

Experimental Cauda Equina Compression Induces HSP70 Synthesis in Dog

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Summary

The heat shock protein 70 (HSP70) is a key component of the stress response induced by various noxious conditions such as heat, oxygen stress, trauma and infection. In present study we have assessed the consequences of the compression of lower lumbar and sacral nerve roots caused by a multiple cauda equina constrictions (MCEC) on HSP70 immunoreactivity (HSP70-IR) in the dog. Our data indicate that constriction of central processes evokes HSP70 up-regulation in the spinal cord (L7, S1-Co3) as well as in the corresponding dorsal root ganglion cells (DRGs) (L7-S1) two days following injury. A limited number of bipolar or triangular HSP-IR neurons were found in the lateral collateral pathway (LCP) as well as in the pericentral region (lamina X) of the spinal cord. In contrast, a high number of HSP70 exhibiting motoneurons with fine processes appeared in the ventral horn (laminae VIII-IX) of lumbosacral segments. Concomitantly, close to them a few lightly HSP70-positive neuronal somata or cell bodies lacking the HSP70-IR occurred. In the DRGs, HSP70 expression was mildly up-regulated in small and medium-sized neurons and in satellite cells. On the contrary, DRGs from intact or sham-operated dogs did not reveal HSP70 specific neuronal staining. In conclusion, we have demonstrated that the MCEC in dogs mimicking the cauda equina syndrome in clinical settings evokes expression of HSP70 synthesis in specific neurons of the lumbo-sacro-coccygeal spinal cord segments and in small and medium sized neurons of corresponding DRGs. This suggests that HSP70 may play an active role in neuroprotective processes partly by maintaining intracellular protein integrity and preventing the neuronal degeneration in this experimental paradigm.

Key words

Cauda equina constriction • Heat shock proteins • Spinal cord • Dog

Introduction

The occurrence of heat shock proteins (HSPs) is nowadays understood as a universal cellular response to a

variety of injuries, including stroke, epilepsy, trauma and neurodegenerative diseases (Gower *et al.* 1989, Gass *et al.* 1995, Brosnan *et al.* 1996). It is well known that the stress proteins are capable of inducing tolerance by

modulating the pathways of stress-induced apoptosis leading to a unique protective effect, first detected following thermal stress or cerebral ischemia (Mosser *et al.* 1997, Buzzard *et al.* 1998). Surprisingly, ischemic preconditioning has been shown to increase the amount of preserved tissue after traumatic brain injury, but not following spinal cord trauma (Ondrejčák *et al.* 2004). The 70-kD stress protein is one of the most frequently studied proteins because of its relatively low level or absence in nonstressed cells and its dramatic elevation following stimulation (Sloviter and Lowenstein 1992). For example, the exposure of brain tissue to short-lasting, noninjurious, conditioning ischemia is effective in inducing HSP70 in those neuronal pools that display an increased resistance to a subsequent injurious interval of ischemia that is referred as induced ischemic tolerance (Čížková *et al.* 2004). Indeed, previous studies have indicated that thermal stress, global or focal cerebral ischemia induce immediate-early gene *c-fos* (Yang *et al.* 2000) and also HSP70 overexpression in a variety of cell populations, such as neurons, glia and endothelial cells (Ikeda *et al.* 1994, Burda *et al.* 2003). Recent advances in molecular biology and particularly in gene manipulation have led to the development of rats genetically modified to overexpress HSP72 (Čížková *et al.* 1999), that are suggested to elucidate the molecular mechanisms leading to preconditioning, or eventually identifying potential therapeutic targets for preventing the post-ischemic brain/spinal cord damage.

To verify the involvement of HSP70 in the repair of injury of the sensory and motor neurons, an experimental constriction injury of the lumbosacral central processes of the dorsal root ganglia neurons resulting in the cauda equina syndrome (CES) was studied in dogs. The fully developed CES is accompanied by sensory and motor disorders such as low-back pain, saddle anesthesia, and motor weakness of lower extremities leading sometimes to paraplegia or bladder dysfunction. These clinical symptoms are related to a sustained stimulation of the cutaneous, muscular and visceral nociceptive afferents (Maršala *et al.* 1995, Orendáčová *et al.* 2001a,b). Thus, the stress protein expression in the neurons of corresponding spinal cord segments could be expected. This hypothesis was tested in the present study using an immunocytochemical analysis of HSP70 expression in the lower lumbosacral and coccygeal spinal cord segments and corresponding DRGs following CES two days after the surgery.

Methods

Adult mongrel dogs (n=4) of both sexes (weighing 8-10 kg) were lightly anesthetized with sodium pentobarbital (15 mg/kg, i.v.), endotracheally intubated and artificially ventilated on a respirator with halothane (1-2 %) in a mixture of oxygen and nitrous oxide (1:1). Lumbar laminectomy was performed and the dural sac of the exposed cauda equina comprising dorsal and ventral roots of L7, S1-S3, Co1-Co5 segments was tied by four loose ligatures with about 2 mm spacing. Thus, the central processes of the L7-Co5 DRG neurons were permanently constricted. Sham-operated dogs (n=4) underwent the cauda equina exposure, without ligation. Two days after the surgery both groups of dogs were deeply anesthetized with sodium pentobarbital (70 mg/kg i.v.) and transcardially perfused with heparinized saline followed by perfusion with 4 % paraformaldehyde in phosphate buffered saline (pH 7.4). The spinal cords and the corresponding DRGs (L7, S1-S3) were removed and transverse cryostat sections (30 µm) throughout the entire lumbar (L), sacral (S) and coccygeal (Co) spinal cord segments and DRGs were cut. Free floating sections were immediately processed for immunocytochemical staining with monoclonal antiserum for HSP70 (the inducible form HSP70/HSP72; StressGen Biotech Corp.) using *elite* ABC Vector kit. Briefly, non-specific protein binding was blocked by incubation in the 7 % normal horse serum for 2 h followed by incubation with a primary mouse antibody against HSP70 (dilution 1:1000), in PBS containing 0.2 % TX-100 during the night at 4 °C. The procedure was continued by rinsing in the PBS, incubation with secondary biotinylated anti-mouse IgG (dilution 1:200 in PBS) for 2 h at room temperature, washing in PBS and reacting with ABC reagents. The color reaction was developed in a filtered solution of 0.05 % 3,3-diaminobenzidine tetrahydrochloride (DAB) in PBS containing 0.02 % hydrogen peroxide. The staining reaction was stopped in distilled water, the sections were then washed, mounted on the gelatine-coated slides, dried, dehydrated and coverslipped with Permount. On some sections, the primary antibody was omitted from the staining procedure to determine the specificity of the immunocytochemical reaction.

Results

In the present study, using the immunocyto-

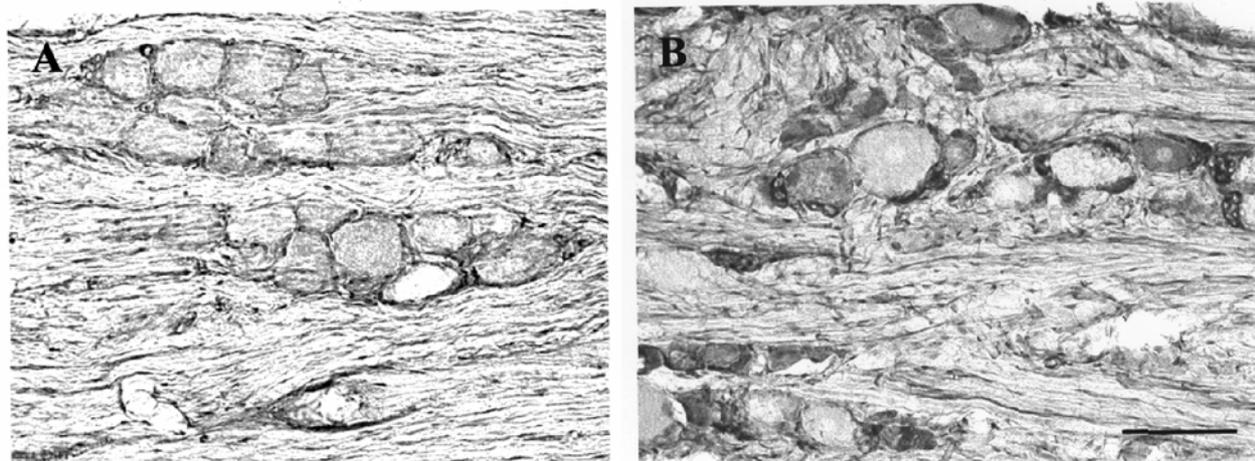


Fig. 1. (A-B) HSP70-IR in the transverse section taken from DRGs (S1) of sham-operated (A) or following MCES two days after the surgery (B). Modest HSP70 expression appeared in the small and medium-sized neurons, and satellite glial cells following nerve root constriction. Bars A, B = 100 μ m.

chemical method, we were able to map the segmental and laminar distribution of the HSP70-IR as indices of the injury in this clinically relevant model of somatovisceral pain.

In the sham-operated dogs, no specific neuronal HSP70-IR could be detected in the DRGs or the spinal cord. The cell bodies, adjacent glia and nerve fibers of sacral DRGs were free of HSP-IR (Fig. 1A). Similarly, the dorsal horn (laminae I-IV) and the lateral collateral pathway (LCP) (Fig. 2A), the pericentral region (lamina X) (Fig. 2B) as well as the ventral horn (laminae VIII-IX) (Fig. 2C) completely lacked HSP70-positive neuronal cells.

On the contrary, MCEC induced mild HSP70-IR in the small or medium-sized cell bodies of sacral DRGs (Fig. 1B). HSP70 positivity was also clearly detectable in the satellite cells surrounding the DRGs cells. Similarly, constriction of nerve roots evoked a significant HSP70 up-regulation in neuronal population throughout the entire sacrococcygeal (S1-Co3) spinal cord at two days following injury.

The lateral portion of the dorsal horn outlining the lateral collateral pathway (LCP) contained a few mainly bipolar or triangular HSP70 positive perikarya with elongated dendrites oriented rostrocaudally (Fig. 3A). These intensely stained neurons were detected bilaterally primarily throughout the S1-S3 segments (Figs 3B and 3C). The superficial laminae I-II as well as deep dorsal horn laminae III-V lacked HSP70-positive neuronal elements (Fig. 3A). Only a few densely labeled neurons were scattered laterally from the central canal, in

lamina X (Fig. 3D). These neurons resemble to large intensely stained NADPH diaphorase (NADPHd)-exhibiting somata, which were described in this region (Maršala *et al.* 1999, Maršala and Jalč 2000).

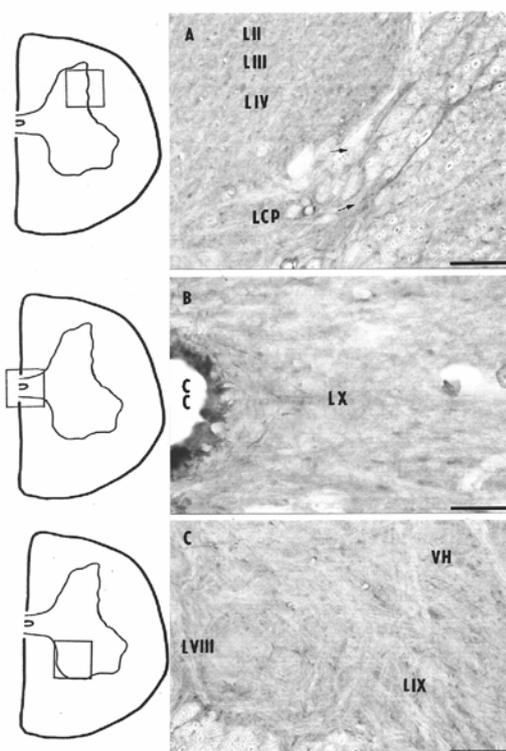


Fig. 2. (A-C) HSP70-IR in the transverse sections through spinal cord S1 segment in sham-operated dogs two days after the surgery. (A) The dorsal horn (laminae I-IV), the lateral collateral pathway (LCP), (B) the pericentral region (lamina X), as well as the (C) ventral horn completely lacked the HSP70 positive neuronal cells. Bars A, C = 100 μ m, B = 50 μ m.

On the other hand, the most pronounced somatic HSP70-IR appeared in the ventral horn, in laminae VIII-IX (Fig. 4A) throughout L7-Co5 spinal cord segments. Concurrently, significant differences in the population of the motoneurons expressing HSP70 were noted. Some motoneurons were intensely HSP70 stained, clearly depicting their cell bodies and dendritic arborizations,

while others were stained quite lightly, allowing the somata to be identified and some did not exhibit any HSP70 immunoreactivity (IR) (Figs 4A, 4A' and 4B). In addition, the dorsal as well as the ventral horn, and the pericentral region revealed a high number of small HSP70 intensely stained elements that may represent activated glial cells (Figs 3A, 3D and 4A-B).

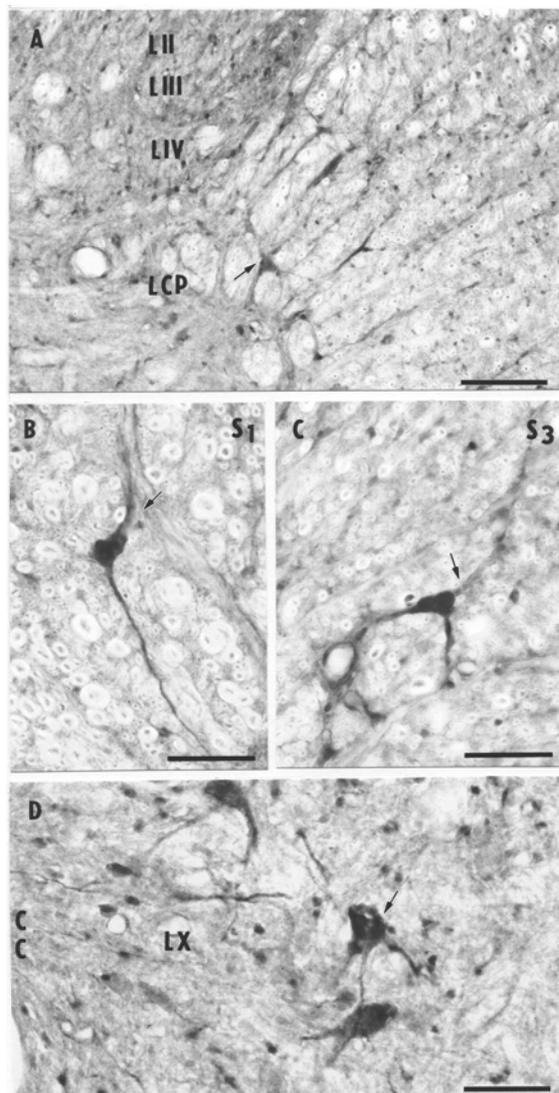


Fig. 3. (A-D) HSP70-IR in the transverse spinal cord sections through S1-S3 segment in dogs two days after cauda equina constriction injury. (A-C) Note, the bipolar or triangular HSP70 positive perikarya with elongated dendrites oriented rostrocaudally in the LCP (arrow) of the S1(B), S3(C) segment. (D) The intensely HSP70 labeled neurons located laterally from the central canal in lamina X (arrow) could be seen. Bars A, B, C = 100 μ m, D = 50 μ m.

Discussion

In the present study, we have examined the influence of MCEC, characterized as a somatovisceral

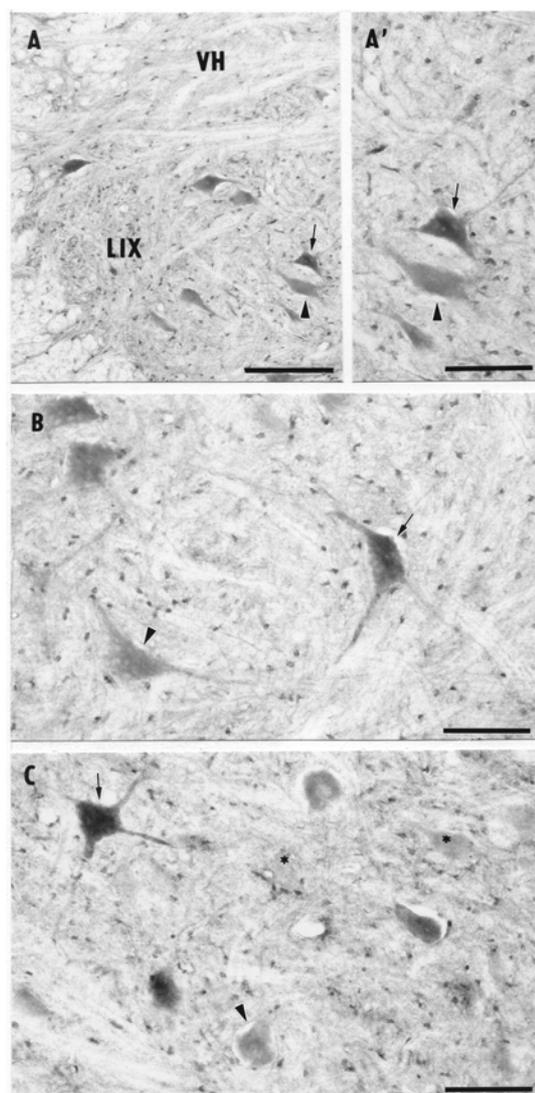


Fig. 4. (A-B) HSP70-positive neurons in the ventral horn of S1 and S3 segments following MCEC. Note the different degree of HSP-IR in the motoneurons; intensely HSP70 stained motoneurons with their dendritic arborizations (arrows), lightly stained neuronal somata (arrowheads)(A,A',B) or HSP70-negative moto-neurons (asterisk) (C), the intensely stained small elements in the neuropil occurred throughout the ventral horn. Bars A, B = 100 μ m.

pain model in dogs (Maršala *et al.* 1995) on HSP70 protein synthesis. This experimental procedure allows the constriction of the dorsal as well as ventral roots of L7-Co5 segments. The constrictions of long dorsal root

afferents forming the cauda equina compromise, functionally divergent spectra of low-threshold myelinated ($A\beta$ and $A\gamma$) mechanoreceptive afferents, high-threshold $A\delta$ mechanoreceptive afferents, $A\delta$ heat nociceptive afferents and C polymodal nociceptive afferents that are particularly distributed in the range of laminae I-V (Ralston and Ralston 1979, 1982). Thus, a sustained constriction of the central multimodal afferents along with the constriction of somatomotor and visceromotor axons of the ventral roots provides a reliable model for studying stress, pain or inflammatory responses in the dorsal and ventral horn of the corresponding spinal cord segments. The present study considerably broadens our previous observations and clearly shows that a sustained constriction of the cauda equina that significantly enhances the expression of c-Fos (Orendáčová *et al.* 2000, 2001a,b, Lukáčová *et al.* 2004) causes an up-regulation of the inducible HSP70-IR in neurons throughout the affected spinal cord two days after the surgery. Constriction injury or axotomy of peripheral nerves is typically followed by axonal regeneration, whereas after central rhizotomy axonal regeneration is limited (Oblinger and Lasek 1984). The absence of the cell body response after lesion to the central nerve roots has been suggested to be related to the reduced regenerative capacity of the central axons (Lieberman 1971). Thus, this phenomenon might be a reasonable explanation for interpretation of present findings revealing only modest HSP70-IR in subpopulation of small and medium-sized DRGs neurons two days after MCEC.

Surprisingly, the dorsal horn was free of the HSP70-IR neurons and only a few bipolar HSP70-positive neurons outlining the lateral collateral pathway (LCP) in the white matter were observed. Recent data suggest that the intensity, frequency, as well as the duration of applied stimulus is the key determinant in neuronal HSP70 induction (Čížková *et al.* 2004). Therefore, stimulus, even if sufficient to induce transient neuronal depolarization, is not always effective in inducing neuronal HSP70 expression in secondary dorsal horn neurons. Our previous experiments using the model of MCEC in the dog showed that Lissauer's tract and the LCP include a large proportion of degenerated sacral dorsal root afferents (Maršala *et al.* 1995), represented mainly by C polymodal nociceptive afferents (Nadelhaft *et al.* 1980). Thus, it is apparent that HSP70-positive bipolar or triangular neurons of the LCP, are located close to the trajectory of those afferent axons known to be

subjected to a sustained stimulation in the MCEC model. Similarly, the appearance of HSP70-IR neurons in the pericentral region (lamina X) could be explained by activation of $A\delta$ mechanoreceptive afferents, which reveal broader distribution and can influence not only nociceptive neurons in the superficial laminae I-II but also the wide-dynamic-range neurons occurring in the deep dorsal horn layers (Price and Dubner 1997). However, the phenotypic differences among NADPH-exhibiting pools in pericentral region may have some relevance with regard to the alteration of NADPHd activity of lamina X neurons following multiple sublethal ischemic insults (Maršala and Jalč 1999, 2000) as well as the variation of nitric oxide synthase (NOS) and HSP70 expression following MCEC (Maršala *et al.* 2003). Significantly more HSP70-IR neurons were detected in the ventral horn and in the laminae VIII-IX. Some of them were densely HSP70-IR thus outlining their cell bodies and dendritic arborizations, some neurons were stained quite lightly, thus displaying their somata, while others completely lacked HSP70-IR. This variability among motoneurons expressing HSP70 shows that even a homogenous population of motoneurons may react in a different manner to the constriction of ventral roots. It has been suggested that the specific HSP70 response is usually associated with the intensity of the cellular stress and can serve as a sensitive and reliable indicator of the cell injury (Tanno *et al.* 1993, Welch 1992). Thus, the motoneurons might be variously susceptible to the stress or they might be exposed to a different intensity of the external stressors which probably lead to a heterogeneous HSP70 expression (Ikeda *et al.* 1994, Sloviter and Lowenstein 1992). However, considerable region-dependent differences in the catalytic activity of nitric oxide synthase (cNOS) that occurs in the ventral horn gray matter areas may also contribute to the different motoneuronal susceptibility (Maršala *et al.* 2003, Lukáčová and Pavel 2000). To our knowledge, there have been only a few studies dealing with an inducible HSP70 involvement in the peripheral nerve injuries. Tedeschi and Ciavarra (1997) clearly showed that peripheral axotomy is associated with the *in vivo* induction of the inducible form of the stress proteins (HSP70) in DRGs, but not its constitutive form (HSC70). Similarly, the HSP70 mRNA was shown to be rapidly and transiently induced in the hamster facial nucleus neurons following the nerve injury (New *et al.* 1989). However, most studies have been monitoring the modulation of a low molecular HSP27, which is constitutively expressed in

sensory and motor neurons, acts as a specific cellular inhibitor of apoptosis in fibrosarcoma and monoblastoid cells, confers resistance to a heat shock, and enhances the cell survival in response to a TNF α -induced cell death (Mehlen *et al.* 1996a,b, Samali *et al.* 1996, Plumier *et al.* 1997). In this context, Costigan *et al.* (1998) have shown that the transection of the sciatic nerve in rats results in the upregulation of the HSP27 mRNA and protein in the corresponding DRG as well as in the spinal cord but without equivalent changes in the expression of the HSP56, HSP60, HSP70 or HSP90 (Costigan *et al.* 1998). Except HSP70 expressing neurons we were also able to detect small HSP70-IR elements localized in the superficial dorsal horn (laminae I-III), pericentral region (lamina X) and the ventral horn (laminae VIII, IX). Morphometric measurements of these structures (2.4-2.8 μ m) suggest that they probably represent glial elements, but this has to be confirmed by further immunocytochemical analysis.

Therefore, the present data broaden the scope of the possible roles played by the inducible HSP70 in neurodestructive or neuroregenerative processes that develop following a sustained peripheral constriction injury. In addition, there is still a number of relevant questions to be answered. Further work will be required to investigate the potential involvement of glial elements, such as microglia, astrocytes and oligodendroglia, in

relation to the cauda equina constriction injury. Since the astrocytes are localized in an intimate contact with neurons, it could be suggested that they could be a potential sensitive element responding to any perturbation of neuronal homeostasis (Murray *et al.* 1990, Vernadakis 1996, Ridet *et al.* 1997, Čížková *et al.* 1997). Another important focus for future studies is the application of highly sensitive double-stained immunocytochemical methods displaying the viability of those motoneurons that express or lack the synthesis of the inducible HSP70 in correlation with the ensuing reaction of all glial types including microglia, astrocytes and oligodendroglia.

Abbreviations

CES – cauda equina syndrome
 DRGs – dorsal root ganglion cells
 HSP – heat shock protein
 HSP-IR – heat shock protein-immunoreactivity
 HSPs – heat shock proteins
 MCEC – multiple cauda equina constrictions
 NOS – nitric oxide synthase

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References

- BROSNAN CF, BATTISTINI L, GAO YL, RAINE CS, AQUINO DA: Heat shock proteins and multiple sclerosis: a review. *J Neuropathol Exp Neurol* **55**: 389-402, 1996.
- BURDA J, HREHOROVSKÁ M, BONILLA LG, DANIELISOVÁ V, ČÍŽKOVÁ D, BURDA R, NÉMETHOVÁ M, FANDO JL, SALINAS M: Role of protein synthesis in the ischemic tolerance acquisition induced by transient forebrain ischemia in the rat. *Neurochem Res* **28**: 1213-1219, 2003.
- BUZZARD KA, GIACCIA AJ, KILLENDER M, ANDERSON RL: Heat shock protein 72 modulates pathways of stress-induced apoptosis. *J Biol Chem* **273**: 17147-17153, 1998.
- COSTIGAN M, MANNION RJ, KENDALL G, LEWIS SE, CAMPAGNA JA, COGGESHALL RE, MERIDITH-MIDDLETON J, TATE S, WOOLF CJ: Heat shock protein 27: developmental regulation and expression after peripheral nerve injury. *J Neurosci* **18**: 5891-5900, 1998.
- ČÍŽKOVÁ D, RAČEKOVÁ E, VANICKÝ I: The expression of B-50/GAP-43 and GFAP after bilateral olfactory bulbectomy in rats. *Physiol Res* **46**: 487-495, 1997.
- ČÍŽKOVÁ D, DILLMANN W, MESTRIL R, ČÍŽEK M, MARŠALA M: Regional distribution of HSP72 in brain and spinal cord in transgenic rats. *Biologia* **54**: 93-101, 1999.
- ČÍŽKOVÁ D, CARMEL JB, YAMAMOTO K, KAKINOHANA O, SUN D, HART RP, MARŠALA M: Characterization of spinal HSP72 induction and development of ischemic tolerance after spinal ischemia in rats. *Exp Neurol* **185**: 97-108, 2004.
- GASS P, PRIOR P, KIESSLING M: Correlation between seizure intensity and stress protein expression after limbic epilepsy in the rat brain. *Neuroscience* **65**: 27-36, 1995.

- GOWER DJ, HOLLMAN C, LEE KS, TYTELL M: Spinal cord injury and the stress protein response. *J Neurosurg* **70**: 605-611, 1989.
- IKEDA J, NAKAJIMA T, OSBORNE OC, MIES G, NOWAK TS: Coexpression of c-fos and hsp70 mRNAs in gerbil brain after ischemia: induction threshold, distribution and time course evaluated by in situ hybridization. *Brain Res Mol Brain Res* **26**: 249-258, 1994.
- LIEBERMAN AR: The axon reaction: a review of the principal features of perikaryon responses to axon injury. *Int Rev Neurobiol* **14**: 49-124, 1971.
- LUKÁČOVÁ N, PAVEL J: Catalytic nitric oxide synthase activity in the white and gray matter regions of the spinal cord of rabbits. *Physiol Res* **49**: 167-173, 2000.
- LUKÁČOVÁ N, KAFKA J, ČÍŽKOVÁ D, MARŠALA M, MARŠALA J: The effect of cauda equina constriction on nitric oxide synthase activity. *Neurochem Res* **29**: 429-439, 2004.
- MARŠALA J, ŠULLA I, JALČ P, ORENDÁČOVÁ J: Multiple protracted cauda equina constrictions cause deep derangement in the lumbosacral spinal cord circuitry in the dog. *Neurosci Lett* **193**: 97-100, 1995.
- MARŠALA J, KAFKA J, LUKÁČOVÁ N, ČÍŽKOVÁ D, MARŠALA M, KATSUBE N: Cauda equina syndrome and nitric oxide synthase immunoreactivity in the spinal cord of the dog. *Physiol Res* **52**: 481-496, 2003.
- MARŠALA J, JALČ P: Short-term changes of NADPHd-exhibited neuronal pools in the spinal cord of rabbit after repeated sublethal ischemia. *Physiol Res* **48**: 34P, 1999.
- MARŠALA J, JALČ P: Short-term changes of NADPHd-exhibited neuronal pools in the spinal cord of rabbit after repeated sublethal ischemia. *Physiol Res* **49**: 157-165, 2000.
- MEHLEN P, KRETZ-REMY C, PREVILLE X, ARRIGO AP: Human hsp27, Drosophila hsp27 and human alphaB-crystallin expression-mediated increase in glutathione is essential for the protective activity of these proteins against TNFalpha-induced cell death. *EMBO J* **15**: 2695-2706, 1996a.
- MEHLEN P, SCHULZE-OSTHOFF K, ARRIGO AP: Small stress proteins as novel regulators of apoptosis: heat shock protein 27 blocks Fas/APO-1- and staurosporine-induced cell death. *J Biol Chem* **271**: 16510-16514, 1996b.
- MOSSER DD, CARON AW, BOURGET L, DENIS-LAROSE C, MASSIE B: Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Mol Cell Biol* **17**: 5317-5327, 1997.
- MURRAY M, WANG SD, GOLDBERGER ME, LEVITT P: Modification of astrocytes in the spinal cord following dorsal root or peripheral nerve lesions. *Exp Neurol* **110**: 248-257, 1990.
- NADELHAFT I, DE GROAT WC, MORGAN C: Location and morphology of parasympathetic preganglionic neurons in the sacral spinal cord of the cat revealed by retrograde axonal transport of horseradish peroxidase. *J Comp Neurol* **193**: 265-281, 1980.
- NEW GA, HENDRICKSON BR, JONES KJ: Induction of heat shock protein 70 mRNA in adult hamster facial nuclear groups following axotomy of the facial nerve. *Metab Brain Dis* **4**: 273-279, 1989.
- OBLINGER MM, LASEK RJ: A conditioning lesion of the peripheral axons of dorsal root ganglion cells accelerates regeneration of only their peripheral axons. *J Neurosci* **4**: 1736-1744, 1984.
- ONDREJČÁK T, VANICKÝ I, GÁLIK J: Ischemic preconditioning does not improve neurological recovery after spinal cord compression injury in the rat. *Brain Res* **995**: 267-273, 2004.
- ORENDÁČOVÁ J, MARŠALA M, ŠULLA I, KAFKA J, JALČ P, ČÍŽKOVÁ D, TAIRA Y, MARŠALA J: Incipient cauda equina syndrome as a model of somatovisceral pain in dogs: spinal cord structures involved as revealed by the expression of c-fos and NADPH diaphorase activity. *Neuroscience* **95**: 543-557, 2000.
- ORENDÁČOVÁ J, ČÍŽKOVÁ D, KAFKA J, LUKÁČOVÁ N, MARŠALA M, ŠULLA I, MARŠALA J, KATSUBE N: Cauda equina syndrome. *Prog Neurobiol* **64**: 613-637, 2001a.
- ORENDÁČOVÁ J, MARŠALA M, ČÍŽKOVÁ D, KAFKA J, RAČEKOVÁ EN, ŠULLA I, VANICKÝ I, MARŠALA J: Fos protein expression in sacral spinal cord in relation to early phase of cauda equina syndrome in dogs. *Cell Mol Neurobiol* **21**: 413-419, 2001b.
- PLUMIER JC, KRUEGER AM, CURRIE RW, KONTOYIANNIS D, KOLLIAS G, PAGOULATOS GN: Transgenic mice expressing the human inducible Hsp70 have hippocampal neurons resistant to ischemic injury. *Cell Stress Chaperones* **2**: 162-167, 1997.
- PRICE DD, DUBNER R: Neurons that subserve the sensory-discriminative aspects of pain. *Pain* **3**: 307-338, 1997.

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- RALSTON HJ, RALSTON DD: The distribution of dorsal root axons in laminae I, II and III of the macaque spinal cord: a quantitative electron microscope study. *J Comp Neurol* **184**: 643-684, 1979.
- RALSTON HJ, RALSTON DD: The distribution of dorsal root axons to laminae IV, V, and VI of the macaque spinal cord: a quantitative electron microscopic study. *J Comp Neurol* **212**: 435-448, 1982.
- RIDET J L, MALHOTRA SK, PRIVAT A, GAGE FH: Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci* **20**: 570-577, 1997.
- SAMALI A, COTTER TG: Heat shock proteins increase resistance to apoptosis. *Exp Cell Res* **223**: 163-170, 1996.
- SLOVITER RS, LOWENSTEIN DH: Heat shock protein expression in vulnerable cells of the rat hippocampus as an indicator of excitation-induced neuronal stress. *J Neurosci* **12**: 3004-3009, 1992.
- TANNO H, NOCKELS RP, PITTS LH, NOBLE LJ: Immunolocalization of heat shock protein after fluid percussive brain injury and relationship to breakdown of the blood-brain barrier. *J Cereb Blood Flow Metab* **13**: 116-24, 1993.
- TEDESCHI B, CIAVARRA RP: Differential effects of axotomy on the in vivo synthesis of the stress-inducible and constitutive 70-kDa heat-shock proteins in rat dorsal root ganglia. *Brain Res Mol Brain Res* **45**: 199-206, 1997.
- VERNADAKIS A: Glia-neuron intercommunications and synaptic plasticity. *Prog Neurobiol* **49**: 185-214, 1996.
- WELCH WJ: Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev* **72**: 1063-1081, 1992.
- YANG LC, ORENDÁČOVÁ J, WANG V, ISHIKAWA T, YAKSH TL, MARŠALA M: Transient spinal cord ischemia in rat: the time course of spinal FOS protein expression and the effect of intranschemic hypothermia (27 °C). *Cell Mol Neurobiol* **20**: 351-365, 2000.
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