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Identification of substance P receptors on fibroblast-like cells derived from the periosteum: an *in vitro* cell culture study

Periost kökenli fibroblast benzeri hücreler üzerinde P maddesi alıcılarının gösterilmesi: *İn vitro* hücre kültürü çalışması

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Objectives: Substance P (SP) is a neuromediator that influences development, growth, and formation of bone. Substance P receptors have not been specified on osteoblast-like cells. It is assumed that the neural network within the periosteum may be the site of neuroendocrine control mechanism of bone growth and repair. The aim of this study was to identify SP receptors on fibroblast-like cells derived from the periosteum.

Materials and methods: Fibroblast-like cells were derived from the periosteum of six patients who underwent femoral derotation and shortening osteotomy for developmental dislocation of the hip. Periosteal tissue was removed from the osteotomy site and the cells were suspended in cell culture media and grown on 24-well macroplates. Polyclonal SP antibodies were added and anti-rabbit peroxidase conjugate (whole molecule) was used as a second antibody. SP reactive sites on cell surface were screened by addition of substrate (3-amino-9-ethyl carbazole+dimethylformamide + 3% hydrogen peroxide). Surface and/or intracellular staining was evaluated under microscope.

Results: Fibroblast-like cells showed immunostaining for SP receptors. The presence of SP-sensitive binding sites were identified on fibroblast-like cells derived from the periosteum.

Conclusion: Substance P receptors shown on fibroblast-like cells derived from the periosteum are very likely to have a modulating effect in periosteal bone growth and healing.

Key words: Cells, cultured; bone development/physiology; fibroblasts; osteogenesis; periosteum; substance P.

Amaç: P maddesi (SP), kemiğin gelişimi, iyileşmesi ve yeniden şekillenmesinde görev alan sinir kökenli düzenleyicilerdendir. P maddesi alıcıları (reseptörleri) osteoblast benzeri hücreler üzerinde daha önce gösterilmemiştir. Periost içindeki periferik sinir ağının kemiğin büyümesi ve onarılmasını kontrol eden mekanizmalardan biri olduğu öngörülmektedir. Bu çalışma, periost kaynaklı hücre kültüründe, fibroblast benzeri hücrelerde bulunan SP alıcılarını tanımlamak için düzenlendi.

Gereç ve yöntem: Fibroblast benzeri hücreler, gelişimsel kalça displazisi nedeniyle rotasyon-kısaltma osteotomisi uygulanan altı hastanın periostlarından elde edildi. Yaklaşık 1.0 cm x 1.5 cm periost dokusu osteotomi bölgesinden alındı. Hücreler kültür otamında 24 hücreli makroplaklarda çoğaltıldı. Makroplaklara poliklonal SP antijenleri eklendi. Anti-tavşan proksidaz konjugatı (tam molekül) ikincil antijen olarak kullanıldı. P maddesine hassas bölgeler substrat (3-amino-9-etil karbazol + dimetilformamide + %3 hidrojen peroksit) kullanılarak işaretlendi. Hücre içi veya üzeri boyanma mikroskopla değerlendirildi.

Bulgular: Laboratuvar ortamında gerçekleştirilen bu hücre kültürü çalışmasında, periost kökenli fibroblast benzeri hücrelerin membranlarında P maddesine hassas bölgeler saptandı.

Sonuç: Periost kökenli fibroblast benzeri hücrelerin üzerinde gösterilen SP alıcıları büyük olasılıkla kemiğin periosteal büyüme ve iyileşme süreçlerinde düzenleyici etkiye sahiptir.

Anahtar sözcükler: Hücre kültürü; kemik gelişimi/fizyoloji; fibroblast; osteogenez; periost; P maddesi.

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Bone formation and repair are under the strict control of the immune, hematopoietic and neuroendocrine systems.[1-5] (Fig. 1). Osteocytes, cells of the cambium layer of the periosteum, and the lining cells that cover the entire endosteal surface of the bone identify and respond to biophysical (mechanical loading, magnetic field, electrical stimulation, etc.) and/or biochemical incentives.[6] Incentives presented to these cells act as systemic and local mediators and initiate a stage- and dose-dependent growth, formation, and repair process in bone.[7] Blood-borne mediators such as transforming growth factor (TGF)[8] and fibroblast growth factor (FGF),[9] and neuroendocrine effective ones such as substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY) may be involved in regulating various stages of these processes.[10] The presence of intense sensory fibers of the periosteum[11] is well-known and this tissue retains its capacity for bone formation and remodeling throughout life.[12-14]

Substance P is a mammalian undecapeptide (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) that belongs to the tachykinin family.[15] Neural and non-neural sources of SP are derived from the preprotachykinin-I gene.[16] This neuromediator is mainly found in the electron-dense, large storage vesicles of unmyelinated, high threshold C-type, and small, myelinated A-δ type fibers.[17] Some biological responses of SP include motor control, contraction and endothelium-dependent relaxation of vascular smooth muscles, vasodilatation, and sensory neurotransmission. Wide tissue distribution of SP and interaction with its ligands are associated with diverse responses such as immunological responses, histamine release, plasma extravasation, inflammation, fibroblast[18] and lymphocyte[19] proliferation, and potentially, nerve regeneration and tissue repair.[20] Furthermore, it presents amino acid homology with FGF[21] and stimulates lymphokine (IL-1, IL-6, TNF-α) production.[22] Most SP immunoreactive fibers have been found close to or within blood vessel walls,[23,24] suggesting regulation of intraosseous blood flow.[25-27]

Research into SP immunoreactivity in the musculoskeletal system is not new. This neuromediator has been identified in the joint capsule,[28-31] ligaments,[28,31] meniscus,[32] synovial membrane,[28,29,32] subacromial bursa,[33] subchondral region of the long bones,[17] intervertebral discs,[23] and developing skeleton.[24] Furthermore, bone marrow space contains extended numbers of SP fibers,[16,17,32,34-36]

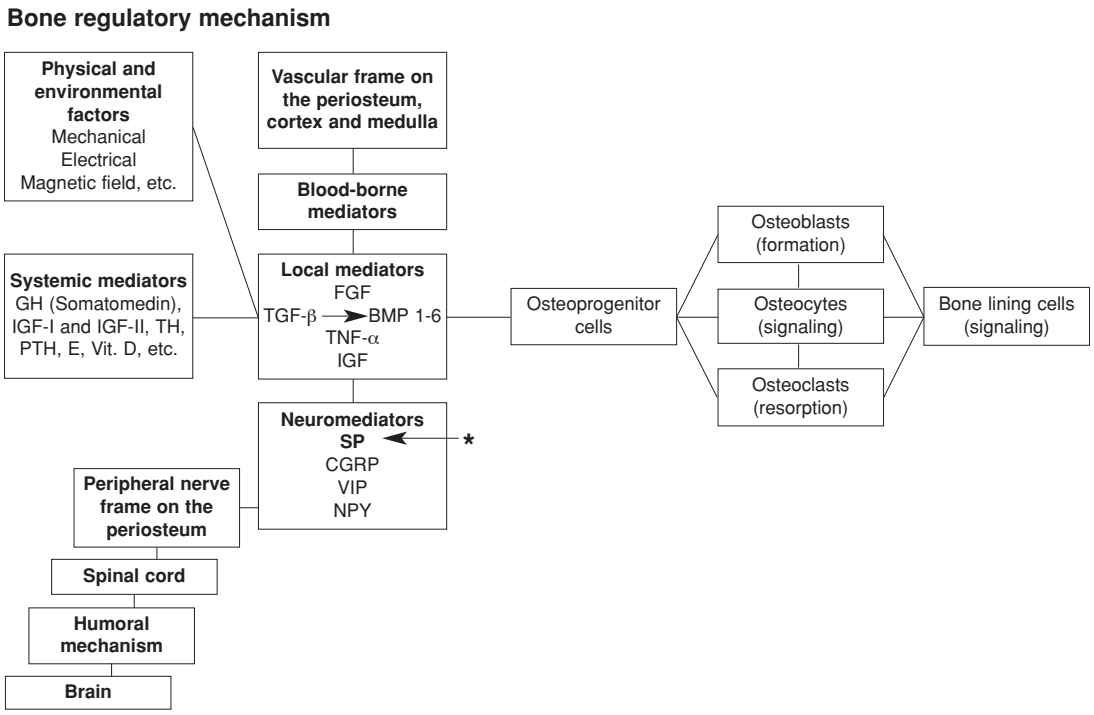

Fig. 1. Mechanism of bone regulation and substance P (*).

while bone graft incorporation results in a significant increase in SP immunoreactivity.[37] Pathological conditions such as degenerative,[29,38] rheumatoid,[39] mycobacterial,[40] and adjuvant arthritis[36,41] are associated with increased SP immunoreactivity.

The concentration of SP varies significantly in the periosteum, bone marrow and cortical bone, the first having the largest, the last having the least concentration.[26] Dense SP immunoreactive fibers of the periosteum are well-defined.[11,25,26,28,32,42] Thus, research into SP immunoreactivity in recent years has concentrated on the cortical bone[10] and bone marrow,[17,32,35,36] but not on the periosteum. Substance P receptors could not be identified in bone-derived UMR 106-1, Saos-2 and MC3T3-E1 cells,[10] indicating that SP has almost no direct effect on the skeleton. Gronblad et al.[43] early in 1984, and Hill and Elde[44] in 1991 demonstrated dense SP-like immunoreactivity in human and rat periosteum. It is assumed that the neural network containing periosteum may be the site of neuroendocrine control for bone growth and repair.

In this study, SP-sensitive receptors on fibroblast-like cells derived from the periosteum were identified in cell culture.

MATERIALS AND METHODS

Collagenase Type IA-S (product no: C 9722), fetal calf serum (FCS) (product no: F 4135), anti-rabbit IgG (whole molecule) peroxidase conjugate (product no: A 6154), BGJb medium (Fitton-Jackson Modification) with L-glutamine (product no: B 6644), Dulbecco's modified Eagle's medium (DMEM) (product no: D 5523), trypsin (product no: T 4799), and antibiotics (penicillin-streptomycin-amphotericin B) (product no: A 7292) were purchased from Sigma Chem. Co., USA. T-25 and T-75 tissue culture flasks were purchased from Corning (product no: 430168, 430720). 24-well macroplates were purchased from Costar (product no: 3512). Substance P antiserum for immunocytochemistry (Rabbit; Code: RPN. 1572) was purchased from Amersham International, England.

Periosteal (fibroblast-like) cell culture: Periosteal tissues were obtained from six patients undergoing femoral derotation and shortening osteotomy for the treatment of developmental dislocation of the hip. The mean age of the patients (5 girls, 1 boy) was 3.2±1.4 years (range 2 to 6 years).

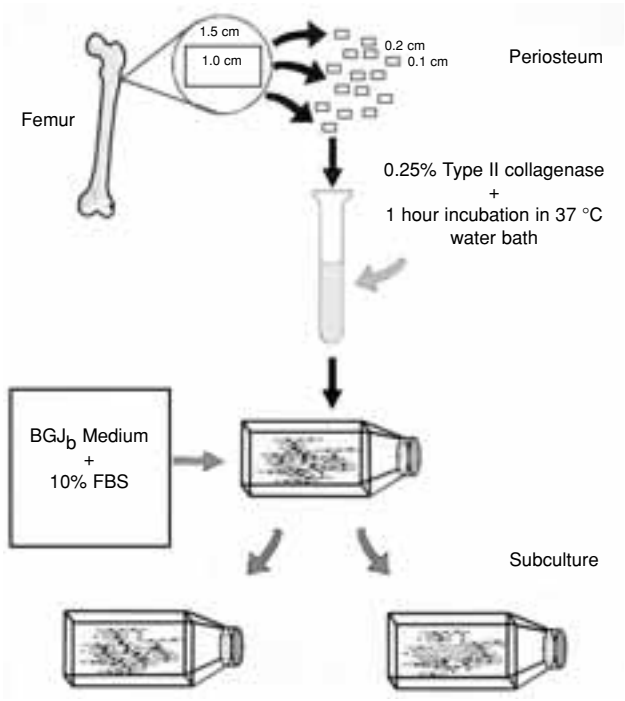

Fig. 2. Cell culture steps.

Approximately 1.0 cm x 1.5 cm of periosteal tissue was removed from the osteotomy site (Fig. 2), after which it was immediately transferred into phosphate buffered saline (PBS) and minced into small pieces aseptically. The minced pieces were either digested using type II collagenase as previously described by Nakahara et al.[14] or directly attached to the bottom of tissue culture flasks allowing direct cell growth from the tissue using FCS (Fig. 3, 4). DMEM or BGJb mediums supplemented with 10% FCS and antibiotics were used to grow the cells.

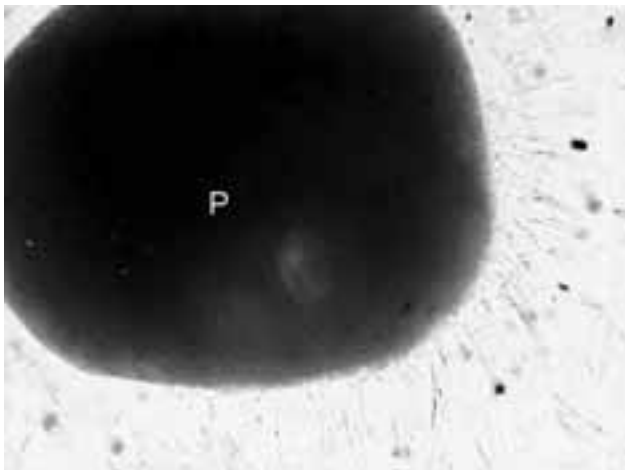

Fig. 3. Fibroblast-like cells (*) grown from the periosteal tissue (x4).

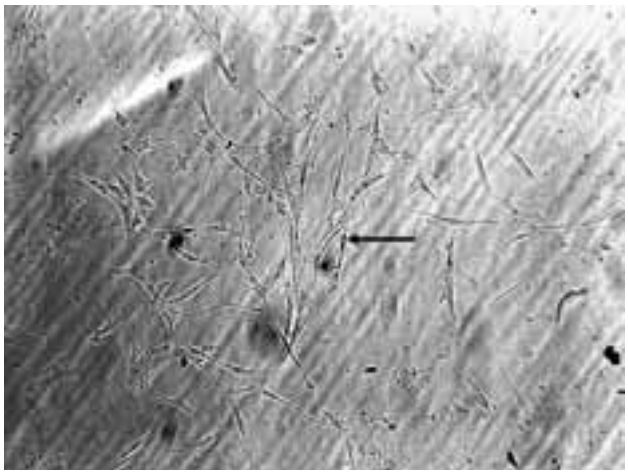

Fig. 4. Spindle-like fibroblastic cells (arrow) in culture, having osteogenic/chondrogenic potential (x10).

Additional growth factors like TGF or FGF were not included in the medium. The cells were monitored by daily microscopic examinations and they were subcultured when 80-90% of cell confluence was obtained. Further cell cultures were obtained by splitting cells into two. Cells were identified by their spindle-like morphology under light microscopy and by alkaline phosphatase staining.[13]

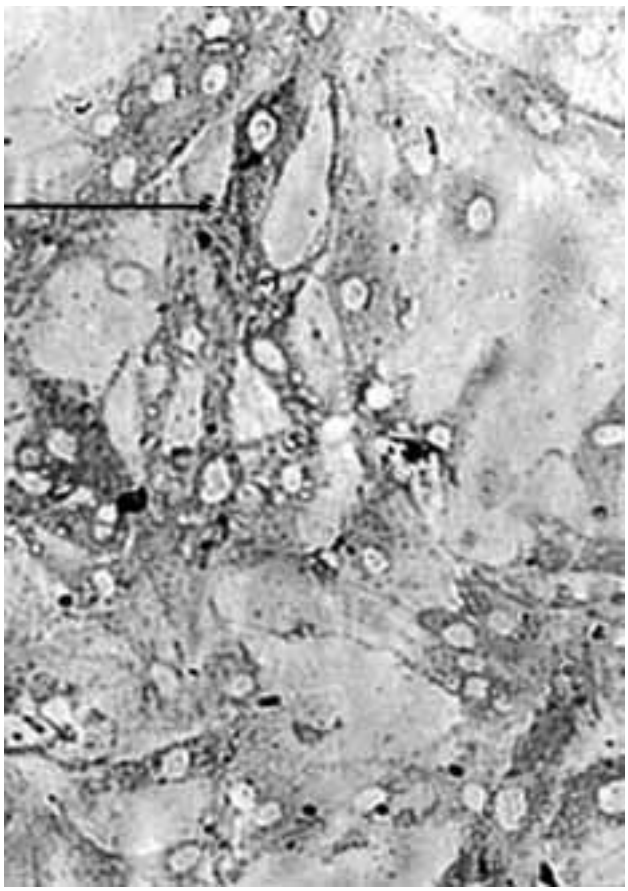

Fig. 5. Substance P-positive stained cells (arrow) (x40).

Immunohistochemical detection of SP-reactive sites in cultured periosteal cells: The second passages of the cells were grown in 24-well macroplates. Each well was inoculated approximately with 10^5 cells. The cells were maintained in BGJ_b medium containing 1.0, 0.1, 0.01, and 0.001 μM of SP and incubated at 37 °C in 95% humidified air + 5% CO_2 for 24 hours. At the end of this period, the SP containing medium was discarded and the cells were washed several times with PBS containing 0.05% of Tween 20. The cells were fixed by heat at 80 °C instead of chemical fixation to prevent possible blockade of the SP-sensitive binding sites. Afterwards, polyclonal SP antibody diluted to the range of 1:20 to 1:200 was added to the wells and the system was reincubated for three hours with shaking at room temperature. Anti-rabbit peroxidase conjugate (whole molecule) was used as a second antibody and incubation for this step was performed as described previously.[14] SP-reactive sites on the cell surface were screened by addition of substrate (3-amino-9-ethyl carbazole + dimethylformamide + 3% hydrogen peroxide). Staining was evaluated by screening the intracellular and/or surface accumulation of brown-reddish product on the microscope.

RESULTS

Two different cell types, fibroblast-like and epithelial-like cells, were morphologically distinguished as described by Uchida et al.[13] SP-reactive sites were detected on fibroblast-like cells after immunostaining (Fig. 5). Immunoreactivity was most apparent at 1.0 and 0.1 μM concentrations of SP. Immunoreactivity was not detected in epithelial-like cells.

DISCUSSION

Findings of our cell culture studies revealed the presence of SP-sensitive binding sites (or SP-like immunoreactivity) on fibroblast-like cells derived from the periosteum. The type of binding sites is not defined in this study. Currently, tachykinin receptors are divided into three homologous types: SP-preferring neurokinin (NK) receptor, neurokinin A-preferring NK_2 receptor, and neurokinin B-preferring NK_3 receptor.[16,20] Neurokinin A and B NK receptors present a wide tissue distribution.

Substance P has at least two types of receptors in the central nervous system (SP-P and SP-E).[45] The receptors of fibroblast-like cells derived from the periosteum are, therefore, speculated to be of neurokinin A- or B-preferring NK type. Thus, further studies are essential to define the receptor type and intracellular pathways of SP-mediated periosteal bone formation.

Growth factors for the stimulation of bone formation are presented in the literature.[2,8,9,45-52] Neouromediators, like growth factors, are produced in very small amounts and create their effect in a very short time following their release before being inactivated. Single injection of these mediators may give limited information on their effect on the tissue because of this short period of activity. The neuromediator should be provided in a consistent manner for longer periods to evaluate its long-term effects. Ekelund et al.[34] reported that neuropeptides are involved in the regulation of secondary developmental processes in bone, such as mineralization and growth, and not in the early phase of induction. This is supported by the observation of transitional effect of SP on the periosteum in a week, demonstrated by the neuron-specific protein gene product (PGP) 9.5.[11]

We suggest that SP may play an active role in the initial stages of periosteal bone formation. Furthermore, this neuromediator is involved in the early organization of new bone formation.

Whether SP directly affects periosteal osteogenic cells or arranges bone formation by regulating the blood flow is yet unknown. The stimulatory effect of SP on hematopoiesis in bone marrow stroma is well demonstrated.[53,54] This effect was mediated by adherent cells, abolished by SP-antagonists, and partially reduced by anti-IL-1, IL-3, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF).[53] The relation between SP-immunoreactivity and blood vessels is not investigated in the current study. Thus, synthesis of an extracellular organic matrix of cartilage and woven bone by new chondroblasts and osteoblasts indicates a direct effect of SP on cortical bone.

In conclusion, periosteum-derived fibroblast-like cells present SP-sensitive binding sites and this neuromediator may have an early transient stimulatory effect in periosteal bone healing.

REFERENCES

1. Cruess RL. Physiology of bone formation and resorption. In: The musculoskeletal system - embryology, biochemistry, and physiology. New York: Churchill Livingstone; 1982. p. 219-52.
2. Joyce ME, Jingushi S, Scully SP, Bolander ME. Role of growth factors in fracture healing. In: Clinical and experimental approaches to dermal and epidermal repair: normal and chronic wounds. Philadelphia: Willey-Liss; 1991. p. 391-416.
3. Buckwalter JA, Glimcher MJ, Cooper RR, Recker R. Bone biology-Part II: Formation, form, modeling, remodeling, and regulation of cell function. J Bone Joint Surg [Am] 1995; 77:1276-89.
4. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, et al. Novel regulators of bone formation: molecular clones and activities. Science 1988;242:1528-34.
5. Trippel SB. Biologic regulation of bone growth. In: Brighton CT, Friedlaender GE, Lane JM, editors. Bone formation and repair. Rosemont, IL: American Academy of Orthopaedic Surgeons; 1994. p. 39-60.
6. Scutt A, Mayer H, Wingender E. New perspectives in the differentiation of bone-forming cells. Biofactors 1992;4:1-13.
7. Mundy GR, Boyce B, Hughes D, Wright K, Bonewald L, Dallas S, et al. The effects of cytokines and growth factors on osteoblastic cells. Bone 1995;17(2 Suppl):71-5.
8. Lind M, Schumacker B, Soballe K, Keller J, Melsen F, Bunger C. Transforming growth factor-beta enhances fracture healing in rabbit tibiae. Acta Orthop Scand 1993;64:553-6.
9. Kato T, Kawaguchi H, Hanada K, Aoyama I, Hiyama Y, Nakamura T, et al. Single local injection of recombinant fibroblast growth factor-2 stimulates healing of segmental bone defects in rabbits. J Orthop Res 1998;16:654-9.
10. Bjurholm A. Neuroendocrine peptides in bone. Int Orthop 1991;15:325-9.
11. Hukkanen M, Konttinen YT, Santavirta S, Paavolainen P, Gu XH, Terenghi G, et al. Rapid proliferation of calcitonin gene-related peptide-immunoreactive nerves during healing of rat tibial fracture suggests neural involvement in bone growth and remodelling. Neuroscience 1993;54:969-79.
12. Pechak DG, Kujawa MJ, Caplan AI. Morphology of bone development and bone remodeling in embryonic chick limbs. Bone 1986;7:459-72.
13. Uchida A, Kikuchi T, Shimomura Y. Osteogenic capacity of cultured human periosteal cells. Acta Orthop Scand 1988;59:29-33.
14. Nakahara H, Bruder SP, Goldberg VM, Caplan AI. In vivo osteochondrogenic potential of cultured cells derived from the periosteum. Clin Orthop Relat Res 1990;(259):223-32.
15. Chang MM, Leeman SE, Niall HD. Amino-acid sequence of substance P. Nat New Biol 1971;232:86-7.
16. Rameshwar P, Poddar A, Zhu G, Gascon P. Receptor

induction regulates the synergistic effects of substance P with IL-1 and platelet-derived growth factor on the proliferation of bone marrow fibroblasts. J Immunol 1997;158:3417-24.
17. Imai S, Tokunaga Y, Maeda T, Kikkawa M, Hukuda S. Calcitonin gene-related peptide, substance P, and tyrosine hydroxylase-immunoreactive innervation of rat bone marrows: an immunohistochemical and ultrastructural investigation on possible efferent and afferent mechanisms. J Orthop Res 1997;15:133-40.
18. Nilsson J, von Euler AM, Dalsgaard CJ. Stimulation of connective tissue cell growth by substance P and substance K. Nature 1985;315:61-3.
19. Payan DG, Brewster DR, Goetzl EJ. Specific stimulation of human T lymphocytes by substance P. J Immunol 1983;131:1613-5.
20. Breeman WA, VanHagen MP, Visser-Wisselaar HA, van der Pluijm ME, Koper JW, Setyono-Han B, et al. In vitro and in vivo studies of substance P receptor expression in rats with the new analog [indium-111-DTPA-Arg1] substance P. J Nucl Med 1996;37:108-17.
21. Nakanishi S. Mammalian tachykinin receptors. Annu Rev Neurosci 1991;14:123-36.
22. Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science 1988;241:1218-21.
23. Ashton IK, Walsh DA, Polak JM, Eisenstein SM. Substance P in intervertebral discs. Binding sites on vascular endothelium of the human annulus fibrosus. Acta Orthop Scand 1994;65:635-9.
24. Sisask G, Bjurholm A, Ahmed M, Kreicbergs A. Ontogeny of sensory nerves in the developing skeleton. Anat Rec 1995;243:234-40.
25. Bjurholm A, Kreicbergs A, Schultzberg M, Lerner UH. Neuroendocrine regulation of cyclic AMP formation in osteoblastic cell lines (UMR-106-01, ROS 17/2.8, MC3T3-E1, and Saos-2) and primary bone cells. J Bone Miner Res 1992;7:1011-9.
26. Ahmed M, Srinivasan GR, Theodorsson E, Bjurholm A, Kreicbergs A. Extraction and quantitation of neuropeptides in bone by radioimmunoassay. Regul Pept 1994;51:179-88.
27. Lundgaard A, Aalkjaer C, Bjurholm A, Mulvany MJ, Hansen ES. Vasorelaxation in isolated bone arteries. Vasoactive intestinal peptide, substance P, calcitonin gene-related peptide, and bradykinin studied in pigs. Acta Orthop Scand 1997;68:481-9.
28. Nixon AJ, Cummings JF. Substance P immunohistochemical study of the sensory innervation of normal subchondral bone in the equine metacarpophalangeal joint. Am J Vet Res 1994;55:28-33.
29. Buma P, Verschuren C, Versleyen D, Van der Kraan P, Oestreicher AB. Calcitonin gene-related peptide, substance P and GAP-43/B-50 immunoreactivity in the normal and arthrotic knee joint of the mouse. Histochemistry 1992;98:327-39.
30. Kido MA, Kiyoshima T, Kondo T, Ayasaka N, Moroi R, Terada Y, et al. Distribution of substance P and calcitonin gene-related peptide-like immunoreactive nerve fibers in the rat temporomandibular joint. J Dent Res 1993;72:592-8.
31. el-Bohy A, Cavanaugh JM, Getchell ML, Bulas T, Getchell TV, King AI. Localization of substance P and neurofilament immunoreactive fibers in the lumbar facet joint capsule and supraspinous ligament of the rabbit. Brain Res 1988;460:379-82.
32. Hukkanen M, Konttinen YT, Rees RG, Santavirta S, Terenghi G, Polak JM. Distribution of nerve endings and sensory neuropeptides in rat synovium, meniscus and bone. Int J Tissue React 1992;14:1-10.
33. Gotoh M, Hamada K, Yamakawa H, Inoue A, Fukuda H. Increased substance P in subacromial bursa and shoulder pain in rotator cuff diseases. J Orthop Res 1998;16:618-21.
34. Ekelund A, Ahmed M, Bjurholm A, Nilsson O. Neuropeptides in heterotopic bone induced by bone matrix in immunosuppressed rats. Clin Orthop Relat Res 1997;(345):229-38.
35. Manske JM, Sullivan EL, Andersen SM. Substance P mediated stimulation of cytokine levels in cultured murine bone marrow stromal cells. In: Atassi MZ, Bixler GS, editors. Immunobiology of proteins and peptides. Vol. 8, New York: Plenum Press; 1995. p. 53-64.
36. Ahmed M, Bjurholm A, Theodorsson E, Schultzberg M, Kreicbergs A. Neuropeptide Y- and vasoactive intestinal polypeptide-like immunoreactivity in adjuvant arthritis: effects of capsaicin treatment. Neuropeptides 1995;29:33-43.
37. Madsen JE, Wang JS, Hukkanen M, Nordsletten L, Konttinen YT, Santavirta S, et al. Sensory nerve ingrowth during bone graft incorporation in the rat. Acta Orthop Scand 1996;67:217-20.
38. Levine JD, Moskowitz MA, Basbaum AI. The contribution of neurogenic inflammation in experimental arthritis. J Immunol 1985;135(2 Suppl):843-7.
39. Levine JD, Collier DH, Basbaum AI, Moskowitz MA, Helms CA. Hypothesis: the nervous system may contribute to the pathophysiology of rheumatoid arthritis. J Rheumatol 1985;12:406-11.
40. Ahmed M, Bjurholm A, Schultzberg M, Theodorsson E, Kreicbergs A. Increased levels of substance P and calcitonin gene-related peptide in rat adjuvant arthritis. A combined immunohistochemical and radioimmunoassay analysis. Arthritis Rheum 1995;38:699-709.
41. Ahmed M, Bjurholm A, Srinivasan GR, Lundeberg T, Theodorsson E, Schultzberg M, et al. Capsaicin effects on substance P and CGRP in rat adjuvant arthritis. Regul Pept 1995;55:85-102.
42. Hukkanen M, Konttinen YT, Rees RG, Gibson SJ, Santavirta S, Polak JM. Innervation of bone from healthy and arthritic rats by substance P and calcitonin gene related peptide containing sensory fibers. J Rheumatol 1992;19:1252-9.
43. Gronblad M, Liesi P, Korkala O, Karaharju E, Polak J. Innervation of human bone periosteum by peptidergic nerves. Anat Rec 1984;209:297-9.

44. Hill EL, Elde R. Distribution of CGRP-, VIP-, D beta H-, SP-, and NPY-immunoreactive nerves in the periosteum of the rat. Cell Tissue Res 1991;264:469-80.
45. Kuraishi Y, Hirota N, Sugimoto M, Satoh M, Takagi H. Effects of morphine on noxious stimuli-induced release of substance P from rabbit dorsal horn in vivo. Life Sci 1983;33 Suppl 1:693-6.
46. Nakamura K, Kawaguchi H, Aoyama I, Hanada K, Hiyama Y, Awa T, et al. Stimulation of bone formation by intraosseous application of recombinant basic fibroblast growth factor in normal and ovariectomized rabbits. J Orthop Res 1997;15:307-13.
47. Beck LS, Ammann AJ, Aufdemorte TB, Deguzman L, Xu Y, Lee WP, et al. In vivo induction of bone by recombinant human transforming growth factor beta 1. J Bone Miner Res 1991;6:961-8.
48. Nielsen HM, Andreassen TT, Ledet T, Oxlund H. Local injection of TGF-beta increases the strength of tibial fractures in the rat. Acta Orthop Scand 1994;65:37-41.
49. Marcelli C, Yates AJ, Mundy GR. In vivo effects of human recombinant transforming growth factor beta on bone turnover in normal mice. J Bone Miner Res 1990;5:1087-96.
50. Aspenberg P, Thorngren KG, Lohmander LS. Dose-dependent stimulation of bone induction by basic fibroblast growth factor in rats. Acta Orthop Scand 1991;62:481-4.
51. Mackie EJ, Trechsel U. Stimulation of bone formation in vivo by transforming growth factor-beta: remodeling of woven bone and lack of inhibition by indomethacin. Bone 1990;11:295-300.
52. Kawaguchi H, Kurokawa T, Hanada K, Hiyama Y, Tamura M, Ogata E, et al. Stimulation of fracture repair by recombinant human basic fibroblast growth factor in normal and streptozotocin-diabetic rats. Endocrinology 1994;135:774-81.
53. Rameshwar P, Ganea D, Gascon P. In vitro stimulatory effect of substance P on hematopoiesis. Blood 1993; 81:391-8.
54. Rameshwar P, Gascon P. Substance P (SP) mediates production of stem cell factor and interleukin-1 in bone marrow stroma: potential autoregulatory role for these cytokines in SP receptor expression and induction. Blood 1995;86:482-90.