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Hexoprenaline activates potassium channels of human myometrial myocytes

N. E. Adelwoehrer[1] and W. Mahnert[2]

[1]Department of Gynecology and Obstetrics, and [2]Institute for Medical Physics and Biophysics, University of Graz, Austria

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Summary. Hexoprenaline is a β-adrenergic agent used for tocolysis after the 26th week of pregnancy. The purpose of the present study was to demonstrate the site of action of hexoprenaline on the membrane of single isolated smooth muscle cells. The main action of β-mimetics on the cell is hyperpolarization of the cellular membrane, i.e. β-mimetics have similar effects as K^+-ions (Standen et al., 1989). Our results indicate a prolonged and significantly enhanced activity of K^+-channels in the cell membrane, as may also be demonstrated by the use of the K^+-channel activator Calcitonin-gene related peptide (CGRP). In control experiments under physiological conditions, we observed a large conductance K^+-channel with 158 pS. The channel was voltage dependent and Ca^{++} sensitive indicating that it belongs to the class of big conductance Ca^{++}-activated K^+-channels (BK_{Ca}). Hexoprenaline and CGRP both increased the open probability (P_o) of the channel measured with the patch clamp system in the cell attached configuration. Hexoprenaline was also an activator of the BK_{Ca} in the presence of Nitrendipine, indicating that the activation of the Ca^{++} sensitive channel is not an indirect effect of Ca^{++} currents via L-type Ca^{++} channels.

Key words: Ionic channels – Hexoprenaline – Calcitonin gene related peptide (CGRP) – Human myometrium

Introduction

Electrophysiologic studies with the patch clamp technique on isolated intact myocytes are widely used to demonstrate the activity of the membrane channels and to analyze their behavior with different antagonists and agonists. Single cell experiments have led to a multitude of results especially on smooth muscle cells. Smooth muscle cells of different origin, i.e. different organs or different species, seem to act and react in different ways. However, it is increasingly important to develop methods of analyzing pharmacological reactions in a non invasive manner and to

Correspondence to: N. E. Adelwoehrer, Department of Gynecology and Obstetrics, University of Graz, Auenbruggerplatz 14, A-8010 Graz, Austria

demonstrate clearly the site of action on the cell membrane of drugs commonly used, in this case β-adrenergics which are employed to arrest preterm labor.

The presence of mRNA encoding the K^+-channel has been reported to vary during the normal menstrual cycle of rats (Boyle et al., 1987). Similiar changes may occur in humans. An increasing number of K^+-channels will modulate the membrane potential to a more negative value, i.e. hyperpolarization. We have recently demonstrated the contervice interaction between hexoprenaline and K^+-ions on stripes of human myometrium (Tritthart et al., 1991).

CGRP may activate K^+-channels even if it is not yeat established which particular side of action is responsible for activation (Nelson et al., 1990). We found that CGRP is also an activator of the large Ca^{++}-activated K^+-channel (BK_{Ca}) in human myocytes.

Material and methods

Strips of myometrium were obtained from uteri removed at hysterectomy for prolapse in premenopausal women. The strips were transferred to a modified Hank's solution (in mM: NaCl 132.1, KCl 2.7, $CaCl_2$ 2.5, $MgCl_2$ 1.15, $NaHCO_3$ 24, NaH_2PO_4 0.42, D-gluocose 5.6 equilibrated with 5% CO_2 and 95% O_2 to a pH 7.4).

Single myometrial cells were isolated as described elsewhere (Driska et al., 1986; Silberberg et al., 1989; Tritthart et al., 1991). The tissue was cut into small pieces and incubated in calcium free solution (this is also the basic solution for enzyme solutions in mM: NaCl 140, KCL 5, HEPES 10, D-glucose 15, pyruvate 2, BSA 1%) for 30 min. After crude dispersion of the tissue in enzyme solution containing papain (40 U/ml), ethylendiamintetraceticacid (27 μM), dithiotreitol (0.1 mg/ml) for 5 min, the tissue was reincubated in collagenase solution (1 mg/ml) for 15 min. Cells were collected after 3 consecutive incubations for 30 min in enzyme solution containing collagenase (1 mg/ml) and Soybean Trypsin Inhibitor (0.5 mg/ml). Cells were cultivated in M 199 at 37°C or stored in "KB-Medium" (Klöckner and Isenberg, 1985) at 0°C.

Membrane currents were recorded in the cell attached and excised inside out configurations (Hamill et al., 1981) employing a path clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, California). Experiments were made at 20–22°C. Patch pipettes were pulled from borosilicate capillary tubes (Hilgenberg, Malsfeld, Germany) and had a resistance between 4 and 8 MegaOhm when filled with extracellular medium. In cell attached experiments on intact cells bathed in a solution containing high K^+-concentrations (Zeroing solution) the potential was expressed relative to the cell membrane potential assumed as 0 mV. Current records were stored on video tape with a VR-10 digital data recorder (list electronic, Eberstadt, Germany) at a sampling rate of 5 kHz. Data were analyzed using custom made software (Pclamp, Axon instruments). Histograms of amplitude and open time were constructed from idealized events. Amplitude histograms were fitted to Gaussian distribution. Open time histograms were fitted to exponential probability density functions.

The pipette solution contained in mM: NaCl 137, KCl 5.4, $MgCl_2$, 2, $CaCl_2$ 2, D-glucose 10; HEPES 10; and the bath solution KCl 117, HEPES 10, EGTA/Mg/Ca 11/1/2 for the inside out configuration.

In cell attached experiments the cell membrane was depolarized with a "zeroing solution" in mM: K^+-glutamate 105, KCl 89, HEPES 20, $MgCl_2$ 2 and EGTA 2 μM (pH was adjusted to pH 7.4 with KOH) and the patch potential was assumed to be near zero.

Hexoprenaline and CGRP (CGRP-1, human, Peninsula laboratories Inc.) were disolved in water and pH was adjusted to 7.4. Hexoprenaline was a gift of the CL-Pharma AG (Linz, Austria).

Results

The potassium channels of human myometrium had a conductance of 158 pS ± 5 between −20 mV in $[K_o^+]/[K_i^+]$ of 5.4/140 (Fig. 1) resembling the large conductance Ca-activated K^+-channels in other tissues. Hexoprenaline and CGRP activated the big conductance Ca-activated K^+-channel (BK_{Ca}) in cell attached experiments.

Current traces are shown at different potentials before and after application of 1 μM hexoprenaline and after application of 0.1 μM CGRP (Fig. 2). An increasing number of channel openings and a prolongation of the mean open times lead to an 2.5-fold increase of the open probability for hexoprenaline and about an 8-fold increase for CGRP.

The effect of hexoprenaline is not caused by activation of inward calcium currents by L-type Ca^{++} channels, thus activating the BK_{Ca}, as hexoprenaline is effective when cells were preincubated with 1 μM mitrendipine (increase of the open propability about 2-fold) (Fig. 3).

Discussion

The human myometrium, like other smooth muscle tissues, possesses a large conductance to potassium ions. At least three types of potassium channels exit in the membrane of human myometrial cells (Mahnert et al., 1992). CGRP was described as a very potent K^+-agonist, that activates ATP sensitive K^+channels by phosphorylation in a cAMP-dependent manner (Nelson et al., 1990). In our studies CGRP activates the BK_{Ca} and hexoprenaline seems to act in a very similar way, as indicated by the histograms of mean open and closed times. This effect may explain the demonstrated effect on stripes, i.e. that an inactivation of spontaneous activity by hexoprenaline is antagonized by K^+-ions (Tritthart et al., 1991). If, as demonstrated above, hexoprenaline acts in the sense of increased repolarisation of the cell membrane, then an increase in free K^+-ions in the surrounding solution should lead to a contrary effect, i.e. depolarisation.

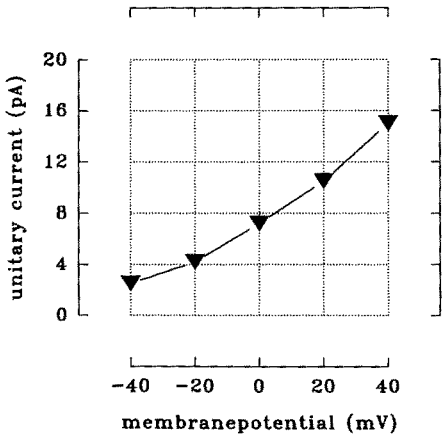

Fig. 1. Current-voltage relationship of single-channel registrations of the large conductance Ca^{++}-activated K^+-channel in the inside-out configuration

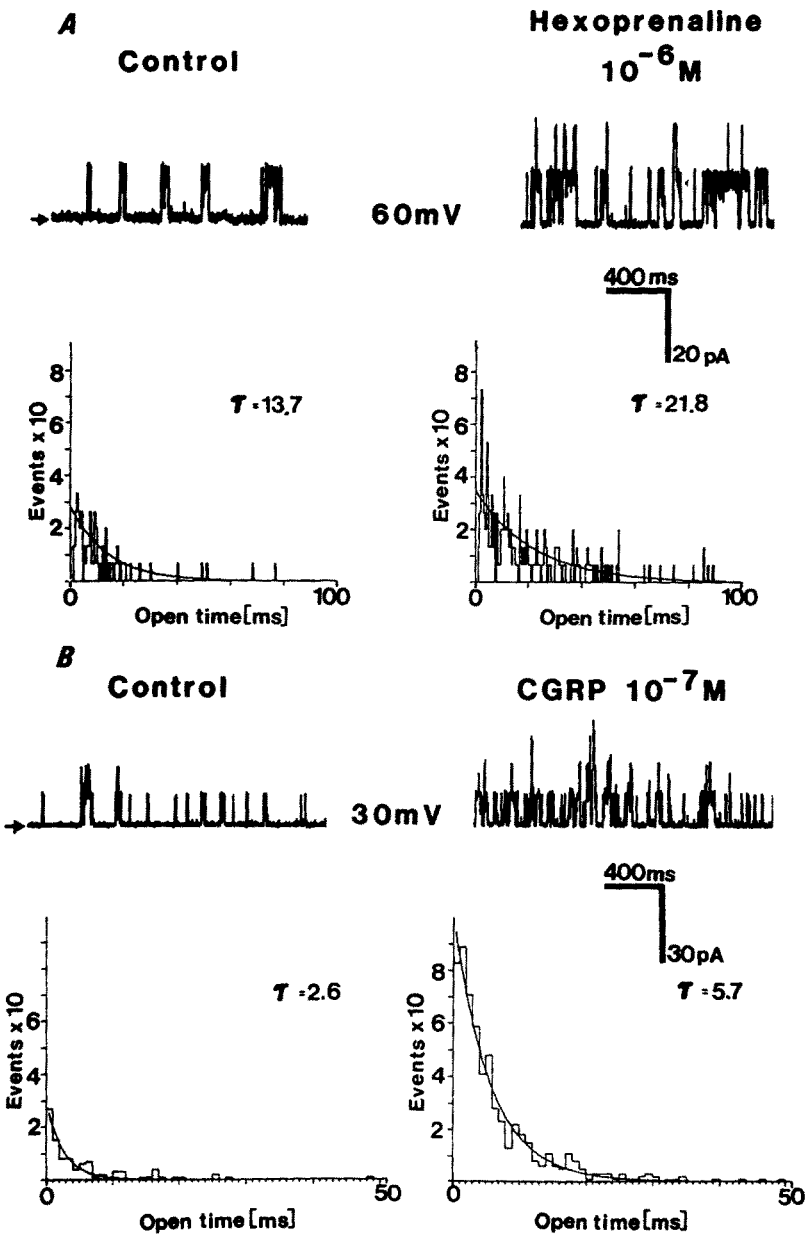

Fig. 2A, B. Increase of the activity of the large conductance Ca^{++}-activated K^+-channel by hexoprenaline and CGRP. **A** Current traces recorded in a cell-attached configuration before and after application of 1 μM hexoprenaline to the bath at a potential of 60 mV. **B** Records of single channels in a cell-attached patch before and after application of 10^{-7} M CGRP to the bath at a potential of 40 mV

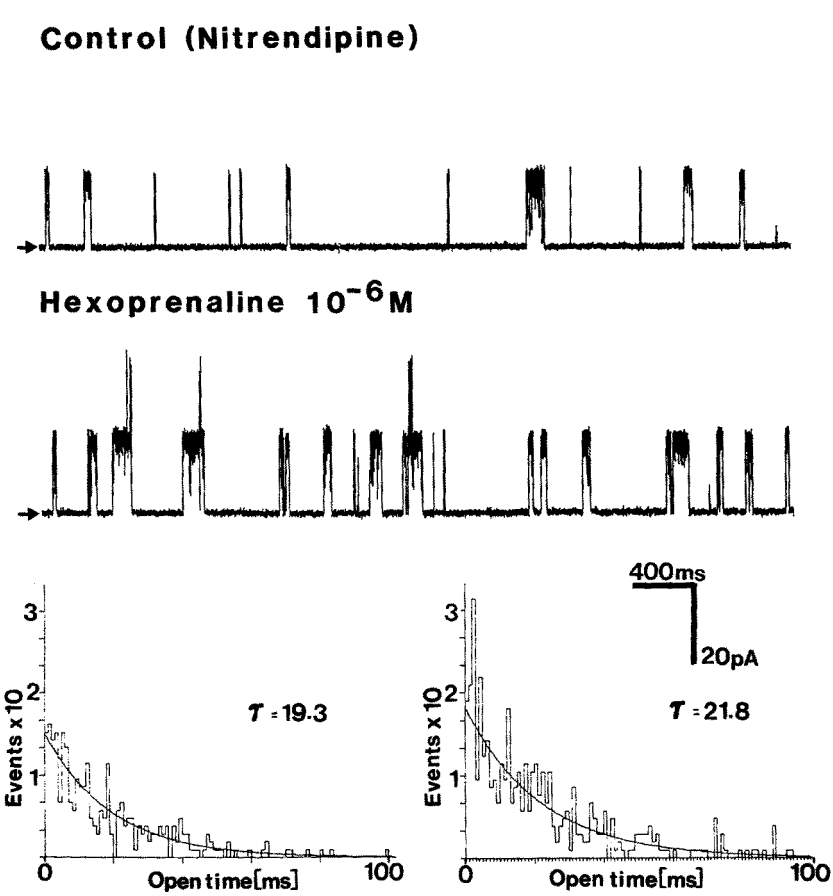

Fig. 3. Increase of the activity of the large conductance Ca^{++}-activated K^+-channel by hexoprenaline in presence of nitrendipine. Current traces obtained from a cell preincubated with 1 μM nitrendipine before and after application of 1 μM hexoprenaline

The concept, that an increase in progesterone level leads to an increase of potassium channels in the cell membrane also indicate a major role of K^+-ions in damping uterine contrations during pregnancy. Hexoprenaline as a β-mimetic drug, activates the BK_{Ca} and therefore can reduce spontaneous uterine activity.

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