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Endothelial P2Y receptors induce hyperpolarisation of vascular smooth muscle by release of endothelium-derived hyperpolarising factor

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Abstract

The effects of P2Y receptor agonists on smooth muscle membrane potential in isolated ring segments of rat mesenteric artery were examined by intracellular microelectrodes. In the presence of inhibitors of nitric oxide-synthase and cyclo-oxygenase, the selective $P2Y_1$ receptor agonist adenosine 5′-*O*-thiodiphosphate (ADPβS) induced endothelium-dependent membrane hyperpolarisations, which were abolished by a combination of the K^+ channel inhibitors charybdotoxin and apamin, providing direct evidence that ADPβS releases endothelium-derived hyperpolarising factor (EDHF). 2-MethylthioATP and ATP, each which stimulates both endothelial P2Y receptors and P2X receptors on the smooth muscle cells, also elicited hyperpolarisation, but only after desensitisation of P2X receptors with αβ-methylATP indicating that simultaneous activation of P2X receptors may counteract the action of EDHF. In conclusion, activation of endothelial P2Y receptors induce release of EDHF.

Keywords: Membrane potential; K^+ channel; Purinoceptor; Vascular endothelium

1. Introduction

ATP and other nucleotides are released from sympathetic nerves, platelets and endothelial cells (Gordon, 1986). These endogenous substances induce both vasoconstriction and vasodilatation in many vascular beds via activation of P2 receptors (Burnstock, 1990). Four of the recently cloned P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$) have been shown to mediate vascular effects (Harden et al., 1998). It has been suggested that activation of P2Y receptors induces endothelium-dependent vasodilatation mediated by a factor distinct from nitric oxide (NO) and prostacyclin (Ralevic and Burnstock, 1991; Saiag et al., 1996; You et al., 1997; Malmsjö et al., 1998). In the rat mesenteric artery, P2Y receptor-induced vasodilatation is antagonised by a combination of the K^+ channel inhibitors charybdotoxin and apamin (Malmsjö et al., 1998), which prevents hyperpolarisation and relaxation mediated by endothelium-derived hyperpolarising factor (EDHF) in different blood vessels (Corriu et al., 1996; Zygmunt and Högestätt, 1996; Chataigneau et al., 1998; Zygmunt et al., 1998). However, no study has provided direct evidence that EDHF is released by activation of endothelial P2Y receptors. We therefore performed microelectrode experiments to examine the effects of P2Y receptor agonists on the membrane potential in isolated ring segments of rat mesenteric artery.

2. Materials and methods

2.1. Experimental procedure

Female Sprague–Dawley rats (200 g) were anaesthetised by inhalation of CO_2 and killed by a cardiac cut. The mesenteric artery was cut into cylindrical segments

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(2 mm long) and mounted in a tissue bath containing a bicarbonate-based physiological salt solution (PSS) at a temperature of 37°C. The PSS was continuously gassed with 5% CO_2 in O_2, resulting in a pH of 7.4. The arterial segments were then allowed to stabilise at a tension of 2 mN for 1 h (for details, see Zygmunt et al., 1994). The endothelium was removed by perfusion for 5 s with 0.1% Triton X followed by another 5 s of perfusion with a PSS using a fine needle (Malmsjö et al., 1998).

Recordings of membrane potential were made with glass microelectrodes filled with 0.5 M KCl (tip resistance 60–150 MΩ). The microelectrode was advanced from the adventitial side of the artery by a motor driven micromanipulator (Burleigh Instruments, USA). A silverchloride pellet immersed in the bath solution was used as reference electrode. The microelectrode was connected to an Axoprobe-1A amplifier (Axon Instruments, USA), and the membrane potential was displayed on a pen recorder (Model BD 41, Kipp and Zonen, Holland) and oscilloscope (Model 5040, Kikusui, Japan). Successful impalements were characterised by a sudden negative shift in voltage, followed by a stable negative potential for at least 5 min (for details, see Zygmunt et al., 1994).

2.2. Drugs

Acetylcholine, αβ-methylATP (αβ-MeATP), adenosine-triphosphate (ATP), 2-methylthioATP (2-MeSATP), adenosine 5′-*O*-thiodiphosphate (ADPβS), *N*ω-nitro-L-arginine (L-NOARG), charybdotoxin and apamin (Sigma, USA). Indomethacin (Confortid®, Dumex, Denmark). The stock solutions were dissolved in 0.9% saline.

2.3. Calculations and statistics

Data are presented as mean ± S.E.M., and *n* denotes the number of impalements from three animals.

3. Results

All experiments were performed in the presence of L-NOARG ($10^{-3.5}$ M) and indomethacin (10^{-5} M) to eliminate any contribution of NO and prostaglandins, respectively (Zygmunt et al., 1998). In the presence of these enzyme inhibitors the resting membrane potential of the smooth muscle cells was -52 ± 1 mV ($n = 9$).

Both ACh and ADPβS, each at a concentration of 10^{-5} M, induced smooth muscle hyperpolarisation (Figs. 1 and 2), which amounted to 14 ± 1 mV ($n = 9$) and 11 ± 4 mV ($n = 6$), respectively. However, these agents failed to induce hyperpolarisation when both charybdotoxin ($10^{-7.5}$ M) and apamin (10^{-6} M) were present in the bath solution (Fig. 2). Instead, ADPβS and ACh caused a small transient depolarisation under these conditions (Fig. 2).

Addition of αβ-MeATP (10^{-7} M) induced a transient depolarisation amounting to 7 ± 2 mV ($n = 5$; Fig. 2). After 10 min in the continuous presence of αβ-MeATP, the resting membrane potential returned to its initial value (± 1 mV, $n = 5$). Subsequent addition of 2-MeSATP (10^{-5} M) induced a hyperpolarisation amounting to 6 ± 1 mV ($n = 4$; Figs. 1 and 2). However, no hyperpolarisation was

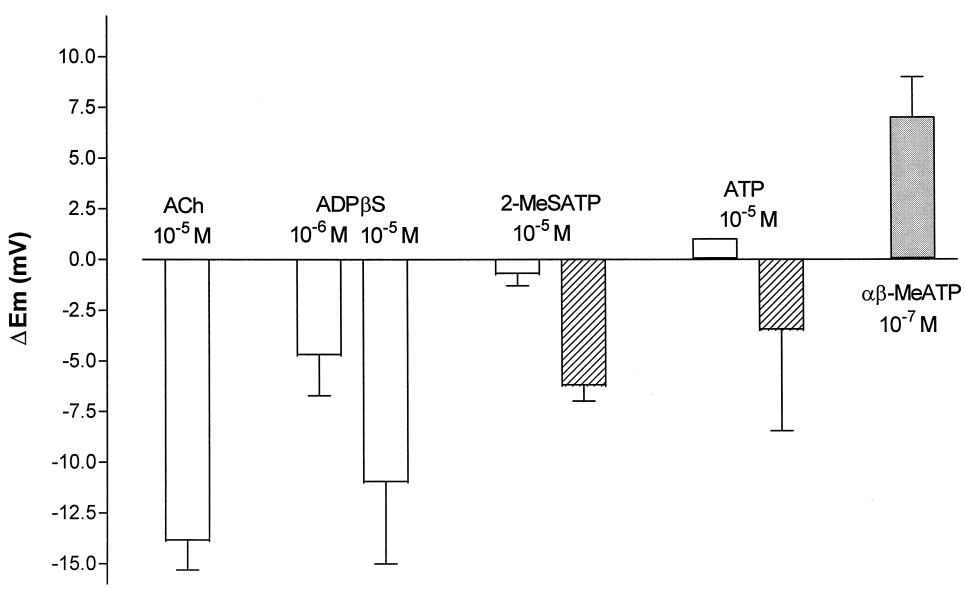

Fig. 1. Hyperpolarisation of smooth muscle cells induced by acetylcholine (10^{-5} M) and the P2Y receptor agonists ADPβS (10^{-6} and 10^{-5} M), 2-MeSATP (10^{-5} M) and ATP (10^{-5} M) in the presence of L-NOARG ($10^{-3.5}$ M) and indomethacin (10^{-5} M). For 2-MeSATP and ATP, results are presented both in the absence (white bars) and in the presence of prior P2X receptor desensitisation with 10^{-7} M αβ-MeATP (hatched bars). The depolarisation elicited by 10^{-7} M αβ-MeATP is shown in grey. Data are presented as mean ± S.E.M.

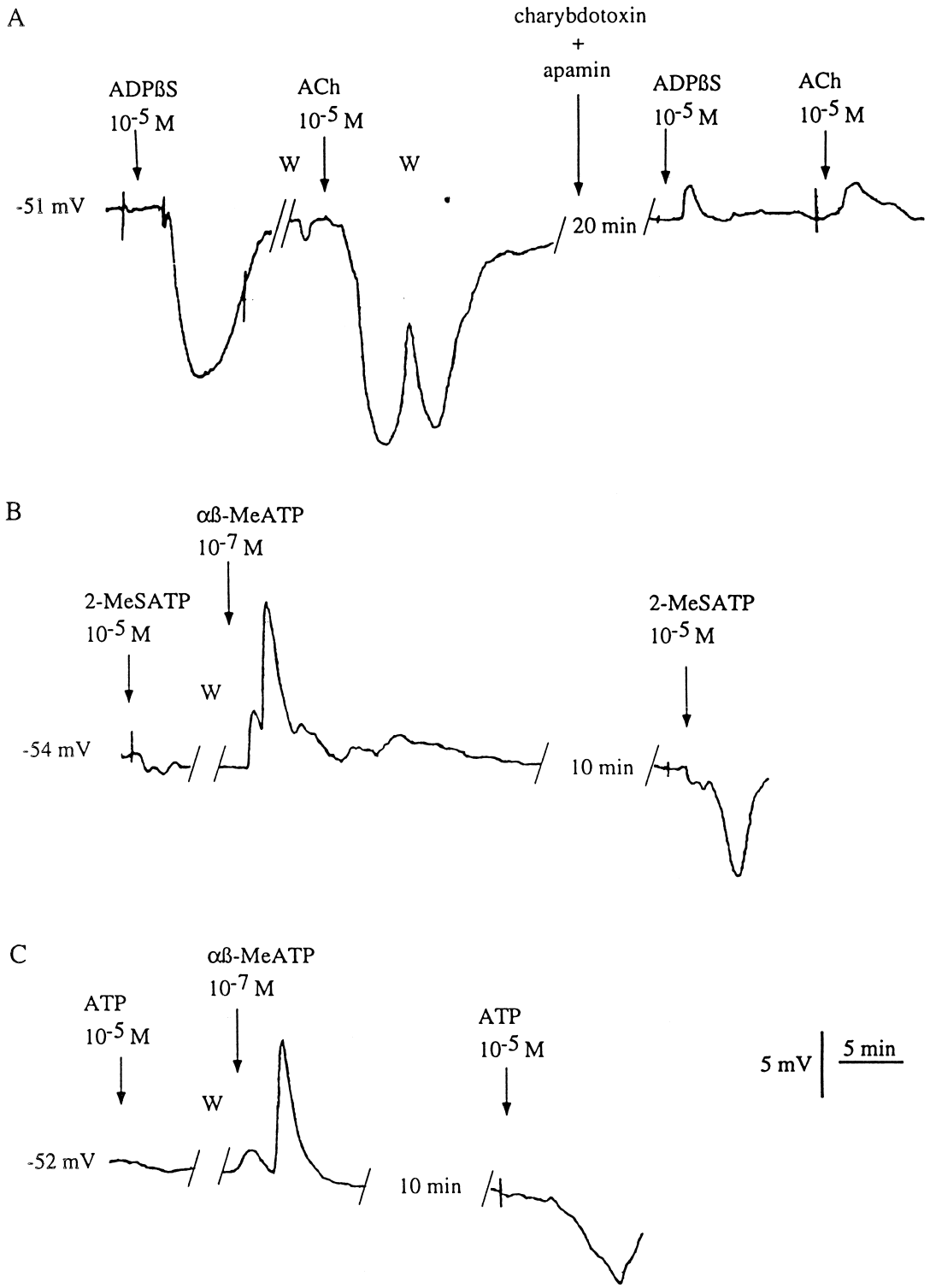

Fig. 2. Traces showing hyperpolarisation of smooth muscle cells at resting membrane potential in the presence of L-NOARG ($10^{-3.5}$ M) and indomethacin (10^{-5} M). (a) ADPβS and acetylcholine, each at a concentration of 10^{-5} M, induced hyperpolarisations which were blocked by a combination of charybdotoxin ($10^{-7.5}$ M) and apamin (10^{-6} M). These potassium channel inhibitors were added 20 min before the addition of ADPβS and acetylcholine, and were present throughout the experiment. 2-MeSATP (b) and ATP (c) only induced hyperpolarisation after desensitisation of P2X receptors with 10^{-7} M αβ-MeATP, the application of which depolarised smooth muscle cells. W denotes washout of drugs.

obtained in preparations in which P2X receptors were not desensitised with αβ-MeATP (Fig. 2). ATP (10^{-5} M) also did not cause smooth muscle hyperpolarisation in the absence of αβ-MeATP ($n = 3$; Fig. 2). Hyperpolarisation could, however, be revealed in two of three cells (3 and 7 mV) after desensitation of the P2X receptor with αβ-

MeATP (10^{-7} M, Fig. 2). At 10^{-6} M we saw a slight hyperpolarisation (1 mV) for both ATP and 2-MeSATP after desensitation and no depolarisation when the P2X receptors were still active. However, this difference was not significant.

Denudation totally abolished the hyperpolarisation to acetylcholine (1 ± 0 mV), ADPβS (0 ± 1 mV), 2-MeSATP (-1 ± 1 mV) and ATP (0 ± 1 mV), $n = 3$, all agonists 10^{-5} M.

4. Discussion

The present study shows that stimulation of endothelial P2Y receptors induces endothelium-dependent hyperpolarisation of vascular smooth muscle via release of EDHF. Although it has been demonstrated that ATP and ADP elicit smooth muscle hyperpolarisation in cat cerebral, guinea-pig and rabbit coronary, and rabbit carotid arteries (Brayden and Wellman, 1989; Chen and Suzuki, 1991; Keef et al., 1992), it is unclear whether EDHF is the mediator of these responses since inhibitors of NO synthase and cyclo-oxygenase were not present in these studies. Indeed, NO and cyclo-oxygenase metabolites mediate endothelium-dependent smooth muscle hyperpolarisation in rabbit mesenteric, guinea-pig coronary and carotid, and rat hepatic arteries (Parkington et al., 1993; Murphy and Brayden, 1995; Corriu et al., 1996; Zygmunt et al., 1998). All experiments in the present study were therefore performed in the presence of both L-NOARG and indomethacin, conditions under which EDHF is the sole mediator of acetylcholine-induced hyperpolarisation in rat mesenteric arteries (Chataigneau et al., 1998). We confirm the finding made by Chataigneau et al. (1998) that Acetylcholine-induced hyperpolarisation of rat mesenteric artery in the presence of L-NOARG and indomethacin is completely inhibited by the combination of charybdotoxin and apamin.

In the present study, the selective $P2Y_1$ receptor agonist ADPβS released EDHF, since this agent induced a smooth muscle hyperpolarisation which was blocked by charybdotoxin plus apamin and abolished by endothelial denudation. Furthermore, in rat mesenteric arteries, we have found that stimulation of P2Y receptors triggers endothelium-dependent relaxations, which are inhibited by this toxin combination in the presence of L-NOARG and indomethacin (Malmsjö et al., 1998). In addition to ADPβS, the $P2Y_1$ receptor is a target for 2-MeSADP, 2-MeSATP, ADP and ATP, the latter being least potent (Harden et al., 1998). However, 2-MeSATP and ATP also stimulate P2X receptors on smooth muscle leading to depolarisation (Evans et al., 1998). In the present study, activation of such receptors by the selective P2X receptor agonist αβ-MeATP induced a transient depolarisation. Only after prolonged exposure to αβ-MeATP, a commonly used approach to desensitise these receptors (O'Connor et al., 1990), could a hyperpolarisation be revealed in response to 2-MeSATP. The less stable agonist ATP elicited hyperpolarisation after desensitisation of P2X receptors in two out of three preparations. It is thus possible that activation of P2X receptors on smooth muscle cells counteracts the hyperpolarising effect of EDHF when evoked by 2-MeSATP and ATP.

We conclude that stimulation of endothelial $P2Y_1$ receptors induce smooth muscle hyperpolarisation via release of EDHF.

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References

Brayden, J.E., Wellman, G.C., 1989. Endothelium-dependent dilation of feline cerebral arteries: role of membrane potential and cyclic nucleotides. J. Cereb. Blood Flow Metab. 9, 256–263.

Burnstock, G., 1990. Dual control of local blood flow by purines. In: Biological actions of extracellular ATP. Ann. NY Acad. Sci. 603, 31–45.

Chataigneau, T., Feletou, M., Thollon, C., Villeneuve, N., Vilaine, J.-P., Duhault, J., Vanhoutte, P.M., 1998. Cannabinoid CB1 receptor and endothelium-dependent hyperpolarization in guinea-pig, rat mesenteric and porcine coronary arteries. Br. J. Pharmacol. 123, 968–974.

Chen, G., Suzuki, H., 1991. Endothelium-dependent hyperpolarization elicited by adenine compounds in rabbit carotid artery. Am. J. Physiol. 260, H1037–H1042.

Corriu, C., Feletou, M., Canet, E., Vanhoutte, P.M., 1996. Endothelium-derived factors and hyperpolarization of the carotid artery of the guinea-pig. Br. J. Pharmacol. 119, 959–964.

Evans, R.J., Surprenant, A., North, R.A., 1998. P2X receptors. In: Turner, J.T., Weisman, G.A., Fedan, J.S. (Eds.), The P2 Nucleotide Receptors. Human Press, Totowa, NJ, 2, pp. 43–61.

Gordon, J.L., 1986. Extracellular ATP: effects, sources and fate. Biochem. J. 233, 309–319.

Harden, T.K., Nicholas, R.A., Schlater, J.B., Lazarowski, E.R., Boyer, J.L., 1998. Pharmacological selectivities of molecularly defined subtypes of P2Y receptors. In: Turner, J.T., Weisman, G.A., Fedan, J.S. (Eds.), The P2 Nucleotide Receptors. Human Press, Totowa, NJ, 5, pp. 109–134.

Keef, K.D., Pasco, J.S., Eckman, D.M., 1992. Purinergic relaxation and hyperpolarisation in guinea pig and rabbit coronary artery: role of the endothelium. J. Pharmacol. Exp. Ther. 260, 592–600.

Malmsjö, M., Edvinsson, L., Erlinge, D., 1998. P2U-receptor mediated endothelium-dependent but nitric oxide independent vascular relaxation. Br. J. Pharmacol. 123, 719–729.

Murphy, M.E., Brayden, J.E., 1995. Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. J. Physiol. (Lond) 486, 47–58.

O'Connor, S.E., Wood, B.E., Leff, P., 1990. Characterisation of P2X-receptors in rabbit isolated ear artery. Br. J. Pharmacol. 101, 640–644.

Parkington, H.C., Tare, M., Tonta, M.A., Coleman, H.A., 1993. Stretch revealed three components in the hyperpolarization of guinea-pig coronary artery in response to acetylcholine. J. Physiol (Lond) 465, 459–479.

Ralevic, V., Burnstock, G., 1991. Effects of purines and pyrimidines on the rat mesenteric arterial bed. Circulation Res. 69, 1583–1590.

Saiag, B., Hillaire-Buys, D., Chapal, P., Pape, B., Allain, H., Loubatières-Mariani, M.M., 1996. Study of the mechanisms involved in adenosine-5′-*O*-(2-thiodiphosphate) induced relaxation of the thoracic aorta and pancreatic vascular bed. Br. J. Pharmacol. 118, 804–810.

You, J., Johnsson, T.D., Childres, W.F., Bryan, R.M., 1997. Endothelial-mediated dilatations of rat middle cerebral arteries by ATP and ADP. Am. J. Physiol. 273, H1472–H1477.

Zygmunt, P.M., Högestätt, E.D., 1996. Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. Br. J. Pharmacol. 117, 1600–1606.

Zygmunt, P.M., Waldeck, K., Högestätt, E.D., 1994. The endothelium mediates a nitric oxide-independent hyperpolarization and relaxation in the rat hepatic artery. Acta. Physiol. Scand. 152, 375–384.

Zygmunt, P.M., Plane, F., Paulsson, M., Garland, C.J., Högestätt, E.D., 1998. Interactions between endothelium-derived relaxing factors in the rat hepatic artery: focus on regulation of EDHF. Br. J. Pharmacol. 124, 992–1000.