PAF activation of a voltage-gated R-type Ca^{2+} channel in human and canine aortic endothelial cells

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By the use of fura-2 and digital imaging techniques, $[\text{K}]_o$ depolarization or PAF (10^{-9} M) were shown to induce a sustained increase of $[\text{Ca}]_i$ in human or canine single aortic vascular endothelial cells (VEC) that was insensitive to nifedipine but sensitive to (–)-PN200-110 or to lowering of $[\text{Ca}]_o$. The PAF-induced effect on $[\text{Ca}]_i$ was blocked by the PAF receptor antagonist, WEB2170. Our results suggest that $[\text{K}]_o$ depolarization and PAF increase $[\text{Ca}]_i$ via the activation of R-type Ca^{2+} channels.

Keywords: Endothelial cells; Ca^{2+} channel; PAF; calcium blockers; calcium

Introduction A sustained increase of intracellular free calcium ($[\text{Ca}]_i$) in vascular endothelial cells (VEC) after stimulation by diverse vasoactive agents is dependent on Ca^{2+} influx (Sturek *et al.*, 1991). However, the ionic channels responsible for this sustained increase of $[\text{Ca}]_i$ are not known (Takeda & Klepper, 1990). The difficulty in identifying the Ca^{2+} channel type that is responsible for the sustained increase of $[\text{Ca}]_i$ in VEC has been attributed to damage of these cells during enzymatic dispersion and/or to the use of long term culture preparations (Sturek *et al.*, 1991). Recently, in our laboratory, we have reported the presence of a new type of Ca^{2+} channel (R-type), in several cell types, that is responsible for the sustained increase of $[\text{Ca}]_i$ (Bkaily, 1991; Bkaily *et al.*, 1991; 1992).

The present study was undertaken in order to verify if the voltage-dependent steady-state R-type Ca^{2+} channel exists in VEC and if platelet-activating factor (1-*O*-alkyl-2-acetyl-sn-glycero-3-phosphocholine; PAF)-induced stimulation of $[\text{Ca}]_i$ (Bregestovski & Ryan, 1989) is mediated via activation of this type of channel.

Methods VEC were isolated as single cells from the luminal surface of human and adult mongrel dog aortae by use of 0.1% trypsin solution as previously described for vascular endothelial cells (Sturek *et al.*, 1991); the single cells were left to attach (within 1 h) to a glass cover slip that fitted a bath chamber (1 ml) of 20 mm diameter (Bkaily *et al.*, 1992). The work was performed in accordance with the requirements of the institutional review committee for the use of human material. The technique used to load the VEC with fura-2 and the Ca^{2+} measurement technique in single cells using an Imagescan microfluorometer (Photo Technology International Inc., Princeton, NJ, U.S.A.) are identical to those previously described for vascular smooth muscle cells (Bkaily *et al.*, 1992).

Freshly isolated VEC were superfused (5 ml min^{-1}) with Tyrode solution containing (in mM) either: NaCl 130, KCl 5, CaCl_2 2, HEPES 10 and glucose 10 or NaCl 105, KCl 30, CaCl_2 2, HEPES 10 and glucose 10. The solutions were buffered to pH 7.4 with Tris base and the osmolality was adjusted with sucrose to 310 mOsm (Advanced DigiMatic Osmometer 3DII, Massachusetts, U.S.A.). The dual L-(Striessnig *et al.*, 1986) and R-type (Bkaily *et al.*, 1992) Ca^{2+} channel blocker (–)-isradipine (PN200-110) was obtained from Sandoz Canada Inc. PAF was obtained from Bachem (U.S.A.) and WEB2170 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]-diazepin-2-yl]-1-(4-mor-

pholimyl)-1-propanone; Casals-Stenzel, 1987) was a generous gift of H. Heuer (Boehringer-Ingelheim, Germany). The fura-2AM was purchased from Molecular Probes Inc. (Eugene, OR, U.S.A.) and all other substances were purchased from Sigma (St Louis, MI, U.S.A.).

Data used in the text and figures are expressed as means $\pm$ s.e.mean of *n* different observations. Statistical comparisons between controls and each experimental interaction were made by paired Student's *t* test. *P* values of < 0.05 were considered to be statistically significant. All experiments were carried out at 28°C.

Figure 1 Blockade of the sustained increase of $[\text{Ca}]_i$ induced with high $[\text{K}]_o$ by PN200-110 in human aortic vascular endothelial cells. (a–b) control (5 mM $[\text{K}]_o$) digital colour image maps of basal steady state free $[\text{Ca}]_i$ at 1 min (170 nM) and 5 min (170 nM) recording. (c) Increase in $[\text{Ca}]_i$ taken at 1 min (280 nM) in presence of high $[\text{K}]_o$ (30 mM). (d–e) Sustained increase in $[\text{Ca}]_i$ taken at 2 min (d, 560 nM) and 5 min (e, 560 nM) in presence of high $[\text{K}]_o$. (f–k) Superfusion with high $[\text{K}]_o$ containing 10^{-7} M PN200-110 rapidly (f, at 1 min, 262 nM) and progressively blocked the sustained increase of $[\text{Ca}]_i$ induced with high $[\text{K}]_o$. Addition of 30 mM EGTA did not further decrease the $[\text{Ca}]_i$ (156 nM).

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Results The basal level of $[Ca]_i$ of human and canine VEC in normal K^+ (5 mM) solution was 135 ± 24 nM ($n = 12$) 154 ± 27 nM ($n = 12$) respectively. Sustained depolarization with 30 mM KCl induced (within 1 min) a steady-state increase of $[Ca]_i$ in both human (from 132 ± 21 to 506 ± 63 nM, $n = 5$) and canine (from 156 ± 21 to 281 ± 33 nM, $n = 5$) VEC that was, as in vascular smooth muscle cells (VSMC) (Bkaily *et al.*, 1992), insensitive to the L-type Ca^{2+} blocker nifedipine (Figure 2) but sensitive to the dual Ca^{2+} L- and R-type blocker PN200-110 (Figure 1, an example out of 8 experiments and Figure 2) and to the Ca^{2+} chelator EGTA (Figure 2). As for high $[K]_o$, superfusion with a Tyrode solution containing 10^{-9} M PAF induced (within 1 min) a sustained increase of $[Ca]_i$ of both human and canine VEC (Figure 2) that was insensitive to nifedipine (10^{-5} M) but significantly decreased by PN200-110 (10^{-7} M). The remaining increase of $[Ca]_i$ was completely blocked by 30 mM EGTA (Figure 2). The PAF-receptor antagonist, WEB2170 (10^{-5} M) significantly decreased the PAF-induced sustained increase of $[Ca]_i$ (Figure 2).

Discussion Our study demonstrates that the mean basal resting $[Ca]_i$ in human and canine aortic VEC is similar, and comparable to values obtained in endothelial cells from bovine aorta (Sturek *et al.*, 1991) and human umbilical cords (Jacob *et al.*, 1988). Our results show that a voltage-sensitive Ca^{2+} channel does exist in VEC and that this channel is similar to the nifedipine-insensitive and PN200-110-sensitive R-type Ca^{2+} channel described in heart and vascular smooth muscle cells in response to endothelin-1 and insulin (Bkaily *et al.*, 1991; 1992). This channel in human and canine VEC seems also to be responsive to PAF. Since the PAF receptor antagonist, WEB2170, reversed the effect of PAF, it may suggest that the effect of PAF on the R-type Ca^{2+} channel is mediated via a receptor-dependent mechanism which remains to be determined. Our results also show that high $[K]_o$ or PAF induced a 4 fold increase of steady-state $[Ca]_i$ in human VEC and a 2 fold increase in canine VEC. These species differences in response to sustained depolarization or PAF could be due to dependence of the steady-state increase of $[Ca]_i$ on mechanisms that are responsible for Ca^{2+} sequestration and/or efflux.

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Figure 2 High $[K]_o$ (30 mM) depolarization (■) and PAF (10^{-9} M, ▨)-induced a sustained increase of $[Ca]_i$ in both human (H) and canine (C) vascular endothelial cells that was completely blocked by 30 mM EGTA (▩) (a, c and d). The high $[K]_o$ or PAF-induced sustained increase of $[Ca]_i$ in both preparations is insensitive to 10^{-5} M nifedipine (▩) but sensitive to 10^{-7} M PN200-110 (▧) (Hb, c and Cb, c) and the PAF receptor antagonist, WEB2170 (10^{-5} M, ▧) (Hd and Cd for PAF only; effects against high $[K]_o$ are not shown). The control steady-state $[Ca]_i$ basal level is □. The number of experiments (n) in Ha, Ca, Hb, Cb, Hc, Cc, Hd and Cd are respectively 5, 5, 4, 4, 3, 4, 4, and 3. * $P < 0.01$ compared to control values only. Substances were added sequentially at intervals of approximately 7 min in the order indicated.

We suggest that the presence of an R-type Ca^{2+} channel that could be gated by voltage or a receptor in VEC may play an important role in excitation-secretion coupling mechanisms in the VEC and subsequent release of vasoactive factors such as endothelium-derived relaxing factor and endothelin-1.

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