agents, attributable to the variations of ionic flux like Ca^{2+} and K^{+} (**2**). In contrast, VSMCs of neointima induced by mechanical injury show hyporeactivity. Some authors address this hyporeactivity to the expression of inducible nitric oxide synthase, but the inhibition of this enzyme did not completely reverse the vascular hyporeactivity (**3**).

In our previous study we have observed that after angioplastic injury, in rat carotid artery, the vascular tissue was hyporeactive to the phenylephrine (PE)-induced stimulation, and the initial contraction disappeared to subsequent stimulations. Our hypothesis is that the mechanical endothelial damage, and the consequent inflammatory process, can impair Ca^{2+} inflow induced by α_1 stimulation.

The purpose of this study was to evaluate if any alteration of Ca^{2+} flux (intra- and/or extra-cellular) could be implied in the vascular hyporeactivity induced by angioplasty.

Endothelial denudation of commune right carotid artery was performed in ketamine and xilazine anaesthetised male Wistar rats by repeated inflations (three times) of a 2F Fogarty arterial embolectomy balloon catheter (**4**). At 14 and 28 days after angioplasty, injured and uninjured carotid arteries were removed, placed in Ca^{2+}-free Hanks' solution and cleaned of connective tissue. VSMCs were isolated via enzymatic dispersion from arteries and loaded with intracellular fluorescent Ca^{2+} indicator FURA 2 by incubation with the membrane-permeant acetoxymethyl ester form of FURA 2-AM (**5**). VSMCs in suspension were transferred to a spectrofluorimeter and intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ was monitored by alternately exciting FURA 2-AM at 340 and 380 nm. Maximum and minimum $[Ca^{2+}]$i were determined at the end of each experiment by treating the cells with 1 µM ionomycin and 15 mmol/l EGTA respectively. The ratios of 340/380 nm were used to calculate $[Ca^{2+}]$i according to Grynkiewicz et al.(**6**). To observe the intra- and extra-cellular calcium fluxes the experiments were performed both in absence and in presence of calcium in the buffer. We measured the effects of PE (0.3 µM) or KCl (60mM) on $[Ca^{2+}]$i

in FURA-2AM loaded VSMCs obtained from injured and uninjured carotid arteries 14 and 28 days after angioplasty balloon.

At 14 days after angioplasty basal $[Ca^{2+}]i$, in Ca^{2+}-free buffer, were significantly lower ($P<0.05$) in cells collected from injured carotid arteries compared to cells isolated from uninjured carotid arteries, while, in Ca^{2+}-containing buffer, no significant differences in basal $[Ca^{2+}]i$ between the cells isolated from injured and uninjured carotid arteries were observed.

After stimulation with PE the percent increase in $[Ca^{2+}]i$ was significantly lower ($P<0.05$ and $P<0.0001$) in VSMCs isolated from injured vessels compared to cells collected from uninjured vessels either in absence than in presence of extracellular Ca^{2+}.

To verify the involvement of voltage operated channels (VOC) in the impairment of Ca^{2+} flux, in injured carotid arteries, KCl was used. The percent of increase in $[Ca^{2+}]i$ was significantly lower in VSMCs collected from injured carotid arteries compared to cells isolated from uninjured arteries both in absence and in presence of extracellular Ca^{2+} ($P<0.005$ and $P<0.001$ respectively).

At 28 days after balloon angioplasty no significant differences in basal $[Ca^{2+}]i$ between VSMCs collected from injured and uninjured carotid arteries were observed, both in absence and in presence of Ca^{2+} into the buffer. After stimulation with PE the percent increase in $[Ca^{2+}]i$ was significantly lower ($P<0.05$) in VSMCs isolated from injured carotid arteries compared to cells collected from uninjured arteries in absence of extracellular Ca^{2+}. In Ca^{2+}-containing buffer the percent increase in $[Ca^{2+}]i$ was significantly lower ($P<0.005$) in cells collected from injured vessels compared to cells isolated from uninjured vessels, but we observed a significant improvement ($P<0.005$) in percent increase in $[Ca^{2+}]i$ compared to cells isolated from injured carotid arteries at 14 days.

The percent increase in $[Ca^{2+}]i$ after KCl stimulation was significantly lower ($P<0.005$) in VSMCs collected from injured carotid arteries compared to cells isolated from uninjured vessels in absence of extracellular calcium.In contrast, in Ca^{2+}-containing buffer no significant differences in percent increase in $[Ca^{2+}]i$ between cells isolated from injured and uninjured vessels were observed. Furthermore the percent increase in $[Ca^{2+}]i$ was significantly higher ($P<0.005$) in VSMCs isolated from injured carotid arteries at 28 days compared to cells isolated from injured vessels 14 days after angioplasty.

These results may indicate that the hyporeactivity to PE and KCl response could be related to an impairment of postreceptorial mechanism of transduction rather than to the alteration of α_1-adrenoreceptors. Furthermore, this hypothesis is, in part, confirmed by the fact that after 28 days an improvement of contraction as well as an increase in Ca^{2+} flux was observed, suggesting that phenotypic properties of VSMCs were ameliorated.

References

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