

– Abstract –

## Changes of Immunoreactivity for CGRP and SP according to the Distance between the DRG and Injury Site of Neuropathic Rat

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**Objectives** : Peripheral nerve injury sometimes leads to neuropathic pain and depletion of calcitonin gene related-peptide (CGRP) and substance P (SP) in the spinal cord. This study was performed to see whether the distribution of immunoreactivity for CGRP and SP in dorsal horn and dorsal root ganglia (DRG) was related to the distance between DRG and injury site.

**Methods** : We compared two groups of rats; one group was subjected to unilateral inferior and superior caudal trunk transections at the level between the S3 and S4 spinal nerves (S34 group) and the other group at the levels between S1 and S2, between S2 and S3, and between S3 and S4 spinal nerve (S123 group).

**Results** : The immunoreactive area of SP and CGRP in the S1 dorsal horn was smaller in the S123 group than in the S34 group, whereas that in the S3 dorsal horn was not significantly different between the two groups. The number of SP- immunoreactive DRG cells decreased as compared to the sham group's in all DRGs of experimental groups except the S1 DRG of the S34 group.

**Conclusion** : This study shows that the amounts of SP and CGRP in the dorsal horn and DRG following neuropathic injury inversely decrease according to the distance between the DRG and injury site.

**Key Words** : Peripheral nerve injury, Substance P, Calcitonin gene-related peptide

(spontaneous burning pain) (allodynia)  
(hyperalgesia)

(neuropathic pain)

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trunk) .<sup>1</sup> (inferior caudal 3,4 (n=13) 1~2 mm S123 . Sham (n=9)

.<sup>2</sup> 2. 1, 1, 4, 8 12 가

가 가 1) (mechanical allodynia) (5.5 × 15, 6.5 × 18 cm, )

가 von Frey hair(0.5 gm; 4.9 mN, 2.0 gm; 19.6 mN) 10 10

.<sup>3</sup> substance P(SP) calcitonin gene-related peptide(CGRP) 2) (cold allodynia)

Bennett 4 가 5 5

가 ,<sup>5,6</sup> Kim Chung<sup>7</sup> SP CGRP가 sham

Bennett<sup>4</sup> SP CGRP<sup>8</sup> 15 15

,<sup>9,10</sup> 3) (warm allodynia) 40

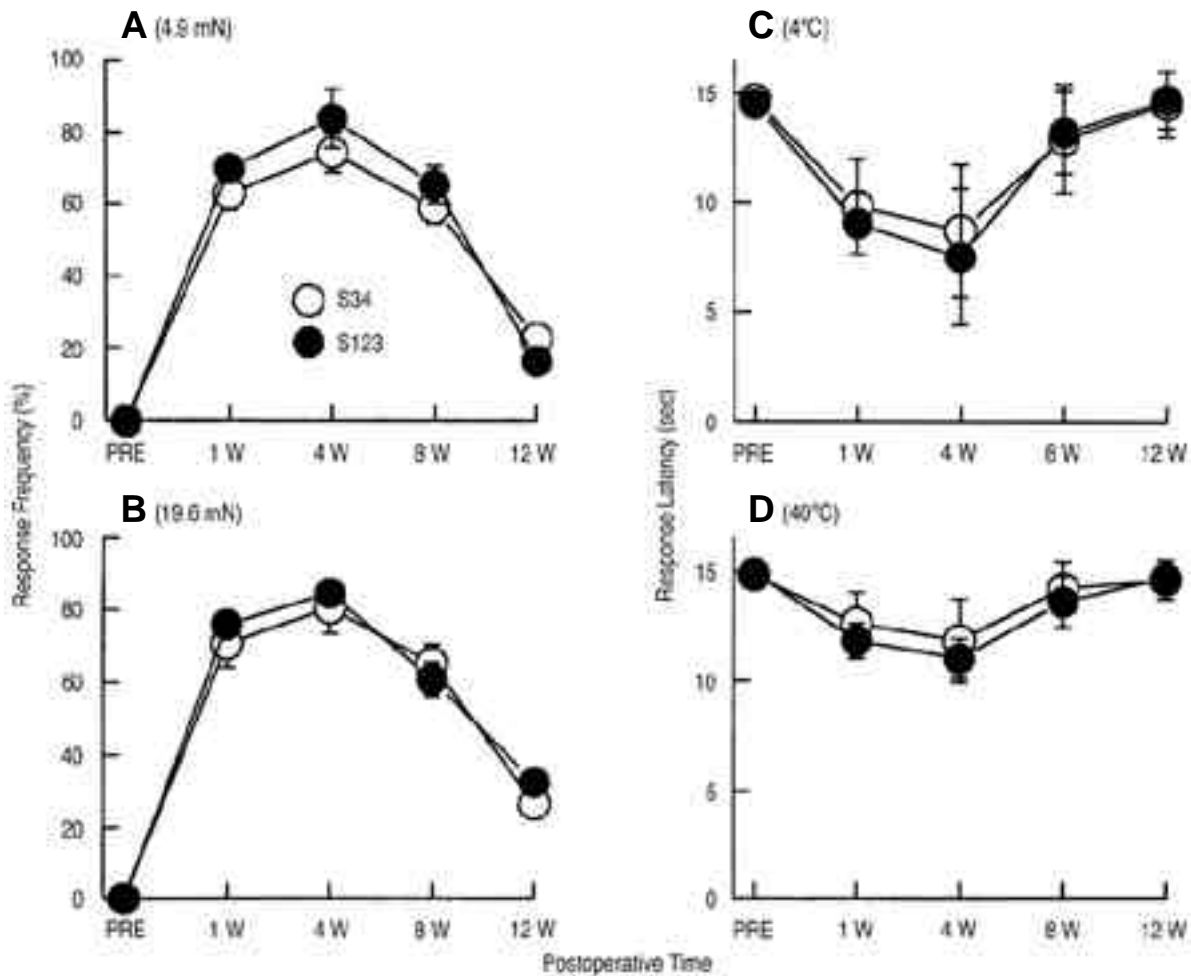
CGRP가 SP 가 가

.<sup>1</sup> 3. 1) 12 1

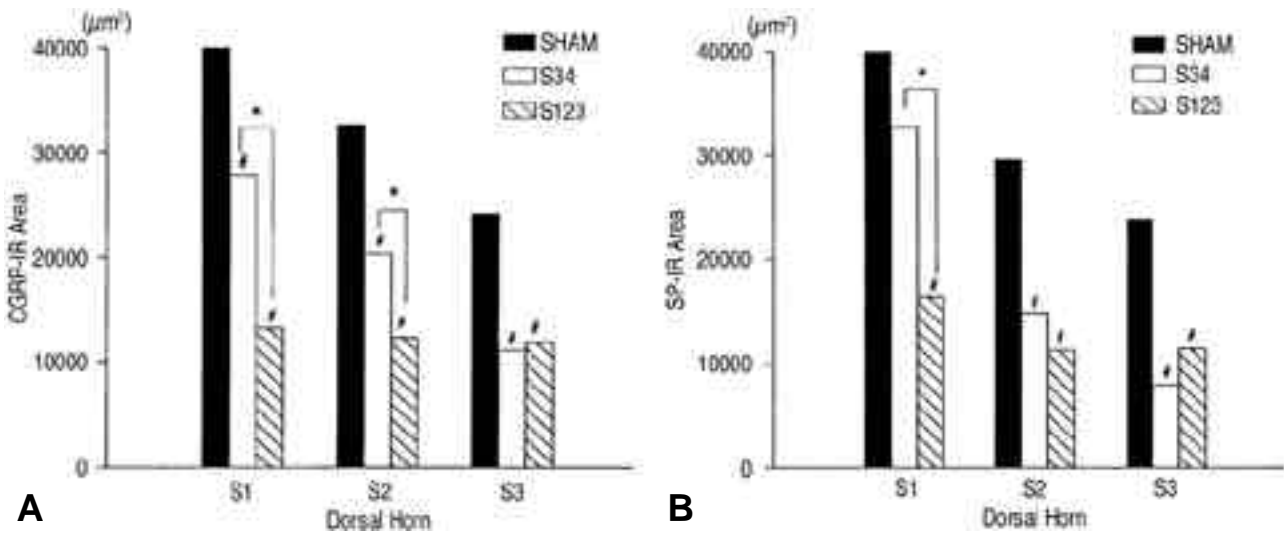
150~200 gm (Sprague-Dawley) 4% Sodium pentobarbital(Nembutal 60 mg/kg) halothane 가 heparin

(superior, inferior caudal trunk) 4% paraformaldehyde, 0.1% picric acid 3,4 1~2 mm S34 , 1, 2, (n=13) , 1,2 , 2, 3 , 3

sucrose 4 12 , 30% 7  
 2~3 가 가 (p<0.05).  
 m 18 μm, 10 μm 가 .  
 3) 2) 12 7 가  
 SP CGRP ABC(avidin-biotin- 8 가  
 horseradish peroxidase complex) (p<0.05). 7  
 0.02% Triton X-100 4 가  
 1% BSA(bovine serum albumin) (p<0.05).  
 10% NGS(normal goat serum) 1 가 .  
 , 4 1:60,000 1:160,000  
 SP, CGRP 1 (Peninsular, Bel- 2.  
 mont, CA, USA) 48 , PBS(phosphate  
 buffered saline) Biotin 1) 1  
 2 (Vector, Burlingame, CA, USA) 1 (1) CGRP SP  
 1%, 3% NGS ABC(Vector, CGRP SP  
 Burlingame, CA, USA) 1  
 3, 3'-Diaminobenzidine(DAB) S34 S123  
 .<sup>11</sup> ( ) CGRP SP Sham  
 4) CGRP  
 substance P CGRP CGRP Rexed I II  
 (image analyzer, Analytical Measuring S34 S123 1, 2, 3  
 System, UK) CGRP CGRP  
 SP CGRP (p<0.05, Fig. 2).  
 S34 S123 1, 2  
 mark(1970) <sup>13</sup> Koenig- CGRP 3 가  
 (p<0.05, Fig. 2).  
 4. 가 가 CGRP  
 Mean±S.E.M SP  
 Friedman Analysis Mann Whitney U-test, SP SP Rexed I II  
 CGRP paired t-test p 0.05 S34 2, 3 S123  
 1, 2, 3 CGRP  
 가 SP  
 (p<0.05, Fig. 2).  
 S34 S123 1 SP  
 1. (Fig. 1) 3 가 2,  
 (p<0.05, Fig. 2).  
 1) 1 가 가 SP  
 S123 S34

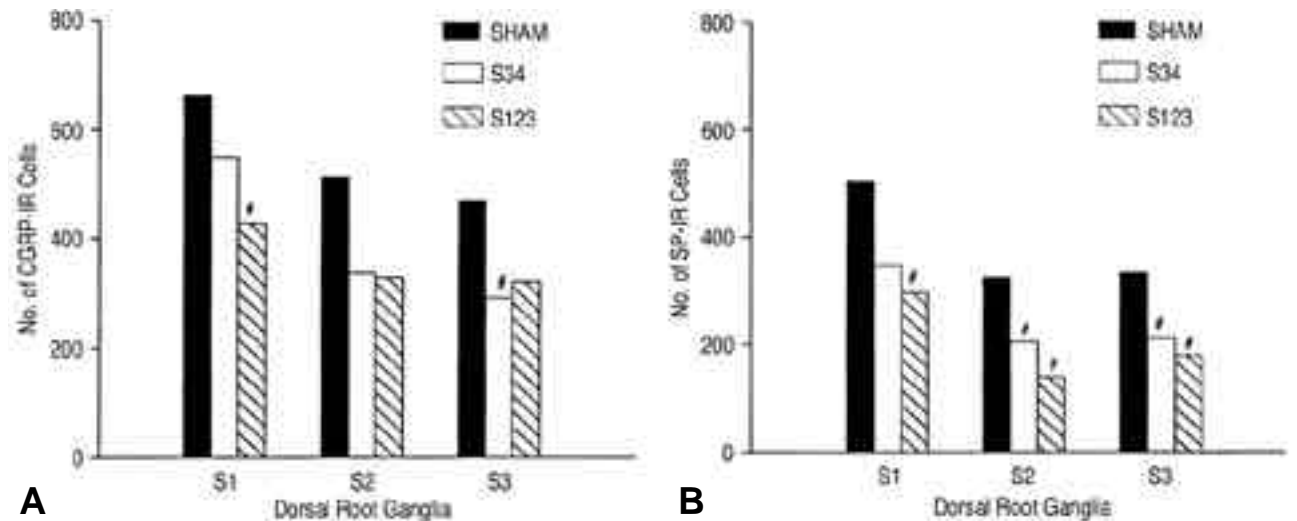


**Fig. 1.** Tail responses to mechanical (A,B) and thermal stimuli (C,D) of 12-week group. \* indicates significant difference between the pre- and post-injury value ( $p < 0.05$  by Friedman Analysis). Between the S34 ( $n=5$ ) and S123 ( $n=5$ ) groups, no significant difference was detected in either pre- or post-injury scores ( $P < 0.05$ , Mann-Whitney U-test).

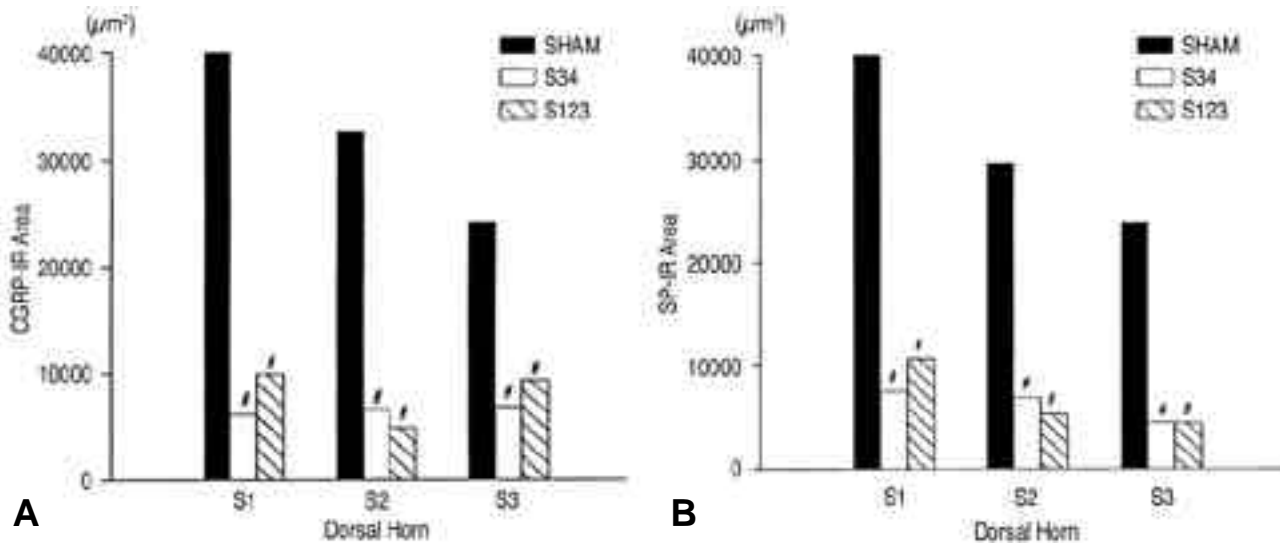


**Fig. 2.** Histograms illustrating changes in CGRP-IR (A) and SP-IR (B) from the S34 and S123 groups on 1 week post-surgery. There is a significant decrease in staining area in all experimental groups relative to the sham group (#;  $p < 0.05$ ). \* indicates significant difference on ipsilateral side between the S123 and S34 groups ( $p < 0.05$  by paired t-test).

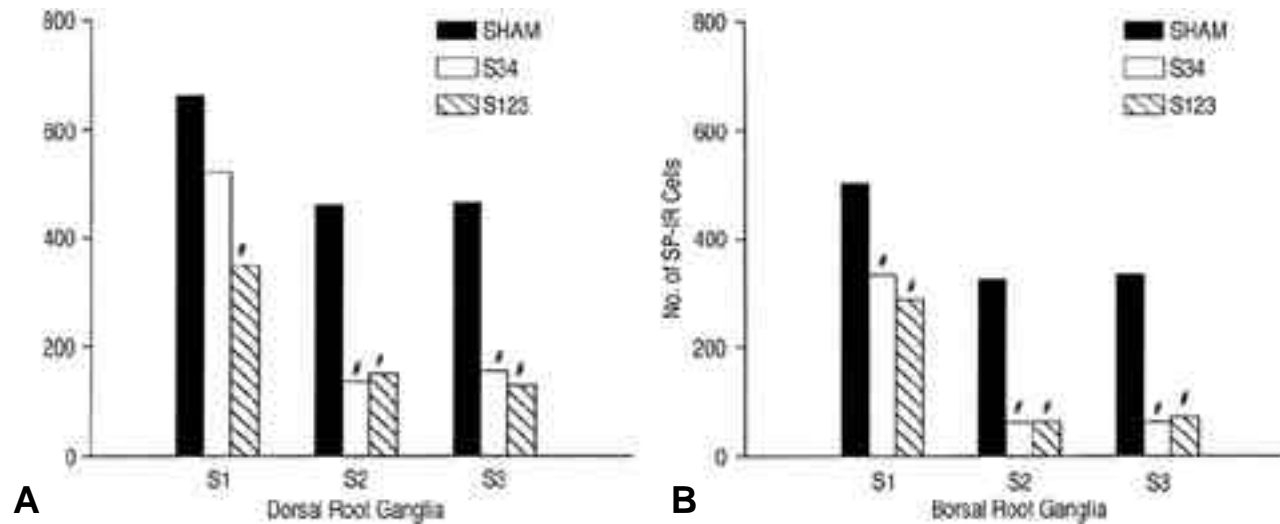
(2) CGRP SP SP CGRP 가 12  
 S34 S123 ( ) CGRP S34 S123 1  
 Sham CGRP SP 1, 1,  
 가 ,  
 CGRP 2, 3  
 S34 S123 1, 2, 3 1  
 CGRP (p<0.05, Fig. 4).  
 S34 S123 1, 2, 3  
 CGRP SP  
 1 S34 3 S123  
 가 가 가  
 (p<0.05, Fig. 3). S34 S123  
 가 가 가  
 SP CGRP  
 S34 S123 (p<0.05, Fig. 5).  
 가 가 CGRP  
 가 가 가  
 SP  
 S34 S123 1, 2, 3  
 가 가 SP  
 (p<0.05, Fig. 3). S34 S123 1, 2, 3  
 가 가 SP  
 (p<0.05, Fig. 5). S34  
 SP 가  
 S123 가  
 가 가  
 2) 12  
 (1) CGRP SP  
 CGRP  
 12 1 S34  
 S123 1, 2  
 , 1, 2, 3  
 1 CGRP  
 (p<0.05, Fig. 4).  
 가  
 (neuroma)  
 ) 가



**Fig. 3.** Histograms illustrating changes in CGRP-IR (A) and SP-IR (B) from the S34 and S123 groups on 1 week post-surgery. # indicates significant difference between the sham group (n=6) and experimental groups (p<0.05 by paired t-test). There is no significant difference on ipsilateral side between the S123 and S34 groups.



**Fig. 4.** Histograms illustrating changes in CGRP-IR (A) and SP-IR (B) area from the S34 and S123 groups on 12 week post-surgery. There is a significant decrease in staining area in all experimental groups relative to the sham group (#;  $p < 0.05$  by paired t-test).



**Fig. 5.** Histograms illustrating changes in CGRP-IR (A) and SP-IR (B) cells from the S34 and S123 groups on 12 week post-surgery. There is a significant decrease in staining cells in all experimental groups relative to the sham group (#;  $p < 0.05$  by paired t-test).

Bonica<sup>1</sup>,<sup>14</sup> Lee<sup>15,16</sup>,<sup>17</sup>  
가 가 가  
가 가 Bennett<sup>4</sup> Seltzer<sup>18</sup>  
Kim Chung<sup>7</sup>  
(sympathetically maintained pain, SMP) 가  
(sympathetically independent pain, SIP) 가  
Kim<sup>3</sup> Kim<sup>2</sup>

(trunk)

1

SP CGRP 가

SP

CGRP

SP, CGRP 가

, SP CGRP

가 가 가

(distance hypothesis).<sup>34</sup>

Bennett<sup>4</sup> Seltzer<sup>18</sup>

Kim Chung<sup>7</sup> SP, CGRP

가<sup>19</sup>

, Kim Chung<sup>7</sup> <sup>35</sup>

가

SP, CGRP

CGRP

,<sup>36</sup> SP

가

SP CGRP<sup>20</sup> SP

SP 11 laminae I, II<sup>37</sup>

lamina I, II<sup>21,22</sup> , SP

capsaicin SP가

가

CGRP calcitonin<sup>23</sup> 37

sauer's laminae I-III, V, X Lis- 가

C A 가 가 SP, CGRP가

가

SP CGRP<sup>21,22</sup> capsaicin SP, CGRP

60%<sup>23</sup> CGRP 가

SP

가<sup>27</sup> CGRP SP, CGRP<sup>10,39</sup>

SP CGRP 가

,<sup>28</sup> GAP-43(growth-associated protein-43), GAL(galanin), NPY(neuropeptide Y), VIP (vasoactive intestinal poly-peptide) 가 SP CGRP 가

가

Bennett<sup>4</sup> S34 S123

GAP-43, GAL, NPY, VIP mRNA 가 . S34 (

, CGRP, SP mRNA<sup>32</sup> 1 - 2 ) 3, 4

SP CGRP 가 1, 2, 3

<sup>5,6</sup> laminae I, II SP 가 2.6, 2.0, 1.5 cm , S123

가 , 1, 2 , 2, 3 , 3, 4

<sup>33</sup> 가 1, 2, 3

가 1.5 cm

CGRP  
가  
가  
1, 2  
, SP  
가  
가  
S123  
가  
가  
S34  
1  
가  
가  
SP  
가  
가  
1)  
SP CGRP  
1 12  
가  
2)  
1 SP CGRP  
가 가  
가  
가  
S123 S34  
가  
가  
S123 S34  
가  
가  
SP  
CGRP가  
가  
7~14  
30~60  
SP, CGRP mRNA가  
14  
20  
32  
mRNA  
1 12 SP, CGRP  
7  
1 가 4 , 8  
가, 12  
12 SP,  
CGRP 1

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