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大鼠牙髓组织缓激肽与脑啡肽的交互作用[1]

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Interaction between bradykinin and enkephalins in rat dental pulp

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ABSTRACT The content of bradykinin (BK)-like peptides in rat dental pulp was significantly increased 1, 6 and 24 h after cavity formation at the neck of incisor. We have reported that enkephalin (EK)-like peptides in rat dental pulp were increased by cavity formation or BK. In the present study, the mechanism of the production of EK enhanced by BK was investigated using benzoyl-L-arginine-2-naphthylamide (BANA), a synthetic subtrate. BK and its products cleft by carboxypeptidase B, des-Arg^9-BK and arginine (Arg), activated the degradation of BANA. It is suggested that these substances may enhance the processing of enkephalins from precursor proteins. The activating effects were inhibited by EGTA. The BANA-degrading enzymes in lysosomal fraction were activated by BK, des-Arg^9-BK and Arg, but the enzymes in supernatant were activated by Arg only. On the other hand, morphine and met-EK inhibited the production of BK-like peptides by trypsin from plasma kininogen. It is suggested that BK is cleft

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by carboxypeptidase B in pulp cell to des-Arg⁹-BK and Arg, which activate the lysosomal or soluble EK processing enzymes, and then the produced EK inhibits the production of BK from plasma kininogens in the pulp.

KEY WORDS dental pulp; bradykinin; EGTA; benzoyl-L-arginine-2-naphthylamide (BANA); lysosomes; methionine-enkephalin; morphine

提要 切牙牙颈钻孔造成的损害性刺激增加牙髓缓激肽(bradykinin, BK)样物质含量。BK及其羧肽酶B分解产物均可激活牙髓BANA分解酶，提示这些物质可促进牙髓中脑啡肽从前体蛋白的生成。这些激活作用均被EGTA抑制。该酶主要分布于牙髓细胞的溶酶体及可溶性成分中。另一方面，吗啡和甲啡肽抑制BK样物质从血浆激肽原的生成。

关键词 牙髓；缓激肽；EGTA；甲苯-L-精氨酸-2-萘酰胺；溶酶体；蛋氨酸脑啡肽；吗啡

牙颈钻孔引起大鼠牙髓损害性刺激后，牙髓中内源性镇痛物质脑啡肽(enkephalin, EK)含量显著增加；离体牙髓实验中，致痛物质缓激肽(bradykinin, BK)可增加EK的生成和释放[1-3]。本文在确认牙髓受损害刺激后BK含量增加的基础上，探讨BK促进EK生成的机理。以柱层析(Sephadex G 75)作牙髓组织酶分离，然后分别以*Nα*-benzoyl-L-arginine-β-naphthylamide (BANA)和牙髓EK前体蛋白的粗提出物作底物，发现分离液中BANA分解酶活性与EK生成酶活性的峰平行，该酶在性质上与cathepsin B相似(未发表资料)，故本文以BANA作底物间接观察EK生成酶的特点；还初步探讨了EK对BK生成的影响。

MATERIALS AND METHODS

切牙牙颈钻孔对牙髓BK含量的影响 SD系大鼠204±SD 22 g, ♂, (购自日本动物株式会社)，以乌拉坦10 g/kg, ip麻醉，于上下切牙牙颈部以牙科电动钻机各钻一深达牙髓的孔，造成牙髓性疼痛刺激。钻孔后1，6，24，72 h处死大鼠取出牙髓，置于HCl 0.1 mol/L匀浆化后离心，100 000×g，上清部分冰冻干燥后以抗BK抗血清作BK样物质含量的放射免疫学测定。

牙髓BANA分解酶实验

1 *完整牙髓* 正常大鼠牙髓在37℃含氨肽酶抑制剂bestatin 20 μmol/L和血管紧张素转换酶(ACE)抑制剂captopril 1 μmol/L的Hanks液(pH 7.4)中，与BANA及BK等一起温浴30 min后，90℃加热4 min中止反应，冷却后之反应液以荧光光度计(岛津RF-540)测定分解产物β-naphthylamine的量(ex 338 nm; em 410 nm)。TPCK处理的胰蛋白酶用作标准酶，BANA分解酶活性以相对于胰蛋白酶的量表示。

2 *牙髓匀浆及各亚细胞成分* 大鼠牙髓在含蔗糖0.25 mol/L的磷酸缓冲液(pH 7.4)中匀浆化后，按分次离心法[4]分离各亚细胞成分。匀浆及各段成分按上述方法分别与BANA及BK等温浴30 min后，加热中止反应，离心后上清作BANA分解酶活性测定。

阿片样物质对BK生成的影响 以肝素抗凝的正常大鼠血浆以56℃热处理5 h，离心后上清部分用作血浆激肽原。热处理血浆30 μl，胰蛋白酶(激肽生成酶之一)20 μg及吗啡等阿片样物质一起在1.5 ml Hanks液(pH 7.4)中37℃温浴2 h后，以90℃ 20 min中止反应，冷却后以60% 乙醇沉淀蛋白质，上清部分冰冻干燥后作BK样物质含量的放射免疫学测定。

BANA和TPCK处理的胰蛋白酶为美国Sigma公司产品；BK, des-Arg⁹-BK, Arg，甲啡肽为Peptide Institute Inc(日本大阪)产品；ethylketociazocine methanesulfonate (EKC)为美国Sterling-Winthrop研究所产品。

RESULTS

牙颈钻孔对牙髓BK样物质含量的影响 钻孔后1，6，24 h，牙髓BK样物质含量显著增加，72 h后恢复至正常水平(Tab 1)。

Tab 1. Influence of cavity formation on the content of bradykinin (BK)-like peptides in rat dental pulp *in vivo*. Number of rats in parentheses. $\bar{x}\pm SD$. $^{*}P>0.05$, $^{***}P<0.01$.

Time after cavity formation (h)	BK-like peptide (pmol/g tissue)	
	Control	Cavity formation
1	51± 3(5)	98± 7(6)***
6	52±14(8)	73±11(8)***
24	50±10(8)	83±10(8)***
72	48±16(8)	54±12(7)*

Tab 2. Effects of BK and des-Arg^9-BK on benzoyl-L-arginine-2-naphthylamide (BANA)-degrading enzyme activity in rat dental pulp *in vitro*. $\bar{x}\pm SD$. $^{**}P<0.05$, $^{***}P<0.01$.

	Activity equivalent to trypsin (μg/g tissue)	
μmol/L	BK	des-Arg^9-BK
0	3± 4(4)	8± 9(12)
0.1	16± 8(4)**	47± 7(3)***
1.0	29±11(4)**	51±13(10)***
10.0	34± 6(4)***	68± 4(3)***

Tab 3. Effects of BK (1 μmol/L), des-Arg^9-BK (1 μmol/L) and arginine (Arg, 1 μmol/L) on BANA-degrading enzyme activities of rat dental pulp in presence or absence of EGTA. $\bar{x}\pm SD$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* control in absence of EGTA; $^{\dagger}P>0.05$, $^{\dagger\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* EGTA 0 mol/L.

	Activity equivalent to trypsin (μg/g tissue)	
	EGTA 0 mol/L	EGTA 2.5 mol/L
Control	13±9(10)	9±12(11)†
BK	22±2(5)**	17± 4(5)††
des-Arg^9-BK	33±7(4)***	13± 4(4)†††
Arg	30±3(4)***	13± 3(4)†††

BK 对牙髓 BANA 分解酶活性的影响

1 完整牙髓 BK(0.1-10 μmol/L)及其羧肽酶 B 分解产物 des-Arg^9-BK (0.1-10 μmol/L)浓度依存性增强 BANA 分解酶活性，后者作用比 BK 更强(Tab 2)；Arg 亦有显著的增强作用(Tab 3)。BK 及其衍生物的激活作用被钙螯合剂 EGTA (2.5 mmol/L)显著地抑制(Tab 3)。

2 牙髓匀浆和各亚细胞成分 BK 10 μmol/L，des-Arg^9-BK 10 μmol/L 和 Arg 10 μmol/L 对牙髓匀浆 BANA 分解酶活性均有增强作用。对溶酶体成分中的 BANA 分解酶活性，该三物质均有很强的增强作用，而对上清中的酶活性仅 Arg 有明显的增强作用。des-Arg^9-BK 对微粒体中酶活性亦有显著的增强作用(Tab 4)。

阿片样物质对 BK 生成的影响 对于大鼠血浆激肽原在胰蛋白酶作用下生成 BK 样物质，甲啡肽 10 μmol/L 和吗啡 10 μmol/L 有明显的抑制作用，κ-阿片受体激动剂 EKC 则无显著抑制作用(Tab 5)。

Tab 4. Subcellular distribution of BANA-degrading enzymes in rat dental pulp cells and their activation by BK, des-Arg^9-BK and Arg. $n=4$-8, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Fraction	Activity equivalent to trypsin (μg/mg protein)			
	Control	BK 10 μmol/L	des-Arg^9-BK 10 μmol/L	Arg 10 μmol/L
Homogenate	0.28±0.11	0.46±0.10**	0.41±0.04**	0.76±0.06***
Nuclear fraction	0.68±0.24	0.75±0.30*	0.40±0.29*	0.58±0.20*
Mitochondrial fraction	3.78±0.99	2.61±1.11*	4.95±1.68*	2.15±0.63*
Lysosomal fraction	3.61±0.74	11.95±1.18***	29.78±3.50***	13.54±1.25***
Microsomal fraction	0.82±0.44	0.66±0.32*	2.21±0.38***	0.42±0.27*
Supernatant	0.74±0.36	0.60±0.19*	0.42±0.29*	3.43±0.75***

Tab 5. Effects of met-enkephalin (ME), morphine and ethylketocyclozocine (EKC) on the production of BK-like peptides by trypsin (20 μg) from rat heated plasma *in vitro*. Number of samples in parentheses, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$ *vs* control.

	BK-like peptides (μmol/L heated plasma)		
Control	2.11±0.38(4)		
	0.1 μmol/L	1.0 μmol/L	10.0 μmol/L
Morphine	2.10±0.28(8)*	1.86±0.32(7)*	1.63±0.16(7)**
ME	1.96±0.06(4)*	1.72±0.26(4)*	1.11±0.32(4)**
EKC	1.70±0.25(4)*	2.02±0.37(4)*	2.14±0.42(4)*

DISCUSSION

损害性刺激可引起牙髓BK样物质含量增高[5]。本文以放射免疫学方法进一步加以证实。Kudo等发现的损害性刺激及BK促进牙髓EK的生成和释放[1,2]，是否由于激活EK生成酶，本文应用BANA对此作了探讨，结果表明BK可增强BANA分解酶活性。BK在细胞内可被酶分解，故还应考虑BK的酶分解产物起的作用。由于实验中以bestatin抑制氨肽酶及captopril抑制ACE，羧肽酶B分解产物的作用当予重视。本文发现，BK的羧肽酶B分解产物des-Arg^9-BK和Arg亦与激活BANA分解酶有关，甚至比BK本身更强，提示BK经羧肽酶分解后起作用。三者的激活作用均可被EGTA抑制。EGTA的抑制作用可被Ca^{2+}浓度增高所拮抗[6]，表明受BK及其衍生物激活的BANA分解酶是Ca^{2+}依赖性酶。

牙髓各亚细胞成分的酶活性分布结果表明，BK，des-Arg^9-BK和Arg均增强溶酶体成分中的BANA分解酶作用；在上清液中仅Arg有明显增强作用，这提示牙髓中EK生成酶可能存在于溶酶体和细胞可溶性成分中，溶酶体中的该酶可被BK及des-Arg^9-BK和Arg激活；可溶性酶则被Arg激活。此外des-Arg^9-BK尚可激活微粒体的BANA分解酶，表明在蛋白质合成，加工及输送部位也有受BK激活的该酶。溶酶体在EK生成中的作用，BK及其分解产物是通过受体还是直接激活EK生成酶，有待探讨。

此外，吗啡等阿片样物质抑制牙髓因损害性刺激引起的BK释放的作用，一般认为这是由于抑制血管通透性从而减少激肽原及激肽生成酶的游离[3]，本文表明吗啡及甲啡肽直接抑制激肽生成酶亦是一种可能的机理。

以上结果提示，损害性刺激促进BK生成，BK进入牙髓细胞后，被羧肽酶B分解为des-Arg^9-BK和Arg，后两者在Ca^{2+}存在下激活溶酶体或其他部位的EK生成酶，使EK大量生成，EK则可直接抑制BK生成酶活性，减少BK的生成。

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