PHYSIOLOGY

Acetylcholine and ATP Hyperpolarize Endothelium via Activation of Different Types of Ca^{2+}-Activated K^+ Channels

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Experiments on rat thoracic aorta showed that ATP and acetylcholine hyperpolarize endothelial cell via selective activation of low (SK) and intermediate (IR) conductance Ca^{2+}-activated K^+ channels, respectively. It was hypothesized that ATP- and acetylcholine-activated Ca^{2+}-signals are spatially separated and generated in plasma membrane regions with predominant localization of SK- and IR-channels, respectively.

Key Words: *endothelial cells; membrane potential; Ca^{2+}-activated K^+-channels*; *acetylcholine; ATP*

Acetylcholine (ACH), ATP, and some other endothelium-dependent vasodilators induce Ca^{2+} release from intracellular depot and hyperpolarization of endothelial cells (HEC) mediated by activation of Ca^{2+}-activated K^+ channels (K(Ca) channels) [2,4,5,7,9]. HEC plays a dual physiological role: first, it stimulates Ca^{2+} entry into endothelial cells thereby affecting Ca^{2+}-dependent intracellular processes (in particular, NO synthesis, [2,8,9]) and second, HEC spreads to vascular smooth muscle via gap junctions and contributes to NO-independent vascular relaxation [1,6].

Low and intermediate conductance K(Ca) channels (SK- and IR-channels, respectively) were previously identified in endothelial cells of rat thoracic aorta [7]. It is known that ACH induces HEC via selective activation of IR channels.

Our aim was to identify channels (SK or IR) mediating ATP-induced hyperpolarization of intact endothelial cells.

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MATERIALS AND METHODS

Membrane potential of rat aorta endothelium was recorded using a modified method [4]. The rats aged from 3 weeks to 2 months were narcotized with ether. The thoracic aorta was isolated and the adipose tissue was removed. The aorta was cut into 3-4 mm segments and stored in modified Krebs solution containing (in mM): 118.3 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 11.1 $C_2H_{12}O_6$, 0.02 phenol red, oxygenated with 95% O_2 and 5% CO_2. To prevent bacterial contamination, 50 μg/ml gentamicin was added to the solution. Before recordings, the segments were cut longitudinally, attached (epithelium upside) to the bottom of experimental chamber (volume 100-150 μl), and perfused with Krebs solution at a rate of 0.1-0.2 ml/min.

Membrane potential was measured using the perforated patch clump technique. Pipette was filled with following solution (in mM): 140 KCl, 10 NaCl, 10 HEPES-KOH, pH 7.3. Nystatin (200 μg/ml) was added to this saline immediately before filling the pipette. All experiments were carried out at 20-22°C.

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RESULTS

For identification of ionic channels responsible for hyperpolarization, we examined the effect of various K(Ca) channel blockers on hyperpolarization induced by ACH and ATP. Previously we showed that charybdotoxin in a concentration of about 100 nM blocks IR-channels in endothelial cells of intact vessels, but does not affect SK-channels [7]. Charybdotoxin in a concentration of 300 nM blocked ACH-induced HEC, but had no effect on ATP-induced HEC even in very high concentrations (up to 500 nM, Fig. 1, *n*=5). Thus, in contrast to ACH-induced hyperpolarization, ATP-evoked hyperpolarization was not associated by activation of IR-channels.

Apamin, another blocker of K(Ca) channels, blocks SK-channels on endothelial cells, but has no effect on IR-channels [7]. In our experiments, apamin (10 nM) blocked ATP-induced HEC, but produced no effect on ACH-induced HEC (Fig. 2, *n*=7). Blockade of ATP-induced HEC was reversible, but long-term (>20 min) washout of the preparation was needed to restore hyperpolarization. These data indicate that ATP-induced HEC is related to opening of SK-type Ca^{2+}-activated K^+-channels.

To test this hypothesis, d-tubocurarine, another blocker of Ca^{2+}-activated K^+-channels, was used. We previously showed that d-tubocurarine (100 μM) selectively blocks SK-channels on endothelial cells [7]. At this concentration, it does not affect IR-channels, whose amplitude is slightly attenuated only when the blocker is used in millimolar concentrations. Here d-tubocurarine attenuated ATP-induced HEC at low concentrations (20-100 μM), but did not affect ACH-induced HEC (*n*=4).

These results suggest that ATP-induced HEC is associated with selective activation of SK-type Ca^{2+}-activated K^+-channels.

At the same time, these findings corroborate our previous conclusion that ACH-induced HEC is associated with activation of IR-channels [7]. Thus, despite both ACH and ATP increase intracellular concentration of Ca^{2+} ions in intact endothelium [9], they activate different types of Ca^{2+}-activated K^+-channels. Similar phenomenon was observed in experiments on cultured endothelial cells from pig coronary artery, where bradykinin blocked predominantly BK-channels, while substance P selectively inhibited SK-channels [3].

We previously showed that IR-channels on endothelial cells are activated at intracellular calcium concentrations of 0.1-1 μM (EC_{50}=0.34 μM), while activation of SK-channels of these cells occurs at significantly higher concentrations (>0.5 μM) [7]. We also showed that in this preparation, ATP produces a more pronounced increase in intracellular calcium concen-

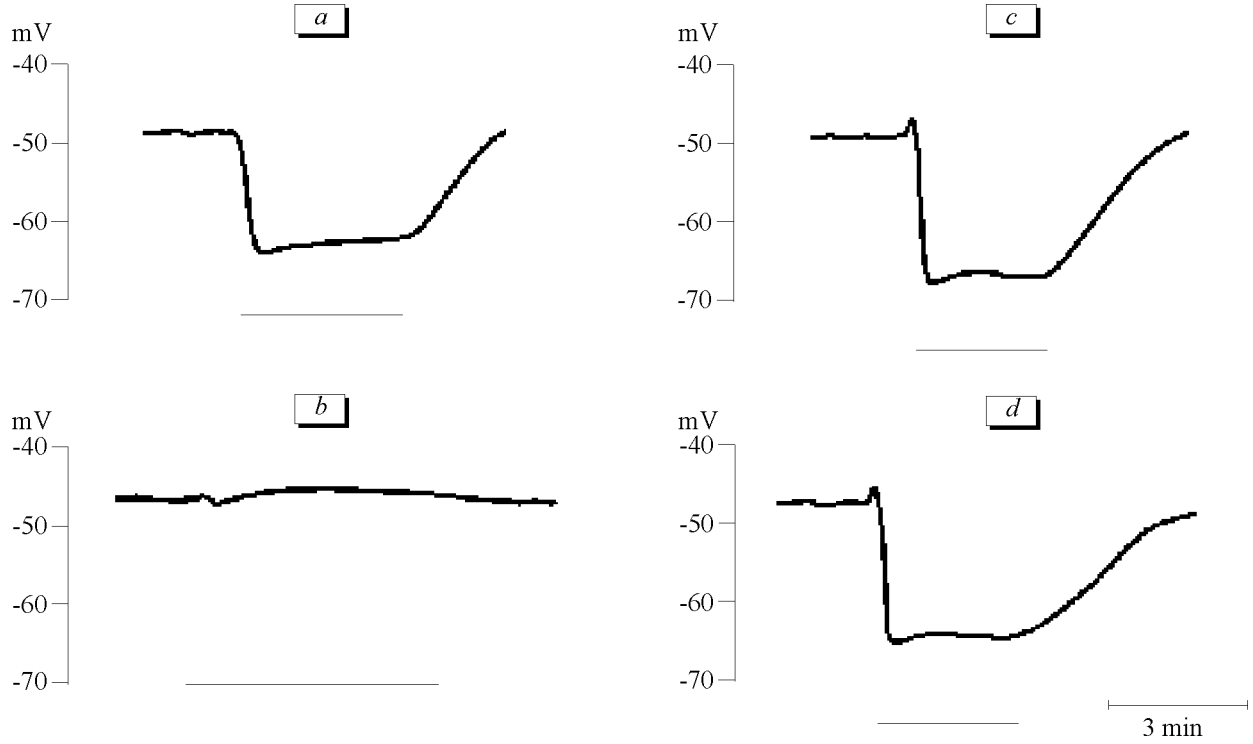

Fig. 1. Effect of charybdotoxin (300 nM) on hyperpolarization of endothelial cells from intact rat aorta induced by acetylcholine (*a*, *b*) and ATP (*c*, *d*). Here and in Fig. 2: *a*, *c*) control; *b*, *d*) corresponding blockers. Marks under curves show periods of application of ATP (100 μM) and ACH (10 μM).

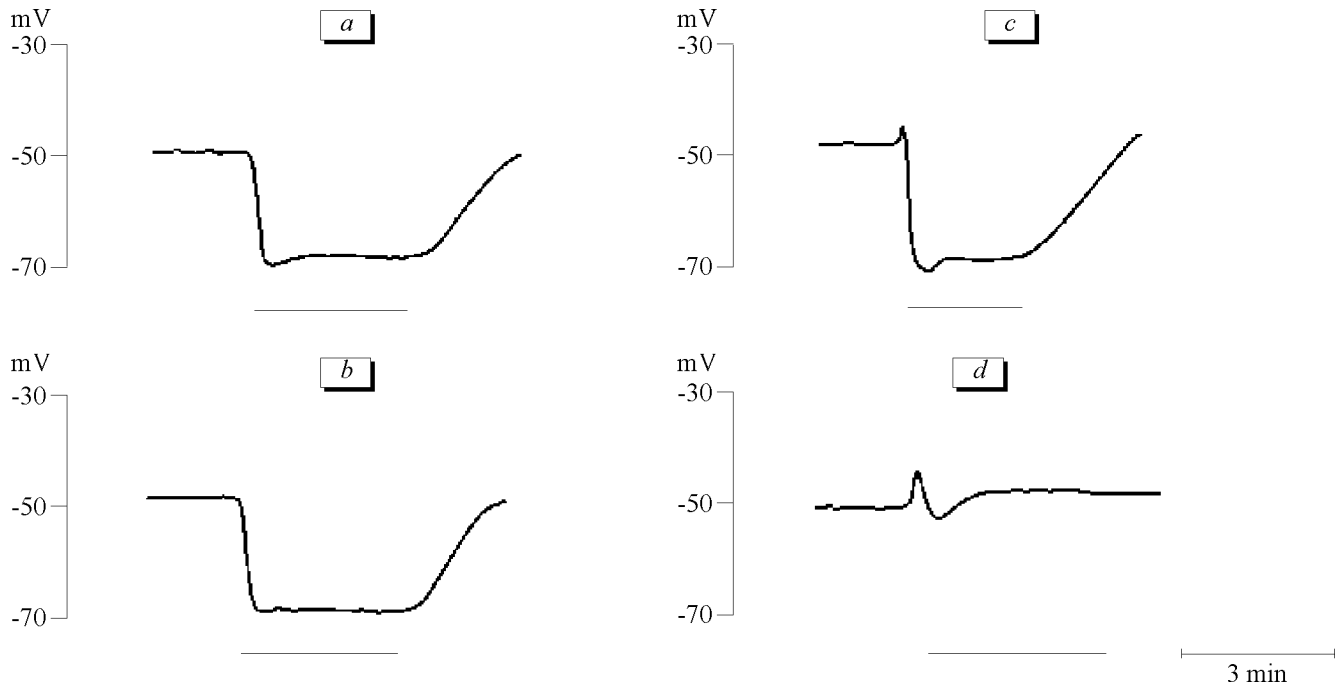

Fig. 2. Effect of apamin (10 nM) on hyperpolarization of endothelial cells from intact rat aorta induced by acetylcholine (*a*, *b*) and ATP (*c*, *d*).

tration than ACH. It can be hypothesized that ACH increased intracellular calcium concentration to a level sufficient for activation of IR-channels, but too low for opening of SK-channels. However, the capacity of ATP to activate SK-channels selectively without opening IR-channels is an unexpected finding, because activation of SK-channels occurs at calcium concentration corresponding to complete activation of IR-channels.

This contradiction can be explained by a hypothesis that ATP increases concentration of Ca^{2+} ions only in membrane regions containing SK-channels, but virtually does not change it near IR-channels. In other words, the Ca^{2+} signals induced by different agonists and the corresponding Ca^{2+}-activated K^+-channels are spatially separated.

We previously showed that the density of IR-channels in the luminal membrane of endothelial cells is extremely low and insufficient for generation of hyperpolarizing current [7]. However, it is unlikely that the close correlation of IR-channel pharmacology and ACH-induced hyperpolarization is accidental. We hypothesized that IR-channels are primarily localized in subluminal membrane of the endothelium, which is not accessible for patch-clamp recording. It can be further assumed that ATP generates Ca^{2+}-signal selectively at the luminal membrane of endothelial cells, while has no effect on intracellular calcium concentration at the subluminal membrane. Similar compartmentalization of Ca^{2+}-signals can be associated with predominant location of ATP receptors on the luminal surface of the epithelium. This spatial separation of Ca^{2+}-signaling corresponds to natural functional polarization of the endothelium.

REFERENCES

1. J. L. Beny and C. Pacicca, *Am. J. Physiol.*, **266**, H1465-H1472 (1994).
2. R. Busse, H. Fichtner, A. Luckhoff, and M. Kohlhardt, *Ibid.*, **255**, H965-H969 (1988).
3. M. Frieden, M. Sollini, and J. Beny, *J. Physiol.*, **519**, 361-371 (1999).
4. S. M. Marchenko and S. O. Sage, *Ibid.*, **462**, 735-751 (1993).
5. S. M. Marchenko and S. O. Sage, *Am. J. Physiol.*, **266**, H2388-H2395 (1994).
6. S. M. Marchenko and S. O. Sage, *Ibid.*, **267**, H804-H811 (1994).
7. S. M. Marchenko and S. O. Sage, *J. Physiol.*, **492**, 53-60 (1996).
8. B. Nilius, F. Viana, and G. Droogmans, *Annu. Rev. Physiol.*, **59**, 145-170 (1997).
9. Y. M. Usachev, S. M. Marchenko, and S. O. Sage, *J. Physiol.*, **489**, 309-317 (1995).