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肾上腺皮质激素对豚鼠腹腔神经节细胞的快速膜效应[1]

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Rapid membrane effects of adrenocorticoids on celiac ganglion cells of guinea pig[1]

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ABSTRACT Intracellular recording technique was used to determine the effects of corticosterone, dexamethasone, aldosterone and cholesterol on the resting membrane potentials and membrane resistance of cells in isolated celiac ganglion of guinea pigs. Hyperpolarization was elicited by corticosterone in 15 out of 83 cells, but was not caused by dexamethasone, which depolarized the membrane in 2 out of 18 cells. In addition, depolarization was also elicited by corticosterone in 2 out of 83 cells. Aldosterone and cholesterol caused no detectable changes of membrane potential and membrane resistance. It is suggested that the membrane effects of adrenocorticoids, which obviously could not be explained by traditional genomic mechanism for their short latency, may indicate the existence of membrane receptors for adrenocorticoids in celiac ganglion neurons of guinea pigs.

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KEY WORDS adrenal cortex hormones; membrane receptors; membrane potentials; sympathetic ganglia

提要 本实验应用细胞内记录技术，观察3种肾上腺皮质激素类药物及胆固醇对离体腹腔神经节细胞电活动的快速影响。结果表明，皮质酮对细胞的快速膜效应以超极化为主，地塞米松则是去极化，醛固酮与胆固醇不表现快速膜效应。结果提示，豚鼠腹腔神经节细胞膜上可能存在皮质酮与地塞米松的两种不同膜受体。

关键词 肾上腺皮质激素；膜受体；膜电位；交感神经节

肾上腺皮质激素对一些神经元膜的电生理特性具有快速作用[1-3]。因其潜伏期仅数分钟甚至数秒以内，故难以用基因机理来解释[4]，被称为快速膜效应(膜效应)。我室以往的工作表明[5,6]，皮质醇对豚鼠腹腔神经节细胞及海马神经元具有膜效应。为进一步明确其它肾上腺皮质激素类药物是否对豚鼠腹腔神经节细胞产生膜效应，并在同一个神经节甚至同一个细胞上比较它们作用的差异，本实验采用离体神经节细胞内记录技术对皮质酮、地塞米松、醛固酮及胆固醇进行了研究。

MATERIALS AND METHODS

豚鼠，体重273±SD 34 g，♀♂不拘。断头处死并放血后迅速分离腹腔神经节，用细铜丝将神经节固定于32-35℃浴槽内，以O_2饱和的Kreb's溶液灌流。溶液成分为(mmol/L)：NaCl 117；KCl 4.7；$CaCl_2$ 2.5；$MgCl_2$ 1.2；$NaHCO_3$ 25；NaH_2PO_4 1.2；葡萄糖11.5。以O_2 95%和CO_2 5%的混合气充分饱和[7]。采用尖端直径小于1 μm、内充KCl 3 mol/L、阻抗20-50 MΩ的玻璃微电极穿刺细胞，通过微电极放大器记录膜电位并向微电极输送波宽200 ms、频率0.1-0.2 Hz的恒定跨膜超极化电流以监测膜电阻，用电子自动电位差计(频响上限15 Hz。)持续描记膜电位与膜电阻变化。所有记录均取自膜静息电位大于40 mV、动作电位大于55 mV的细胞[8]。

实验用药品为皮质酮(Sigma公司)、地塞米松(信谊制药厂)、醛固酮及胆固醇(Fluka公司)，用乙醇溶解，然后用溶液稀释，乙醇终浓度0.1 mmol/L。给药方式采用以三向开关控制的灌流给药。

RESULTS

激素溶液的灌流时间一般持续1-3 min，灌流开始后0.5-3 min内可出现膜电位与膜电阻的变化，其时程随激素灌流时间的延长而增长，在停止激素灌流后膜电位与膜电阻变化迅速恢复到原水平；如再次灌流同一种激素，这种变化仍可被重复。下述结果取材于121个记录稳定的细胞。由于一个细胞常接受两种以上激素溶液灌流，因此，下述分别接受各种激素灌流的细胞的数目有部分是重复的。

皮质酮的快速膜效应 83个细胞接受皮质酮(1-1000 nmol/L)溶液灌流。有15个细胞对皮质酮的反应为超极化(6.6±3.2 mV)，其中8个细胞伴膜电阻增大28±17%。6个细胞不伴膜电阻变化，1个细胞伴膜电阻减小20%；另有2个细胞对皮质酮的反应为去极化(2.5-13 mV)，其中1个伴膜电阻减小25%；其余66个细胞对皮质酮不敏感。Fig 1示3个细胞对皮质酮的不同反应。在皮质酮浓度为1-100 nmol/L的变化范围内，受试的4个细胞的超极化幅度随皮质酮浓度增大而增大(Fig 2)，即具有一定的量-效关系，产生膜效应的最低浓度为1 nmol/L。皮质酮浓度在0.1-1 μmol/L时，所有细胞的反应均不表现量-效关系，4个对皮质酮反应为超极化的细胞在接受第二次施药时，施药时间被延长至近第一次施药的2倍，观察到超极化随施药时间延长而延长，幅度基本保持不变，一旦停药，电位迅速恢复原水平。

皮质酮产生超极化的幅度与该细胞膜静息电位及超极化时所伴膜电阻变化的%无显著相关(直线相关检验，$n=9$，$P>0.05$)。对皮质酮的反应为超极化的细胞的膜静息电位、膜电阻、动作电位幅度与对皮质酮不敏感的细胞的相应数值无显著性差异(t检验，$P>0.05$)。

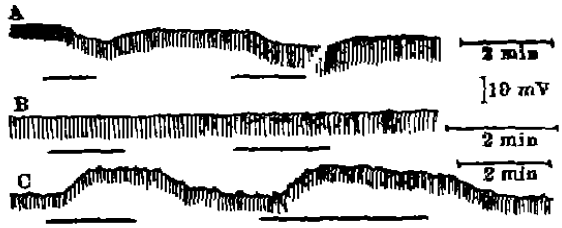

Fig 1. Three types of membrane potential changes elicited by corticosterone (CS, 0.1 μmol/L, black bars) from 3 different cells. A) Hyperpolarization (hyperpol) accompanied by an increase of membrane resistance; B) no detectable change of membrane potential and membrane resistance; C) depolarization not accompanied by significant change of membrane resistance. Resting membrane potentials of cells A), B) and C) were −47, −45 and −53 mV, respectively. Downward deflexions were hyperpolarizing potentials elicited by hyperpolarizing current pulses of 200 ms duration applied at frequency of 0.2 Hz.

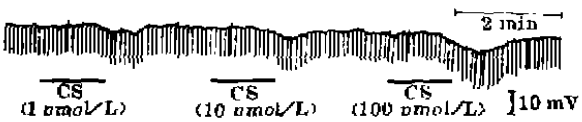

Fig 2. The relationship between dose and response of CS. Hyperpol was elicited by different concentrations of CS from one neuron with resting membrane potential of −54 mV. For downward deflexions see Fig 1.

地塞米松快速膜效应 18 个细胞接受地塞米松(0.1 μmol/L)溶液灌流，有 2 个细胞产生去极化反应(3-11 mV)，其中 1 个伴膜电阻下降(20%)；其余 16 个细胞对皮质酮不敏感.

皮质酮与地塞米松快速膜效应的比较 为了比较同一个细胞对皮质酮与地塞米松的反应，本实验共观察了 13 个细胞在先后灌流皮质酮与地塞米松(与给药次序无关)时膜电位的变化。13 个细胞中有 5 个对皮质酮的反应为超极化，但这 5 个细胞对地塞米松均不敏感(Fig 3)；另 8 个细胞对皮质酮与对地塞米松均不敏感。统计学表明，这 13 个细胞对皮质酮与对地塞米松的反应相差显著(McNemar 检验，$P<0.05$)。

Fig 3. Hyperpol by CS being not mimicked by dexamethasone (Dex) on one neuron. A) CS (1 nmol/L) caused a hyperpol of 6 mV with no change of membrane resistance; B) higher concentration of Dex (100 nmol/L) caused no change of membrane potential and membrane resistance. The resting membrane potential of this neuron was −52 mV. For downward deflexions see Fig 1.

醛固酮无快速膜效应 接受醛固酮(0.1-10 nmol/L)溶液灌流的细胞有 36 个。在灌流之后 3 min 内，这些细胞的膜电位与膜电阻没有发生明显变化。因此，醛固酮在这 36 个细胞中不表现快速膜效应，与皮质酮使 83 个细胞中的 15 个产生超极化的快速膜效应相比，二者存在显著差别(χ^2 检验，$P<0.05$)。

胆固醇无快速膜效应 考虑肾上腺皮质激素中有胆固醇分子基本结构，为排除这一因素，我们测试了胆固醇对神经节细胞的影响。接受胆固醇(0.1 μmol/L)溶液灌流的细胞共 21 个，在灌流胆固醇溶液期间及灌流之后 3 min 内，这些细胞的膜电位与膜电阻未出现明显变化。

乙醇对照 由于制备肾上腺皮质激素溶液时均先用乙醇溶液激素，为排除溶剂本身对神经元的影响，我们测试了乙醇(0.1 mmol/L)对神经节细胞的影响。接受乙醇溶液灌流的细胞有 35 个。在灌流期间及灌流之后 3 min 内这些细胞的膜电位与膜电阻没有发生明显变化。因此，所试乙醇浓度在这 35 个细胞中不表现快速膜效应，与皮质酮使 83 个细胞中的 15 个产生超极化的快速膜效应相比，两者存在显著差别(χ^2 检验，$P<0.05$)。

DISCUSSION

我室以往的工作表明[5,6]，糖皮质激素对离体海马神经元及腹腔神经节细胞电活动有快

速作用，本实验进一步支持这一结论．在本实验中，醛固酮、胆固醇及乙醇对神经元膜电位无影响。因此，由于乙醇溶剂对膜直接作用或者由于肾上腺皮质激素分子结构上共同的因子引起细胞膜物理、生化特性变化继而产生膜效应的可能性可以排除[9,10]。

现已公认[11]，糖皮质激素胞液受体分为二型，Ⅰ型受体与皮质酮、醛固醛亲和性高，Ⅱ型受体与地塞米松有较高亲和性。如果皮质酮是因先与胞液受体结合而后不经基因机制直接产生膜效应，那么醛固酮或地塞米松二者之一应能模拟皮质酮的膜效应，但本实验结果恰恰是不能模拟，因此，皮质酮的膜效应可能不是由于皮质酮与胞液受体结合后产生的。皮质酮膜效应时程短暂，一旦细胞外液中皮质酮被清除膜效应即消失，表明皮质酮与其特异结合位点结合(以产生膜效应)后的解离较为容易。这与 Towle 等报告[12]的大鼠脑突触浆膜特异结合的解离常数较高的结果不悖。

在本实验中，皮质酮的膜效应以超极化为主。我室另有实验表明，在低钙高镁溶液阻断突触传递的条件下，糖皮质激素的超极化膜效应依然存在，这表明此效应不依赖于突触前机理[13]。因此，受微电极穿刺的交感神经节细胞膜上可能存在皮质酮的膜受体。另外，本实验中有 2 个细胞出现去极化反应，其原因尚不清楚。但离体腹腔神经节的细胞可能接受该神经节内其他神经元的支配[14]，因此不能排除皮质酮作用于突触前神经元继而引起突触后神经元去极化的可能性。

Towle 等报告[12]，地塞米松与大鼠脑突触浆膜的特异结合与皮质酮的类似。但 Suyemitsu 等的工作表明[15]，大鼠肝细胞膜上存在着天然糖皮质激素皮质酮与皮质醇的高亲和性特异结合位点，但不存在合成糖皮质激素的特异结合位点。本实验中皮质酮的超极化效应不能被地塞米松所模拟，地塞米松只有去极化效应。这提示交感神经节细胞膜上可能存在着皮质酮与地塞米松二种不同的膜受体。本实验中的 13 个细胞先后接受皮质酮和地塞米松的灌流，其中 5 个对皮质酮反应为超极化的细胞对地塞米松不反应，这至少可以说，我们未能发现皮质酮与地塞米松的可能膜受体共存于同一细胞的证据。

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Prophylactic effects of *m*-nisoldipine and nisoldipine on reperfusion arrhythmias exacerbated by free radical generating system in Langendorff heart of rat

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ABSTRACT Xanthine-xanthine oxidase (X-XOD, 500 μmol/L + 100 nmol/L) free radical generating system was perfused 10 min prior to coronary artery ligation until the end of the experiment. It exacerbated the reperfusion ventricular fibrillation, reduced the activities of superoxide dismutase and catalase and increased the contents of malondialdehyde in Langendorff heart of rats. *m*-Nisoldipine or nisoldipine (0.05 μmol/L) was perfused 10 min prior to coronary artery ligation until the end of the experiment. They prevented reperfusion arrhythmias exacerbated by X-XOD and decreased the free radicals generated by X-XOD.

KEY WORDS *m*-nisoldipine; nisoldipine; nifedipine; reperfusion; arrhythmia; xanthines; xanthine oxidase; free radicals

It has been suggested recently that regional reperfusion after only a few minutes of ischemia resulted from the ligation of left anterior descending coronary artery in heart of rats can induce reperfusion arrhythmia and free radical formation, and *m*-nisoldipine (*m*-Nis) or nisoldipine (Nis) prevents reperfusion arrhythmias and inhibits free radical formation in heart of rats[1]. Xanthine-xanthine oxidase (X-XOD) pathway was an important source of superoxide radical upon reperfusion[2]. This may be converted to the more reactive hydroxyl radical and then to other free radical species[3]. Thus, these free radicals may cause membrane damage leading to local electrophysiological derangements that trigger serious ventricular arrhythmias[4]. However, it hasn't yet been reported that reperfusion arrhythmia in rats are exacerbated by X-XOD and calcium antagonists prevent this reperfusion arrhythmias.

In order to further research the relations of calcium antagonists, free radicals and reperfusion arrhythmias, the reperfusion-arrhythmogenic effects of the free radicals resulted from the addition of X-XOD to the perfusion fluid and prophylactic effects of m-Nis and Nis on reperfusion arrhythmias exacerbated by X-XOD free radical generating system in Langendorff heart of rats were investigated in this paper.

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