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－在留中国人研究者研究助成－

１６ 年 ３ 月 １０ 日

財団法人 日中医学協会
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１．研究テーマ

血管平滑筋の酸素による収縮弛緩機序

２．本年度の研究業績

(1) 学会・研究会等における発表 (有) ・ 無 (学会名・演題)

1) American Heart Association Scientific Sessions 2003,
November 9-12, 2003, Orlando, Florida
Decreases in intracellular H2O2 cause contraction of the rabbit ductus arteriosus.

2) 第６８回日本循環器学会
Oxygen causes contraction of the ductus arteriosus by decreasing intracellular H2O2 generation.

(2) 学会誌等に発表した論文 有 ・ (無) (雑誌名・論文名)

3．今後の研究計画

本研究では酸素による動脈管収縮の機序を調べた。今後はさらに研究を発展させ、肺動脈や動脈管に存在する酸素感受性カリウムチャンネルのクローニングを行い、その遺伝子発現や、分子生物学的な特徴を明らかにする予定である．またそのカリウムチャンネルの酸素への感受性をアフリカツメガエル卵母細胞を用いて電気生理学的に調べる予定である。

4．指導責任者の意見

呉　桂栄は、未熟な血管、ことに動脈管の平滑筋や内皮細胞の機能の研究を行ってきた。特に、平滑筋細胞が酸素を感受する機構についての研究を行ってきた。研究態度も真面目で、日夜、寸暇を惜しんで研究を行った。本研究は日本国内はもちろん世界的にもすぐれた独創的な研究となった。特に、アメリカ心臓病学会の発表論文に採択され、発表し、高い評価を得た。臨床的にも重要な研究であるので、今後ますます研究を発展させていって欲しい。

指導責任者氏名 中西敏雄 ㊞

5．研　究　報　告　書

別紙「研究報告書の作成について」に倣い、指定の用紙で作成して下さい。

研究発表または研究状況を記録した写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

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—日中医学協会助成事業—

血管平滑筋の酸素による収縮弛緩機序
—特に動脈管の収縮弛緩におけるカリウムチャンネルの役割—

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Abstract

The ductus arteriosus (DA) constricts after birth when blood O2 lebels increase in mature newborns. Its mechanism is unknown. As an O2 sensor of DA, O2-sensitive Kv and KATP have been implicated. The purpose of the present study was to investigate the role of O2-sensitive Kv and KATP in the contraction of the smooth muscle cells of the DA. DA was isolated from the fetal rat at 19 or 21 days of gestation (term: 22 days). The force of isometric contraction of intact rings was measured. Kv and KATP currents were measured using single smooth muscle cells and patch clamp techniques. The vessel contraction and K currents were measured by changing solution from an anoxic (PO2 35 mmHg) to an oxygenated solution (PO2 150 mmHg) or to a solution containing Kv channel inhibitor 4-aminopyridine (4AP, 5mM), KATP channel opener pinacidil (300uM), or the KATP blocker glybenclamide (Glyb, 4 uM). In the DA, O2, 4AP, and Glyb caused significant contraction in the mature (21 day) fetus. In the DA of the premature (19 day) fetus, O2 and Glyb did not induce significant contraction and only 4AP caused contraction. KATP, which was activated by pinacidil and inhibited by Glyb, was detected in the DA. KATP was present in the DA of mature fetus and the value was greater than in the DA of premature fetus. The data suggest that 1) In the DA, both O2-sensitive K channels, which close with O2, and KATP are underdeveloped in the premature fetus, 2) in addition to O2-sensitive Kv, KATP may also be important as an O2-sensor in the DA.

Key Words Ductus arteriosus, K channel, Oxygen, Ductal contraction

Introduction:

The ductus arteriosus (DA) normally closes after birth. The rise in arterial Po_2 that occurs after birth following initiation of respiration is important in causing ductal constriction. Oxygen has been shown to cause ductal constriction in the isolated ductal preparation. The

mechanisms of the O_2-induced ductal constriction remain unclear. We showed previously that glybenclamide, an inhibitor of the ATP-dependent K channel (K_{ATP}), caused ductal contraction and that cromakalim, an opener of K_{ATP}, antagonized the O_2-induced ductal contraction (1). We hypothesized that O_2 might close the K_{ATP}, resulting in the membrane depolarization. Tristani-Firouzi et al (2), however, showed that there was a voltage-dependent K channel in the DA, which closed with O_2, and they hypothesized that the voltage-dependent K channel might be an O_2 sensor. Physiological importance of ATP-sensitive K channel (K_{ATP}) and voltage-dependent K channel (Kv) in the constriction of the ductus arteriosus (DA) remains unclear. In the present study, we investigated developmental changes in K_{ATP} in smooth muscle cells of the DA in the rat fetus.

Materials and methods:

Experiments were performed using rat fetuses at either 19 or 21 days of gestation. Contractile force of the DA was examined, as described previously (3, 4). Voltage-clamp experiments were performed in the whole-cell configuration of the patch-clamp techniques using freshly isolated single cells. The pipette (internal) solution contained (in mM) KCl 20, KOH 120, $MgCl_2$ 2, EGTA 10, HEPES 5, Mg-ATP 1, and aspartate 60 (pH 7.4). The holding potential was set to -70 mV and K currents were evoked either by a series of voltage steps ranging from -80 to +60 mV with a duration of 200 ms at a rate of 0.1 Hz or by a ramp pulse from -120 mV to 60 mV with a duration of 225 ms. K currents activated by 1-10 mM pinacidil, an opener of K_{ATP} channels, and that inhibited by 4 mM glybenclamide were considered to be K_{ATP} currents (5).

Results:

Membrane depolarization caused by high KCl induced ductal contraction in all age groups studied. In the 21-day fetuses, O_2 did cause contraction and increases in $[Ca]_i$. In the 19-day fetus, however, O_2 did not cause significant contraction nor changes in $[Ca]_i$. These data suggested that in the premature fetus, the contractile system, including membrane depolarization, [Ca]i increase and its activation of contractile proteins, is already functioning, but the O_2-sensing mechanism is underdeveloped. We then investigated developmental changes in K_{ATP} in smooth muscle cells of the DA in the rat fetus. Glybenclamide caused significant contraction in the DA of the 21-day fetal rat, but not in the 19-day fetus. Glybenclamide significantly decreased the membrane potential by 33 mV in the fetus at 21 days of gestation and the decrease was greater than in the fetus at 19 days of gestation (by 8 mV). Voltage-clamp experiments were performed in the whole-cell configuration of the

patch-clamp techniques. Pinacidil, an opener of K_{ATP} channels, and glybenclamide, an inhibitor of K_{ATP} channels, were used to detect K_{ATP} currents. K currents activated by 10 uM pinacidil in the fetus at 21 days of gestation was significantly greater than in the fetus at 19 days of gestation. The glybenclamide-inhibited K current density after activation by 10 uM pinacidil was similar to the pinacidil-activated K current. The glybenclamide-inhibited K current density was significantly greater in the fetus at 21 days than in the fetus at 19 days of gestation. These data support the hypothesis that K_{ATP} is one of the O_2 sensors in the DA and that O_2-induced contraction of the DA may not occur because K_{ATP} is underdeveloped in the premature fetus.

We next studied voltage-gated, O2-sensitive K channels (Kv) in the DA. 4-aminopyridine (4AP), a blocker of Kv, caused ductal contraction both in the 19-day and 21-day fetus. Under hypoxic conditions (PO2 35 mmHg), K currents sensitive to 4-AP were present both in the 19-day and 21-day fetus.

In the PA, Kv 1.2, Kv1.5, Kv2.1, and/or KV3.1 may open with O2. Beta-subunits may modulate gating properties of the alpha-subunits. The DA and the main pulmonary artery were isolated from the fetal rat at 21- day of gestation. mRNA levels of Kv alpha-subunits (Kv1.2, Kv1.5, Kv2.1, Kv9.3) and beta-subunits (Kvbeta1.1, Kvbeta2, Kvbeta3) were estimated using RT-PCR. No significant differences in the levels of mRNA of Kv 1.2, 1.3, 1.5 and 2.1, nor in the levels of beta-subunits among DA and PA were observed.

Discussion:

The present study showed that K_{ATP} was underdeveloped in the DA of the premature fetus. The data support the hypothesis that K_{ATP} is one of the O_2 sensors in the DA and that O_2-induced contraction of the DA may not occur because K_{ATP} is underdeveloped in the premature fetus. In the DA, K_{ATP} is an important O2 sensor in the DA. Kv is present in the DA. Although alpha and beta units of Kv specifically expressed in the DA have not been detected, Kv may be also important for ductal contraction.

References:

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