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甲基黄酮醇胺的抗心律失常与抗脂质过氧化作用

顿 文、杨立志、周尔凤、梁月琴 (山西医学院药理教研室，太原 030001，中国)

Anti-arrhythmia and anti-lipid peroxidation effects of methylflavonolamine

DUN Wen, YANG Li-Zhi, ZHOU Er-Feng, LIANG Yue-Qin (*Department of Pharmacology, Shanxi medical college, Taiyuan 030001, China*)

ABSTRACT Effects of methylflavonolamine (MFA) on arrhythmias induced by myocardial reperfusion were studied with rat hearts *in situ* and *in vitro*. In pentobarbital-anesthetized rats, MFA (20 mg · kg^{-1}, iv) pretreatment reduced the incidence of reperfusion-induced ventricular fibrillation after left descending coronary artery ligation (15 min) and reperfusion (3 min) (28.6% *vs* 85.7% in control, $P<0.05$). Malondialdehyde (MDA) production (85 ± 9 nmol / g wet wt) was inhibited in myocardium from the reperfused area in comparison with control (133 ± 15 nmol / g wet wt). In isolated rat hearts with local ischemia (15 min) and reperfusion (1 min), MFA 5 μmol · L^{-1} (perfused 10 min prior to coronary artery ligation) prevented reperfusion-induced arrhythmias (0% *vs* 85.7% in control, $P<0.01$). In myocardium from the reperfused area, superoxide dismutase (SOD) and catalase (Cat) activity was increased and xanthine oxidase (XOD) activity, MDA production and nonesterified fatty acids (NEFA) contents were decreased. The results show that MFA prevents reperfusion-induced arrhythmia by inhibiting lipid peroxidation and regulating the metabolism of NEFA.

KEY WORDS methylflavonolamine; myocardial reperfusion injury; arrhythmia; lipid peroxidation; nonesterified fatty acids

提要 甲基黄酮醇胺(MFA (20 mg · kg^{-1}, iv)预防大鼠结扎冠脉(15 min)和再灌注(3 min)所致的心律失常，减少心肌再灌注区丙二醛(MDA)生成。缺血前 10 min 给予离体灌流大鼠心脏 MFA 5 μmol · L^{-1} 可抗缺血再灌注心律失常，显著保护心肌再灌注区 SOD 和 Cat 活性的降低，抑制 XOD 活性的升高，减少 MDA 生成，防止再灌注区心肌非脂化脂肪酸(NEFA)堆积，提示 MFA 预防再灌注心律失常与其抗脂质过氧化作用有关。

关键词 甲基黄酮醇胺；心肌再灌注损伤；心律失常；脂质过氧化；非脂化脂肪酸

甲基黄酮醇胺(methylflavonolamine, MFA)为一黄酮类化合物，其对多种实验性心律失常模型均有对抗作用[1]，对心肌缺血具有保护作用[2]，在离体豚鼠心房肌呈负性变频和变力作用[3]，抑制血小板聚集[4]，并经离体血管条和心肌微电极实验证实为钙拮抗剂[5,6]，目前认为再灌注心律失常是心脏性猝死的主要原因。据 Brooks 等报道[7]钙通道阻断剂维拉帕米(verapamil, Ver)有较好的抗再灌注心律失常作用。为此本文进一步研究 MFA 对大鼠再灌注心律失常预防作用和对脂质过氧化(lipid peroxidation)及非脂化肪肪酸(nonesterified fatty acids, NEFA)的影响，以探讨其作用原理。

MATERIALS AND METHODS

药物 MFA 系上海医药工业研究院提供，实验前用双蒸水配成 20 mmol · L^{-1} 的母液，于 4℃保存，使用期限不超过 3 d.

麻醉大鼠再灌注心律失常模型制备及 MDA 测定 Sprague-Dawley 大鼠 20 只，♂，体重 285 ± SD 29 g 随机分为两组，戊巴比妥钠 60 mg · kg^{-1} ip 麻醉，iv 肝素 200 IU / 鼠，颈动脉插管记录血压，距胸骨左缘 2 mm 纵行开胸，人工呼吸，暴露心脏于胸腔外，用 000 号丝线 2 根，同时穿过左冠脉前降支(在左心房下缘 2 mm 处进针，肺动脉圆锥左缘出针)，将心脏放回胸腔内，稳定 15 min 后(15 min 内，有 6 只大鼠因发生心律失常或

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血压低于 9.2 kPa，弃之不用)，将长 1 cm，外经约 1 mm，一侧面剪成槽状的聚乙烯管置于与左冠脉前降支平行位置，用穿过冠脉的一根丝线将冠脉和聚乙烯管一起结扎，实现再灌注(这种剪法可防止剪线时损伤心肌)．在结扎冠脉前 5 min 分别由舌下 iv MFA (20 mg·kg^{-1})和相应容量的生理盐水．结扎 15 min 和再灌注 3 min 期间内连续记录标准Ⅱ导程心电图．再灌注 3 min 后，取心，用 NS 冲洗，随后将另一根穿过冠脉的丝线结扎，以稀释的碳素墨水从主动脉逆行灌流[8]，再灌注区(reperfused area，R)无色，正常区(normal area，N)出现黑色，将 R 区和 N 区分别以 NS 冲洗，吸干表面水份，称重，用 pH 7.4，0.05 mol·L^{-1} 的磷酸盐缓冲液制备 10%心肌匀浆，用硫代巴比妥酸比色法[9]测定丙二醛(malondialdehyde，MDA)含量．

离体大鼠心脏再灌注心律失常模型制备 Sprague-Dawley 大鼠 25 只，♂，体重 298±SD 17 g，制备 Langendorff 心脏，以通入 95% O_2+ 5% CO_2 的 Krebs-Henseleit 缓冲液(KHB)持续灌注，pH 7.35±0.05，温度 37±0.5℃，灌注压 7.8 kPa．将两铂丝电极分别置于右心室游离壁和主动脉根部，记录心表面电图(有自发心律失常的弃之不用)，根据麻醉动物方法制备再灌注心律失常模型．在结扎冠脉前 10 min 分别将含 MFA 5 μmol·L^{-1} 和等容量溶剂的 KHB 液连续灌流心脏直至实验结束．再灌注 1 min 后，取心肌 R 区和 N 区，按邻苯三酚自氧化法[10]测定超氧化物歧化酶(superoxide dismutase，SOD)，Nelson 改良法[11]测定过氧化氢酶(catalase，Cat)，比色法[12]测定黄嘌呤氧化酶(xanthine oxidase，XOD)活性，硫代巴比妥酸比色法[9]测定 MDA 含量，蛋白定量用酚试剂比色法[13]．

将测定 NEFA 的心肌称重，用 Folch 氯仿甲醇提取法[14]，提取心肌全部脂质，然后用铜试剂比色法[15]测定心肌 NEFA 含量．

RESULTS

MFA 对再灌注心律失常的影响 (Tab 1) 在麻醉大鼠，与对照组比较，MFA 20 mg·kg^{-1} 明显降低再灌注室颤(VF)发生率，延长 VF 潜伏期，缩短 VF 持续时间，降低大鼠死亡率．在 Langendorff 大鼠心脏 MFA 5 μmol·L^{-1} 于缺血前 10 min 灌注能完全阻止 VF 的发生．

Tab 1. Effects of methylflavonolamine (MFA) on reperfusion arrhythmias in rat hearts. $n=7$, $\bar{x}\pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

MFA	Incidence of VF (%)	Onset of VF (s)	Duration of VF (s)	Mortality (%)
Anesthetized rats				
Control	85.7	11±6	46±31	57.1
20 mg·kg^{-1}	28.6**	85±7***	9.0±1.4**	0**
Isolated hearts of rat				
Control	85.7	5±4	26±14	
5 μmol·L^{-1}	0***	0	0	

MFA 对麻醉大鼠再灌注心肌脂质过氧化作用的影响 N 区和 R 区心肌脂质过氧化产物 MDA 测定表明，对照组分别为 79±10 和 133±15 nmol·g^{-1} wet wt ($n=7$, $P<0.01$)；MFA 组则使 R 区 MDA 生成显著降低，由对照组 133±15 降至 85±9 nmol·g^{-1} wet wt ($n=7$, $P<0.01$)，两组 N 区无显著差异 ($n=7$, $P>0.05$)．

MFA 对离体灌流大鼠再灌注心肌自由基生成和 NEFA 含量的影响 对照组 R 区 SOD 和 Cat 活性显著低于 N 区，XOD 活性和 MDA 含量明显高于 N 区．MFA 组两区 3 种酶活性和 MDA 含量均无明显差异；但 R 区 SOD 和 Cat 活性均高于对照组，XOD 活性和 MDA 含量低于对照组 R 区．对照组 R 区 NEFA 含量显著高于 N 区，MFA 组两区无显著差异；但 MFA 组 R 区 NEFA 含量低于对照组 R 区(Tab 2)．

Tab 2. Effects of MFA 5 $\mu mol \cdot L^{-1}$ on myocardial superoxide dismutase (SOD), catalase (Cat) and xanthine oxidase (XOD) activities and the contents of malondialdehyde (MDA) and nonesterified fatty acids (NEFA) in ischemic and reperfused rat hearts *in vitro*. N: normal area. R: reperfused area. $n=7$, $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* N. $^{+}P>0.05$, $^{+++}P<0.01$ *vs* control.

			Control	MFA
SOD	(IU / 100 mg protein)	N	193 ± 25	194 ± 27^{+}
		R	115 ± 13***	194 ± 18^{**+++}
Cat	($nmol \cdot mg^{-1}$ protein $\cdot min^{-1}$)	N	930 ± 70	923 ± 32^{+}
		R	620 ± 80***	936 ± 56^{*+++}
XOD	(IU / g protein)	N	8.7 ± 2.4	9.2 ± 1.9^{+}
		R	19 ± 4***	9.3 ± 1.5^{*+++}
MDA	($nmol \cdot mg^{-1}$ protein)	N	1.32 ± 0.18	1.33 ± 0.16^{+}
		R	2.49 ± 0.20***	1.36 ± 0.15^{*+++}
NEFA	($\mu mol \cdot g^{-1}$ wet wt)	N	3.17 ± 0.18	3.22 ± 0.19^{+}
		R	4.57 ± 0.22**	3.24 ± 0.18^{*+++}

DISCUSSION

我们在整体和离体试验证明，MFA 对心肌缺血再灌注心律失常具有对抗作用，这一作用与 MFA 抗氧自由基形成和减少非脂化脂肪酸在心肌细胞内堆积有关. MFA 抑制缺血再灌注心肌 XOD 活力升高，减少了氧自由基的生成途径；保护 SOD 和 Cat 活力，增强了内源性氧自由基清除系统的功能；减少了氧自由基作用于膜脂质生成的脂质过氧化产物 MDA，最终，降低再灌注心律失常的发生率.

在心肌缺血期间，细胞内游离钙浓度增高，Ca^{2+}可以激活一种催化黄嘌呤脱氢酶转变为黄嘌呤氧化酶的蛋白水解酶，使黄嘌呤氧化酶活性升高，加强了黄嘌呤氧化酶生成氧自由基的途径；而氧自由基引起的膜脂质过氧化反应，可使膜对 Ca^{2+}的通透性增加，进一步加剧细胞内钙聚集；心肌细胞内 Ca^{2+}浓度增高还可激活磷脂酶，水解磷脂和甘油三酯，释放 NEFA，形成恶性循环，加速细胞内一系列病理改变. MFA 已经证明为钙拮抗剂，通过减少缺血再灌注心肌细胞内 Ca^{2+}浓度，抑制黄嘌呤氧化酶生成，同时降低心肌能量消耗，保护内源性氧自由基清除剂 SOD 和 Cat 活性，发挥抗脂质过氧化的作用. MFA 的钙拮抗作用，还可抑制磷脂酶，减少 NEFA 形成. 本实验结果提示，MFA 抗氧自由基形成，抑制脂质过氧化以及减少 NEFA 堆积，最终抗缺血再灌注心律失常作用是其抗钙作用的一个重要方面. 与 Ca^{2+}的密切关系有待研究.

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人胃平滑肌不同亲和性毒蕈碱受体的分布[1]

郭政东、李 智、阮 英、丛 华、张克义 (中国医科大学药理教研室，沈阳 110001，中国)

Distribution of muscarinic receptors of different affinities in smooth muscle of human stomach[1]

GUO Zheng-Dong, LI Zhi, RUAN Ying, CONG Hua, ZHANG Ke-Yi (*Department of Pharmacology, China Medical University, Shenyang 110001, China*)

ABSTRACT Muscarinic receptors of high and low affinity were found in the fundus and the body of human stomach through the contraction experiment combined with ligand method *in vitro*. The 2 types of muscarinic receptors with different affinity regulated respectively the contractions of the longitudinal and the circular muscle of human gastric fundus and gastric body. However, in the antrum exists only one kind of muscarinic receptors of high affinity, which regulated the contractions of the longitudinal and the circular muscles of human stomach.

The contractile force of the longitudinal muscle induced by exogenous ACh in the fundus and that in the body of human stomach were found to be similar to each other. The contractile force of the circular muscle in the body was found to be the strongest, and the contractile force of both longitudinal and circular muscles in antrum was weaker.

KEY WORDS smooth muscle; stomach; radioligand assay; muscarinic receptors; quinuclidinyl benzilate

提要 用离体收缩实验和放射配体结合实验相结合的方法，发现人胃底、胃体部平滑肌上有高低两种不同亲和性的 M 受体，分别支配纵、环肌的收缩；而胃窦部只存在一种高亲和性的 M 受体，支配纵、环肌的收缩. 外源性 ACh 引起的人胃底、胃体部纵肌收缩力相近；胃体部环肌收缩力最强；胃窦部纵环两肌收缩力均较弱.

关键词 平滑肌；胃；放射配位体测定；毒蕈碱受体；奎纽定二苯羟乙酸盐

近年来对胃平滑肌 M 受体亚型的研究日渐增加，认为豚鼠、大鼠、猪胃平滑肌上都存在 M 受体亚型[1-4]. 对于人胃平滑肌 M 受体亚型的研究迄今未见报道. 本项研究的目的是检定人胃平滑肌不同亲和性 M 受体的分布.

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