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Cardiac responses induced by bradykinin activation of canine ganglial plexus between aorta and pulmonary artery

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ABSTRACT To study the function of bradykinin-sensitive cardiac neurons, bradykinin (50 μg) was injected into the ganglial plexus between aorta and pulmonary artery (A-PGP) in 33 anesthetized open-chest dogs. Either positive or negative inotropic and chronotropic responses were elicited. Control injections of 0.1 ml saline into A-PGP and injections of bradykinin (50 or 100 μg) into the right marginal ganglial plexus did not elicit any cardiac response. After acute decentralization, bradykinin (50 μg) was again injected into the same locus of A-PGP. Some positive responses were still induced, while negative ones were completely abolished. These data suggested that bradykinin can directly activate the efferent neurons associated with sympathomimetic activation, and indirectly activate them by stimulation of afferent neurons.

KEY WORDS bradykinin; heart; ganglia; efferent neurons; afferent neurons

Cardiac neurons in canine ganglial plexuses have been assumed to contain only parasympathetic efferent neurons[1,2]. However, anatomical and physiological evidences have suggested that afferent[3,4], local circuit[5,6], and efferent postganglionic sympathetic neurons[3] are also present in the cardiac ganglia. A number of peptides are known to be involved in cardiovascular regulation[7-9], including bradykinin[7]. It is unknown if peptides can modify neurons on the heart such that cardiodynamics are changed. Furthemore, bradykinin is known to activate axons of cardiac afferent neurons, while little is known about the function of bradykinin sensitive cardiac neurons of ganglial plexuses. Thus, in the present experiments bradykinin was injected into the cardiac ganglial plexus between aorta and pulmonary artery (A-PGP) to determine whether this peptide can modify neurons in the ganglial plexus and, if so, how the cardiodynamics may altered by such modification.

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MATERIALS AND METHODS

Dog preparation A total of 33 mongrel dogs of either sex, weighing 15.2± *s* 1.3 kg, was tranquilized with sodium thiopental (12 mg·kg^{-1}, iv) and anesthetized with alpha chloralose (100 mg·kg^{-1}, iv). During the experiment, alpha chloralose (25 mg·kg^{-1}, iv) was injected as a bolus every 1-2 h. Following intubation, positive-pressure ventilation was maintained using a Bird Mark 7a ventilator. A bilateral thoracotomy was performed through the 4th intercostal space and the pericardium was incised to expose the heart. Walton-Brodie strain gauge arches were sutured to the right and left atria. Miniature solid-state pressure transducers (Konigsberg Instruments, Model p16) were inserted into the midwall region of the right ventricular conus, as well as into the ventral wall of the left ventricle to record regional intramyocardial pressure (IMP)[10]. Left ventricular chamber pressure was measured using a Bently Trantec Model 800 transducer connected to a Cordis #7 catheter which was inserted into that chamber via a femoral artery. All data, including electrocardiogram and arterial pressure, were recorded on a curvilinear 8-channel Beckman dynograph or an Astro-Med, Model MT 9500 8-channel rectilinear recorder. The abdomen was opened and the adrenal arteries and veins were occluded bilaterally with clamps before injection of bradykinin.

Bradykinin injection Normal saline (0.1 ml) was

injected via a 30 gauge needle into A–PGP. Bradykinin (50 μg / 0.1 ml saline, peptide content 82%, 88F–5885, Sigma Chem Co) was injected via the same needle which remained in the ganglial plexus during the experiment. In order to mark the injection site, a fine metal wire (0.1 mm wide, 4 mm long), bent at one end to form a hook, was inserted into the tip of the needle. Following all injections, when the needle was removed, the wire remained with its end hooked in the tissue. When right marginal ganglial plexus was studied, bradykinin (100 μg / 0.2 ml saline) was used since preliminary experiments had demonstrated that 50 μg did not elicit cardiac responses.

Acutely decentralization operation After bradykinin had been injected into the ganglial plexuses, the heart was acutely decentralized by cutting the cervical vagosympathetic complexes and by eliminating all the connections between both the stellate ganglia and the spinal cord by cutting the vertebral nerves, T_1 and T_2 rami, and thoracic sympathetic chains immediately caudal to the stellate ganglia bilaterally. Thereafter, bradykinin was injected again into the same sites via the same needle 30 min later.

Anatomical investigation At the conclusion of the pharmacological experiment, the hearts were fixed in 10% formalin for 7 d. The collections of the fat pads between aorta and pulmonary artery were stained by 0.02% methyline blue in phosphate buffer for 15 min and then washed with phosphate buffer (pH = 7.4) for 2 h. Microdissection of the collections was carried out with a Wild M400 dissecting microscope to look for ganglia at the sites of injections marked with metal wires.

Data analyses Heart rate (HR), right atrial force (RAF), left atrial force (LAF), right ventricular conus intramyocardial pressure (RVC IMP), left ventricular ventral intramyocardial pressure (LVV IMP), left ventricular chamber pressure (LVP), and carotis communis artery pressure (CCAP) were measured and their $\bar{x} \pm s$ were calculated for the period immediately prior to injection and when maximal response was elicited by chemical stimulation. The cardiac responses were then evaluated by comparing data obtained immediately prior to each intervention with maximal changes elicited following each intervention using t test for paired data. The frequency of responses was evaluated by comparing enumeration data obtained after decentralization with those before decentralization.

RESULTS

Bradykinin given in heart with intact innervation Control injections of saline (0.1 ml) into A–PGP and injections of bradykinin (50 or 100 μg) into right marginal ganglial plexus did not elicit any change in cardiac rate or force. Injections of bradykinin (50 μg) into A–PGP induced cardiac responses in 31 / 33 dogs. Positive or negative responses were detected after injections, but more frequent positive responses were induced, especially on inotropic parameters. Usually, when bradycardia was elicited, suppressions of RAF, LAF, RVC IMP, and LVV IMP occurred; and when tachycardia was elicited, RAF, LAF, and IMP were augmented. However, augmentations of RAF, LAF, or IMP were also accompanied by bradycardia in 3 dogs. The changes of LVP were consistent with those of LVV IMP. Ventricular tachycardia was induced in 2 dogs following injection of bradykinin into A–PGP.

Bradykinin given in acutely decentralized heart After acute decentralization, bradykinin injections into A–PGP via the needle which had been remaining in the locus still elicited some positive responses in 16 dogs, but less frequently than those in intact hearts, while all negative responses were abolished after decentralization (Tab 1).

Anatomical finding After the pharmacological experiments, anatomical dissection of the fat pads between aorta and pulmonary artery which had been identified pharmacologically by bradykinin revealed the presence of ganglia or ganglial plexuses in the

Tab 1. Cardiac responses to bradykinin (50 μg) into ganglial plexus between aorta and pulmonary artery. $\bar{x} \pm s$ $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control; $^{+}P > 0.05$, $^{++}P < 0.05$, $^{+++}P < 0.01$ *vs* the number of sympathetic responses before decentralization.

	Control	Bradykinin	Sympathetic n	Sympathetic Control	Sympathetic Bradykinin	Parasympathetic n	Parasympathetic Control	Parasympathetic Bradykinin
Intact hearts (n=31)								
HR	21± 3	22± 4*	15	22± 4	24± 4***	10	21± 4	18± 3***
RAF	100	110± 34*	22	100	127± 21***	9	100	70± 20***
LAF	100	104± 32*	16	100	127± 22***	10	100	71± 22***
CIMP	3.1± 0.9	3.9± 1.3***	21	3.5± 1.3	5.1± 2.1***	5	3.2± 1.5	3.3± 0.9**
VIMP	13± 3	14± 5**	21	13± 3	16± 5***	10	13± 4	11± 4***
LVP	14± 4	14± 5*	19	14± 4	16± 5***	10	13± 4	10± 4***
AP	13± 3	14± 5*	19	13± 3	15± 4***	10	13± 3	10± 4***
Decentralized hearts (n = 16)								
HR			8++	19± 5	22± 5**			
RAF			12+++	100	133± 24**			
LAF			12+	100	120± 18**			
CIMP			13++	2.7± 0.7	4.3± 1.6**			
VIMP			13++	10± 3	14± 5**			
LVP			8+++	14± 4	15± 5**			
AP			8+++	12± 4	13± 5**			

HR = heart rate ; RAF = right atrial force (%); LAF = left atrial force (%); CIMP = right ventricular conus intramyocardial pressure (kPa); VIMP = left ventral ventricular intramyocardial pressure (kPa); LVP = left ventricular chamber pressure (kPa); AP = arterial pressure (kPa).

immediate vicinity to the active locus identified by the wire.

DISCUSSION

Canine intrinsic cardiac ganglia are located in some specific fat pads on the surfaces of atria and ventricles[5,6,11,12,13], including right marginal ganglial plexus, but A–PGP have never been investigated. In the present experiments, injections of bradykinin resulted in positive or negative cardiac responses in all regions of intact and decentralized heart at different frequencies. It appeared that the cardiac neurons stimulated by bradykinin can innervate the sinus, right, and left atria as well as the right and left ventricles in different tones. Either positive or negative regional responses were elicited depending on dominant innervation of either sympathomimetic or vagomimetic active neurons sensitive to bradykinin in A–PGP. After acute decentralization, thus blocking the afferent passage, tachycardia, and positive inotropic responses still be induced. These data implied that bradykinin can directly activate efferent neurons associating with sympathomimetic responses. The decrease of frequency of positive responses and the absence of all negative responses indicated that these reactions having been elicited in intact hearts by bradykinin stimulation of afferent neurons were abolished by decentralization.

Bradykinin is a vasodilator agent which undergoes rapid degradation on the way through the pulmonary vasculature[14]. When left ventricular intra–myocardial pressure was depressed, it might be due, in part, to systemic vascular hypotension. However, hypotension would unlikely have induced the bradycardia or negative artial inotropic

changes. Furthermore, when bradykinin was injected into A–PGP or right marginal ganglial plexus, no systemic vascular pressure changes were observed or occurred earlier than other parameters. These data suggested that negative inotropic responses were not due to a reduction in systemic vascular resistance and presumably were from an indirect activation of efferent parasympathetic neurons in cardiac ganglial plexuses.

In summary, bradykinin can modify cardiac neurons in canine ganglial plexuses such that it directly modifies the efferent sympathetic neurons or indirecly modifies the sympathetic and parasympathetic neurons by activation of afferent neurons, and thus, modulates the cardiac rate and force.

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31-40 (4)

缓激肽刺激主动脉与肺动脉之间的神经节丛后所引起的心脏反应

R971.9

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摘要 缓激肽(50 μg)注射入犬主动脉与肺动脉之间的神经节丛，引起心脏各部位的正性或负性变时性和变力性反应。急性心脏去神经支配后，缓激肽再注射于同样部位，某些正性反应仍被引出，而负性反应被消除。表明缓激肽可直接刺激心脏表面节后交感神经元，也可靠刺激传入神经元间接兴奋心脏表面节后交感和副交感神经元。

关键词 缓激肽；心脏；神经节；传出神经元；传入神经元

35-38 (5)

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3,4-二氨基吡啶诱发去甲肾上腺素释放及 B-50 (GAP-43) 磷酸化[1]

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R365.2

3,4-DAP

3,4-Diaminopyridine-evoked norepinephrine release and B-50 (GAP-43) phosphorylation

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ABSTRACT 3,4-Diaminopyridine (3,4-DAP 100 μmol·L^{-1}) evoked [^{3}H]norepinephrine ([^{3}H]NE) release in rat hippocampal slices preincubated with [^{3}H]NE and superfused with medium with or without Ca^{2+}. Phorbolester 4β-phorbol 12, 13, dibutyrate 1 μmol·L^{-1}) enhanced and polymyxin B (100 μmol·L^{-1}) inhibited the release of [^{3}H]NE under both conditions. The neuron-specific protein B-50 is a major presynaptic substrate of protein kinase C and involved in exocytosis. Using *in situ* protein phosphorylation analyzed by SDS-PAGE and autoradiography, we observed that B-50 phosphorylation was significantly decreased by 3,4-DAP in the presence of extracellular Ca^{2+} and completely inhibited by removal of extracellular Ca^{2+}. It was suggested that B-50 phosphorylation was not involved in 3,4-DAP-evoked [^{3}H]NE release.

KEY WORDS calcium; 3,4-diaminopyridine; norepinephrine; B-50 (GAP-43); protein kinase C

摘要 在胞外有 Ca^{2+}或无 Ca^{2+}时, 3,4-二氨基吡啶(3,4-DAP)都能诱发大鼠海马释放去甲肾上腺素, 咐啡醇基酯(phorbol ester)或多粘菌素 B 对此诱发释放有加强或抑制作用. 在胞外有 Ca^{2+}时, 3,4-DAP 显著地减弱 B-50 磷酸化, 除去胞外 Ca^{2+}, B-50 磷酸化完全被抑制. 结果表明, B-50 磷酸化不参与 3,4-DAP 诱发海马去甲肾上腺素释放机制.

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关键词 钙; 3,4-二氨基吡啶; 去甲肾上腺素; B-50 (GAP-43); 蛋白激酶 C

3,4-二氨基吡啶(3,4-diaminopyridine, 3,4-DAP)能诱发海马释放去甲肾上腺素(norepinephrin, NE). 蛋白激酶 C (PKC)的激活剂 phorbol ester (4β-phorbol 12, 13-dibutyrate, 4β-PDB)能加强这一诱发释放[1]. 位于突触前的 PKC 的特异性底物蛋白 B-50 (GAP-43)磷酸化参与 4-aminopyridine (4-AP)诱发神经递质释放以及 PKC 加强递质释放的机制[2]. 本研究观察在细胞外有钙或无钙时, 3,4-DAP 诱发海马去甲肾上腺素的释放, 4β-PDB 对这一诱发释放的加强作用以及脑片中 B-50 (GAP-43)磷酸化, 以考察 B-50 磷酸化是否参与 3,4-DAP 诱发海马释放去甲肾上腺素及其调制机制.

MATERIALS AND METHODS

药品和试剂 [^{3}H]NE (1-(7,8-^{3}H) norepinephrin, [^{3}H]NE, Amersham); 3,4-二氨基吡啶(3,4-diaminopyridine, 3,4-DAP), 咐啡醇基酯(4β-pborbol 12, 13-dibutyrate, 4β-PDB), 多粘菌素 B (polymyxin B sulfate, PMB) (Sigma); Soluene 350, Insta-gel XF, Hionic-fluor (Packard), desipramine (Merck). 同位素标记的 n-[^{32}P]Na_3PO_4 ($^{32}P_i$, 无载体, 中国原子能研究院).

[^{3}H]NE 诱发释放实验 Sprague-Dawley 大鼠, ♂, 体重 250± s 25g, 断头, 取全脑, 投入 4℃ 生理溶液, 在 6-8℃ 下分离海马, 用 McIlwain 组织切片机, 沿板状器方向制备厚 0.4 mm 的脑片. 用生理溶液淋洗后, 加入 2 ml 含[^{3}H]NE (1.6 PBq·mol^{-1}, 0.1 μmol·L^{-1})的生理溶液, 37℃ 保温 30 min, 再用

生理溶液淋洗 3 次将脑片随机转入容量 1 ml 的灌流小室，每室 1 片，以 0.7 ml· min^{-1} 流速进行表面灌流. 生理溶液成分(mmol· L^{-1})：NaCl 118，KCl 4.8，$CaCl_2$ 1.3，$MgSO_4$ 1.2，$NaHCO_3$ 25，KH_2PO_4 1.2，葡萄糖 11，抗坏血酸 0.57，EDTA-Na_2 0.03，用 95% O_2+5% CO_2 饱和，加 NaOH 调 pH 至 7.4. 灌流液中另加 desipramine 1 μmol· L^{-1}. 灌流 45 min 后，将流出液收集入闪烁杯内，每 5 min 一份，加入闪烁液 Insta-gel XF 4 ml，用液体闪烁计数仪测[^{3}H]含量. 脑片用 Soluene 350 0.25 ml 溶解后，加 Hionic-fluor 5 ml，然后测定[^{3}H]含量.

在灌流开始后 60 min，脑片接受刺激(引入 3,4-DAP 100 μmol· L^{-1})持续 10 min，以诱发[^{3}H]NE 释放，在刺激之前 15 min 和 45 min 加入 4β-PDB 和 PMB，以测试它们对诱发释放的影响，每次同时进行对照实验. 药物的作用以计算刺激所诱发释放的^{3}H(包括刺激开始后 10 份样品中[^{3}H]含量减去基础释放量)占脑片[^{3}H]总含量的%来评价，所有结果均以 $\bar{x}\pm s$ 表示，用 t 检验测定组间差别的显著性[3].

体内 B-50 磷酸化实验[4] 大鼠海马脑片 0.4 mm，每管 3 片，加 2 ml(含或不含 Ca^{2+})生理溶液，34℃ 保温 30 min. 然后将脑片移入 900 μl(含 3.7 MBq $^{32}P_i$)生理溶液保温 90 min. 各管分别加入 4β-PDB 1 μmol· L^{-1}，3,4-DAP 200 μmol· L^{-1} 或 4β-PDB 1 μmol· L^{-1}+ 3,4-DAP 200 μmol· L^{-1}，4β-PDB 于 3,4-DAP 之前 15 min 加入，空白对照为不加任何药物. 3,4-DAP 作用 10 min 后，立即用 4℃ 生理溶液(含或不含 Ca^{2+})洗去药物，并将反应管插入冰浴以终止反应. 生理溶液成份：NaCl 124，KCl 5，$MgSO_4$ 1.3，$NaHCO_3$ 26，*D*-glucose 10，含或不含 $CaCl_2$ 2 (mmol· L^{-1})，用 95% O_2+5% CO_2 饱和，调 pH 至 7.4.

从反应管中取出脑片，加入 160 μl 蛋白变性溶液包含：Tris-HCl 62.5 mmol· L^{-1} (pH 6.8)，2.3% 十二烷基磺酸钠(SDS)，10% 甘油，5% β-巯基乙醇和痕量溴酚兰制备匀浆，通过 SDS-PAGE 分离组织内的标记蛋白[5]. 下胶(分离胶，110 mm× 160 mm× 1.2 mm)含有 10% 丙烯酰胺和 0.27% 双丙烯酰胺(Bis)；上胶(堆胶)含有 4.4% 丙烯酰胺和 0.12% BiS. 电泳缓冲液的成分为 Tris-HCl 0.05，EDTA 0.002，甘氨酸 0.375 mol· L^{-1}，0.1% SDS，pH 8.3. 取组织匀浆 20 μl(经定量测定含蛋白约 35 μg)进行电泳. 用作分子量测定的标准蛋白，分子量为：牛血清白蛋白 67 000，卵清蛋白 43 000，胰凝乳蛋白酶元 A 25 000 和核糖核酸酶 A 13 700. 电泳电压：60 V，电泳 30 min，然后将电压调至 125 V，电泳 3-4 h，直到染料前沿到达离凝胶底边约 1 cm 处时停止电泳. 凝胶经染色，真空干燥后，用放射自显影技术显示磷酸化蛋白电泳图谱.

RESULTS

1 在细胞外有 Ca^{2+} 条件下，将 3,4-DAP 引入脑片周围灌流液中，[^{3}H]NE 释放明显升高，10 min 后撤去刺激，释放即逐渐下降，并恢复到基础水平. 在细胞外无 Ca^{2+} 条件下，3,4-DAP 亦能诱发[^{3}H]NE 释放，10 min 后撤去刺激，释放继续上升至高峰，然后逐步下降，并恢复到基础水平(Fig 1，下部). 有 Ca^{2+} 组的释放量明显大于无 Ca^{2+} 组. 两组之差(即由胞外 Ca^{2+} 进入胞内所引起的 Ca^{2+}-依赖性释放)为组织[^{3}H]总含量的 3.20%.

2 4β-PDB 加强 3,4-DAP 诱发[^{3}H]NE 释放. 无论在细胞外有无 Ca^{2+} 条件下，若刺激之前 15 min 加 4β-PDB 1 μmol· L^{-1}，3,4-DPA 诱发[^{3}H]NE 释放量显著增加，细胞外有 Ca^{2+} 组仍高于胞外无 Ca^{2+} 组，两组之差为组织[^{3}H]总量的 3.67% (Fig 1，上部)与不加 PDB 时 (Fig 1，下部) 3.20% 相比，无显著性差别 ($P>0.05$).

3 PMB 抑制 3,4-DAP 诱发[^{3}H]NE 释放. 无论在细胞外有或无 Ca^{2+} 条件下，若在刺激之前 45 min 加入 PMB 100 μmol· L^{-1}，3,4-DAP 诱发[^{3}H]NE 释放都受抑制，前者抑制了 58%，后者抑制了 94% (Tab 1).

4 在细胞外有 Ca^{2+} 和无 Ca^{2+} 时 B-50 磷酸化. 在细胞外有 Ca^{2+} 条件下，B-50 磷酸化

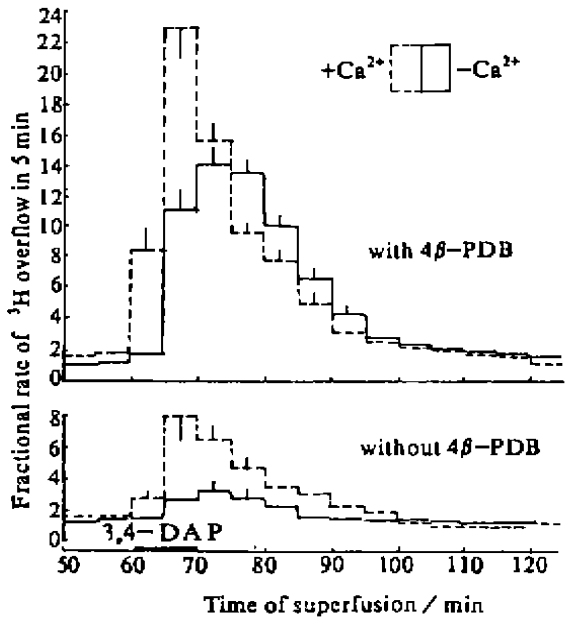

Fig 1. 3,4–Diaminopyridine (3,4–DAP)–evoked ^3H overflow in the presence or absence of extracellular Ca^{2+} with or without 4β–phorbol 12, 13 dibutyrate (4β–PDB). ^3H overflow was induced by addition of 3,4–DAP 100 μmol· L^{-1} for 10 min after 60 min superfusion. 4β–PDB 1 μmol· L^{-1} was added 15 min before stimulation. n= 6–10 slices from 2–4 rats. $\bar{x}\pm s$.

Tab 1. Effects of polymyxin B (PMB) on 3,4–DAP–evoked [^{3}H]NE release in the presence and absence of extracellular Ca^{2+}. Hippocampal slices preincubated with [^{3}H]NE, superfused with medium (with or without Ca^{2+}) and stimulated by addition of 3,4–DAP 100 μmol· L^{-1} for 10 min. PMB 100 μmol· L^{-1} was added 45 min before the stimulation. n= 5–10 slices from 2–4 rats. $\bar{x}\pm s$. $^{***}P<0.001$ *vs* controls.

		3,4–DAP–evoked ^3H overflow (% of tissue ^3H)		
	n	Ca^{2+} 1.3 mmol· L^{-1}	n	Ca^{2+} 0 mmol· L^{-1}
Control	10	7.04± 0.64	10	3.14± 0.34
PMB	5	3.10± 0.32***	5	0.16± 0.12***

结果(Fig 2, 左). 从左到右分别为: (0) 标准 B–50 蛋白磷酸化后的电泳定位(见箭头处); (1) 不加任何药物时, 磷酸化的 B–50 蛋白在标准蛋白相当的部位上被显示; (2) 加 4β–PDB 1 μmol· L^{-1}, B–50 磷酸化有所加强; (3) 加 3,4–DAP 200 μmol· L^{-1} 10 min, B–50 磷酸化被抑制; (4)和(5) 4β–PDB 1 μmol· L^{-1} 和 3,4–DAP 200 μmol· L^{-1} 同时使用, B–50 磷酸化程度比单独使用 3,4–DAP 时略加强. 在细胞外无 Ca^{2+}条件下, 脑片内的蛋白磷酸化普遍明显下降, 特别是 B–50 磷酸化几乎被完全抑制(Fig 2, right).

Fig 2. Effects of 3,4–DAP and 4β–PDB on B–50 protein phosphorylation *in vivo*. In the presence or absence of extracellular Ca^{2+} hippocampal slices were incubated with or without 3,4–DAP 100 μmol· L^{-1} or/ and 4β–PDB 1 μmol· L^{-1}. *In situ* protein phosphorylation was analyzed by SDS–PAGE (10% gel) and autoradiography. The position of B–50 is indicated with an arrow. (0) standard B–50; (1) control; (2) 4β–PDB 1 μmol· L^{-1}; (3) 3,4–DAP 200 μmol· L^{-1}; (4) and (5) 4β–PDB 1 μmol· L^{-1}+ 3,4–DAP 200 μmol· L^{-1}.

DISCUSSION

1989 年我们首次报道了 3,4-DAP 可以作为化学刺激物制造动作电位诱发神经递质释放模型，以研究递质释放及其调节机制. 我们以往的实验结果[6]已提示其作用机制大致如下: (1) 阻断 I_DK^+电流; (2) Na^+通道开放, Na^+进入细胞内; (3) 细胞外 Ca^{2+}进入细胞内(在胞外无 Ca^{2+}时，进入膜内的 Na^+引起内源 Ca^{2+}释放); (4) 最后诱发神经递质释放. 现观察到用 4β-PDB 激活蛋白激酶 C, 3,4-DAP 诱发海马释放去甲肾上腺素显著加强. 用 PMB 抑制 PKC, 能使这一诱发释放下降，而且 PDB 和 PMB 的作用主要在 Ca^{2+}不依赖释放部分. 说明蛋白激酶 C 参与 Ca^{2+}不依赖释放机制. 然而, PKC 激活后加强胞吐的机制尚未得到阐明. 位于突触前的蛋白激酶 C 的特异性底物蛋白 B-50 (GAP-43)参与胞吐机制，认为 B-50 磷酸化中介 4-AP 刺激去甲肾上腺素释放机制[7,8]. 我们观察到在细胞外有钙条件下，3,4-DAP 对 B-50 磷酸化显示了抑制作用,这与 Heemskerk 等[9] 1987 年报道的结果相一致，而 4β-PDB 无论在有无 3,4-DAP 的情况下都能加强 B-50 磷酸化. 除去细胞外 Ca^{2+}，脑片中许多种蛋白(包括 B-50)的磷酸化都受到很大抑制，而 3,4-DAP 诱发[^{3}H]NE 释放仍非常明显，4β-PDB 对这一诱发释放的加强作用亦仍清晰可见，提示 B-50 磷酸化并不参与 3,4-DAP 诱发去甲肾上腺素释放或 4β-PDB 加强这一诱发释放的机制，特别是在细胞外无 Ca^{2+}条件下.

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猪冠状动脉上的苯环利定受体[1]

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Phencyclidine receptors in porcine coronary artery[1]

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ABSTRACT By using radioligand assay, there was a phencyclidine (Phe) binding site in porcine coronary artery. This binding was specific, reversible, saturable, and stereoselective. The Scatchard analysis showed that the binding site was a single class, with dissociation constant (K_d) and maximum binding (B_{max}) of 27.7± 6.9 nmol· L^{-1} and 0.82± 0.15 pmol/mg protein, respectively. The displacement experiments revealed that the [^{3}H]Phe binding was displaced by nonradioactive Phe, TCP (Phe receptor agonists), and dextrophan (its antagonist). d-INN, a ligand of sigma receptor, had a weaker activity of displacement. These showed a dose-dependent manner. Both etorphine, an agonist of opioid receptor, and *N*-methyl-*D*-aspartic acid (NMDA), an excitatory amino acid, failed to displace the binding. These results suggest that the Phe receptors exist in the porcine coronary artery.

KEY WORKS phencyclidine; drug receptors; coronary vessels; radioligand assay

摘要 本文采用放射受体结合法研究猪冠状动脉上的苯环立定(Phe)受体，发现猪冠状动脉上存在 Phe 特异结合部位，Scatchard 分析表明，结合呈单位点，K_d 为 27.7± 6.9 nmol· L^{-1}，B_{max} 为 0.82± 0.15 pmol/mg protein. 取代实验表明 Phe 及 TCP, dextrophan 和 sigma 受体配基 *d*-INN 均有取代作用，两类药物取代作用呈量-效关系，本实验结果提示猪冠状动脉上存在的特异结合位点是 Phe 受体.

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关键词 苯环利定; 药物受体; 冠状血管; 放射配位体测定

动物的心房[1]、心室[2]、兔耳血管[3]及肠系膜血管上[4]有 Phe 受体，并参与心血管功能的调节. Phe 促进脑内单胺类递质的释放[5]并抑制其重摄取[6]，与脑缺血等疾病有关[7]. 冠状动脉上是否也存在 Phe 受体，Phe 对冠脉功能有无调节作用，至今未见文献报道. 本文研究猪冠状动脉上的 Phe 受体，为其功能找到受体基础.

MATERIALS AND METHODS

[^{3}H]Phencyclidine (0.91 TBq· $mmol^{-1}$)，放射纯度 >95%，上海医科大学放射药学教研室提供；Phe，etorphine 由上海医科大学药学院合成；*N*-(1-2-thienyl cyclohexyl)-3,4-piperidine (TCP)，军事医学科学院赠；*N*-allylnormetazocine (INN)由美国 NIDA 的 Dr Richard L HANKS 赠；dextrophan，美国 Prof Avram GOLDSTEIN 赠；*N*-methyl-*D*-aspartic acid (NMDA)，美国 Du Pont 公司惠赠.

新鲜的猪冠状动脉取自上海龙华肉联加工厂. XHF-1 高速分散器由上海兴华电子仪器厂生产.

猪冠状动脉匀浆蛋白的制备 Tris 5 mmol· L^{-1} 缓冲液(pH 7.4, 4℃)中分离猪冠状动脉左降支 6.5 cm，右支第一分支处开始分离 8 cm，去掉血管周围结缔组织及脂肪，洗去血液，剪碎，在玻璃匀浆管内匀浆 5 min，然后在高速分散器上捣碎 10 000 rpm 1 min，匀浆液离心 30 000× *g* 反复 3 次，测定沉淀层蛋白含量.

匀浆蛋白的饱和分析及 Scatchard[8]分析 用 [^{3}H]Phe 和冠状动脉匀浆蛋白进行反应，反应分总结合管(TB)和非特异结合管(NSB)，TB 管加入[^{3}H]Phe，匀浆蛋白，NSB 管还须加入 100 μmol· L^{-1} 的非标记

Phe. 反应容量为 0.5 ml, 投料在冰浴中进行. 反应管在 4℃ 放 1 h. 用 GF/C 玻璃纤维滤膜过滤反应液, 用冰冷缓冲液快速冲洗滤膜 3 次, 每次 5 ml. GF/C 滤膜使用前先浸入含 0.01% polyethyleni-mine 缓冲液内 2 h, 以减少滤膜自身吸附. 滤膜用红外线烘干, 在液体闪烁计数仪(LKB 公司生产)上计数. 根据实验要求, 匀浆蛋白和[^{3}H]Phe 投料如下:

采用匀浆蛋白 0.05, 0.15, 0.25, 0.35, 0.45, 0.6 mg 与[^{3}H]Phe 20 nmol·L^{-1} 进行反应.

采用 0.25 mg 的匀浆蛋白与[^{3}H]Phe 3, 6, 19, 38, 51, 76, 126, 152 mmol·L^{-1} 进行反应.

[^{3}H]Phe 结合竞争性抑制反应 采用 0.25 mg 匀浆蛋白和[^{3}H]Phe 20 nmol·L^{-1} 作结合反应, 用 0.01, 0.1, 0.5, 1, 5, 10, 100 μmol·L^{-1} 的 Phe, TCP, dextrophan, *d*-, *l*-INN etorphine 和 NMDA 取代这种结合, 计算各药物的抑制%, 求出抑制特异结合达 50%时所需浓度(IC_{50}).

RESULTS

匀浆蛋白饱和分析 用[^{3}H]Phe 20 nmol·L^{-1} 与猪冠状动脉匀浆蛋白作特异结合, 在 0.1–0.4 mg 范围内, 结合随蛋白浓度增加而增加, 达 0.5 mg 时呈饱和状态. 在 0.25 mg 蛋白处有较大的 SB/NSB 比值 (2.2 : 1), 故在本文 Scatchard 分析及取代反应中均采用 0.25 mg 匀浆蛋白.

Scatchard 分析 特异结合随[^{3}H]Phe 浓度增大而增加, 到达 150 nmol·L^{-1} 时, 呈饱和趋势(n=3) (Fig 1). 说明[^{3}H]Phe 结合反应具有饱和性. 根据 Scatchard 分析, [^{3}H]Phe 和匀浆蛋白的特异结合是单一位点, 计算机处理数据, 求得解离常数(K_d)和最大结合量(B_{max})分别为 27.7± 6.9 nmol·L^{-1} (n=3) 0.82± 0.15 pmol/mg protein (n=3).

[^{3}H]Phe 与受体结合的竞争性抑制 几种配基均可取代[^{3}H]Phe 的特异结合, 从取代强度看, 以 Phe 配基 Phe、TCP、dextrophan

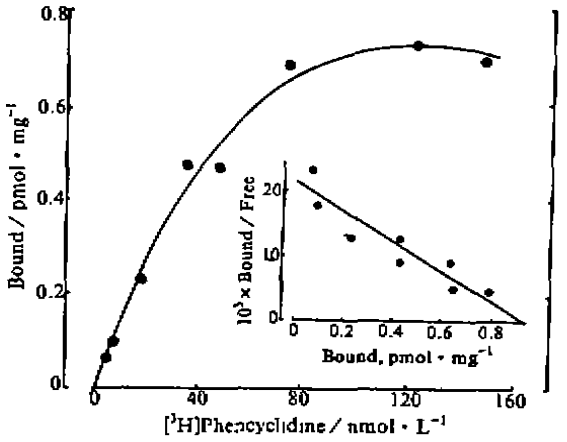

Fig 1. Saturation of the specific binding of [^{3}H]Phe to porcine coronary artery. (Inset) Scatchard analysis.

较强, 而 sigma 受体配基 INN 较弱, 其中右旋体的取代作用强于左旋体, 左旋体取代作用极弱. NMDA 和 etorphine 几无取代作用 (Tab 1).

Tab 1. Potencies of unlabeled ligands in inhibition of [^{3}H]Phencyclidine binding.

Drugs	n	IC_{50}/ μmol·L^{-1}	Relative potency
Phencyclidine	3	0.34± 0.20	1.00
TCP	3	0.56± 0.43	0.61
Dextrorphan	3	4.03± 1.05	0.08
d-INN	3	5.78± 2.71	0.06
l-INH	3	15.99± 5.54	0.02
Etorphine	3	>100	–
NMDA	3	>100	–

DISCUSSION

本实验中[^{3}H]Phe 与冠状动脉匀浆蛋白的结合具有饱和性, 一系列受体配基竞争取代实验表明这一结合是特异的, 可逆的. INN 的取代作用说明了该结合的立体专一性. 由此证明该结合具有受体结合的特点, 即特异性、