Evoked thalamic neuronal activity following DRG application of two nucleus pulposus derived cell populations: an experimental study in rats

E. Nilsson · K. Larsson · B. Rydevik ·
H. Brisby · I. Hammar

Received: 24 May 2012 / Revised: 18 October 2012 / Accepted: 9 January 2013 / Published online: 24 January 2013
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Abstract

Purpose To investigate the effects on evoked thalamic neuronal activity of application of notochordal cells and chondrocyte-like cells derived from nucleus pulposus (NP) onto a dorsal root ganglion (DRG) and to compare these effects with a previously reported increased thalamic activity induced by NP.

Methods Nucleus pulposus was harvested from tail discs of adult rats and the disc cells were separated into two cell populations, notochordal cells and chondrocyte-like cells. The two cell populations were applied separately, or in combination, to the L4 DRG of anaesthetised female Sprague–Dawley rats during acute electrophysiological experiments. In control experiments, cell suspension medium was applied on the DRG. Recordings from the contralateral thalamus were sampled for 40 min while electrically stimulating the ipsilateral sciatic nerve at above A δ -fibre thresholds.

Results Application of notochordal cells resulted in a decrease in evoked thalamic activity within 10 min while chondrocyte-like cells did not induce any changes during the 40 min of recording. The difference in evoked thalamic activity 40 min after notochordal and chondrocyte-like cell application, respectively, was statistically significant. Neither an increased concentration of chondrocyte-like

cells alone nor a combination of the two cell populations induced any changes in thalamic activity.

Conclusions Separate exposure of the DRG to the two NP-derived cell populations induced different effects on evoked thalamic activity, but none of the tested cell samples induced an increase in neuronal activity similar to that previously observed with NP. This indicates a high complexity of the interaction between NP and nervous tissue.

Keywords Notochordal cells · Chondrocyte-like cells · Nucleus pulposus · Thalamus · Rat · Disc herniation

Introduction

Lumbar disc herniation is a common cause of radiculopathy and sciatic pain. Such pain is believed to be the result of both mechanical compression of nerve roots by disc tissue [1, 2] and an inflammatory reaction in and around nerve roots following leakage of nucleus pulposus (NP) from the inner core of the disc [3–5].

NP, an aggrecan-rich gel-like tissue consisting of a relatively low cell number in a surrounding matrix, contains at least two different cell populations, one of small (17–23 μ m) chondrocyte-like cells and one of large (25–85 μ m), highly vacuolated notochordal cells [6]. Viable chondrocyte-like cells have been demonstrated in human NP from individuals of high ages [7] while it has previously been proposed that notochordal cells disappear within the first three decades of life [8]. Weiler et al. [9], however, recently observed cells with notochordal phenotype present in elderly human NP. Both notochordal cells and chondrocyte-like cells are present in rat NP after skeletal maturity [10].

It has been shown that both notochordal cells and chondrocyte-like cells inhibit neurite outgrowth from

E. Nilsson (✉) · I. Hammar
Department of Physiology, Institute of Neuroscience and
Physiology, University of Gothenburg, PO Box 432,
405 30 Gothenburg, Sweden
e-mail: elin.nilsson@physiol.gu.se

K. Larsson · B. Rydevik · H. Brisby
Department of Orthopaedics, Institute of Clinical Sciences,
University of Gothenburg, Gothenburg, Sweden

dorsal root ganglion (DRG) in vitro [11] and that the notochordal cells also reduce the diameter of the growing neurites [12]. In these studies, Larsson et al. demonstrated that the effects are dependent on the total number of cells as well as on the ratio between the cell types. A similar inhibitory effect on neurite outgrowth was also found in the same in vitro model following application of naïve NP [13].

While NP application on nerve roots and DRG in rats has been shown to increase ectopic firing in the DRG and dorsal root [14, 15] and to enhance responses of dorsal horn wide dynamic range neurones to peripheral noxious stimuli [16, 17], effects at supraspinal levels have been less investigated. The thalamus is the main relay structure for sensory information from the spinal cord to the cerebral cortex, and an increase in excitability has been shown in the ventral posterior lateral (VPL) thalamic nucleus in rats following nociceptive stimulations to the hind paw [18]. Brisby and Hammar [19] reported an increase in evoked activity in the rat VPL within 20 min of DRG exposure to NP. The contribution of individual cell populations present in NP to this effect was, however, not evaluated. The aim of the present study was to examine the changes in evoked neuronal activity in the rat VPL in vivo following DRG exposure to two cell populations derived from NP, notochordal and chondrocyte-like cells and to compare these effects to the previously reported increase in evoked thalamic activity of NP [19].

Materials and methods

In total, 55 adult female Sprague–Dawley rats (Charles River, Germany) were used for acute electrophysiological experiments and as NP donors. Evoked contralateral thalamic neuronal activity was investigated after ipsilateral L4 DRG exposure to notochordal and/or chondrocyte-like cells or cell suspension medium. The electrophysiological procedures and analyses were performed in a blinded manner. All animals were euthanized by a lethal intraperitoneal injection of pentobarbital sodium (300 mg/kg, APL, Sweden). All experimental procedures were approved by the local animal research ethics committee.

Cell sorting by flow cytometry

NP from 13 tail discs from each donor rat was harvested and placed in F12 medium (Invitrogen, Sweden). The cell-sorting procedure was performed according to the method described earlier by Larsson et al. [11]. Briefly, the cells were separated from the surrounding matrix and the cell clusters were dispersed by incubation with 0.1 % trypsin (Invitrogen, Sweden) for 20 min at 37 °C. The cell

Fig. 1 Methodology. **a** Flow cytometry of NP cells. X axis indicates forward scatter (FSC) = cell size and Y axis indicates side scatter (SSC) = cell granularity. B is the gate for chondrocyte-like cells and A is the gate for notochordal cells. The gap between B and A is constructed to avoid overlap of the two cell populations. **b** Representative histological section at the location of the recording site in the VPL marked with an electrolytic lesion. The electrode track and recording site are indicated by a dotted vertical line

suspension was filtered through a filter (70 µm) just before flow cytometry and cell viability was determined using 2 µg/ml propidium iodide. The cells were sorted by size [11] into two populations with fluorescence-activated cell-sorting technique (FACSARIA, BD, Biosciences, San Jose, CA, USA), (Fig. 1a). The two cell populations, notochordal cells (large cell size 25–85 µm) and chondrocyte-like cells (small cell size 17–23 µm), respectively, were collected and placed in F12 medium either separately or in combination, and the cell vials were stored in a water bath at 37 °C for a maximum time of 4 h.

Five experimental groups were used in this study (the cell concentrations are expressed as number of cells $\pm$ 10 %/60 µl of F12 medium): (1) 25,000 notochordal cells (n = 6), (2) 25,000 chondrocyte-like cells (n = 6), (3) 150,000 chondrocyte-like cells (n = 6), (4) a combination of 1,500 notochordal and 28,500 chondrocyte-like cells (in total 30,000 cells, ratio 1/20, n = 6) and as control (5) F12 medium (n = 7).

Acute electrophysiological experiments

The procedure has previously been described by Brisby and Hammar [19]. Briefly, anaesthesia was induced with a mixture of fentanyl (Leptanal 272 µg/kg, Janssen-Cilag AB, Sweden) and medetomidine hydrochloride (Domitor-Vet 545 µg/kg, Orion Pharma, Finland; i.p.) and maintained by intermittent administration of α -chloralose (dose 5–30 mg/kg, Rhône-Poulenc Santé, France; i.v.). Atropine (0.5 mg/kg, Mylan AB, Sweden; s.c.) was given during the preliminary dissection to limit respiratory tract mucous secretion. Intermittently administrated small volumes

(0.1 ml; i.v.) of Ringer Acetate and buffer (containing sodium bicarbonate and glucose) were given throughout the experiment. The animals were tracheotomised and attached to a respirator, with neuromuscular transmission blocked by pancuronium bromide (Pavulon total dose 0.3 mg/kg, Organon, the Netherlands; i.v.). The heart rate was monitored via subcutaneous electrodes and the rectal temperature maintained at 36–38 °C by servo-controlled infrared lamps.

The left sciatic nerve was transected at knee level and mounted on a pair of silver hook stimulating electrodes in a paraffin pool created by skin flaps. The left L4 DRG was exposed for the application of cell solution or F12 medium and a laminectomy was performed at Th11–12 exposing the spinal cord and used for cord dorsum records of ascending volleys. A craniotomy was made and the dura was removed to enable electrode insertion into the thalamus.

Stimulation and recording

The sciatic nerve was stimulated using a short train of impulses (3 stimuli, 0.2-ms duration, 400 Hz) delivered at intervals of 2 Hz. Stimulation intensities are expressed in multiples of threshold (T) for the most sensitive fibres in the nerve [20]. A glass micropipette filled with 2 M NaCl was positioned in the VPL (target measured in millimeters from Bregma: posterior −2.5 to 3.5, lateral 3.0, horizontal −6, Fig. 1b) where low-intensity sciatic stimulation (2 T) only evoked scarce neuronal responses while higher-intensity stimulation (20–50 T) evoked maximal response (corresponding to A β and A β together with A δ fibres activation, respectively, but excluding the higher threshold and slower conducting C-fibres [20]). Neuronal responses evoked by sciatic nerve stimulation were sampled every 10 min in multiple series of 10–20 consecutive stimuli evaluated within a time window of 25–30 ms. At the onset of each experiment, a series of baseline records from the contralateral VPL while stimulating the ipsilateral sciatic nerve were sampled and used to compare changes in subsequent records. Thereafter, the cell suspension or F12 medium was applied on the DRG and evoked thalamic activity was recorded for 40 min.

Analyses

The original data records from the electrophysiological experiments as well as averages of 10–20 consecutive records were stored online using a software system designed by E. Eide, T. Holmström and N. Pihlgren (University of Gothenburg). The mean number of evoked responses obtained as baseline was set to 100 % and data obtained in each series thereafter presented as a

percentage $\pm$ SEM of these initial values. Statistical analyses were made with SPSS Statistics software package (SPSS Inc., Chicago). Kruskal–Wallis test was used to compare the number of responses evoked at different time points between groups, and paired *t* test was used to compare the changes in number of evoked responses between time points in the individual groups with a significance level of $p < 0.05$.

Results

The cell viability was above 80 % in all experiments (mean viability: 84 % for notochordal cells, 97 % for chondrocyte-like cells). The cell suspension medium did not induce any change in evoked thalamic activity during the 40 min of recording (Fig. 2).

25,000 notochordal cells and 25,000 chondrocyte-like cells

Application of 25,000 notochordal cells on the DRG resulted in a statistically significant decrease in evoked thalamic activity to 76 % (SEM $\pm$ 9) within 10 min. The decreased activity lasted throughout the recording period of 40 min (Fig. 3a). In contrast, application of 25,000 chondrocyte-like cells did not evoke any statistically significant changes in thalamic activity during the 40 min of recording (Fig. 3b). Comparing the mean number of evoked thalamic responses after 40 min for each of these populations revealed a statistically significant difference between notochordal and chondrocyte-like cells (Fig. 3c).

150,000 chondrocyte-like cells

Similar to the lower amount of chondrocyte-like cells, applying 150,000 such cells on the DRG did not result in any changes in evoked thalamic activity compared to

Fig. 2 Control experiment. Mean number of evoked responses in VPL in percent of baseline during 40 min of recording with F12 medium ($n = 7$, $\pm$ SEM)

Fig. 3 Mean number of evoked responses in the VPL nucleus of the thalamus following application of 25,000 notochordal or chondrocyte-like cells on the DRG. Averaged data during 40 min of recordings with **a** notochordal cells ($n = 6$), **b** chondrocyte-like cells ($n = 6$) and **c** both groups presented in the same graph for comparison. * $p < 0.05$, $\pm$ SEM

baseline records (Fig. 4). Further, no statistically significant differences in evoked thalamic responses were found at any time point during 40 min of recordings when comparing the results to those obtained when DRGs were exposed to 25,000 chondrocyte-like cells.

Combination of notochordal cells and chondrocyte-like cells

Applying 30,000 cells in a combination containing both notochordal cells and chondrocyte-like cells to the DRG (1,500 notochordal cells and 28,500 chondrocyte-like cells, ratio 1/20) did not induce any changes in evoked thalamic activity during 40 min of recording (Fig. 5).

Fig. 4 Mean number of evoked responses following application two different numbers of chondrocyte-like cells. Average number of responses during 40 min at of either 25,000 ($n = 6$) or 150,000 ($n = 6$) chondrocyte-like cells. Data presented as $\pm$ SEM

Discussion

The results of the present study demonstrate that when applied separately to the DRG, the two cell populations affect the evoked neuronal thalamic activity differentially. While DRG exposure to 25,000 notochordal cells resulted in a decrease in evoked thalamic activity, neither application of 25,000 chondrocyte-like cells, the higher amount of 150,000 chondrocyte-like cells, nor the application of a combination of both cell types induced any change. None of the groups showed an increase in neuronal activity as previously observed with NP [19]. The contribution of the cell suspension medium may be negligible as the medium per se did not induce any change in evoked responses when applied on its own.

The majority of the small cells retrieved after sorting by cell size are cells with a chondrocyte-like phenotype, but there might also be some other cell types among the small cells as shown by Ichimura et al. [21] who described a morphologically heterogeneous population of small cells in rat NP and Kim et al. [22] who described the presence of a population of small notochordal cells with chondrocytic

Fig. 5 Effects following application of a combination of cells. Averages of number of evoked responses during 40 min of recordings after application of 30,000 cells in a combination of notochordal and chondrocyte-like cells ($n = 6$). Data presented as $\pm$ SEM

phenotype in rabbit NP, possibly similar to those found in elderly human discs [10].

It has previously been shown that the notochordal cells can affect nervous tissue negatively in vitro by inhibiting neurite outgrowth [11] and reducing neurite diameter [12] of cultured DRGs. In the present study, in which the changes in evoked activity in the thalamus were investigated in vivo, notochordal cells rapidly induced a decrease in evoked thalamic activity. Although the cellular mechanisms underlying this effect could not be investigated in this model, it is reasonable to assume that the changes in evoked thalamic activity following stimulation of the sciatic nerve could result from altered excitability either in the soma or axons of the DRG neurones, or at the synapses in the dorsal horn of the spinal cord, or both.

In contrast to the effects of notochordal cells, chondrocyte-like cells did not induce any changes in evoked thalamic activity and this lack of effect was observed irrespective of whether the DRG was exposed to 25,000 or 150,000 chondrocyte-like cells. The lower number of cells, i.e. 25,000 notochordal and 25,000 chondrocyte-like cells, were chosen since the approximate same cell numbers have been used previously albeit in a different model [11]. The higher number, i.e. 150,000 chondrocyte-like cells, was used to investigate possible effects of a substantially larger amount of cells, thereby, also increasing the amounts of different cell types constituting the more heterogeneous population of small NP cells. However, the lack of effects indicates that the chondrocyte-like cells alone may not induce the increase in evoked thalamic activity previously observed with NP [19] but it cannot be excluded that these cells may play a role in the complex pathophysiology in, e.g. disc herniation and sciatica.

It is possible that the previously reported effects following application of NP in vivo depended on the presence and interactions between different cell populations. An inhibition in neurite outgrowth in cultured DRGs has been reported following application of a combination of 1,500 notochordal and 28,500 chondrocyte-like cells (ratio 1/20) [11] as well as NP [13]. The same cell amount and cell ratio as that used in vitro was, therefore, also applied on the DRG in vivo in the present investigation to evaluate whether the effect would resemble the increase in evoked neuronal responses following acute application of NP previously observed in vivo [19]. However, in the present model, this combination of cell types did not induce any change in evoked thalamic activity. It is possible that the cell amount and/or cell ratio applied in vivo was not optimal to induce thalamic responses such as those observed after application of NP [19]. An additional reason for the lack of effect could be the more complex tissue composition of NP than a combination of solely two NP-derived cell populations used in this study. Cytokines or other factors, either present

in the matrix of NP or released by the cells in NP may be involved in the pathophysiological mechanisms behind the effects of NP on the nervous system. The cells in the nucleus pulposus can produce several cytokines [23, 24] including TNF- α which is suggested to be a factor of importance in the pathophysiology of disc herniation and sciatic pain and has, e.g. been shown to increase neuronal excitability in primary nociceptive neurons when applied locally [25].

Furthermore, in the present study, all cells were applied as the primary intervention on the DRG while the facilitating effects reported by Brisby and Hammar [19] were shown following application of NP to the DRGs not only as a primary intervention but also on DRGs following prior exposure to adipose tissue. The possible contribution to the NP evoked effect by first applying adipose tissue and thereafter NP was not evaluated in their study leaving a possibility that the reported potent increase in evoked neuronal activity is a combined effect (adipose tissue and NP) and might, therefore, not be fully compatible to the lack of effects after application of the combination of cells in the present investigation.

In the present study, we have demonstrated different effects on evoked thalamic activity following DRG exposure to two different cell populations derived from NP. The reduction in evoked thalamic activity after application of notochordal cells was an interesting observation. The clinical relevance of this observation is, however, difficult to assess, since the number of notochordal cells in human adult nucleus pulposus is limited [8, 9]. The findings indicate a high complexity of the cellular interactions involved in NP-related changes in nervous tissue as seen in association with disc herniation, radiculopathy and sciatic pain.

Acknowledgments The authors thank Jytte Grännsjö and Katarina Junevik for excellent technical assistance and Valter Sundh at EpiStat, Sahlgrenska Academy, for statistical support. This work was supported by the Foundation in memory of Sigurd and Elsa Golje, Wilhelm and Martina Lundgren Foundation, Dr. Felix Neubergh Foundation, Västra Götaland ALF Grants and the Swedish Research Council Grant No. 2010-2594.

Conflict of interest None.

References

1. Hu SJ, Xing JL (1998) An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. *Pain* 77(1):15–23 pii:S0304395998000670
2. Rydevik B, Brown MD, Lundborg G (1984) Pathoanatomy and pathophysiology of nerve root compression. *Spine (Phila Pa 1976)* 9(1):7–15
3. Byrod G, Rydevik B, Nordborg C, Olmarker K (1998) Early effects of nucleus pulposus application on spinal nerve root morphology and function. *Eur Spine J* 7(6):445–449

4. Hou SX, Tang JG, Chen HS, Chen J (2003) Chronic inflammation and compression of the dorsal root contribute to sciatica induced by the intervertebral disc herniation in rats. *Pain* 105(1–2):255–264 pii:S0304395903002227
5. McCarron RF, Wimpee MW, Hudkins PG, Laros GS (1987) The inflammatory effect of nucleus pulposus. A possible element in the pathogenesis of low-back pain. *Spine (Phila Pa 1976)* 12(8):760–764
6. Maldonado BA, Oegema TR Jr (1992) Initial characterization of the metabolism of intervertebral disc cells encapsulated in microspheres. *J Orthop Res* 10(5):677–690. doi:10.1002/jor.1100100510
7. Trout JJ, Buckwalter JA, Moore KC (1982) Ultrastructure of the human intervertebral disc: II. Cells of the nucleus pulposus. *Anat Rec* 204(4):307–314. doi:10.1002/ar.1092040403
8. Trout JJ, Buckwalter JA, Moore KC, Landas SK (1982) Ultrastructure of the human intervertebral disc. I. Changes in notochordal cells with age. *Tissue Cell* 14(2):359–369 [pii: 0040-8166 (82)90033-7]
9. Weiler C, Nerlich AG, Schaaf R, Bachmeier BE, Wuertz K, Boos N (2010) Immunohistochemical identification of notochordal markers in cells in the aging human lumbar intervertebral disc. *Eur Spine J* 19(10):1761–1770. doi:10.1007/s00586-010-1392-z
10. Hunter CJ, Matyas JR, Duncan NA (2004) Cytomorphology of notochordal and chondrocytic cells from the nucleus pulposus: a species comparison. *J Anat* 205(5):357–362. doi:10.1111/j.0021-8782.2004.00352.x
11. Larsson K, Runesson E, Junevik K, Rydevik B, Brisby H (2011) Effects of intervertebral disc cells on neurite outgrowth from dorsal root ganglion explants in culture. *Spine (Phila Pa 1976)* 36(8):600–606. doi:10.1097/BRS.0b013e3181d8bca7
12. Larsson K, Brisby H, Johansson BR, Runesson E, Rydevik B (2011) Electron microscopy analysis of neurites extending from dorsal root ganglia in vitro following exposure to intervertebral disc cells. *Cells Tissues Organs*. doi:10.1159/000330696
13. Lidslot L, Olmarker K, Kayama S, Larsson K, Rydevik B (2000) Nucleus pulposus inhibits the axonal outgrowth of cultured dorsal root ganglion cells. *Eur Spine J* 9(1):8–13
14. Chen C, Cavanaugh JM, Song Z, Takebayashi T, Kallakuri S, Wooley PH (2004) Effects of nucleus pulposus on nerve root neural activity, mechanosensitivity, axonal morphology, and sodium channel expression. *Spine (Phila Pa 1976)* 29(1):17–25. doi:10.1097/01.BRS.0000096675.01484.87
15. Takebayashi T, Cavanaugh JM, Cuneyt Ozaktay A, Kallakuri S, Chen C (2001) Effect of nucleus pulposus on the neural activity of dorsal root ganglion. *Spine (Phila Pa 1976)* 26(8):940–945
16. Anzai H, Hamba M, Onda A, Konno S, Kikuchi S (2002) Epidural application of nucleus pulposus enhances nociceptive responses of rat dorsal horn neurons. *Spine (Phila Pa 1976)* 27(3):E50–E55
17. Cuellar JM, Montesano PX, Carstens E (2004) Role of TNF-alpha in sensitization of nociceptive dorsal horn neurons induced by application of nucleus pulposus to L5 dorsal root ganglion in rats. *Pain* 110(3):578–587. doi:10.1016/j.pain.2004.03.029
18. Zhang Y, Wang N, Wang JY, Chang JY, Woodward DJ, Luo F (2011) Ensemble encoding of nociceptive stimulus intensity in the rat medial and lateral pain systems. *Mol Pain* 7:64. doi:10.1186/1744-8069-7-64
19. Brisby H, Hammar I (2007) Thalamic activation in a disc herniation model. *Spine (Phila Pa 1976)* 32(25):2846–2852. doi:10.1097/BRS.0b013e31815b6526
20. Jack JJB (1978) Studies in neurophysiology. Some methods for selective activation of muscle afferent fibers. University Press, Cambridge
21. Ichimura K, Tsuji H, Matsui H, Makiyama N (1991) Cell culture of the intervertebral disc of rats: factors influencing culture, proteoglycan, collagen, and deoxyribonucleic acid synthesis. *J Spinal Disord* 4(4):428–436
22. Kim JH, Deasy BM, Seo HY, Studer RK, Vo NV, Georgescu HI, Sowa GA, Kang JD (2009) Differentiation of intervertebral notochordal cells through live automated cell imaging system in vitro. *Spine (Phila Pa 1976)* 34(23):2486–2493. doi:10.1097/BRS.0b013e3181b26ed1
23. Takahashi H, Suguro T, Okazima Y, Motegi M, Okada Y, Kakiuchi T (1996) Inflammatory cytokines in the herniated disc of the lumbar spine. *Spine (Phila Pa 1976)* 21(2):218–224
24. Yoshida M, Nakamura T, Sei A, Kikuchi T, Takagi K, Matsukawa A (2005) Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model. *Spine (Phila Pa 1976)* 30(1):55–61 (pii:00007632-200501010-00011)
25. Liu B, Li H, Brull SJ, Zhang JM (2002) Increased sensitivity of sensory neurons to tumor necrosis factor alpha in rats with chronic compression of the lumbar ganglia. *J Neurophysiol* 88(3):1393–1399