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Loss of receptor-mediated ^{86}Rb efflux from pig aortic endothelial cells in culture

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The responsiveness of freshly-isolated and subcultured pig aortic endothelial cells to adenosine triphosphate (ATP), bradykinin and ionophore A23187 was compared by monitoring agonist-induced ^{86}Rb efflux. ATP, bradykinin and ionophore A23187 stimulated ^{86}Rb efflux from freshly-isolated cells. ATP and bradykinin, which act via specific receptors, were less effective at inducing ^{86}Rb efflux from subcultured cells but ionophore A23187 was as effective on subcultured as on freshly-isolated cells. These results suggest that pig aortic endothelial cells, when subcultured, lose receptors for ATP and bradykinin and/or develop an abnormality in the coupling-mechanism between receptor-occupation and calcium-mobilization.

Introduction The responsiveness of vascular endothelial cells to a range of vasodilator agents has been studied indirectly by measuring endothelium-dependent relaxation (Furchgott & Zawadzki, 1980; Altura & Chand, 1981; De Mey & Vanhoutte, 1981; Gordon & Martin, 1983). We have recently introduced a technique which can be used to assess directly the responsiveness of endothelial cells to certain stimuli by monitoring calcium-activated potassium efflux, using ^{86}Rb (Gordon & Martin, 1982; 1983).

Endothelial cells can be grown readily in culture (Jaffe, Nachman, Becker & Minick, 1973; Gimbrone, 1976), thus providing large numbers of cells for experimentation. In the experiments described in this study, we compared the ability of a range of agents to induce ^{86}Rb efflux from freshly-isolated and subcultured pig aortic endothelial cells, to determine whether the responsiveness of the cells changed during culture.

Methods Endothelial cells were isolated from the aortae of 1–14 day old pigs by collagenase treatment (Pearson, Carleton, Hutchings & Gordon, 1978), and maintained in culture for up to 18 passages in the present study. ^{86}Rb efflux from superfused monolayers of freshly isolated (tested within 48 h of isolation) and subcultured (5–18 passages) aortic endothelial cells prelabelled with ^{86}RbCl (5–10 μCi ml^{-1}; Amersham International) was measured as previously described (Gordon & Martin, 1983).

Adenosine triphosphate (ATP) and bradykinin triacetate were purchased from Sigma, Dorset; ionophore A23187 was purchased from Calbiochem-Behring, Herts.

Results are expressed as mean values ± s.e.mean (of n replicate experiments).

Results The first-order rate constants for basal ^{86}Rb efflux from prelabelled, superfused monolayers of freshly-isolated and subcultured pig aortic endothelial cells were $1.9 \pm 0.02 \times 10^{-2}$ min^{-1} ($n = 24$) and $1.76 \pm 0.05 \times 10^{-2}$ min^{-1} ($n = 16$) respectively. ATP (10^{-4} M), bradykinin (10^{-7} M) and ionophore A23187 (10^{-5} M) induced maximal stimulations of ^{86}Rb efflux from freshly-isolated cells of 1.8 ± 0.04, 4.4 ± 0.1 and 6.4 ± 0.2 times the basal rate of efflux, respectively ($n = 7–9$). ATP (10^{-4} M) and bradykinin (10^{-7} M) induced much smaller responses in subcultured aortic endothelial cells: 1.1 ± 0.01 and 1.7 ± 0.1 times the basal rate respectively ($n = 6$). Statistical comparisons of the responses to ATP and bradykinin in freshly isolated and subcultured cells were significant at $< 0.1\%$ level. Higher concentrations of ATP and bradykinin did not elicit larger responses in these cells. The ionophore A23187 (10^{-5} M) increased the rate of ^{86}Rb efflux from subcultured cells by 5.8 ± 0.2 times ($n = 5$), a value comparable with that from freshly isolated cells. Representative responses of freshly isolated and subcultured aortic endothelial cells to ATP, bradykinin and ionophore A23187 are shown in Figure 1.

Discussion ATP and bradykinin induce ^{86}Rb efflux from freshly isolated pig aortic endothelial cells by a calcium-activated process (Gordon & Martin, 1982; 1983). These responses were much reduced when endothelial cells were grown in culture, suggesting that in these cells there is an abnormality in some step(s) involved in stimulus-response coupling. The

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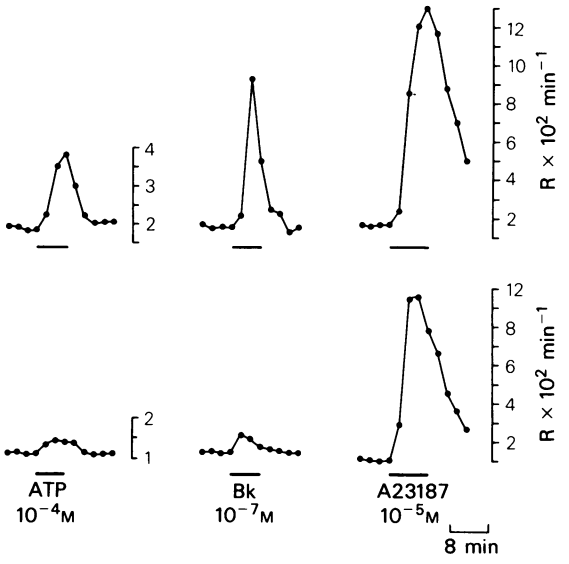

Figure 1 The effects of ATP, bradykinin (Bk) and ionophore A23187 on the rate of ^{86}Rb efflux from freshly isolated (top row) and 5–18th passage subcultured (bottom row) pig aortic endothelial cells. Each point represents the first-order rate constant for efflux (unit of min^{-1}) in 2 min fractions of superfusate; the ordinate values have been multiplied by the factor shown ($\times 10^2$) in order to obtain the units of the scale as displayed. Note that the scale for ATP is different from that for bradykinin and ionophore A23187.

finding that the calcium ionophore A23187 induces a similar degree of stimulation of ^{86}Rb efflux in freshly-isolated and subcultured cells indicates that the calcium-activated potassium efflux mechanism is unchanged in subcultured pig aortic endothelial cells. It therefore appears that subcultured endothelial cells either lack receptors for ATP and bradykinin or have undergone some change in the coupling mechanism(s) that link(s) receptor-occupation and calcium-mobilization. We previously observed in subcultured aortic smooth muscle cells a loss of the ^{86}Rb efflux response to noradrenaline and histamine but not to depolarizing solutions of KCl (Martin & Gordon, 1983a,b). The reduction in sensitivity of subcultured pig aortic endothelial cells is not restricted to stimulation of ion-flux since release of prostacyclin by bradykinin and thrombin is also reduced when the cells are grown in culture (Pearson, Carleton & Hutchings, 1983).

In conclusion, by monitoring ^{86}Rb efflux we have found a loss of responsiveness of subcultured cells to certain agents which act through specific membrane receptors but not to stimuli which increase the intracellular level of calcium directly. Care must be taken therefore when using subcultured pig aortic endothelial cells, since their responses to vasoactive agents may not reflect those of freshly isolated cells or endothelium *in situ*.

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