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Changes in mRNA for CAPON and Dexras1 in adult rat following sciatic nerve transection

Aiguo Shen [a,1], Mengling Chen [a,b,1], Shuqiong Niu [a], Linlin Sun [b], Shangfeng Gao [b], Shuxian Shi [b], Xin Li [b], Qingshan Lv [b], Zhiqin Guo [a], Chun Cheng [a,b,*]

[a] *The Jiangsu Province Key Laboratory of Neuroregeneration, Nantong University, Nantong 226001, People's Republic of China*
[b] *Department of Microbiology and Immunology, Medical College, Nantong University, Nantong 226001, People's Republic of China*

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Abstract

Peripheral nerve transection has been implicated to cause a production of neuronal nitric oxide synthase (nNOS), which may influence a range of post-axotomy processes necessary for neuronal survival and nerve regeneration. Carboxy-terminal post synaptic density protein/Drosophila disc large tumor suppressor/zonula occuldens-1 protein (PDZ) ligand of neuronal nitric oxide synthase (CAPON), as an adaptor, interacts with nNOS via the PDZ domain helping regulate nNOS activity at postsynaptic sites in neurons. And Dexras1, a small G protein mediating multiple signal transductions, has been reported to form a complex with CAPON and nNOS. A role for the physiologic linkage by CAPON of nNOS to Dexras1 has suggested that NO-mediated activation of Dexras1 is markedly enhanced by CAPON. We investigated the changes in mRNA for CAPON, Dexras1 and nNOS in the sciatic nerve, dorsal root ganglia and lumbar spinal cord of adult rat following sciatic axotomy by TaqMan quantitative real-time PCR and in situ hybridization combined with immunofluorescence. Signals of mRNA for CAPON and Dexras1 were initially expressed in these neural tissues mentioned, transiently increased at certain time periods after sciatic axotomy and finally recovered to the basal level. It was also found that nNOS mRNA underwent a similar change pattern during this process. These results suggest that CAPON as well as Dexras1 may be involved in the different pathological conditions including nerve regeneration, neuron loss or survival and even pain process, possibly via regulating the nNOS activity or through the downstream targets of Dexras1.

Keywords: Neuronal nitric oxide synthase; Carboxy-terminal PDZ ligand of neuronal nitric oxide synthase; Protein–protein interactions; Sciatic nerve transection; Real-time PCR; In situ hybridization; Rat

1. Introduction

Nitric oxide (NO), a short-lived free radical with important biological activities, which is synthesized from L-arginine by a family of enzymes termed the nitric oxide synthases (NOS) including endothelial NOS (eNOS), inducible or macrophage NOS (iNOS), and neuronal NOS (nNOS) (Griffith and Stuehr, 1995), appears to have a variety of interesting actions after nerve injury. Previous researches have studied on the effects of axonal injury, especially on neurons in the dorsal root ganglia (DRG) and spinal cord (SC). For example, after ligation of nerve roots, NOS activity was bilaterally decreased in SC and ipsilaterally increased in DRG (Choi et al., 1996). Peripheral axotomy leads to an increase in NOS-positive neurons in the DRG (Shi et al., 1998), while nNOS is over-expressed in DRG neurons and accumulated in regenerating axons after sciatic nerve ligature (Sharma et al., 1998). Lesion-induced NO production has been attributed to have a protect effect on damaged neurons (Cristino et al., 2000; Thippeswamy et al., 2001) and might be associated with neuronal regeneration, however, induction of NO is also suggested to be involved in neuronal death (Zhou and Wu, 2006).

Protein–protein interactions represent an important mechanism in the control of NOS spatial distribution or activity (Alderton et al., 2001; Dedio et al., 2001; Zimmermann et al.,

* Corresponding author at: The Jiangsu Province Key Laboratory of Neuroregeneration & Department of Microbiology and Immunology, Medical College, Nantong University, Nantong 226001, People's Republic of China.
Tel.: +86 513 85051999; fax: +86 513 85051999.
E-mail address: chunchng@yahoo.com (C. Cheng).
[1] Both authors contribute equally to this work.

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2002). nNOS is larger than eNOS and iNOS because of an N-terminal extension that contains a PDZ domain (Cho et al., 1992; Ponting and Phillips, 1995), which interacts with a variety of other proteins including PSD95/93 (Brenman et al., 1996) and Carboxy-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON) (Jaffrey et al., 1998). These interactions are thought to facilitate the targeting of nNOS to distinct intracellular sites. As an adaptor protein for nNOS, CAPON forms a complex with synapsin or Dexras1, and nNOS, which directs and enhances the delivery and specificity of the nitric oxide (NO) signal (Jaffrey et al., 1998, 2002; Fang et al., 2000). Such careful controls are typical of NO signaling (Denninger and Marletta, 1999; Luck et al., 2000; Blottner and Luck, 2001) and add to the complex regulation of nNOS gene transcription and translation (Wang et al., 1999). CAPON interactions via the PDZ domain of nNOS help regulate nNOS activity at postsynaptic sites in neurons (Jaffrey et al., 1998), and neuronal CAPON expression in the facial motor nucleus decreases after axotomy and recovers with reinnervation. These changes suggest neuronal CAPON helps regulate nNOS stability, localization, and possibly expression during synapse formation and muscle reinnervation (Che et al., 2000).

Dexras1, as a novel brain enriched member of the Ras family of small monomeric G proteins, is S-nitrosylated by NO donors (Fang et al., 2000). A role for the physiologic linkage by CAPON of nNOS to Dexras1 has suggested that NO-mediated activation of Dexras1 is markedly enhanced by CAPON (Fang et al., 2000). This implies that NO signaling is regulated by protein–protein interactions that juxtapose nNOS with its targets. However, there has been little characterization of Dexras1, and its downstream targets are not definitively established. Ras family members have been implicated in growth and differentiation of neurons (Curtis and Finkbeiner, 1999). nNOS exhibits a dynamic cell-type specific pattern of expression during development of the embryonic brain (Bredt and Snyder, 1994), and neurons in nNOS−/− mice have impaired dendritic arborization (Inglis et al., 1998). Conceivably, Dexras1-specific effectors may exist, mediating the effects of NO in the nervous system.

The rat sciatic nerve is well characterized with regard to fiber composition (Schmalbruch, 1986). Its motor neurons originate in the anterior horn of the lumbar cord, and its sensory neurons are localized in the L4-L6 DRG. Both afferent and efferent fibers of the sciatic nerve will be cut when subjected to transection. In the present study, we investigated the gene expression patterns of CAPON as well as Dexras1 in adult rat neural tissues, including sciatic nerves, DRG and lumbar spinal cord, and studied their regulation in a state of post-axotomy, mainly to ask whether they are expressed in rat nervous system and explore their potential roles in the physiological or pathological conditions in which NO activity was involved.

2. Materials and methods

2.1. Animals and surgery procedures

Adult Sprague-Dawley (SD) rats, either sex, (weighing 200–220 g, from Experimental Animal Center, Nantong University, China), deeply anesthetized with pentobaibital (50 mg/kg, i.p.), were subject to unilateral sciatic nerve transection. Briefly, the right sciatic nerves were transected at the midthigh level after exposure, and the distal stump was folded into the distal in order to prevent target regenerative sprouting within the 4 weeks of our studies. This work complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animals were kept under standardized laboratory conditions in an air-conditioned room with free access to food and water. All efforts were made to minimize animal suffering, to reduce the numbers of animals used and to apply appropriate euthanasia.

For quantitative real-time PCR analysis, lumbar SC and ipsilateral lumbar DRG (L4-L6) from nerve-injured animals were harvested 0, 6, 12 h, 1, 2 days, 1, 2 and 4 weeks after surgery ($n = 3$ at each time point). Tissues were stored at −80 °C prior to RNA isolation.

For histological studies, the animals were deeply anesthetized and perfused with sterile 0.9% saline and fixed with 4% phosphate-buffered formalin (PH 7.4) by intracardial perfusion at room temperature. The lumbar SC and bilateral lumbar DRG of three rats per time point in each group mentioned above were collected, embedded in optimum cutting temperature (OCT) compound (Sakura), frozen and sectioned (14 μm) on a cryostat, thaw-mounted onto poly-D-lysine-coated slides and stored at −20 °C until use.

2.2. Plasmids and probes preparation

Primers and probes (Table 1) were designed using Primer Premier 5 software (Premier Biosoft) avoiding contaminating genomic DNA amplification by positioning one of the primers or a probe over the exon/intron boundary. Using these primers, cDNA fragments for each were obtained by RT-PCR using newborn SD rat (1day postnatal) brain total RNA. These cDNA fragments were ligated into pGEM-T vector (Promega) respectively, amplified in DH5α, and the plasmids were purified and sequenced using standard procedures. Then standard curves for real-time PCR analysis were ready to be constructed using a series of dilutions of standard samples (the standards were plasmids containing the corresponding gene).

For in situ hybridization (ISH), the plasmids were linearized with the restriction enzymes ApaI or SacI in order to obtain nonradioactive-labeled probes. The digoxigenin (DIG) labeled sense and antisense RNA probes were produced using SP6 or T7 RNA polymerase respectively by in vitro transcription according to its protocol (Boehringer Mannheim). The efficiency of DIG labeling was quantified by dot blotting using a DIG detection kit (Boehringer Mannheim).

For TaqMan quantitative real-time PCR analysis, the probes were labeled with 6-carboxyfluorescein (FAM) at the 5′ end as reporter dye and 6-carboxytetramethylrhodamine (TAMRA) at the 3′ end as quencher dye. The β_2-microglobulin (β_2-M) gene was used as a stable endogenous control, plasmids of which were commercially purchased (Promega). Transcript expression was

Table 1
List of primers and probes used in the present study

	Sequence (5′–3′)	Length (bp)	Accession
CAPON			
FW	GTGGGCAGCCCCTTAGGTA	144	NM_138922
RV	GATGCCTGACTCTCGGAACTT		
TP	CAGCCGAGGATAACCAGCCGAT		
Dexras1			
FW	GCGGCGAAGTCTACCAGTTG	117	XM_340809
RV	TGTCTAAGCTGAACACCAGAATGA		
TP	TCTGGCAATCATCCGTTTCCCG		
β_2-M			
FW	GTCTTTCTACATCCTGGCTCACA	134	NM_012512
RV	GACGGTTTTGGGCTCCTTCA		
TP	CACCCACCGAGACCGAT-GTATATGCTTGC		

FW, forward primer; RV, reverse primer; TP, TaqMan probe dual-labeled with 5′-FAM and 3′-TAMRA.

determined from standard curves mentioned above and corresponding cycles of threshold (C_t) value.

2.3. TaqMan quantitative real-time PCR

Pooled and frozen tissues were first homogenized ($n = 3$ per time point) and total RNA was then extracted using TRIzol (Gibco) according to the manufacturer's instructions. The cDNA samples were then obtained by RT-PCR and stored at −20 °C until use. Control reactions were performed in parallel to exclude contamination from genomic DNA including exclusion of reverse transcriptase or primers from reverse transcriptase reaction. The samples were run at least in triplicate for target gene and endogenous β_2-M controls.

TaqMan quantitative real-time PCR experiments were performed on R-G 3000 (Rotor-Gene) apparatus using following cycling parameters: 3 min hold at 94 °C, then 40 × (20 s at 94 °C and 1 min at 60 °C) cycles. Each reaction (20 μl) contained 1 × real-time PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 μM each of the respective forward and reverse primers, 0.1 μM of labeled TaqMan probes, 1 U of Taq polymerase and 2 μl of plasmids or cDNA samples.

After computing the relative amounts of target gene and endogenous control for one sample, the final amount of target gene in that sample was presented as a ratio between the amount of target gene and amount of endogenous β_2-M control, which are available directly after PCR with the help of the Rotor-Gene software version 6.0. Relative mRNA of the control was normalized as 1.

2.4. Double staining: In situ hybridization combined with immunofluorescence

In situ hybridization (ISH) was performed on cryostat-cut tissue sections (14 μm) as described previously with some modifications (Shen et al., 2002, 2003). Briefly, slide-mounted tissue frozen sections were prehybridized in 50% formamide in 4 × SSC at 42 °C for 2 h and hybridized at 42 °C for 16 h by adding DIG-labeled sense probe (used as negative control) and antisense probes (0.5 μg/ml) in a humidified chamber, then were treated with fluorescein-conjugated or rhodamine-conjugated anti-digoxigenin (1:12, Roche) in 1% BSA overnight at 4 °C. To facilitate the identification of the hybridized cell types, sections were co stained for CAPON or Dexras1 (ISH signals) and specific marker proteins including mouse monoclonal anti-S-100 (1:50, Santa Cruz), mouse monoclonal anti-glial fibrillary acidic protein (GFAP) (1:200, Sigma) or mouse monoclonal anti-neuronal Nuclei (NeuN) (1:600, Chemicon) primary antibodies and FITC- or TRITC-conjugated secondary antibodies (1:200, Jackson immunolab). The stained sections were examined with a fluorescence microscope (Leica), and images were captured with a camera.

For negative controls, apart from the use of the sense probes in place of the antisense probes in hybridization described above, sections which had been pretreated with ribonuclease A (Boehringer Mannheim) (100 μg/ml in 2 × SSC) for 1 h at 37 °C were also used for hybridization with both sense and antisense probes. Moreover, sections lack of primary antibodies for specific marker proteins were done in parallel as negative controls. These controls all produced nonspective background and were devoid of signal.

2.5. Data analysis

Data values were expressed as means ± S.E.W. Analysis of variance (ANOVA) for random measures was preformed followed by Turkey post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Gene expression for CAPON and Dexras1 in normal adult rat

In normal adult rat, either CAPON mRNA or Dexras1 mRNA was initially expressed in sciatic nerve, DRG and lumbar spinal cord (SC) using TaqMan quantitative real-time PCR. More abundant expression for these two molecules was detected in the SC than in the DRG or sciatic nerve (Fig. 1). Potential changes in mRNA for CAPON and/or Dexras1 might occur since alterations of nNOS have been reported in neural tissues after peripheral axotomy.

3.2. Changes in mRNA for CAPON and Dexras1 in the injured sciatic nerve

Transcript expressions for both CAPON and Dexras1 were significantly increased in either proximal or distal stumps of the transected sciatic nerves 1 w after injury (Fig. 2A, B, D, E). At other time points, their mRNA levels also varied, such as a trend to increase for Dexras1 in both proximal and distal stumps at earlier stages. However, there was no statistical significance. Using the double staining method described above, it was shown that increased mRNA for CAPON or Dexras1 were mainly present in the proliferated Schwann cells marked by anti-S-100 staining in the proximal or the distal stumps (Fig. 2G, H). Simultaneously, remarkable upregulation of nNOS mRNA was observed in both proximal and distal stumps 1 w after injury (Fig. 2C, F). These data suggest a potential role for CAPON and Dexras1 in nerve regeneration at

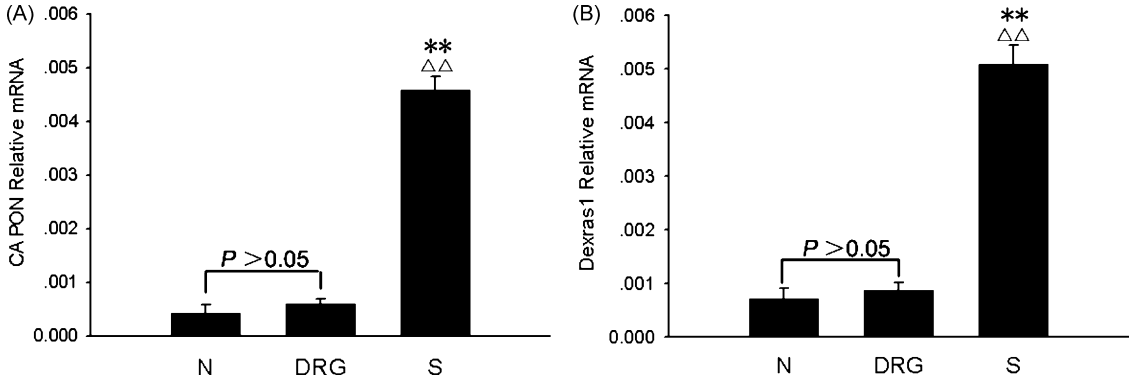

Fig. 1. Analysis on mRNA expression for CAPON and Dexras1 in normal adult rat by TaqMan quantitative real-time PCR. (A) CAPON mRNA expression in rat sciatic nerves (N), lumbar dorsal root ganglia (DRG) and lumbar spinal cord (SC). (B) Dexras1 mRNA expression in rat N, DRG and lumbar SC. Relative mRNA represents a ratio between the amount of target gene and amount of endogenous β_2-M control. $^{**}P < 0.01$, compared with the nerves (N); $^{\triangle\triangle}P < 0.01$ compared with the DRG.

certain time periods, possibly via regulating the enzyme nNOS activity.

3.3. mRNA expressions for CAPON and Dexras1 in DRG following sciatic axotomy

Changes in mRNA for CAPON and Dexras1 were found in the ipsilateral DRG (L4-L6) after sciatic nerve transection. As it was shown, CAPON mRNA trended to increase since 6 h though without significance. However, it decreased on the 1st day and significantly upregulated on the 2nd day of nerve injury, reaching its peak, and recovered to the basal level thereafter (Fig. 3A). In contrast, Dexras1 mRNA in ipsilateral DRG had a significant increase at 6 h, 2 days and 2 weeks after nerve transection (Fig. 3B). Similarities could be found that 6 h or 2 weeks was the time point when both molecules were upregulated. To explore the cellular sources of the increased mRNA for CAPON or Dexras1, in situ hybridization combined with immunofluorescence was used. It revealed that occasionally some GFAP staining satellite glia cells showed CAPON mRNA signals (Fig. 3D, arrowheads); however, a good colocalization between CAPON and NeuN, a marker for neuron, was observed (Fig. 3E, arrows). Similarly, most of Dexras1 positive cells were overlapped with the NeuN staining

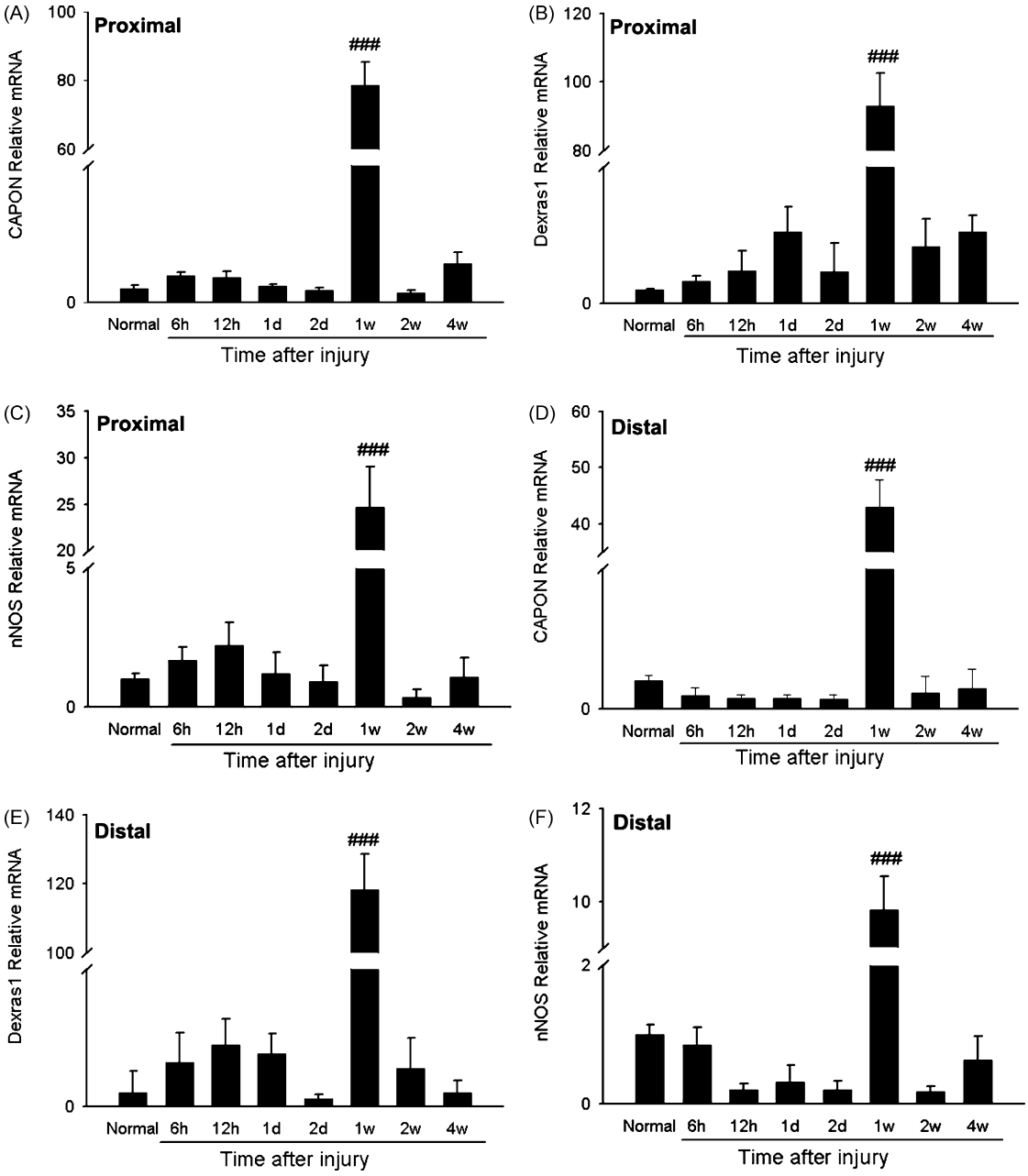

Fig. 2. Gene expression patterns for CAPON, Dexras1 and nNOS in rat injured sciatic nerves. Changes in mRNA were shown in the proximal (A–C) and the distal stumps (D–F) of transected sciatic nerves by real-time PCR. $^{###}P < 0.001$; compared with the normal control. Representative photomicrographs by in situ hybridization combined with immunofluorescence showing colocalization between Dexras1 and S-100, a marker for Schwann cells, in the distal stumps (G-H, longitudinal sections) 1 week after injury. H, the magnification of the framed areas in G. Scale bars: G, 20 μm; H, 100 μm.

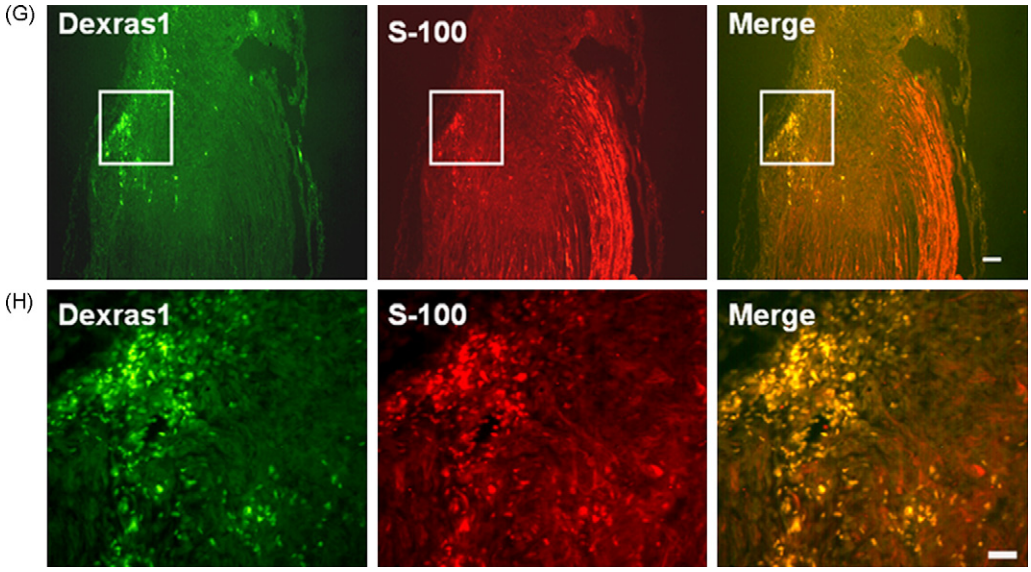

Fig. 2. (*Continued*).

(data not shown) while only a few of the GFAP positive satellite glia cells showed Dexras1 mRNA signals (Fig. 3F, arrowheads). Meanwhile, nNOS was increased markedly 1 d after axotomy in the ipsilateral DRG (Fig. 3C), mainly present in the neurons identified by the marker NeuN (data not shown). While in the contralateral DRG, we did not observe significant differences in mRNA for CAPON or Dexras1 compared with the normal control.

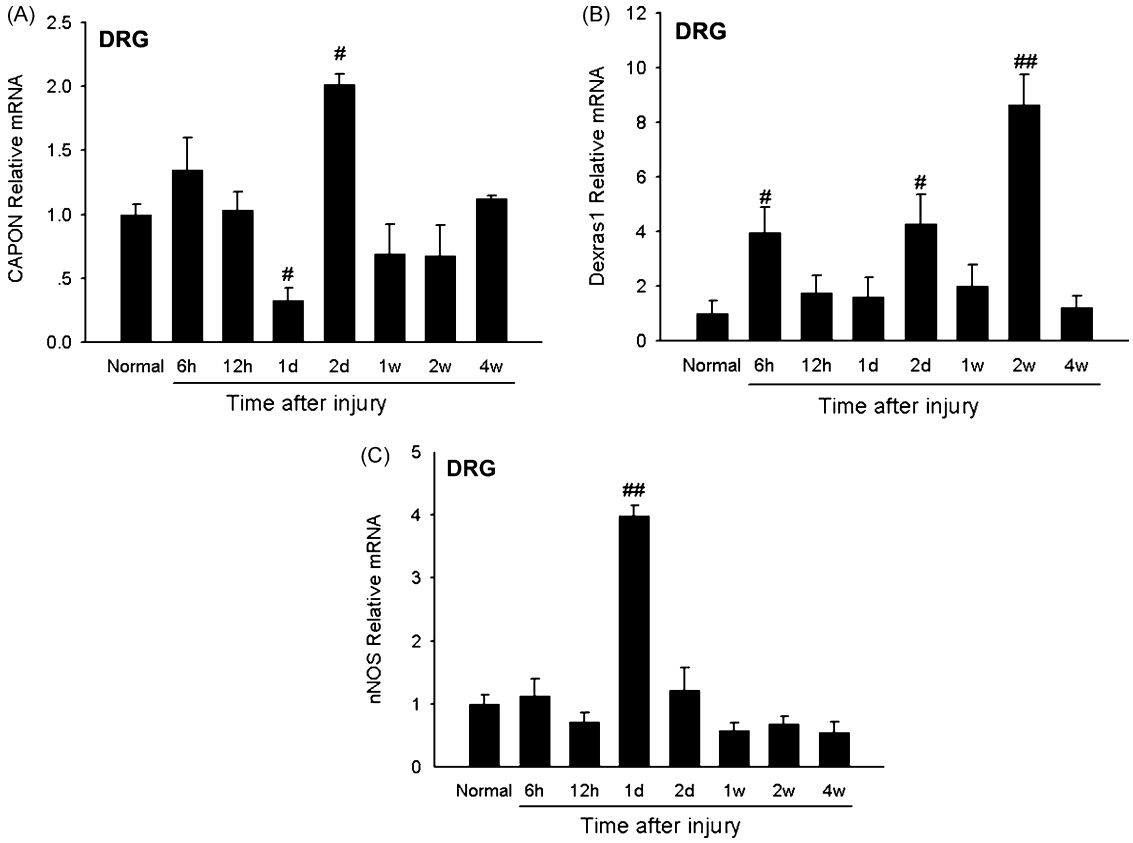

Fig. 3. Gene expression patterns for CAPON, Dexras1 and nNOS in the dorsal root ganglia (DRG, L4-L6) after unilateral sciatic nerve transection. Changes in mRNA in the ipsilateral DRG post axotomy were shown by real-time PCR (A–C). Representative photomicrographs showing the colocalization between CAPON and GFAP (D) or NeuN (E), and the colocalization between Dexras1 and GFAP (F), in the ipsilateral DRG 2 d after nerve injury. Arrows showing the mRNA expression in neurons while arrowheads referring to the gene expression in glia satellite cells. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; compared with the normal control. Scale bars: D and E, 10 μm; F, 20 μm.

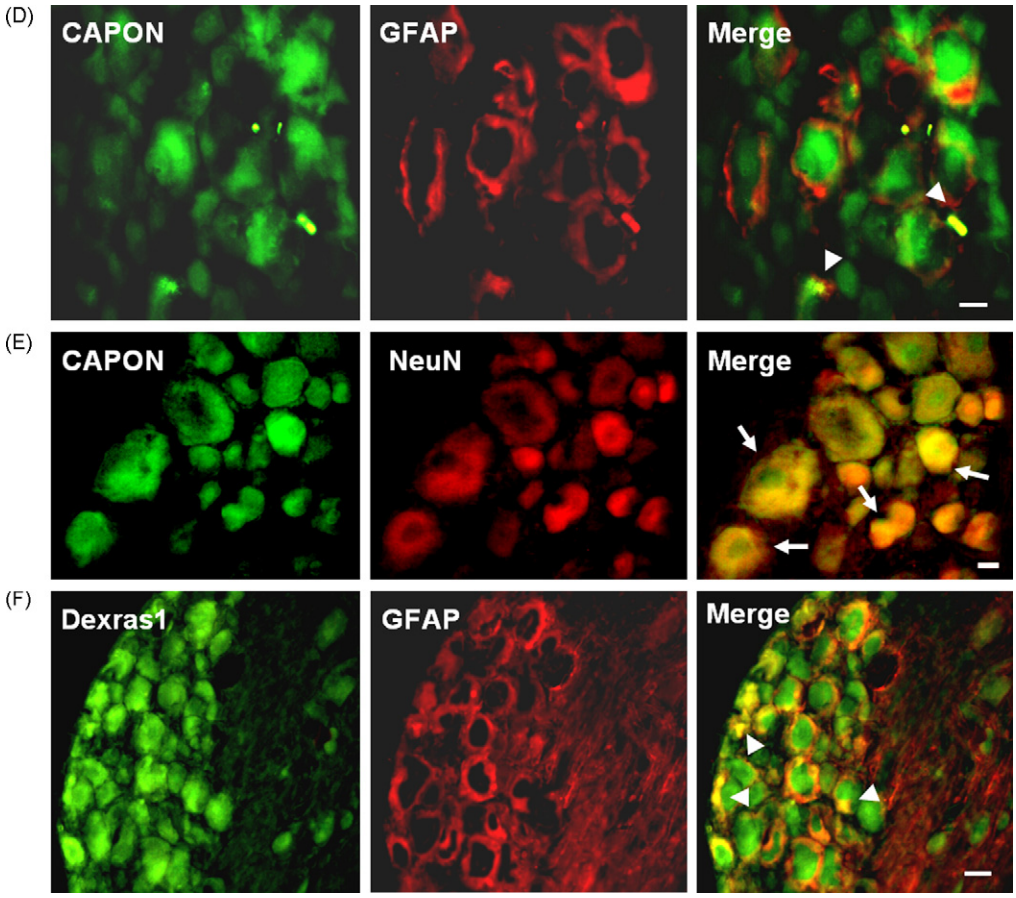

Fig. 3. (*Continued*).

3.4. mRNA expressions for CAPON and Dexras1 in spinal cord following sciatic axotomy

We also examined the mRNA expression patterns for CAPON and Dexras1 in the lumbar spinal cord (SC) following sciatic axotomy. It was found that both CAPON mRNA and Dexras1 mRNA were significantly increased 1 week after nerve injury in the whole segments of lumbar SC (Fig. 4A, B). Histological studies revealed that increased CAPON mRNA were strongly expressed in the superficial laminae of dorsal horns, the central canal and the ventral horns 1 week post surgery; and enhanced Dexras1 mRNA showed a similar distribution pattern except for some faint signals in the central canal. However, weak staining for both molecules was observed in the SC of intact rats (data not shown). In addition, we found that significant spinal upregulation of nNOS mRNA occurred 1 week after axotomy (Fig. 4C).

4. Discussion

Previous studies suggest that injured neurons express a program, which controls axonal regeneration, or induces cell death, presumably involving the sequential expression of genes (Fawcett, 1992). In the present study, we characterized alterations in CAPON and Dexas1 mRNA expression in a rat model with peripheral axotomy, mainly to explore their potential roles in the physiological or pathological conditions in which NO activity was involved. It was found that CAPON mRNA was constitutively expressed in adult rat neural tissues including sciatic nerve, DRG and lumbar spinal cord. Moreover, Dexras1 was constitutively present either. Transcript expression for CAPON and Dexras1 changed similarly, which underwent an upregulation following sciatic axotomy, except for some tiny differences including the time course and distributions described in the results. Thus the involvement of CAPON as well as Dexras1 in post-axotomy processes was under consideration.

Schwann cells are thought to be a crucial factor in peripheral nerve regeneration. The reason that sciatic nerve has a far greater capacity for regeneration than those of the central nervous system is that axons are wrapped by Schwann cells. After axotomy of the peripheral nerve, axons of the injury site undergo Wallerian degeneration, and then Schwann cells proliferate and form columns within basal lamina tubes (bands of Büngner), that act as conduits for regenerating axons, whilst the Schwann cells secrete diffusible neurotrophic factors (Symons et al., 2001). Expressions for CAPON, Dexras1 and nNOS mRNA all significantly increased mainly in proliferated Schwann cells at 1 week after transection, suggests that it might be the vital period for nerve regeneration when Schwann cells are proliferating actively and many regenerating events are undergoing. As the Schwann-cell proliferation or nerve

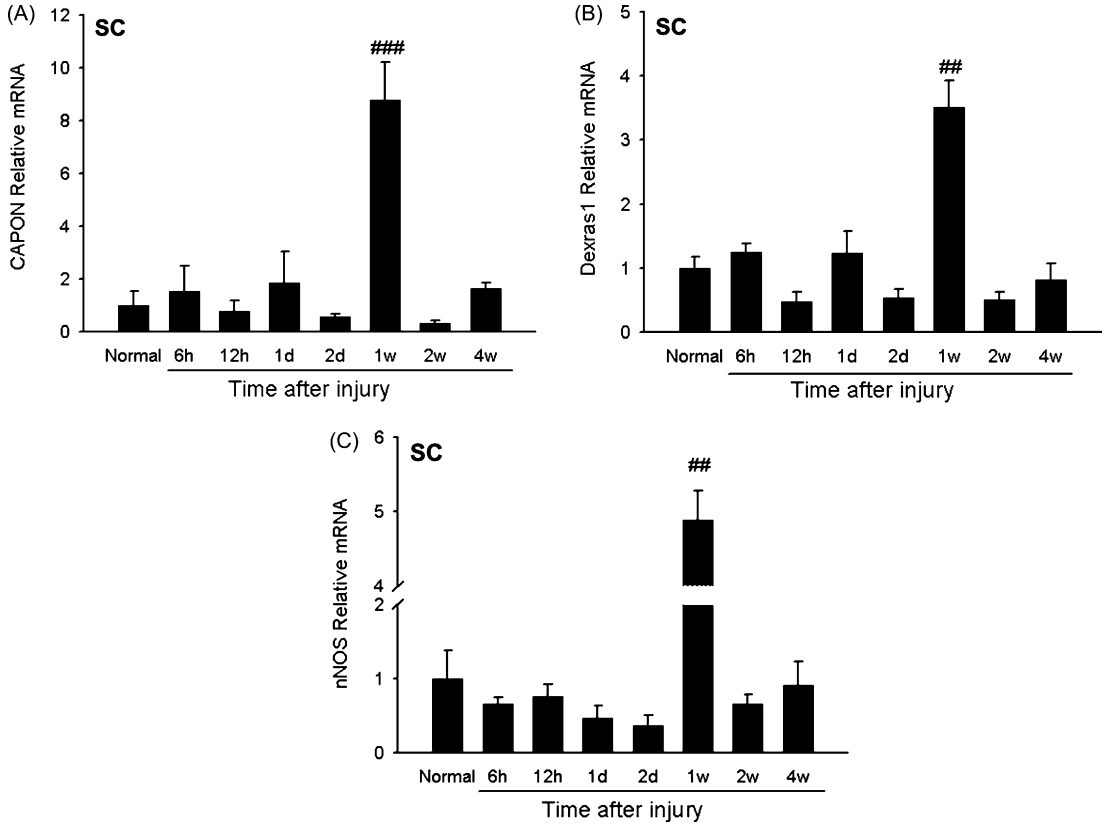

Fig. 4. Gene expression patterns for CAPON, Dexras1 and nNOS in the lumbar spinal cord (SC) after sciatic axotomy. Changes in mRNA in the lumbar SC post axotomy were shown by real-time PCR (A–C). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; compared with the normal control.

regeneration worked not merely at 1 week after transection, it seems to be difficult to interpret why at other time points the transcript expressions did not significantly change, an alternative explanation might be that there were other agents rather than CAPON, Dexras1 or nNOS playing a dominant role.

Lesion-induced NO production has been attributed to have a protect effect on damaged neurons (Thippeswamy et al., 2001) and might be associated with neuronal regeneration (Cristino et al., 2000). NO has been found to influence the elimination of misdirected axons, the so-called pruning (Ernst et al., 2000). Furthermore, NO is involved in path-finding and establishment of appropriate synaptic connection supporting cell survival in this way (Van Wangenen and Rehder, 2001). NO signals within or between cells through posttranslational covalent modification of proteins. The most studied example is the binding of NO to heme in soluble guanylyl cyclase, leading to activation of the enzyme and increased formation of cGMP (Denninger and Marletta, 1999). NO can also directly modify cysteine residues in proteins, leading to the formation of a nitrosothiol adduct (Stamler et al., 1997). One might anticipate that physiologic targets of NO should be closely juxtaposed to NOS, with the juxtaposition established by appropriate targeting proteins. One of such targeting proteins, CAPON, contains a C-terminal domain that binds to the PDZ domain of nNOS, as well as an N-terminal phosphotyrosine-binding (PTB) domain that can bind Dexras1 (Jaffrey et al., 1998; Fang et al., 2000). Thus, the axtomy-induced gene upregulation of CAPON as well as Dexras1 in proliferated Schwann cells may play an important role in nerve regeneration since nNOS changed with the similar spatio-temporal pattern simultaneously. However, the exact functions for CAPON or Dexras1 in nerve regeneration need further investigations.

Damage to sciatic nerve may cause the loss of the neurons in the corresponding DRG or the spinal cord. Previous studies have demonstrated that deficiency of nNOS leads to an enhanced cell loss in the lumbar DRG or promotes apoptotic cell death of spinal cord neurons after peripheral nerve axotomy (Keilhoff et al., 2002, 2004); moreover, knockdown of de novo nNOS gene expression by nNOS antisense oligos (AS-ODN) aggravates the motor neuron loss induced by spinal root avulsion (Zhou and Wu, 2006). Thus expression of lesion-induced nNOS in lumbar DRG or spinal neurons may play a favorable role for neuronal survival after peripheral nerve injury. However, it cannot rule out that crosstalk might occur between nNOS and key molecules that are responsible for the death of injured neurons. Our data showed both CAPON and Dexras1 mRNA increased in the neurons of lumbar DRG or the spinal cord, suggesting that they may take part in the processes in which nNOS regulates the death or survival of the damaged neurons since nNOS mRNA was altered meanwhile and interactions among them have already been identified (Fang et al., 2000).

Peripheral nerve lesion varying etiologies may cause chronic pain and many studies have demonstrated an important role of

NO in the development and maintenance of pathological pain (Guhring et al., 2000; Levy et al., 2000; MaihÖfner et al., 2000). Here, peripheral axotomy induced a significant increase in the number of CAPON- or Dexras1-expressing cells in lumbar DRG and the spinal cord dorsal horn, specifically in superficial laminae, which is one of the regions involved in the processing of nociception and thermoreception (Hunt et al., 1992). The importance of Dexras1 as a physiologic target of nNOS is established by its activation by NMDA receptor-stimulated nNOS activity and by the finding that Dexras1 activation is diminished in the brains of mice harboring a targeted genomic deletion of nNOS (nNOS−/−) (Fang et al., 2000). Thus, present data imply that CAPON and/or Dexras1 might be involved in the pain process.

Because NO reacts relatively nonspecifically with cysteines to form nitrosothiol adducts, which are capable of modulating protein activity (Jaffrey et al., 2001), anchoring nNOS to specific proteins may deliver neurally generated NO selectively to its physiologic targets. Indeed, the linkage of nNOS to Dexras1 by CAPON mediates NO-dependent activation of Dexras1 (Fang et al., 2000). There has been little characterization of Dexras1, and its downstream targets are not definitive. However, it has been reported that Desras1, as a GTP-binding protein, is capable of exerting important regulatory effects on G_i-mediated signal transduction to the Erk-1/2 MAP kinase cascade (Graham et al., 2002). Previous studies have shown that the ERK pathway is essential for nerve regeneration (Ogata et al., 2004; Wiklund et al., 2002), neuronal survival or death (Almeida et al., 2005; Volosin et al., 2006), and persistent hyperalgesia (Sammons et al., 2000; Ji et al., 2002; Galan et al., 2003). Since the ternary complex of nNOS, CAPON, and Dexras1 provides a system that is uniquely suited for targeting NO to Dexras1 for S-nitrosylation, leading to Dexras1 activation; Dexras1-specific downstream effectors may exist, possibly via the ERK pathway, mediating the effects of NO in the nervous system.

In summary, we studied the gene expression patterns of CAPON and Dexras1 in adult rat following sciatic axotomy. It revealed that CAPON, as one of the nNOS-interacting proteins, upregulated at the transcript level in the injured nerves, corresponding DRG or lumbar spinal cord at certain time points after sciatic nerve transection. Moreover, Dexras1, a physiologic target of nNOS, altered in mRNA as well with a similar pattern as that of CAPON or nNOS. It has been reported that CAPON forms a complex with Dexras1 and nNOS, which directs and enhances the delivery and specificity of the nitric oxide (NO) signal (Fang et al., 2000). And NO-mediated Dexras1 activation is enhanced by CAPON, which may regulate the Erk-1/2 MAP kinase cascade. Our findings thus suggest that CAPON and Dexras1 may play an important role in different pathological conditions including nerve regeneration, neuron loss or survival and possibly pain process, in which NO or nNOS is involved, probably via regulating the nNOS activity or through the ERK pathway. However, further studies are needed to elucidate the functional link between NO, CAPON, Dexras1 and these post-axotomy processes mentioned above.

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