The action of prostaglandin E₂ and triamcinolone acetonide on the firing activity of lumbar nerve roots

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Summary. *Sciatica, due to lumbar disc herniation, is understood electrophysiologically to be an ectopic firing originating from a nerve root. The recent concept of chemical radiculitis implies the involvement, not only of mechanical compression, but also of chemical mediators which contribute to the generation of ectopic firing. The present study demonstrates that prostaglandin E₂, a chemical mediator of inflammation, provoked the ectopic firing of nerve roots in a canine in vitro model which indicates that it may play a part in the irritation of nerve roots. In contrast, triamcinolone acetonide suppressed the firing induced by prostaglandin suggesting that steroids may be effective in the treatment of root symptoms.*

Résumé. *Une sensation anormale, telle qu'une névralgie sciatique provoquée par la formation d'une hernie discale lombaire, est électrophysiologiquement comprise comme un déclenchement ectopique provenant de la racine du nerf. Une étude récente admet le concept de »radiculite chimique«, ce qui signifie non seulement une compression mécanique mais aussi des médiateurs chimiques ou neuromédiateurs qui contribuent à la génération d'un déclenchement ectopique. L'étude actuelle démontre que la prostaglandine E₂, un médiateur existant avec l'inflammation, est susceptible de provoquer un déclenchement ectopique de la racine du nerf en utilisant un modèle canin in vitro. Ceci permet de suggérer que la prostaglandine E₂ joue un rôle dans l'irritation de la*

racine du nerf. D'autre part, la triamcinolone supprime le déclenchement provoqué par la prostaglandine E₂, ce qui indique une efficacité clinique du stéroïde pour le traitement de symptômes radiculaires.

Introduction

The diagnosis of herniated lumbar discs using imaging techniques has demonstrated that mechanical compression of the nerve roots is an important cause of sciatica [5, 15, 18, 19]. On the other hand, clinical improvement has often occurred, even in the absence of obvious improvement in the root compression depicted on imaging. Furthermore, nerve root compression due to disc herniation does not always cause symptoms [3, 10, 22]. These observations might be explained by the involvement of factors other than mechanical compression.

Sciatica is electrophysiologically understood as ectopic firing, or excessive excitation, originating in a root lesion [21]. Recent studies have suggested that chemical mediators might induce ectopic firing [1, 2, 18]. Marshall et al. showed that intervertebral discs contain inflammatory chemical substances and they proposed the concept of chemical radiculitis [16]. Gertzbein et al. reported the occurrence of an autoimmune reaction in the nerve root when disc material was herniated into the epidural space [10].

In the present study we investigated the potential role of prostaglandin E₂, (PGE₂) which is one of the representative mediators of inflammation

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Fig. 1. Diagram of the in vitro model of a posterior root associated with its root ganglion (PRG). This tissue was maintained in a chamber containing artificial cerebrospinal fluid (aCSF). Recording electrodes (each R1–4 represents a bipolar electrode) arranged in series allowed the measurement of conduction velocity of action potentials.

[11, 14] in ectopic firing. We used an in vitro model in which the nerve root and its ganglion can be maintained under controlled conditions [1, 2] and analysed the responses of the nerve root to PGE₂. We also evaluated the effect of triamcinolone acetonide on PGE₂-induced firing in order to clarify the mechanism of its use in treatment.

Materials and methods

The lumbar nerve roots from 4 adult mongrel dogs (body weight 6 to 10 kg) were resected under intravenous pentobarbital anaesthesia. Sixteen 6th to 7th lumbar roots with their posterior root ganglia were obtained. The specimens were immersed and maintained in a chamber filled with artificial cerebrospinal fluid (aCSF) which contained NaCl (128 mM), KCl (3 mM), NaH₂PO₄ (0.5 mM), CaCl₂ (1.5 mM), Mg SO₄ (1 mM), NaHCO₃ (21 mM) and glucose (30 mM). The pH was 7.4, the temperature 30 °C and the fluid was saturated with 95% oxygen and 5% carbon dioxide.

The chamber was separated into 2 compartments. The proximal stump of the posterior root, where action potentials were to be recorded, was placed in the compartment, which was filled with liquid paraffin, adjacent to the one holding most of the tissue. The stump was divided into 6 to 8 fine nerve fascicles and the recording made from a few of them using 4 bipolar platinum electrodes (Fig. 1).

PGE₂ (Funakoshi, Tokyo, Japan) was applied to the nerve roots by depositing a known amount into the bath in which aeration helped its uniform distribution. The firing activity of the roots provoked by PGE₂ was amplified by an electromyograph (Dantec Counterpoint, Skovlunde, Denmark), drawn by a thermal array recorder (Nihon Koden, Tokyo, Japan) and recorder by a digital tape recorder (TEAC RD-180T, Tokyo, Japan). The conduction velocity of the active unit was measured from recordings from 2 bipolar electrodes arranged in series for the same nerve fascicle as shown in Figure 1. Although most of the recordings contained action potentials

Fig. 2. Representative responses of 4 nerve roots (indicated by 1–4) to PGE₂ (5×10^{-5} M). Note the significantly increased firing activity a few minutes after PGE₂ was applied to the bath (arrow).

Fig. 3. Representative responses of 2 nerve roots (indicated by 1–2) to triamcinolone acetonide (2 mg/ml). Note the rapid inhibition of PGE₂-firing after triamcinolone acetonide was added (arrow).

of multi-units, it was possible to identify each unit using a window discriminator. The conduction time of each action potential between 2 electrodes was measured with a trigger-averaging technique. The conduction velocity was calculated from the conduction time and the distance between the electrodes.

To observe the effect of the steroid on firing activity, triamcinolone acetonide (Bristol Myers Squibb, New York) was added to the same bath while the response to PGE₂ was continuing. The concentrations of PGE₂ and triamcinolone reported here represent those calculated to be present in the bath.

Results

The effect of PGE₂

In aCSF alone almost no spontaneous firings were observed whereas the PGE₂ (5×10^{-5} M) induced significant firing a few minutes after it was applied to the bath (Fig. 2) and this continued to be observed for over 30 minutes. Using this concentration, the 8 nerve roots tested showed a similar response. Half of the 4 tested roots had a recognisable firing response to 10^{-5} M PGE₂ which indicated the threshold concentration. When the PGE₂ was washed out by changing the bath fluid to aCSF alone, the firing activity ceased (data not shown).

The average nerve conduction velocity of action potentials in 21 activated units was 25.5 m/s, and

the highest distribution was obtained in the range of 12.0–35.0 m/s.

The effect of triamcinolone acetonide

This was analysed on the 5×10^{-5} M PGE₂-induced firing activity in 4 nerve roots. While under the effect of PGE₂, 2 mg/ml triamcinolone was added to the bath. In every case, significant suppression of firing was seen immediately after the addition of triamcinolone (Fig. 3). Moreover, firing activity remained suppressed in the presence of triamcinolone.

Discussion

Pain and numbness are subjective symptoms associated with lumbar radiculopathy and are thought to be induced by ectopic firing of the nerve roots. It has been suggested that mechanical causes, such as compression and traction, and local chemical factors around a herniated disc and nerve root were related to the pain caused by ectopic firing [18].

It has, however, been difficult to analyse the effect of each mechanical and chemical factor in relation to the generation of ectopic firing in *in vivo* animal experiments. In this sense, our *in vitro* model has an advantage because the surrounding environment can be controlled by the investigators, and the effects of independent chemical factors can be examined [1, 2].

The effect of PGE₂

PGE₂ is synthesised from arachnidonic acid and is found in various inflammatory processes [11, 14]. There have been few reports describing the relation between PGE₂ and radiculitis because it is not possible to examine the human nervous tissue biochemically. It seems reasonable to assume that PGE₂ will be present in inflamed nerve roots, and it is also possible that it is synthesised in the herniated nucleus pulposus and infiltrates the roots [20].

PGE₂ is a mediator acting on peripheral nociceptors and elicits hyperalgesia associated with inflammation [7, 17], although it is not known how PGE₂ affects the function of nerve roots. The present study has shown that it has an excitatory effect which can be appreciated as root irritation clinically.

It has been shown that action potentials are evoked in the nociceptive afferent fibres when mechanical compression is applied to a root [1, 2,

12, 13]. Further, the posterior root ganglia are sensitive to various chemical substances [1, 2, 4], and they are also the most sensitive part of the root to compression. It is therefore highly probable that PGE₂-induced firing observed in this study originated from these ganglia.

Lateral disc herniation has been described in association with severe neuralgia [15], and we presume that such pain is related to the sensitivity of the posterior root ganglia to mechanical compression as well as to chemical mediators.

The firing induced by PGE₂ had conduction velocities of 12.0–35.0 m/s which suggests that part of the activity was generated by nerve fibres of group III (A δ) involved in the production of pain and numbness. This implies that other chemical factors present around the nerve root should be identified and each examined in this experimental model.

The effect of triamcinolone

Triamcinolone acetonide, a steroid, significantly inhibited ectopic firing provoked by PGE₂ confirming reports which describe the value of steroids administered either by epidural injection, or applied directly to the nerve roots, in patients with sciatica [5, 6, 9]. This is generally ascribed to an anti-inflammatory effect, but in our study no such action was present. Furthermore, since the effect of triamcinolone was seen within a minute after it was added, some mechanism other than an anti-inflammatory response must be considered. Devor et al. reported that ectopic neural discharge in experimental neuroma is rapidly suppressed by locally applied steroids, including triamcinolone, and concluded that this was due to direct action on the neural membrane rather than an anti-inflammatory response [8]. Since the inhibition of firing demonstrated in our study was rapid, it might be based on a similar mechanism.

Future identification of other factors which suppress ectopic firing, using this model, will contribute to better pharmacological treatment of nerve root irritation associated with lumbar disc herniation.

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References

1. Atsuta Y, Sugawara O, Muramoto T, Iwahara T, Takemitsu Y, Harada Y (1993) In vitro analysis of ectopic firing generated in the lumbar nerve root. *J Jpn Spine Res Soc* 4: S 74
2. Atusta Y, Iwahara T, Sugawara O, Muramoto T, Watakabe M, Takemitsu Y (1994) The study of generating and suppressive factors of ectopic firing in the lumbar dorsal root using an in vitro model. *Rinsho Seikei Geka* 29: 441–448
3. Boden SD, Davis DO, Dina TS, Patronas NJ, Wiesel SW (1990) Abnormal magnetic-resonance scans of the lumbar spine in asymptomatic subjects. *J Bone Joint Surg [Am]* 72: 403–408
4. Burchiel KJ (1984) Spontaneous impulse generation in normal and denervated dorsal root ganglia. Sensitivity to alpha-adrenergic stimulation and hypoxia. *Experimental neurology* 85: 257–272
5. Bush K, Cowan N, Katz DE, Gishen P (1992) The natural history of sciatica associated with disc pathology. *Spine* 17: 1205–1212
6. Bush K, Hillier S (1991) A controlled study of caudal epidural injections of triamcinolone plus procaine for the management of intractable sciatica. *Spine* 16: 572–574
7. Davies P, Bailey PJ, Goldenberg MM (1984) The role of arachidonic acid oxygenation products in pain and inflammation. *Ann Rev Immunol* 2: 335–357
8. Devor M, Govrin-Lippmann R, Raber Pnina (1985) Corticosteroids suppress ectopic neural discharge originating in experimental neuromas. *Pain* 22: 127–137
9. Dilke TFW, Burry HC, Grahame R (1973) Extradural corticosteroid injection in management of lumbar nerve root compression. *Br Med J* 2: 635–637
10. Gertzbein SD, Tait JH, Devlin SR (1977) The stimulation of lymphocytes by nucleus pulposus in patients with degenerative disk disease of the lumbar spine. *Clin Orthop* 123: 149–154
11. Harada Y, Tanaka K, Uchida Y, Ueno A, Ohishi S, Yamashita K (1982) Changes in the levels prostaglandins and thromboxane and their roles in the accumulation of exudate in rat carrageenin-induced pleurisy—a profile analysis using gas chromatography-mass spectrometry. *Prostaglandins* 23: 881–895
12. Howe JF, Loesed JD, Calvin WH (1977) Mechanosensitivity of dorsal root ganglia and chronically injured axons: a physiological basis for the radicular pain of nerve root compression. *Pain* 3: 25–41
13. Howe JF (1978) A neurophysiological basis for the radicular pain of nerve root compression. In Bonica JJ, Liebeskind JC, Albe-Fessard DG (eds) *Advances in Pain Research and Therapy*. Vol 3., Raven Press New York
14. Kuehl FA, Egan RW (1980) Prostaglandins, arachidonic acid and inflammation. *Science* 210: 978–984
15. Lindblom K, Rexed B (1948) Spinal nerve injury in dorso-lateral protrusions of lumbar discs. *J Neurosurg* 5: 413–432
16. Marshall LL, Trethewie ER, Curtain CC (1977) Chemical radiculitis, a clinical physiological and immunological study. *Clin Orthop* 129: 61–67
17. Martin HA, Basbaum AI, Kuiat GC et al. (1988) Leukotriene B₄ decreases the mechanical and thermal thresholds of C-fiber nociceptors in the hairy skin of the rat. *J Neurophysiol* 60: 438–445
18. Rydevik B (1989) Spinal nerve root compression. In Szabo RM (eds) *Nerve Compression Syndromes – Diagnosis and Treatment*. Slack Inc, New Jersey
19. Smyth MJ, Wright V (1958) Sciatica and the intervertebral disc. An experimental study. *J Bone Joint Surg* 40 [Am]: 1401–1418
20. Takahashi H, Okajima K, Motegi M, Okada Y, Kakiuchi S (1994) A study of inflammatory cytokines in the herniated disc of the lumbar spine. *J Jpn Orthop Assoc* 68: S 1570 (in Japanese)
21. Torebjörk HE, Ochoa JL, McCann FV (1979) Paresthesia: Abnormal impulse generation in sensory nerve fibers in man. *Acta Physiol Scand* 105: 518–520
22. Wiesel SW, Tsourmas N, Feffer HL, Citrin CM, Patronas N (1984) A study of computer-assisted tomography. I: The incidence of positive CAT scans in an asymptomatic group of patients. *Spine* 9: 549–551