Calcitonin Gene Related Peptide에 의한 치수미세순환 조절

김성교* · 김영경 · 진명욱
경북대학교 치과대학 치과보존학교실 및 치수생물학연구실

ABSTRACT

REGULATION OF PULPAL MICROCIRCULATION BY CALCITONIN GENE-RELATED PEPTIDE

Sung-Kyo Kim*, Young-Kyung Kim, Myoung-Uk Jin
Department of Conservative Dentistry and Laboratory of Pulp Biology,
School of Dentistry, Kyungpook National University

The purpose of this study was to investigate the function of calcitonin gene-related peptide (CGRP) in regulatory mechanism of pulpal microcirculation with the aim of elucidating neurogenic inflammation.

Experiments were performed on twelve cats under general anesthesia. CGRP was administered through the femoral vein to see the systemic influence and through the external carotid artery to see the local effect. Sympathetic nerve to the dental pulp was stimulated electrically and pulpal blood flow (PBF) was measured with a laser Doppler flowmeter on the canine teeth to the drug administration. The paired variables of control and experimental data were compared by paired *t*-test and differences with $p < 0.05$ were considered statistically significant.

Systemic administration of CGRP (0.3 ㎍/㎏) exerted decreases in systemic blood pressure and caused changes in PBF with an initial increase followed by decrease and a more marked second increase and decrease.

Close intra-arterial (i.a.) injection of CGRP (0.03 ㎍/㎏) resulted in slight PBF increase. The effect of CGRP resulted in no significant increase in PBF in the presence of $CGRP_{8-37}$.

The electrical stimulation of the sympathetic nerve alone resulted in PBF decreases. The i.a. administration of CGRP following the electrical stimulation of the sympathetic nerve compensated the decreased PBF. Therefore, CGRP effectively blocked the sympathetic nerve stimulation-induced PBF decrease.

Results of the present study have provided evidences that even though the local vasodilatory function of CGRP are weak, CGRP is effectively involved in blocking the vasoconstriction caused by sympathetic nerve stimulation in the feline dental pulp. 〔J Kor Acad Cons Dent 30(6):470-476, 2005〕

Key words : Dental pulp, Blood flow, Calcitonin gene related peptide (CGRP), Sympathetic nerve stimulation, Neurogenic inflammation, Laser Doppler flowmeter

- Received 2005.7.9., revised 2005.8.24., accepted 2005.8.26. -

Ⅰ. Introduction

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide encoded in the calcitonin gene and is known to play an important role in vasoregulation via vascular leakage and vasomotion[1].

* *Corresponding Author:* ***Sung-Kyo Kim***

Department of Conservative Dentistry and Laboratory of Pulp Biology, School of Dentistry, Kyungpook National University, 188-1, Samdeok-Dong 2-Ga, Jung-Gu, Daegu. Korea, 700-412
Tel : 82-53-420-5935 Fax : 82-53-426-8958
Email : skykim@knu.ac.kr

※This work was supported by the Medical Research Institute grant, Kyungpook National University Hospital (2000).

CGRP positive fibers were found throughout the normal pulp[2]. Inferior alveolar nerve denervation induced a complete loss of CGRP- and SP-immuno reactive fibers in the anterior part of the jaws3). CGRP positive fibers became denser and the number of fibers penetrating into dentinal tubules also increased in inflammatory pulps[2]. Pulpal fibroblasts demonstrated NK1 and NK2 receptors and NK1 and CGRP receptors were identified in the dental pulp[4]. CGRP expression was significantly higher in acute irreversible pulpitis than in healthy pulps[5].

Sympathetic nerve is believed to exert a vasoregulatory function in dental pulp. Its function is mainly vasoconstriction through the release of norepinephrine and neuropeptide Y[6].

For the full understanding of neural reactions to dental injury and inflammation, it needs to include studies of sympathetic, parasympathetic, and sensory fibers and their reactions with pulp[7]. Since there is some evidence that the function of intradental sensory nerve system is affected by activation of the sympathetic nerve[8], there are possible interactions between sensory and sympathetic nerve systems. The possible interaction of vasoregulatory functions needs to be understood between sensory nerve system and sympathetic nerve system. Although some functions of neuropeptides are studied, little is known about their functions in relation to each other.

Therefore, the purpose of this study was to investigate the function of calcitonin gene-related peptide (CGRP) in relation to the sympathetic nerve stimulation in the regulatory mechanism of pulpal microcirculation with the aim of elucidating neurogenic inflammation.

Ⅱ. Materials and Methods

Animal Preparation

All experimental protocols on animal use were approved by the Institutional Animal Care and Use Committee of Kyungpook National University. Twelve cats with average age of 13 months and average weight of 3.7 ㎏ were used. Periapical radiographs were taken of the canine teeth to assure the fully formed apices and to estimate the size of the pulp. Animals were initially anesthetized with intra-peritoneal injection of sodium pentobarbital (35 ㎎/㎏) (Nembutal®, Abbott Lab., N. Chicago, Il., U.S.A.). A femoral artery and a femoral vein were cannulated for the monitoring of systemic blood pressure and for supplemental anesthetics (2 ㎎/㎏ as needed), respectively. A lingual artery was catheterized for the local injection of drugs into the maxillary artery. Air way was maintained through the tracheostomy.

The body temperature was monitored with a thermometer in the stomach, and an electric animal blanket was used to maintain the temperature between 37 and 38°C.

The mandible of animal was stabilized by intermaxillary immobilization by means of a steel rod with dental stone and anchored with a magnetic stand.

Sympathetic Nerve Stimulation

Cervical sympathetic nerve was isolated from vagosympathetic nerve trunk under a binocular epiilluminting surgical microscope (OPMI1®, Zeiss Co., Germany), placed on bipolar silver electrodes and stimulated with a current of 10 Hz, 4 V and 1.5 ms for 3.5 min with a Grass S48 stimulator giving square pulses. A pool was made and the preparation was kept immersed in mineral oil (U.S.P.®, Humco Lab., TX, USA) to prevent drying of the nerve fiber.

Pulpal Blood Flow (PBF) Measurement

Enamel was removed with a dental bur over the cervical third of the canine teeth ipsilateral to the catheterized maxillary artery. To the exposed dentin the laser Doppler flowmeter was placed. The exposed dentinal surface was kept wet with isotonic saline solution to avoid drying.

The PBF was monitored with a laser Doppler flowmetry (PeriFlux PF3®, Perimed Co., Stockholm, Sweden). The blood pressure recording and the laser Doppler recording of PBF (in perfusion unit) were monitored continuously and simultaneously throughout the experiment with a Gould P23 physiological pressure transducer and

a Gould 2400S recorder (Gould Inc., Cleveland, Ohio, USA).

All injections and measurements were made 90 min after the animal preparation since *Liu et al.* (1987) reported that a minimum of 30 - 50 min is required after cavity preparation for PBF to return to the control level.

Administration of Drugs

Systemic administration of CGRP was through the femoral vein to see its systemic influence. To see the local effect, close intra-arterial (i.a.) administration of drugs was performed via a catheter introduced into the lingual artery. The tip of catheter was at the junction of external carotid artery and the lingual artery without interfering blood flow of the external carotid artery.

100 ㎍/㎏ of human CGRP (C-0167, Sigma-aldrich Co., St Louis, Missouri, U.S.A.) and 1.0 mg of human CGRP8-37 (C-2806, Sigma-aldrich Co.) were dissolved in 1 mL of phosphate buffered saline with 1% bovine serum albumin (Sigma-aldrich Co.) respectively. Aliquots of the drugs were made and kept frozen in a freezer of -70˚C until use to minimize degradation of the drugs, and normal saline solution were used to dilute drugs at the time of administration.

CGRP was given at the dose of 0.3 ㎍/㎏ to see the systemic influence. CGRP was given at the dose of 0.03 ㎍/㎏ alone and after $CGRP_{8-37}$ administration to see the local effect. $CGRP_{8-37}$ was given at the dose of 8.0 - 24.0 ㎍/㎏ for 1 min before CGRP administration or sympathetic nerve stimulation. 0.2 mL of normal saline solution was injected instead of drugs through the lingual artery as a control.

In order to minimize the systemic effects of these drugs for the evaluation of local effect, each drug was tried at several dosages, starting with low dose and observed PBF changes and arterial pressure changes. The dosages which alter the PBF without influencing the systemic blood pressure were selected.

The selected dose of drug was given i.a. into the maxillary artery for 1 min followed by a 0.3 mL of isotonic saline flush. To exclude the effects of previously injected drugs or stimulation, at least 90 minutes were passed before the next one.

A stable laser Doppler signal during a recording of 10 - 15 sec before each administration was defined as the control value, the maximum deviation after each administration was used as the experimental value, and PBF values were recorded on a multichannel recording chart until laser Doppler flowmetry readings returned to the control level.

Statistics

All numerical data in the text and tables are expressed as percent change from control and mean ± standard error of the mean (SEM). The paired variables of control and experimental data were compared by paired t-test and differences with $p < 0.05$ were considered statistically significant.

Ⅲ. Results

A) Systemic Influence of CGRP on PBF

Systemic administration of CGRP (0.3 ㎍/㎏) through femoral vein exerted decrease in systemic blood pressure and caused PBF change with an initial increase of 68.85 ± 25.79% followed by decrease to -2.45 ± 9.47% and a second increase of 161.80 ± 17.14% followed by back to the control level (n = 4, Figure 1).

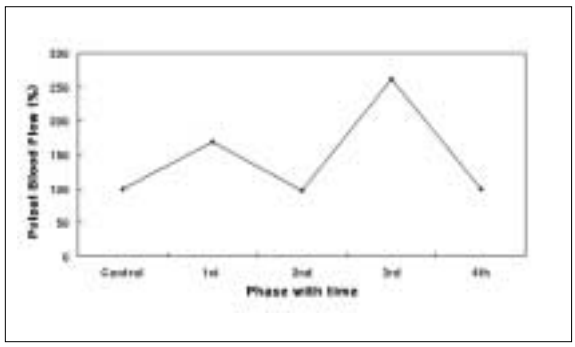

Figure 1. Systemic influence of CGRP on pulpal blood flow (PBF, mean ± SEM). Injection of CGRP (0.3 ㎍/㎏) through the femoral vein exerted decreases in systemic blood pressure and caused significant changes in PBF with 4 phases ($p < 0.05$).

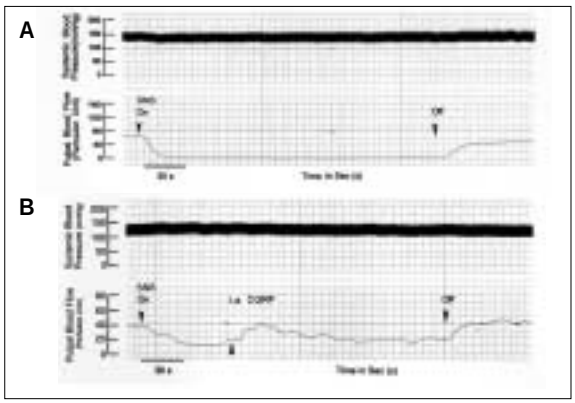

Figure 2. (A) Polygraph recording showing the reduction of pulpal blood flow (PBF) during electrical stimulation of the sympathetic nerve (SNS). Systemic blood pressure did not change. (B) Polygraph recording showing the blocking effect of intra-arterial (i.a.) injection of CGRP (0.03 ㎍/㎏) on SNS-induced PBF decrease.

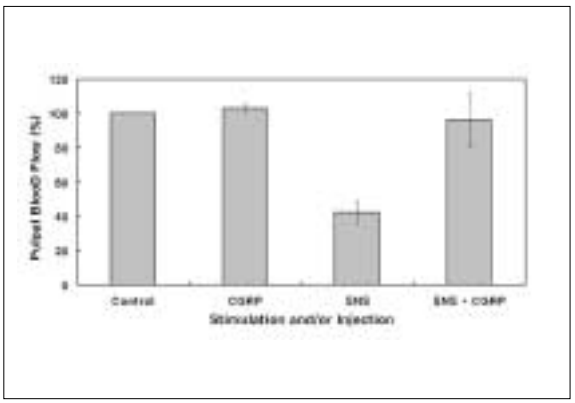

Figure 3. Effect of CGRP alone and during the sympathetic nerve stimulation (SNS)-induced pulpal blood flow (PBF) reduction (mean ± SEM). Intra-arterial injection of CGRP (0.03 ㎍/㎏) effectively recovered the reduced PBF caused by SNS ($p < 0.05$).

B) Local Effect of CGRP on PBF

Close i.a. injection of CGRP (0.03 ㎍/㎏) changed PBF by 2.92 ± 10.51% (n = 25). Intra-arterial injection of $CGRP_{8-37}$ (8.0 - 24.0 ㎍/㎏) caused non-significant change of PBF by 8.54 ± 5.70% (n = 6). Intra-arterial injection of $CGRP_{8-37}$ (8.0 ~ 24.0 ㎍/㎏) following i.a. injection of CGRP (0.03 ㎍/㎏) did not cause any significant change on PBF.

C) Effect of Sympathetic Nerve Stimulation on PBF

Results of percentage changes of PBF in respo- nse to electrical stimulation of sympathetic nerve and i.a. application of CGRP are presented in Figure 3. The electrical stimulation (10 Hz, 4 V and 1.5 ms for 3.5 min) of sympathetic nerve resulted in decreases of PBF by 57.88 ± 22.50% (n = 12).

D) Effect of CGRP on Vasoconstriction induced by Sympathetic Nerve Stimulation

A typical strip-chart recording of systemic blood pressure and PBF in response to sympathetic nerve stimulation and i.a. CGRP is presented in Figure 2. Intra-arterial injection of CGRP (0.03 ㎍/㎏) completely recovered the PBF which was decreased by sympathetic nerve stimulation and induced flow increase by 112.88 ± 23.79% of the amount of the previous decrease (n = 11, Figure 3). This effect of CGRP was not shown when $CGRP_{8-37}$ (8.0 - 24.0 ㎍/㎏) was administered before i.a. CGRP.

Ⅳ. Discussion

In the present study, when CGRP was administered through the femoral vein in a higher dosage than in local injection, it caused significant decrease of systemic blood pressure. The resultant PBF was very fluctuating. Initially the PBF was increased significantly and returned to the control level followed by a second more marked increase and decrease. This hypotensive effect of CGRP was in agreement with previous studies in rats[9,10] and human subjects[11]. This fluctuating PBF in the present study may be explained by the direct influence of the central vasculature and peripheral vasculatures. The first increase may be due to the vasodilating effect on the central vessels[10,12] and the second increase may be the vasodilating effect of CGRP conducted to the dental pulp. In a research by Kim and his colleagues[13], when sodi-

um nitroprusside (SNP), a potent hypotensive agent, was infused intravenously, SNP-induced hypotension was caused by peripheral vasodilation as a result of a direct action on the vessels.

To see the local effect of CGRP in the present study, CGRP was administered close intra-arterially into the dental pulp in a maximum dose that does not exert any significant influence on the systemic blood pressure. The administered CGRP caused only a slight increase of PBF.

Afferent nerves contribute importantly to hemodynamic reaction in the pulp in response to clinical procedures[14]. CGRP is released from capsaicin-sensitive afferents and is a candidate as mediators of plasma protein extravasation and antidromic vasodilation along with substance P[15]. In rats, CGRP was the most active substance in increasing vascular permeability in the pulp[16]. However, in other studies[17,18], SP and CGRP caused weak albumin leakage in the pulp, while the opposite is true in high compliance tissues, such as muscles, suggesting that the vessels in the low compliance environment, such as the pulp, may not be as permeable in response to selected mediators.

PBF was decreased significantly when sympathetic nerve was stimulated in the present study. It is known that neuropeptides such as norepinephrine and neuropeptide-Y are released from the nerve ending when sympathetic nerve is activated and these neuropeptides work as vasodilators in dental pulp[19]. When sympathetic nerve is activated, neuropeptide Y is coreleased with norepinephrine from perivascular sympathetic nerves and exerts vasoconstricton[15].

In the present study, when CGRP was injected to the pulp while PBF was reduced by sympathetic nerve stimulation, the reduced PBF was completely recovered while combined use of CGRP and a CGRP antagonist, $CGRP_{8-37}$, showed no effect. Therefore, exogenously administered CGRP blocked sympathetic nerve function of vasoconstriction. This phenomenon showed two different aspects of CGRP function. When CGRP was administered without activation of sympathetic nerve, it caused only slight vasodilation in the dental pulp. However, when pulpal vessel was severely constricted by sympathetic nerve, administered CGRP caused significant increase of PBF. The result of the present study is indicating that the vasodilatory function of CGRP was more prominent when PBF was attenuated by sympathetic nerve activation compared to when PBF is normal.

Studies explained the mechanism for the peripheral sympathetic vasomotor control in two ways. One is a direct mechanism (vasoconstriction) and the other is an indirect one (e.g., inhibition of exocytosis from afferent fibers)[20]. The function of intradental sensory receptor was reported that it can be strongly affected by activation of the sympathetic nerve, and this effect is most probably indirect[21,22] and due to changes in PBF[8]. In the studies[20], either NE or epinephrine inhibited capsaicin-evoked iCGRP and especially beta (2)-adrenoceptors in dental pulp significantly reduced exocytosis of neuropeptides from capsaicin-sensitive nociceptors[23]. On the other hand, the result of the present study showed that the function of intradental sympathetic nerve can be strongly affected by the neuropeptide from sensory nerve. Therefore, it may be concluded that both nerve systems can be affected by the other nerve systems.

Because the effects of inflammatory mediators on pulpal microcirculatory hemodynamics are complex[17] and a full understanding of neural reactions to dental inflammation should include studies of all kinds of nerve system[7], it is necessary to study the function of the inflammatory mediators in each particular environment especially that of interaction between nerve systems.

Further studies are needed to explain how CGRP works differently in different environment and to understand the interactions between sensory and autonomic nerve systems in order to elucidate neurogenic inflammation in dental pulp.

References

1. Goodman EC, Iversen LL. Calcitonin gene-related peptide: novel neuropeptide. *Life Sci* 16;38:2169-2178, 1986.
2. Cao Z, Zhao M, Hu S. The change of calcitonin gene-related peptide positive fibers in inflammatory pulps. *Zhonghua Kou Qiang Yi Xue Za Zh*i 32:164-166, 1997.
3. Jacobsen EB, Fristad I, Heyeraas KJ. Nerve fibers immunoreactive to calcitonin gene-related peptide, substance P, neuropeptide Y, and dopamine beta-hydroxylase in innervated and denervated oral tissues in ferrets. *Acta Odontol Scand* 56:220-228. 1998.
4. Fristad I, Vandevska-Radunovic V, Fjeld K, Wimalawansa SJ, Hals Kvinnsland I. NK1, NK2, NK3 and CGRP1 receptors identified in rat oral soft tissues, and in bone and dental hard tissue cells. *Cell Tissue Res* 311:383-391. 2003.
5. Caviedes-Bucheli J, Camargo-Beltran C, Gomez-la-Rotta AM, Moreno SC, Abello GC, Gonzalez-Escobar JM. Expression of calcitonin gene-related peptide (CGRP) in irreversible acute pulpitis. *J Endod* 30:201-204, 2004.
6. Kim SK. Role of sympathetic nerve on the control of microcirculation in the feline dental pulp. *J Kor Acad Cons Dent* 21:375-384, 1996.
7. Byers MR, Taylor PE, Khayat BG, Kimberly CL. Effect of injury and inflammation on pulpal and periapical nerves. *J Endod* 16:78-84, 1990.
8. Narhi M. Interaction between the autonomic and sensory nerves in the dental pulp. *Proc Finn Dent Soc* 85:389-393, 1989.
9. Brown MJ, Morice AH. Clinical pharmacology of vasodilator peptides. *J Cardiovasc Pharmacol* 10 (Suppl) 12:S82-87, 1987.
10. Haass M, Skofitsch G. Cardiovascular effects of calcitonin gene-related peptide in the pitched rat: comparison with substance *P. Life Sci* 2:37:2085-2090, 1985.
11. Gennari C, Fischer JA. Cardiovascular action of calcitonin gene-related peptide in humans. *Calcif Tissue Int* 37:581-584, 1985.
12. Marshall I, Al-Kazwini SJ, Roberts PM, Shepperson NB, Adams M, Craig RK. Cardiovascular effects of human and rat CGRP compared in the rat and other species. *Eur J Pharmacol* 16;123:207-216, 1986.
13. Kim S, Fan F-c, Chen RYZ, Simchon S, Schuessler GB, Chien S. Effects of changes in systemic hemodynamic parameters on pulpal hemodynamics. *J Endod* 6:394-399, 1980.
14. Olgart LM. Involvement of sensory nerves in hemodynamic reactions. *Proc Finn Dent Soc* 88 Suppl 1:403-410, 1992.
15. Lundberg JM. Peptidergic control of the autonomic regulation system in the orofacial region. *Proc Finn Dent Soc* 85:239-250, 1989.
16. Maltos KL, Menezes GB, Caliari MV, Rocha OA, Santos JM, Alves DL, Duarte ID, Francischi JN. Vascular and cellular responses to pro-inflammatory stimuli in rat dental pulp. *Arch Oral Biol* 49:443-450, 2004.
17. Kim S, Liu M, Simchon S, Dörscher-Kim JE. Effects of selected inflammatory mediators on blood flow and vascular permeability in the dental pulp. *Proc Finn Dent Soc* 88 Suppl 1:387-392, 1992.
18. Kim S, Dörscher-Kim J. Hemodynamic regulation of the dental pulp in a low compliance environment. *J Endod* 15:404-408, 1989.
19. Kim SK, Ang L, Hsu YY, Dörscher-Kim J, Kim S. Antagonistic effect of D-myo-inositol-1, 2, 6-trisphosphate (PP56) on the neuropeptide Y-induced vasoconstriction in the feline dental pulp. *Arch Oral Biol* 41:791-798, 1996.
20. Hargreaves KM, Jackson DL, Bowles WR. Adrenergic regulation of capsaicin-sensitive neurons in dental pulp. *J Endod* 29:397-399, 2003.
21. Kerezoudis NP, Olgart L, Funato A, Edwall L. Inhibitory influence of sympathetic nerves on afferent nerve-induced extravasation in the rat incisor pulp upon direct electrical stimulation of the tooth. *Arch Oral Biol* 38:483-490, 1993.
22. Kerezoudis NP, Funato A, Edwall L, Olgart L. Activation of sympathetic nerves exerts an inhibitory influence on afferent nerve-induced vasodilation unrelated to vasoconstriction in rat dental pulp. *Acta Physiol Scand* 147:27-35, 1993.
23. Bowles WR, Flores CM, Jackson DL, Hargreaves KM. Beta 2-Adrenoceptor regulation of CGRP release from capsaicin-sensitive neurons. *J Dent Res* 82:308-311, 2003.

국문초록

Calcitonin Gene Related Peptide에 의한 치수미세순환 조절

김성교* · 김영경 · 진명욱

경북대학교 치과대학 치과보존학교실 및 치수생물학연구실

본 연구에서는 감각성 neuropeptide인 CGRP의 치수혈류 조절에 관해 교감신경과의 유기적 관계를 연구함으로써 CGRP의 치수혈류 조절기전을 밝히고자 하였다.

열두 마리의 전신마취된 고양이에서 실험하였으며 CGRP를 혈관을 통해 전신적 또는 국소적으로 투여하였다. 견치에서 치수혈류의 변화를 측정하고 paired *t*-test로 통계분석 하였으며 95% 수준에서 유의성을 검증하였다.

CGRP (0.3 ㎍/㎏)를 전신정맥으로 주사시, 전신혈압에 현저한 영향을 나타내면서 치수혈류는 평균 68.85%의 일차적인 증가와 감소를 보였고 이차적으로 다시 평균 161.8% 증가하였다가 감소하였다.

CGRP를 저용량 (0.03 ㎍/㎏)으로 국소적으로 투여시, 치수혈류는 평균 2.92%의 미약한 증가를 나타내었다.

교감신경을 전기자극시 (10 Hz, 4 V, 1.5 ms), 전신혈압은 영향을 받지 않으면서 치수혈류가 유의하게 평균 57.88% 감소하였다.

교감신경 자극으로 치수혈류가 저하되어 있는 동안 주입한 CGRP는 저하된 치수혈류를 유의하게 회복시켰다. CGRP의 이 치수혈류 증가 효과는 $CGRP_{8-37}$에 의해 효과적으로 차단되었다.

주요어: 치수, 혈류, Calcitonin gene related peptide (CGRP), 교감신경 자극, 신경성 염증, Laser Doppler flowmeter