CHAPTER 60

Central consequences of peripheral nerve damage

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Summary

Peripheral nerve injury of various types, for example complete nerve transection or loose nerve constrictions (Bennett model), results in changes in expression of a large number of molecules in the parent neuronal cell bodies in the sensory dorsal root ganglia (DRGs), as elegantly amplified in recent global expression analyses. These molecules include neuropeptides, seven-transmembrane G-protein-coupled receptors, ion channels, enzymes, and other types of molecules. Peripheral nerve injuries are often associated with neuropathic pain, and it is an important task to find out to what extent these changes are pro- or anti-nociceptive. In general terms, it is thought that down-regulation of certain molecules aims to attenuate excitatory (e.g. pain) transmission in the dorsal horn, whereas up-regulated molecules may have trophic effects and promote survival and regeneration, and perhaps also modulate pain transmission. It has been postulated that nerve injury causes sprouting of large-diameter primary afferents from the deep dorsal horn into laminae I and II, and that this may underlie neuropathic pain. There is now evidence that this phenomenon can also be explained, not by sprouting, but by a nerve injury-induced phenotypic change. Thus the marker used for sprouting, the cholera toxin B (CTB) subunit, is normally taken up only by large-diameter axons. However, after nerve injury, C and Aδ fibres also acquire the ability to bind and transport this molecule, explaining the dense CTB labelling in laminae I and II after nerve injury.

In contrast to the situation after neuropathic pain, virtually none of the changes observed in DRGs can be seen after inflammatory pain, which instead causes up-regulation of, for example, opioid peptides in local dorsal horn neurons in the spinal cord. This suggests the existence of two separate defence systems for inflammatory and neuropathic pain. There are also plastic changes in the dorsal horn neurons, such as expansion and/or creation of novel receptive fields. Peripheral nerve injury may cause ongoing activity, after-discharges, and in some cases hypersensitivity. This overall increased excitatory drive may be combined with a compromised inhibitory control in the dorsal horn. It is hoped that a better understanding of the significance of these changes will lead to novel strategies for the treatment of neuropathic pain.

INTRODUCTION

In the first *Textbook of Pain*, edited by Patrick Wall and Ronald Melzack, and published in 1984, basic aspects of the relation between damage of a peripheral nerve and pain were discussed by Marshall Devor (Devor 1984) as well as in several clinically oriented chapters, including one by John Scadding on peripheral neuropathies (Scadding 1984). Devor's chapter dealt with nerve injury and focused on the pathophysiology and anatomy of the damaged nerve, in particular on processes leading to production of abnormal impulse discharges and pain. In the mid 1970s, Wall & Gutnick (1974) reported massive spontaneous discharges in L4 and 5 dorsal rootlets after producing an experimental neuroma by peripheral lesion of the sciatic nerve. Govrin-Lippmann & Devor (1978) and Scadding (1981) described the time course, showing a lack of discharges during the first few days, followed by spontaneous, high activity for several weeks. It was proposed that the nerve damage or neuroma leads to establishment of ectopically generated, abnormal impulses and amplification of impulse discharges, and that this contributes to chronic neuropathic pain.

At that time, little was known about the dramatic and robust *chemical* changes occurring in DRGs after peripheral nerve damage, presumably contributing to the changes in electrical phenomena monitored by Wall, Devor, and their collaborators, as well as others. In this chapter, we will focus on such chemical changes induced in DRG neurons by nerve injury, including changes in intracellular messengers and their receptors, enzymes, ion channels, and other molecules. We will also discuss the issue of nerve injury-induced sprouting of primary afferents in the dorsal horn.

Much of the early work was carried out on rat after a complete transection of the sciatic nerve, the model introduced by Wall and collaborators, and resulting in robust and reproducible effects. Subsequently, several other 'functional' pain models were introduced (see Chs 10 and 59). Nahin and colleagues were the first to show changes in peptide expression in DRGs in the chronic nerve constriction model (four loose ligatures) developed by Bennett and Xie; these changes are similar to those seen after complete nerve transection (Nahin et al 1994). The purpose and consequences of these changes are still not well understood, but they may contribute to survival and regeneration of the damaged neuron, and also to generation and/or attenuation of pain. It is, however, clear that these changes are not confined to the neurons but also affect satellite and other non-neuronal cells in the DRG. They extend to the spinal cord as transganglionic effects, which we will discuss with regard to both functional and anatomical or chemical consequences. However, we will not deal with events occurring at higher centres. It may be anticipated that a fuller comprehension of these processes may lead to improved understanding of, and ultimately novel treatment strategies for, chronic pain.

GENE EXPRESSION IN DORSAL ROOT GANGLIA AFTER NERVE INJURY

Fifty years ago, Fred Lembeck in Graz suggested that substance P, an 11-amino acid peptide, could be a transmitter in sensory neurons. The presence of this and several other peptides—for example calcitonin gene-related peptide (CGRP), somatostatin, and pituitary adenylate cyclase-activating peptide—in subpopulations of normal DRGs of rats and other species could subsequently be demonstrated.

Early evidence that nerve injury could influence the expression of messenger molecules in DRGs was presented by Jessell, Otsuka and associates, who showed that peripheral transection of the sciatic nerve causes a decrease in substance P levels in the dorsal horn. It

could later be demonstrated that this decrease in fact represents a down-regulation of substance P synthesis in DRG neurons (Nielsch et al 1987). Evidence of nerve injury-induced messenger *up*-regulation in DRG neurons was first obtained for vasoactive intestinal polypeptide (VIP) by Shehab & Atkinson (1986). Subsequently, it has turned out that a very large number of molecules—including several neuropeptides such as substance P, CGRP, neuropeptide Y (NPY), galanin, and pituitary adenylate cyclase-activating peptide (Fig. 60.1A,B); enzymes such as nitric oxide synthase; and receptors for peptide and classical transmitters synthesized in DRG neurons—are regulated by axotomy (see Hökfelt et al 1994, Zigmond et al 1996). This is also seen in several neuropathic pain models; that is, without a true transection.

Importantly, in inflammatory models substance P and CGRP in DRG are up-regulated but no effects on galanin, VIP or NPY have been recorded. Instead, inflammation activates opioid and other peptides in the dorsal horn (see Dubner & Ruda 1992).

The DRG neurons discussed so far are often termed the *peptide-positive* population because of their expression of several peptides. They represent 35–40% of all DRG neurons, have thinly myelinated axons, and are assumed to be involved in nociception.

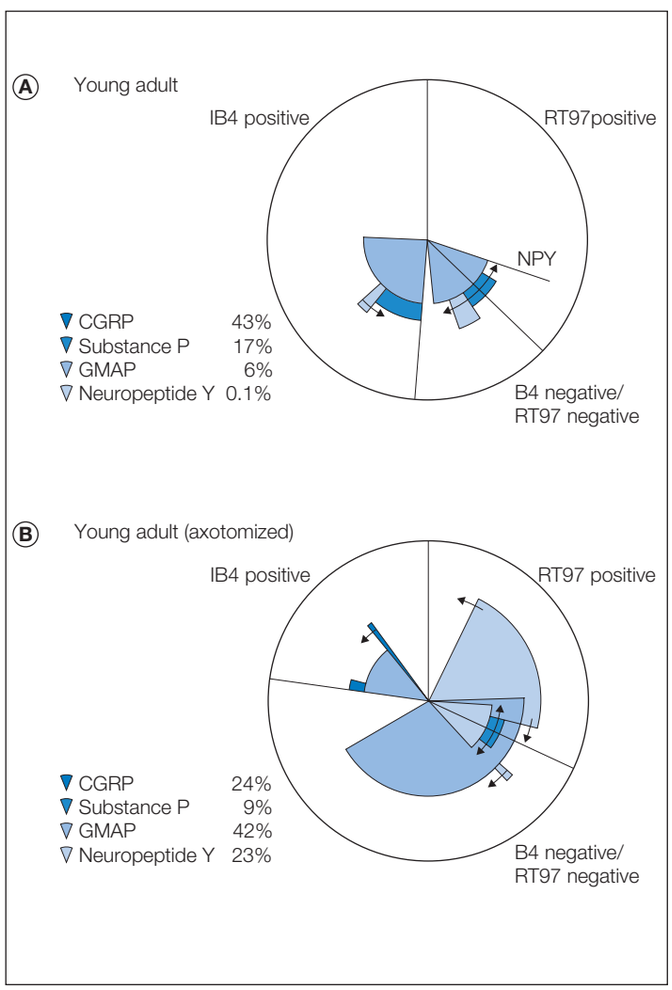
The non-peptide DRG neurons represent a second class. They comprise 20–30% of the DRG neurons and are characterized by several markers, such as the non-lysosomal fluoride-resistant acid phosphatase; the $P2X_3$ purinoreceptor; vanilloid receptor 1 (VR1, now called TRPV1); and receptor components of the glial cell line-derived neurotrophic factor (GDNF), especially RET mRNA. They bind the lectin *Griffonia simplicifolia* isolectin B4 (IB4). Some of these markers, such as TRPV1 and $P2X_3$, are up-regulated after peripheral nerve injury; others are down-regulated. Also, these neurons are small with non-myelinated axons, and they are assumed to represent nociceptors.

The remaining 30–40% of the DRG neurons are large- and medium-sized. These neurons have fast-conducting, myelinated Aα/β-range axons and receive input from peripheral mechanoreceptors.

For a comprehensive account of neuron types, their receptors, and ion channels expressed in DRG (and examples of their regulation), we refer readers to a review by McMahon & Priestley (2004).

SOME METHODOLOGICAL ASPECTS

Most nerve injury studies are based on complete transection of the sciatic nerve of rat at midthigh level, and, in general, percentages of DRG neuron profiles expressing a particular molecule (immunohistochemistry) or a transcript (in situ hybridization) are calculated. It is important to note that this lesion will affect only approximately 70–80% of the L5 DRG neurons projecting into the sciatic nerve,



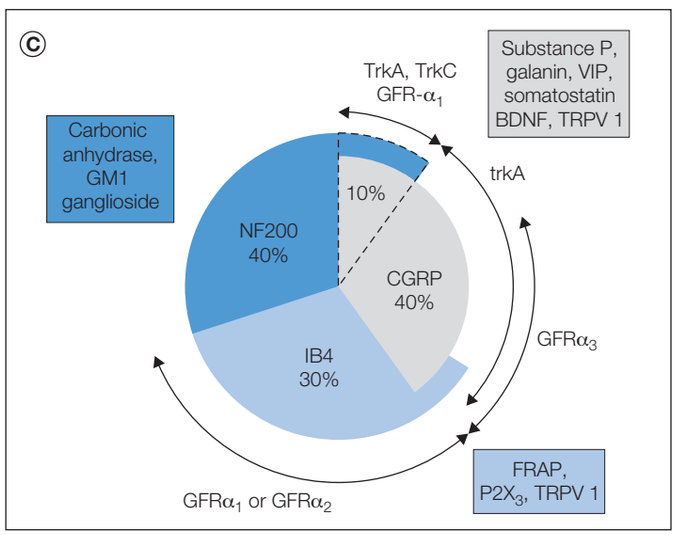


Fig. 60.1 Pie charts summarizing the expression pattern of several neuropeptides, nitric oxide synthase, and Growth-associated protein 43 (GAP-43) in subpopulations of dorsal root ganglion neurons in non-lesioned (**A**) and axotomized (**B**) young adult rats. Arrows in A and B indicate the extent of variability. The main neurochemical populations of dorsal root ganglion neurons, (**C**) as defined by markers for large myelinated neurons (RT97), and small unmyelinated or thinly myelinated axons, predominantly nociceptors, comprising two populations—isolectin B4 (IB4)-positive and calcitonin gene-related peptide (CGRP)-positive—and their relation to several growth factor receptors. BDNF, brain-derived neurotrophic factor; FRAP, fluoride-resistant acid phosphatase; GMAP, galanin message-associated peptide; GFRα, Glial cell line-derived neurotrophic factor receptor α; NF200, neurofilament 200; $P2X_3$, Purinoceptor $2X_3$; TrK, tyrosine kinase receptor; TRPV1, transient receptor potential vanilloid receptor 1; VIP, vasoactive intestinal polypeptide. (A and B from Bergman et al 1999 and C from Priestley et al 2002, with permission of Elsevier Science B.V. (A, B) and National Research Council Canada, Research Press (C).)

because the remaining ones branch off central to the transection (Devor et al 1985). Moreover, an important issue is to what extent the nerve injury causes cell loss, which has not been monitored in most studies. More recently, stereological techniques have been applied, showing that in the rat no significant loss of neurons occurs up to 4 weeks after a lesion at midthigh level (Tandrup et al 2000, but see McKay et al 2002), whereas a lesion of spinal nerves (close to cell bodies) causes a progressive neuronal cell loss (Vestergaard et al 1997). In the mouse, axotomy at midthigh level causes a 24% loss after 7 days and a 50% loss after 28 days ((Shi et al 2001), possibly due to the short distance between lesion and cell bodies in this small animal.

GLOBAL GENE EXPRESSION STUDIES

Global gene expression has been analysed with array techniques monitoring genes expressed in DRGs after peripheral nerve injury as compared with those expressed in non-lesioned ganglia (Costigan et al 2002, Wang et al 2002, Xiao et al 2002). In these analyses many different types of genes showed marked changes (Table 60.1).

Costigan and coworkers found changes in 178 or 240 genes (depending on cut-off) after axotomy (Costigan et al 2002). Wang and associates showed a greater than twofold up-regulation for 102 genes, and for 46 a greater than twofold down-regulation, in a spinal nerve ligation neuropathic pain model (Wang et al 2002). As shown in Fig. 60.2, around 2.3 and 2.8% of the genes have been down- and up-regulated, respectively, if genes with a more than 1.5-fold regulation and $P < 0.05$ are considered. The genes have been categorized as indicated in Fig. 60.2, and bidirectional changes are found in all classes except for translational regulation genes, where no down-regulation was observed. In many cases, approximately the same number of genes go in either direction, although up-regulation dominates in the categories apoptosis, cytoskeleton, and immunologically related genes. Down-regulation prevails in the groups ion channels, neurotransmission, vesicle trafficking, and unknown genes.

Xiao's group examined 7523 genes and expressed sequence tags after axotomy, of which 122 and 51, respectively, were strongly

Table 60.1 Changes in cDNA array ratio for strongly regulated genes in DRGs after peripheral axotomy

	No. of days after peripheral axotomy			
	2	7	14	28
Neuropeptides				
Calcitonin gene-related peptide	↓	↓	↓	–
Substance P	–	↓	↓	–
Cholecystokinin	↑↑	↑↑↑	↑↑↑	↑↑↑
Galanin	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Neuropeptide Y	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Vasoactive intestinal polypeptide	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Receptors				
Adrenoceptor α_{2B}	↓	↓	↓	↓
Metabotropic glutamate receptor 4	↓	↓	↓	↓
Opioid μ receptor	↓	↓	↓	↓
Neuropeptide Y Y_1	↓	↓	–	–
$GABA_A$ α_5	–	–	↑	↑
Nicotinic acetylcholine receptor subtype α_7	–	↑	↑	↑
Adrenoceptor α_{2A}	↑	↑	↑	↑
Purinoceptor $P2Y_1$	↑	↑	↑	↑
Benzodiazepine (peripheral)	↑	↑	↑	↑
Neuropeptide Y Y_2	↑	↑	↑	↑
Neuropeptide Y Y_5	↑	↑	↑	↑
CCK_B	↑	↑	↑	↑
Channels				
Na^+ channel (sensory neuron-specific)	↓	↓	↓	↓
K^+ channel 11 (inward rectifying)	–	↓	↓	↓
K^+ channel RCK4 subunit	↓	↓	↓	↓
Na^+ channel β_2 subunit	–	↑	↑	↑
Na^+ channel III	↑	↑	↑	↑
Ca^{2+} L-type/α_2/δ_1 subunit	↑	↑↑↑	↑↑	–
Enzymes				
Nitric oxide synthase	↑	↑	↑↑	↑↑
Tyrosine phosphatase	↑↑	↑↑	↑↑	↑↑
Tyrosine kinase	–	↑	↑	↑
Synaptic transmission				
Vesicle-associated membrane protein-1	↓	↓	↓	↓
Synaptotagmin IV	↑	↑	↑	↑
Growth-associated proteins				
Basic fibroblast growth factor	↑	↑	↑	↑↑
GAP-43	↑	↑	↑↑	↑
VGF (nerve growth factor-inducible protein)	↑↑	↑↑	↑↑	↑↑
Cytoskeleton or cell mobility				
Neurofilament (high or low molecular weight)	↓	↓	↓	↓
β-Actin (cytoplasmic)	–	–	↓	↓
LIM domain CLP36	↑↑↑	↑↑	↑	–
Tubulin β	↑	↑	↑	↑
Metabolism				
ATPase	↓	↓	↓	↓
Serine protease	↓	↓	↓	↓
Apolipoprotein D	↑	↑	↑	↑
Acyl-CoA oxidase	–	↑	↑	↑
Others				
CDK 109	↓↓	↓	↓	↓
Mast cell protease	↓	↓	↓	↓
Lysozyme	↑	↑	↑	↑
Heat shock 27-kDa	↑↑	↑	–	–
Class II major histocompatibility complex α chain RTLD	–	↑	↑	↑
Glycipan	↑	↑	↑↑	↑
Lipocortin I	↑	↑	↑	–
Lipocortin II	↑	↑	↑↑	↑
Telomerase comp 1	↑	↑	↑	↑

↑↑↑, > 10-fold; ↑↑, 5–10-fold; ↑, 2–5-fold; –, 0.5–2-fold; ↓, 0.2–0.5-fold;↓↓, < 0.2-fold.

(From Xiao et al 2002, with permission of Proceedings of the National Academy of Sciences of the United State of America.)

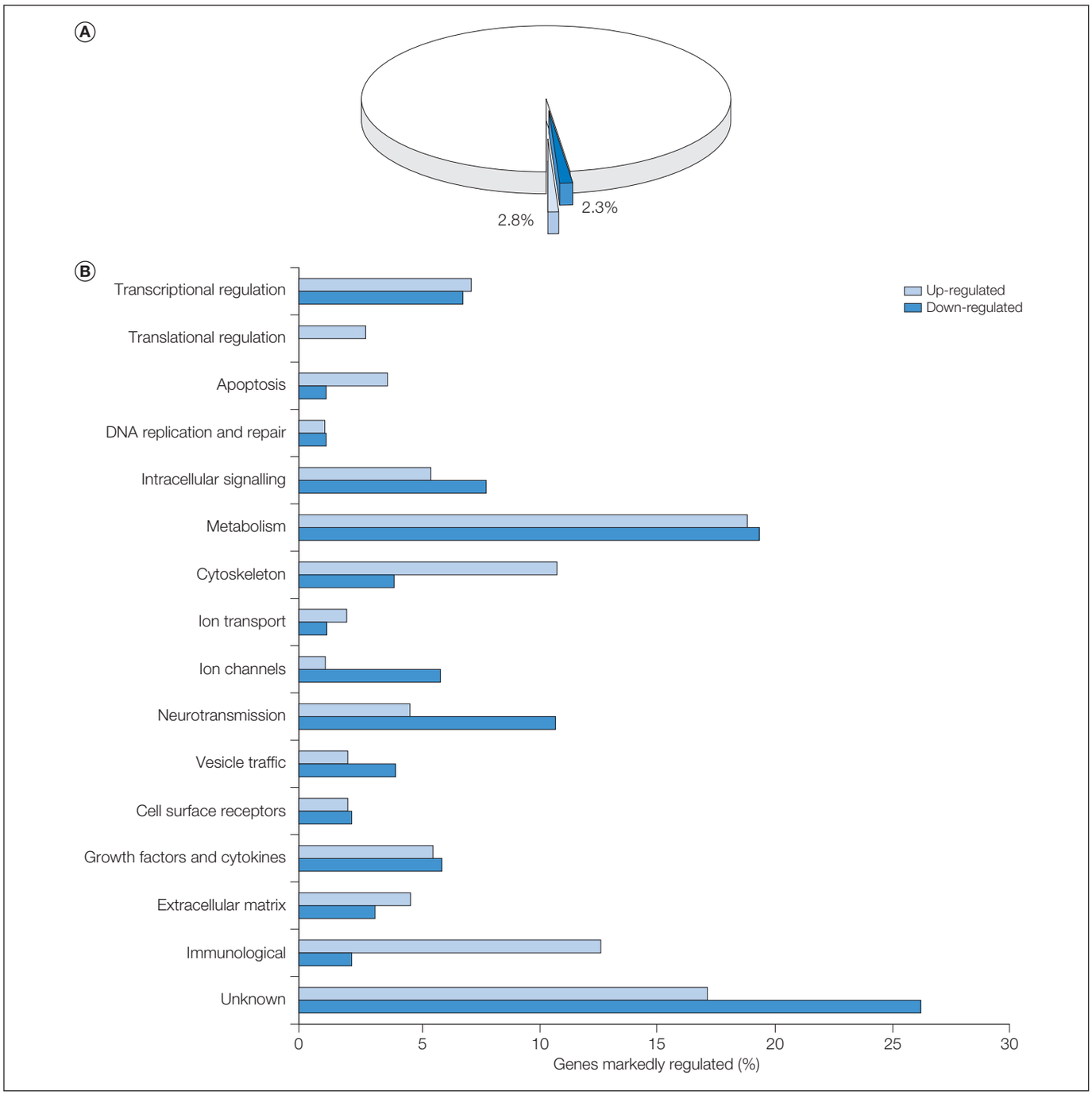


Fig. 60.2 Genes regulated above 1.5-fold (P >0.05) have been classified into functional classes, and the percentage of up- and down-regulated genes are shown for each functional class. (From Costigan et al 2002, with permission of BioMed Central.)

changed (Xiao et al 2002; Table 60.1). In a similar way as in Costigan's study (Costigan et al 2002), several families were identified, although under somewhat different headings and confined to 10 classes (Table 60.1).

Xiao and colleagues used a twofold change in signal intensity as a cut-off line. The identified 122 genes and 51 expressed sequence tags represented 2.3% of the total genes examined. Among these, the vast majority (86%) were not previously identified in DRGs after nerve injury (Xiao et al 2002). Thus these array studies have not only provided evidence for regulatory changes, but also showed the presence of a number of genes so far not identified in DRGs. Interestingly, the changes are long-lasting, often throughout the entire period studied (28 days) (Table 60.1). It is known from other studies that changes in expression can last for long periods, if regeneration

is efficiently prevented. If not, regeneration will take approximately a month in the rat after complete midthigh transection, and most changes then revert to normal. The significance of some of these results has been discussed by Xiao and coworkers (Xiao et al 2002 and references therein) and is summarized here.

Not unexpectedly, neuropeptides belonged to the group of compounds showing the most dramatic changes, in particularly with regard to up-regulation (Table 60.1). One reason is that neuropeptides, in contrast to enzymes and other proteins, are released from the neuron and have to be replaced by ribosomal synthesis in the cell soma. This is reflected in distinct changes in peptide and especially mRNA levels, and can be conveniently recorded in single cells by in situ hybridization or immunohistochemistry. In contrast, most other messenger molecules (e.g. acetylcholine and noradrenaline) are synthesized by enzymes in all parts of the neurons, and efficient transporter molecules allow reuptake and recycling of the transmitter. Down-regulation of excitatory peptides (substance P and CGRP) will attenuate transmission, and some up-regulated peptides (VIP, galanin and NPY) may also affect transmission but in addition improve survival and regeneration (see, for example, Wynick & Bacon 2002).

Compounds of importance for survival—such as heat shock protein 27 and LIM domain protein CLP36—are transiently up-regulated. Also, the transcription factor jun D and eukaryotic initiation factor-4E, a translation initiation factor, were up-regulated. Interestingly, two important growth factors, brain-derived neurotrophic factor (BDNF) and GDNF, were increased less than twofold. However, basic fibroblast growth factor was strongly up-regulated.

With regard to synaptic transmission, most molecules were down-regulated, and only synaptotagmin IV showed an increase. However, synaptotagmin IV can form hetero-oligomers with synaptotagmin I, and this results in less efficient calcium coupling. Thus, in general, the changes in synaptic vesicle proteins tend to work towards attenuation of transmission in the dorsal horn.

The expression of receptors for messenger molecules is often changed. For example, several of the neuropeptide receptors—such as the cholecystokinin B receptor (CCK_B) and the NPY Y_2 and Y_5 receptors, as well as a nicotinic acetylcholine receptor subtype (α_7), a purinoceptor ($P2Y_1$), the α_{2A}-adrenergic receptor, and the benzodiazepine receptor of the peripheral type—were up-regulated, whereas others were down-regulated. Clearly, it will be a difficult puzzle to understand the functional significance of these complex regulations.

CLASSICAL GROWTH FACTORS

Classical growth factors play a major role in the development, physiology and pathophysiology of DRGs and sensory mechanisms. They are often target-derived, and therefore localized in non-neuronal cells, whereas their tyrosine kinase (Trk) receptors (TrkA, TrkB and TrkC) are often present in neurons. Many studies have been devoted to the analysis of these growth factors in non-neuronal cells (including satellite and Schwann cells) and their receptors under normal circumstances and after injury, but this topic will not be discussed here (but see Del Fiacco & Priestley 2001). However, we will address BDNF and its reaction to nerve injury and inflammation, because it is neuronally localized. (GDNF and nerve growth factor, NGF, have, however, in a few studies been suggested to have a neuronal localization.)

Under normal circumstances, BDNF is constitutively expressed in 11–38% of DRG neurons, mainly in small TrkA-positive neurons (Cho et al 1997, 1998, Kashiba & Senba 1999, Mannion et al 1999, Michael et al 1999, Wetmore & Olson 1995, Zhou & Rush 1996). Axotomy of a peripheral nerve remote from the cell body induces up-regulation of BDNF mRNA mainly in medium-sized and large-diameter neurons expressing TrkB and TrkC; that is, NGF-insensitive neurons (Cho et al 1998, Fukuoka et al 2001, Kashiba & Senba 1999, Michael et al 1999). NPY is up-regulated in the same neurons (Kashiba & Senba 1999, Michael et al 1999). BDNF expression in most TrkA cells was, however, unchanged (Michael et al 1997).

In contrast, spinal nerve ligation increases BDNF expression mainly in small-sized neurons (Fukuoka et al 2001), showing a clear differential regulation depending on type of nerve injury. Peripheral tissue inflammation increases BDNF mRNA in DRG neurons (Cho et al 1997), whereas capsaicin causes a marked down-regulation (Kashiba & Senba 1999). Interestingly, BDNF is packaged in large dense-core vesicles (the same vesicles that store neuropeptides) and then transported into the dorsal horn of the spinal cord (Michael et al 1999), suggesting that it can be released and control spinal cord excitability, acting in a transmitter-like fashion (see Thompsen et al 1999). In Fig. 60.1C, the relation of several growth factor receptors and other molecules to some major DRG neuron markers (CGRP, IB4 and neurofilament 200) has been summarized.

SPECIES DIFFERENCES

Most studies on the effect of nerve injury on the expression of various molecules in DRGs have been done on rat, but increasingly mice are also analysed, demonstrating no obvious qualitative differences. However, in other species, differences have been encountered (see Hökfelt et al 1997). Thus in guinea pig the up-regulation of galanin is much more restricted than in rat and mouse, whereas NPY regulation is similar. Also, in cat, galanin up-regulation is very modest. In contrast, up-regulation of galanin is strong in monkey (*Macaca mulatta*), whereas NPY expression is hardly detectable. Recently, human ganglia have been analysed, and galanin is expressed in some 10–15% of the DRG neuron profiles versus around 40–50% CGRP-positive neurons (Landry et al 2003), a proportion that is similar to that found in the monkey. Thus galaninergic mechanism may exist at the spinal level in humans as in certain rodents and a monkey.

ROLE OF VOLTAGE-GATED SODIUM CHANNELS

Voltage-gated sodium channels in DRG neurons play a crucial role in chronic pain. Many subtypes of sodium channel are localized in DRG neurons (see McMahon & Priestley 2004). Based on their sensitivity to tetrodotoxin (TTX), these sodium channels are classified as TTX-sensitive or TTX-resistant subtypes. Both electrophysiological experiments and pain behaviour tests show that TTX-sensitive subtypes of sodium channels play important roles in generating ectopic discharges in injured sensory neurons and in maintaining allodynic behaviours in animal models of neuropathic pain (Liu et al 2001, Lyu et al 2000). Moreover, following peripheral nerve injury, the expression of TTX-sensitive sodium channels $Na_v1.3$ and Na_v2 are up-regulated in primary sensory neurons, while $Na_v1.1$, $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ are down-regulated (Baker & Wood 2001, Kim et al 2002, Lai et al 2003, Xiao et al 2002). These findings suggest that TTX-sensitive sodium channels, especially $Na_v1.3$ and some other sodium channels, are potentially important in generating and maintaining neuropathic pain.

MECHANISMS UNDERLYING INJURY-INDUCED PHENOTYPIC CHANGES

The mechanisms underlying up- and down-regulation of the various molecules in sensory ganglia are only incompletely understood. In several cases, classical neurotrophic factors play a role (see McMahon & Priestley 2004, Priestley et al 2002). A common denominator does not seem to exist, and every molecule may have its own regulatory pathways. These problems have been analysed in vivo in DRGs for substance P, CGRP and galanin, whereas knowledge on VIP and NPY mainly stems from in vitro work and work on cell lines. In the case of the dramatic down-regulation of the two excitatory peptides substance P and CGRP, a clear mechanism has, however, been identified. Thus it was shown early on that NGF is essential for the expression of these two peptides, and that axotomy-induced down-regulation can be reversed by administration of exogenous NGF (see Del Fiacco & Priestley 2001). The nerve transection interrupts the centripetal transport of peripheral, target-derived NGF to the cell body; even if the injury increases NGF production in Schwann cells and fibroblasts around the injured site, this cannot compensate for the loss of NGF produced in the skin (see Del Fiacco & Priestley 2001).

There is some evidence on the mechanisms underlying axotomy-induced regulation of galanin. Galanin up-regulation is markedly impaired in mice lacking the gene for leukaemia inhibitory factor (LIF) (Rao et al 1993). LIF is produced in DRG neurons during development and is up-regulated in Schwann cells after nerve injury (Murphy et al 1993). Also, NGF may play a role by having an antagonistic action on LIF induction. The nerve injury-induced inhibition of NGF transport to the cell bodies may release LIF synthesis and thus enhance galanin production. Also, the cytokine interleukin (IL)-6 may participate, because IL-6 transcript levels increase in DRG neurons and Schwann cells after nerve injury, and because galanin up-regulation is impaired in IL-6-KO mice (Murphy et al 1999). It was therefore suggested that the effect of LIF on galanin synthesis is mediated through IL-6 (Murphy et al 1999).

In the case of VIP, focus has been on ciliary neurotrophic factor, like LIF, a member of the neuropoietic cytokine family (see Pitts et al 2001 and references therein). Ciliary neurotrophic factor and fibroblast transforming growth factor-β induce transcription of VIP through a 180-base pair cytokine response element in the VIP promoter. These molecules act synergistically, whereby ciliary neurotrophic factor induces signal transducer and activator of transcription- and Smad-containing complexes.

With regard to the NPY gene, both NGF and BDNF stimulate the NPY promoter (Minth-Worby 1994, Wernersson et al 1998, Williams et al 1998 and references therein). In vivo studies suggest that members of the fibroblast growth factor family can attenuate NPY up-regulation in large DRG neurons, in agreement with the presence of these growth factors and their receptors on DRG neurons and associated cells (Grothe et al 2001, Oellig et al 1995).

Recently, the focus has turned to GDNF and related molecules, demonstrating they not only partially or completely reverse many of the nerve injury-induced morphological and neurochemical changes described earlier, but also block the associated neuropathic pain state (Gardell et al 2003, Wang et al 2003). These findings open up new venues for understanding mechanisms underlying, and for treatment of, neuropathic pain.

THE ROLE OF THE DRG NEURON CELL SOMA

Our view on the role of the soma of the DRG neurons is at present expanding. As discussed by Devor (1984) in the first edition of this textbook, this part of the neuron was not supposed to be involved in signal generation or processing but should be responsible for the metabolic demands of the neurons. However, DRG neurons may discharge spontaneously due to a high level of excitability, and this is increased by peripheral nerve injury (see Devor et al 1984). Moreover, the DRG somata are not innervated; that is, they do not have nerve endings synapsing on to them, in contrast to sympathetic ganglion neurons and most other neurons, but evidence for chemically mediated cross-excitation in rat DRGs has been reported (Amir & Devor 1996).

There is also both immunohistochemical and electrophysiological evidence for functional peptide receptors on the DRG soma membrane. For example, NPY (Abdulla & Smith 1999), substance P (Abdulla et al 2001) and galanin (Kerekes et al 2003) all distinctly modulate membrane excitability after micropipette application, providing further evidence for a more active role for the DRG soma. Moreover, the demonstration that neuropeptides can be released from the DRG soma (Huang & Neher 1996), and from neuronal soma and dendrites in general (see Ludwig & Pittman 2003, Morris et al 1993), strongly indicates the possibility that signalling molecules can be released intraganglionically and control membrane excitability and neuronal activity, perhaps in particular after nerve lesion, when the levels of some peptides are dramatically increased. Kerekes and colleagues recorded effects on electrical activity from acutely dissociated DRG neurons with galanin at concentrations as low as 10^{-14} M (Kerekes et al 2003). It does not seem unreasonable to assume that such peptide concentrations can be achieved around cell bodies, when they spontaneously discharge after nerve injury.

FUNCTIONAL CONSEQUENCES OF ALTERED GENE EXPRESSION IN DRG

The significance of the dramatic changes in gene expression is only very incompletely understood, not least because many of them have been identified so recently. However, in addition to the overview already presented (under *Global gene expression studies*), we here would like to expand on the significance of some of the profound changes in neuropeptide expression, in particular that of galanin and NPY, because of the large body of evidence that has been collected over the past 15 years or so.

Because peptides penetrate the blood–brain barrier only to a very limited extent, and because, at least for galanin, no small, non-peptide agonists or antagonists have been developed, this type of molecules have to be delivered intrathecally, a route originally described by Yaksh and Rudy. Woolf & Wiesenfeld-Hallin (1986) subsequently used this intrathecal method in conjunction with the (nociceptive) flexor reflex model introduced by Wall and Woolf for studies on CGRP. The role of CGRP and substance P as excitatory messengers is generally accepted, as is the idea that their down-regulation in DRGs after nerve injury attenuates transmission in the dorsal horn.

Galanin is under normal circumstances detectable only in a few DRG neurons, mainly small ones. After axotomy, up to 50% express the peptide, again mainly small neurons but also some medium-sized and large ones, and it seems likely that this up-regulation occurs in those neurons that normally produce CGRP (Villar et al 1989).

Early on, intrathecal application of galanin was shown to have a biphasic effect on the flexor reflex, with facilitation at low and depression at high concentrations, whereby the inhibitory effect was enhanced after sciatic nerve section, thus suggesting both pro- and antinociceptive effects. Meanwhile, three galanin receptors have been identified (GalR1 to R3; see Branchek et al 2000), and it is clear that

GalR1 and GalR2 have a robust expression in DRG, and that GalR1 in addition is present in interneurons in the superficial dorsal horn (see O'Donnell et al 2003). Early work suggested that up-regulated galanin may serve to inhibit increased nociceptive input after nerve injury, and recent studies using a partial nerve injury model or genetically modified mice have largely supported this notion (see Liu & Hökfelt 2002, Wiesenfeld-Hallin & Xu 2001). A reasonable conclusion at the moment, based on a long series of studies in our and other laborabories, seems to be that galanin's pronociceptive effect is mediated via GalR2 receptors located on the primary afferents, whereas the antinociceptive effect is exerted via the GalR1 receptor on dorsal horn interneurons.

With regard to NPY, the situation appears even more complex. NPY can normally be detected only in single DRG neurons; is up-regulated by nerve injury in some 25% of the DRG neurons, almost exclusively the large ones (Wakisaka et al 1991); and is present in numerous local dorsal horn neurons. Intrathecal injection of NPY in normal rats shows an antinociceptive action (Hua et al 1991), but biphasic responses have been recorded in the flexor reflex model (Xu et al 1999), and White (1997) showed a pronociceptive effect of NPY. In mice with genetically deleted NPY Y_1 receptor, the pain threshold is strongly reduced (Naveilhan et al 2001), suggesting a tonic antinociceptive role for this receptor (which, however, is deleted in the entire animal). Also, the NPY receptors show complex regulations after axotomy; the Y_1 receptor, normally present in small DRG neurons, is markedly down-regulated, and the Y_2 receptor strongly up-regulated in large neurons (Zhang et al 1997). Y_1 receptors are also present on dorsal horn neurons. Thus up-regulated NPY in large DRG neurons could be involved in controlling the pain threshold by acting at least at three sites:

- somatic, up-regulated Y_2 autoreceptors on large DRG neurons,
- Y_1 receptors on dorsal horn neurons, and perhaps
- Y_2 receptors on presynaptic primary afferents.

CANCER PAIN

Pain is common in cancer, and perhaps the most serious complication with regard to quality of life. And, just as in neuropathic pain, present treatment alternatives (e.g. for bone pain) are not efficacious. However, comparatively little is known about the relation between cancer pain and the nervous system. It could be guessed that an eroding tumour also damages the nerves, inducing cancer pain of the neuropathic type. However, studies on a murine model show that bone cancer appears to represent a unique pain state neurochemically distinct from the profiles existing in both inflammatory and neuropathic pain (Honore et al 2000). Interestingly, the dramatic phenotypic changes described earlier for inflammatory and neuropathic pain are not seen in bone pain. For example, there are no apparent changes in substance P and CGRP (up-regulated in inflammatory pain and down-regulated in neuropathic pain), and there is a down-regulation of galanin and NPY (up-regulated in neuropathic pain) (Honore et al 2000).

THE SPROUTING PARADIGM

A major hypothesis in our attempts to understand neuropathic pain has been the so-called sprouting theory developed by Woolf and collaborators (see Woolf & Salter 2000). In short, it was proposed that transection of a peripheral nerve induces sprouting of large, myelinated, non-nociceptive, primary afferent fibres from deeper laminae into laminae I and II of the spinal cord; that is, where nociceptive afferents normally terminate. Thus nerve injury may not only induce major chemical alterations in DRG neurons as already described, but also, in fact, profound anatomical changes.

It is well known (see Ch. 4) that the small and large DRG neurons, respectively, give rise to C fibres terminating in laminae I and II, and to A fibres terminating in laminae III and IV. A third group, thin myelinated, nociceptive Aδ afferents, terminate in laminae I and V. It was shown that two retrograde and transganglionic neuronal tracers, CTB subunit and CTB conjugated to horseradish peroxidase, which bind to cell membrane glycoconjugates (especially the monoganglioside GM1), normally are mainly taken up by large neurons and label A fibres in the deep dorsal horn (Robertson & Grant 1985). However, after nerve injury there was a marked increase in CTB-labelled afferents of laminae I and II, and this was interpreted as sprouting of Aβ afferents from deeper laminae and suggested to be an anatomical basis for the development of neuropathic pain (see Woolf & Salter 2000).

However, Tong and associates showed that peripheral nerve injury apparently causes a phenotypic change in small DRG neurons, resulting in a marked expansion of the capacity of DRG neurons to take up and transport CTB (Tong et al 1999), so that now not only large but also almost all small neurons are labelled, presumably due to an up-regulation of the GM1. These findings have now been confirmed in various ways (Bao et al 2002, Hughes et al 2003, Santha & Jancso 2003, Shebab et al 2003). For example, Bao's group injected CTB into the peripheral nerve *before* nerve injury (preinjury labelling) and, as expected, this resulted in labelling mainly of large DRG neurons or Aβ afferents (Bao et al 2002). Two weeks later, after peripheral nerve injury, the distribution of CTB-labelled afferents in the dorsal horn was similar to that seen after injection of CTB into an intact nerve (Fig. 60.3A). Hughes and colleagues performed intra-axonal labelling and could not show any certain sprouting of Aβ afferents in laminae I and II 7–10 weeks after nerve injury (Hughes et al 2003). However, a limited sprouting may occur, because a small number of Aβ afferents may enter the inner lamina II after nerve injury (Bao et al 2002). Taken together, these findings strongly suggest that CTB-labelled fibres in laminae I and II after axotomy mainly represent C fibres, and seriously question the role of anatomical sprouting as an important basis for neuropathic pain.

It should be noted that after peripheral nerve injury, neurons in laminae I and II can be activated by selective electrical stimulation of A fibres, using both the proto-oncogene *c-fos* as a functional marker (Shortland & Molander 1998) and electrophysiology (Kohama et al 2000). However, the structural and molecular mechanisms for this functional reorganization still remain to be elucidated.

In sharp contrast to in the rat, in normal monkey (*M. mulatta*) CTB labels both small and large DRG neurons, and CTB-labelled afferents are present in laminae I and II of the spinal cord. After peripheral nerve injury, more than 70% of the DRG neurons (up from 11%), including both small and large neurons, are labelled by CTB, and the number of CTB-labelled afferents is increased in laminae I and II of monkey spinal cord, with a limited increase in lamina III (Fig. 60.4B). These findings suggest distinct anatomical differences with regard to termination of primary afferents in dorsal spinal cord between rat and monkey, at least in *M. mulatta*. Therefore to understand the mechanisms underlying allodynia, particularly in humans, further studies are needed on the anatomical and neurochemical network in the spinal cord and on its plasticity in response to peripheral nerve injury.

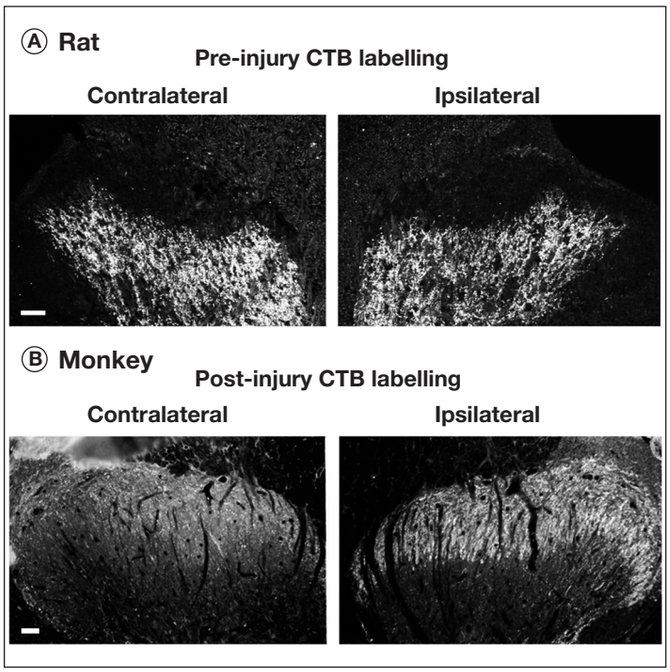


Fig. 60.3 **A** Fourteen days after unilateral sciatic nerve transection, the A fibres labelled with cholera toxin B (CTB) 4 days before nerve injury are distributed in laminae III and IV of the ipsilateral dorsal horn of rat spinal cord, which is similar to that seen in the contralateral dorsal horn. **B** In monkey spinal cord, CTB-labelled afferents are distributed in the contralateral laminae I and II. After nerve injury the number of labelled afferents is increased in the superficial dorsal horn on the ipsilateral side. Bars: 50 μm.

FUNCTIONAL ASPECTS OF SPINAL CORD PLASTICITY AFTER PERIPHERAL NERVE DAMAGE

Injury or disease processes affecting peripheral nerves induce complex direct and secondary changes in the spinal cord, eventually reaching the level of the cerebral cortex, where the somatosensory map related to the injured nerve's peripheral territory becomes reorganized. Here we concentrate on the physiological changes in the spinal cord, including terminals of sensory afferents, in response to peripheral nerve injury, but we will also briefly discuss cell loss and glial responses. Physiologically peripheral injury leads to direct uncoupling of some afferent input to dorsal horn neurons, but also to changes in the properties of afferent terminals, synaptic efficacy and response characterization of dorsal horn neurons, and inhibitory modulation. The result is a complex plasticity beyond a simple reduction in synaptic input, and this plasticity may participate in the development of neuropathic pain.

Alteration in receptive field of dorsal horn neurons

In normal animals, the skin surface of the hind limb is mapped somatotopically in the dorsal horn of lumbar spinal cord. The neurons in the medial region of low lumbar dorsal horn have their receptive fields in the toe–foot region, whereas those in the lateral region correspond to the upper and lower legs (see Devor 1988). Immediately after total transection of major nerves innervating the hind paw, such as the sciatic and saphenous nerves, there is a period of loss of input for neurons in the medial dorsal horn (see Devor 1988). However, within a few days in rats, and a few weeks in cats, many neurons in the medial dorsal horn start to respond to cutaneous stimulation applied to the upper leg, thigh and perineum (see Devor 1988). Similar plasticity has also been found to occur between neurons receiving input from sciatic versus saphenous nerve (see Devor 1988).

In addition to complete nerve section, animal models of partial nerve injury and neuropathic pain have been developed. Although earlier studies using the chronic nerve constriction model did not reveal major abnormalities in receptive field characteristics of dorsal horn neurons (Laird & Bennett 1993), some more recent studies in several partial injury models have shown an expansion of receptive fields of dorsal horn neurons (Suzuki et al 2000, Takaishi et al 1996). These enlargements are often moderate and are time- and modality-dependent (Suzuki et al 2000, Takaishi et al 1996).

There is evidence that such plasticity in receptive field organization is not a result of peripheral changes, such as afferent sprouting or cross-talk between the afferents innervating the two regions, but rather to central changes occurring in the spinal cord (see Devor 1988), with at least two potential underlying mechanisms. One is the growth of new axons or dendrites across previous somatotopic boundaries. Another, more likely, scenario is an increased effectiveness of pre-existing synapses, so-called weak or silent synapses, which have been demonstrated in dorsal horn neurons in both anatomical and electrophysiological studies (see Devor 1988). Thus an increased excitatory drive resulting from, for example, repetitive C-fibre stimulation or reduced spinal inhibitory control can readily modify receptive fields of dorsal horn neurons through alteration in synaptic strength (see Woolf & Salter 2000). As discussed below, peripheral nerve injury induces a cascade of changes in the dorsal horn leading to reduced inhibition and/or increased excitation.

Response characteristics of dorsal horn neurons

Spontaneous activity

It has long been known that, in humans, deafferentated dorsal horn neurons develop spontaneous activity after dorsal root injury, which has also been observed in cat or rat after rhizotomy and peripheral nerve injury (Dalal et al 1999). Thus the percentage of dorsal horn neurons exhibiting ongoing discharges is markedly increased in comparison with normal animals. Deafferentation or loss of input is not, however, a requisite for ongoing activity after axotomy, because in many studies, particularly in those after partial injury, there seems to be no difference in the level of spontaneous activity in neurons with or without peripheral receptive field (Behbehani et al 1994, Chapman et al 1998, Dalal et al 1999, Laird & Bennett 1993, Palecek et al 1992, Pertovaara et al 2001, Takaishi et al 1996, Weng et al 2003). Thus dorsal horn neurons per se can be the source of ongoing activity after nerve injury, but in addition this may result from ectopic discharges generated in the neuroma and DRG, and has also been recorded from rats with experimentally induced diabetic neuropathy, despite lack of evidence for increased ongoing primary afferent activity (Pertovaara et al 2001).

Taken together, increased ongoing activity in dorsal horn neurons, especially such that normally receive nociceptive input, is likely to be an important mechanism in the development of neuropathic pain. Such activity may give rise to spontaneous pain-like behaviours observed in different pain models, and may also contribute to the development of hypersensitivity when occurring in neurons with peripheral receptive field. Importantly, procedures known to alleviate neuropathic pain-like behaviours in animal models have also been shown to reduce such ongoing activity (Yakhnitsa et al 1999).

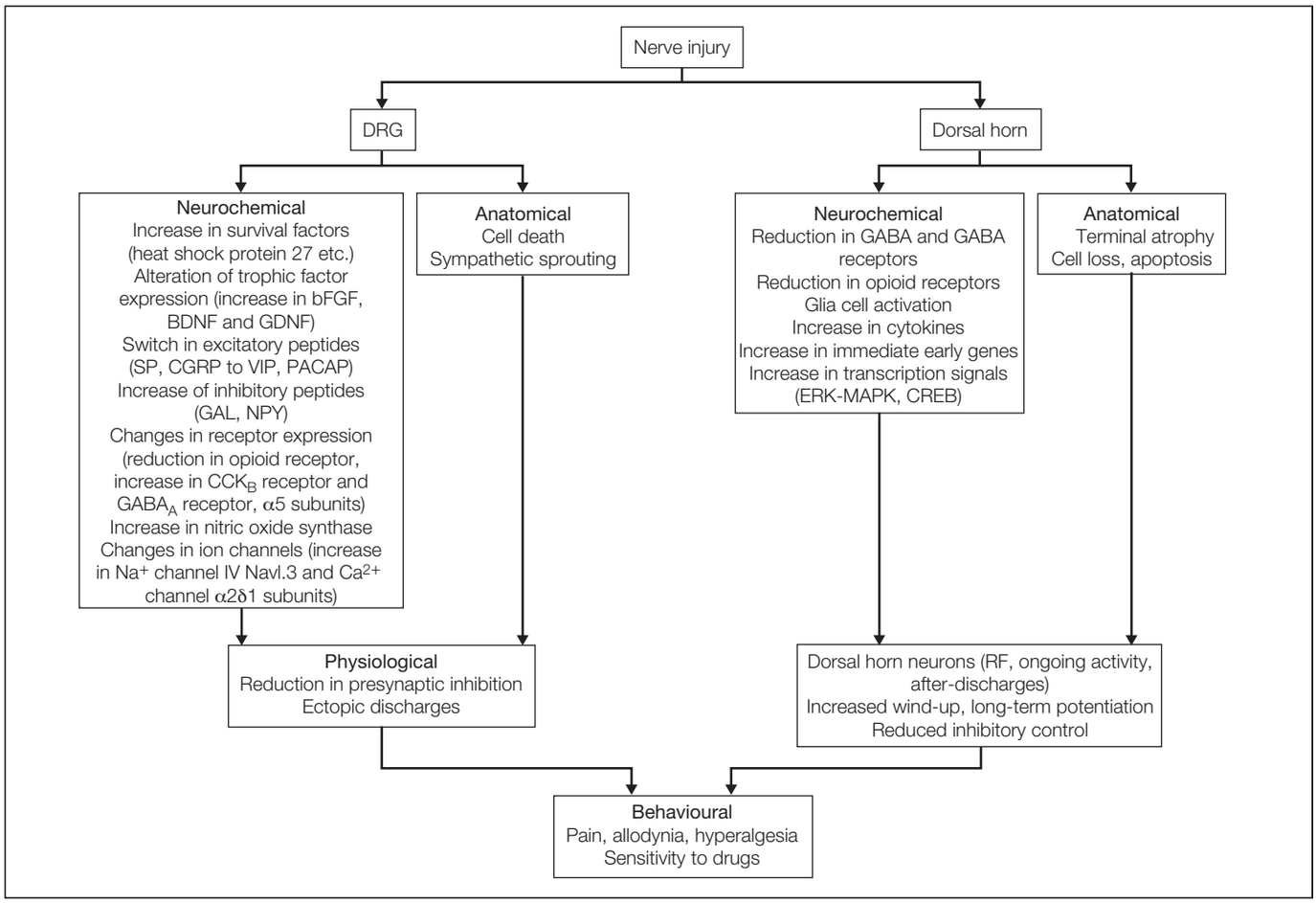


Fig. 60.4 Changes occurring in dorsal root ganglia and dorsal horn in response to peripheral nerve injury, and their functional consequences. Peripheral nerve injury induces complex anatomical, neurochemical and functional changes in the dorsal root ganglion and dorsal horn. Some of these changes may represent survival and regenerative mechanisms, some may be adaptive responses as a consequence of loss of peripheral innervation or afferent input, and some may be defensive mechanisms against excessive nociceptive stimulation. Together, such plasticity changes are important not only in the development of the pain syndrome in nerve-injured patients, but also in the response of such pain to treatments. bFGF, basic fibroblast growth factor; BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; CREB, cyclic AMP response element-binding protein; ERK, extracellular signal-regulated kinase; GAL, galanin; GDNF, glial cell line-derived neurotrophic factor; MAPK, mitogen-activated protein kinase; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating peptide; SP, substance P.

Response of dorsal horn neurons to mechanical and thermal stimulation

In contrast to the consistent observation of increased ongoing activity in dorsal horn neurons following peripheral deafferentation, the responses of these neurons to peripheral electrical, mechanical and thermal stimulation have been found to be normal in the majority of studies. Thus after axotomy the threshold and magnitude of A- and C-fibre responses of dorsal horn neurons to electrical stimulation of the proximal end of the cut nerve did not alter (Wall et al 1981). More remarkably, the majority of studies with partial injury models also failed to demonstrate an increased response of dorsal horn neurons to mechanical and/or heat stimulation, despite the presence of behavioural hypersensitivity in these animals. For example, the threshold and stimulus-response relationship to heat stimulation have been reported to be comparable for dorsal horn neurons recorded from nerve injury versus in sham rats in almost all studies (Chapman et al 1998, Laird & Bennett 1993, Palecek et al 1992, Pertovaara et al 2001, Takaishi et al 1996). The response to mechanical stimulation is variable, with some authors reporting some hypersensitivity (Weng et al 2003, Yakhnitsa et al 1981) and others reduced sensitivity (Chapman et al 1998, Laird & Bennett 1992, Takaishi et al 1996).

Following partial nerve injury, responses of dorsal horn neurons to natural stimulation is very much influenced by the extent of nerve injury as well as the relative involvement of myelinated versus unmyelinated input, and this may contribute to the variable neuronal responses. Overall, the less robust sensory responses of dorsal horn neurons in rats with partial nerve injury suggest that additional mechanisms may account for the allodynia-type pain in animals. These mechanisms may include expansion of receptive field for individual dorsal horn neurons as well as the recruitment of more neurons resulting from activation of previously inactive synapses. In agreement, Colvin and coworkers have shown that in rats with sciatic nerve constriction there is an increase in dorsal horn postsynaptic currents in relation to afferent fibre input measured with surface compound potentials (Colvin et al 1996). Finally, some of the behavioural

changes in animals with partial nerve injury may be caused by direct stimulation of the injured nerve site, which is known to trigger marked responses in dorsal horn neurons after nerve injury (Laird & Bennett 1993).

After-discharges

In addition to spontaneous activity, prolonged after-discharges represent a consistent feature of the dorsal horn neuron response to mechanical stimulation after partial nerve injury (Laird & Bennett 1993, Palecek et al 1992, Takaishi et al 1996, Yakhnitsa et al 1999). This may also be important for pain perception in these animals because of temporal summation, and the behavioural correlate of such after-discharges may be the prolonged paw withdrawal in neuropathic animals following noxious mechanical stimulation. Such after-discharges could be caused by cross-talk of afferent fibres at the injury site or in the DRG, or by increased neuronal excitability in the dorsal horn.

Activity-dependent increase (central sensitization) in spinal excitability in nerve-injured animals

Activity-dependent increase in spinal cord excitability may represent an important mechanism underlying abnormal pain conditions after tissue injury, and may share similar mechanisms with memory formation (see Ji et al 2003). In earlier studies, central sensitization after repetitive activation of C fibres was unaltered or reduced in rats after axotomy in the flexor reflex preparation (Wall & Woolf 1986). Thus the wind-up of dorsal horn neurons after C-fibre stimulation was unchanged, and there was no Aβ fibre-induced wind-up in rats after spinal nerve ligation (Chapman et al 1998). However, more recently Weng's group reported that, in rats with vincristine-induced neuropathy, wind-up occurs at lower-frequency stimulation than in normal animals (Weng et al 2003).

Miletic & Miletic (2000) showed that tetanic stimulation of A fibres in the sciatic nerve at low frequency triggers long-term depression of dorsal horn field potentials, and this was markedly reduced in rats with nerve ligation compared with in normal rats. Moreover, when stimulated at high frequency, A-fibre tetanic stimulation generated a prolonged increase in spinal excitability in nerve-injured rats, in contrast to in normal rats, where a brief facilitation was followed by depression. These results demonstrated spinal plasticity after partial nerve injury, leading to an overall shift in response to A-fibre tetanic stimulation from depression to excitation, which can result from either reduced spinal inhibitory mechanisms or increased excitatory drive.

NMDA receptor activation is one of the principal mechanisms in central sensitization, and its role in neuropathic pain is implicated by a large number of preclinical studies showing that NMDA receptor antagonists are effective in alleviating experimental neuropathic pain (see Woolf & Salter 2000). Following chronic constriction injury, the content of glutamate, aspartate, as well as intracellular calcium is increased in dorsal horn slices (Kawamata & Omote 1996), possibly related to alterations in spinal glutamate transporters (Sung et al 2003). Also, spinal nerve ligation causes facilitation of NMDA-induced currents and calcium transients in rat substantia gelatinosa neurons (Isaev et al 2000). This may be caused by postsynaptic intracellular events subsequent to NMDA receptor activation, such as activation of protein kinase C, because peripheral nerve injury does not seem to alter the level of NMDA receptors or expression of NMDA receptor subunits in the dorsal horn (Mao et al 1995). Furthermore, increased excitatory drive in the spinal cord may also involve activation of AMPA receptors and metabotropic glutamate receptors. Finally, a role for neuropeptides such as VIP and pituitary adenylate cyclase-activating peptide, the synthesis of which is increased in sensory neurons after axotomy, has been suggested (Wiesenfeld-Hallin et al 1990, see Dickinson & Fleetwood-Walker 1999), and they appear to take over the excitatory role of substance P in normal rats in mediating repetitive C-fibre stimulation-induced facilitation of the nociceptive flexor reflex (Wiesenfeld-Hallin et al 1990).

Reduced inhibitory mechanism in the spinal cord

Transmission of sensory information from the periphery to the central nervous system is normally subjected to either pre- or postsynaptic inhibitory control maintained by activity in sensory afferents, dorsal horn interneurons, and descending pathways. Wall and colleagues showed a marked axotomy-induced reduction in the magnitude of the dorsal root potential, a measure of presynaptic inhibition (Devor 1988), later confirmed in several other studies, including models of partial nerve injury (Laird & Bennett 1992). Furthermore, the postsynaptic inhibition of dorsal horn neurons exerted by A-afferent input was also reduced after axotomy (Devor 1988). Such reduction in pre- and postsynaptic inhibition of spinal sensory input was later shown to be paralleled by a decrease in the content of the important inhibitory neurotransmitter GABA (Castro-Lopes et al 1993) as well as GABA receptors (Castro-Lopes et al 1995). More recent work has further demonstrated that partial nerve injury (chronic constriction and spared nerve injury), but not nerve transection, induced a selective loss of GABA-mediated inhibition on superficial dorsal horn neurons resulting from a reduced GABA release (Moore et al 2002).

The endogenous opioid peptides and their receptors represent another important spinal inhibitory system on nociception. Opioids are very effective in treating nociceptive or inflammatory pain, but their effect on neuropathic pain is a matter of debate (Arnér & Meyerson 1988). This may be due to injury-induced loss of μ and δ opioid receptors on afferent terminals of and/or dorsal horn interneurons (Besse et al 1992). In addition, opioid antinociception is also subjected to modulation of several antiopioid systems, including activation of NMDA receptors (Mao et al 1995) and peptides such as cholecystokinin (see Wiesenfeld-Hallin & Xu 2001) and dynorphin (Gardell et al 2004). Interestingly, there is evidence for a coupling between the activity-dependent insertion of opioid δ receptors and the release of pronociceptive neuropeptides (Bao et al 2003), and for dynorphin being a mediator of neuropathic pain (Gardell et al 2004 and references therein).

Nerve injury-induced cell loss in the dorsal horn

More recently, attention has been paid to the possibility that peripheral nerve injury in fact can cause cell loss in the dorsal horn, and that this could contribute to neuropathic pain. Thus Sugimoto's group early on described neurons with signs of degeneration in the lumbar dorsal horn, on both sides, after unilateral constriction of the sciatic nerve, with a greater increase ipsilaterally (Sugimoto et al 1990). However, after sciatic nerve transection there was no evidence for dark (degenerating) neurons. Later, Azkue and colleagues, using the terminal deoxynucleotidyl transferase nick end labelling (TUNEL) technique, demonstrated nuclear fragmentation in neurons in the superficial layers and in the neck of the dorsal horn, probably reflecting apoptotic cell death (Azkue et al 1998). This was seen at 7 days, but not 3 or 14 days, after sciatic nerve transection, and could be prevented with an NMDA receptor antagonist (MK-801), suggesting the involvement of a glutamatergic mechanism, perhaps excessive

glutamate release. Coggeshall and associates, using stimulation with parameters that selectively activate Aβ fibres, then provided evidence that myelinated afferents (A fibres), using glutamate as a transmitter, are responsible for cell death in the dorsal horn (Coggeshall et al 2001). They did not see this after sciatic nerve transection alone, but activation of A fibres in a previously sectioned sciatic nerve caused cell death.

The type of neuron undergoing cell death has not been identified, but GABA neurons represent a large population of dorsal horn neurons, and neuropathic injury induces a decrease in GABA content in the dorsal horn (Castro-Lopes et al 1993). Also, partial nerve injury decreases dorsal horn levels of the GABA-synthesizing enzyme glutamic acid decarboxylase and induces neuronal apoptosis (Moore et al 2002).

Changes in spinal glia after peripheral nerve injury

A role for spinal glia cell activation in chronic pain-processing has been proposed in recent years (Jin et al 2003, Svensson et al 2003, Watkins et al 2001). The number of glia in the spinal cord is increased after peripheral nerve injury. Peripheral nerve injuries, particularly those involving an inflammatory component, activate glia in the dorsal spinal cord, and agents disrupting glia activation reduce pain-related behaviours (Watkins et al 2001). The action of glia probably involves the release of proinflammatory cytokines, because drugs that block the action of cytokines (Watkins et al 2001), ATP (Tsuda et al 2003), and p38 mitogen-activated protein kinase activation (Jin et al 2003, Svensson et al 2003) reduce pain.

CONCLUDING REMARKS

Many changes occur in DRG and the spinal cord in response to peripheral nerve injury, and recent global expression analyses have expanded the number of genes involved. Thus, even if we in this chapter have discussed several molecules, many others have been left out. Some of the changes may play a role in the generation and maintenance of neuropathic pain (Fig. 60.4), but may also have antinociceptive effects. Taken together, exact knowledge of the role of the molecules regulated by nerve injury, and of the mechanisms underlying their regulation, may not only offer an understanding of neuropathic pain but also suggest novel strategies for the treatment of this type of pain. However, the very large number of injury-regulated genes makes it even more difficult to define the mechanisms underlying this type of pain.

The distinctly different patterns of regulation seen after peripheral inflammation (up-regulation of opioid and other peptides in dorsal horn neurons; see Dubner & Ruda 1992) versus nerve injury (regulation of molecules in the DRG neurons) suggest the existence of separate defence systems for these two types of pain, associated with dorsal horn neurons and DRG neurons, respectively (see Hökfelt et al 1997).

In the spinal cord, the terminals of sensory afferents undergo plasticity that reflects changes occurring in their cell bodies in the DRG. The plastic changes in the dorsal horn neurons include expanded or novel receptive fields, ongoing activity, after-discharges, and in some cases hypersensitivity. The overall excitatory drive in the dorsal horn may be increased, and there is strong evidence that inhibitory control in the spinal cord is compromised after nerve injury.

The unique efficacy of morphine in treating inflammatory pain has raised the question of whether there is a single molecule that in a similar way can abolish neuropathic pain. The fact that neurons produce and release multiple messengers (Hökfelt 1991)—a single primary pain afferent may, for example, release glutamate, ATP, nitric oxide, substance P, CGRP and galanin—calls for several antagonists to block transmission. In fact, an NK_1 antagonist has been shown to be unable to antagonize pain in humans (see Rupniak & Kramer 1999). This has led to the dramatic idea to delete entire dorsal horn projection neurons with the help of a conjugate of a toxin coupled to substance P, which is internalized via the NK_1 receptor (Mantyh et al 1997). However, injury-induced neuropathic pain can be reversed by antisense oligonucleotides against the TTX-resistant sodium channel $Na_v1.8$ (Lai et al 2002). In recent studies investigators treated rats with neuropathic pain induced by spinal nerve ligation with systematically administered GDNF and artemin, a member of the GDNF-family (Gardell et al 2003, Wang et al 2003). They not only observed pain reversal but also at least a partial normalization of a number of morphological and neurochemical features, including changes in neuropeptides and the $Na_v1.8$ sodium channel.

Beyond the conventional design of exogenous agonists and antagonists acting (mostly) on seven-transmembrane, G-protein-coupled receptors, transcription factors and other molecules can be targeted to activate endogenous downstream antinociceptive systems. For example, inhibition of the enzyme enkephalinase, which inactivates the opioid peptides methionine- and leucine-enkephalin, represents one approach (Roques & Noble 1995). Furthermore, Cheng and coworkers genetically deleted the transcription factor downstream regulatory element antagonistic modulator (DREAM), which tonically suppresses the opioid peptide dynorphin, and in this way created a mouse with an increased pain threshold in a variety of pain models due to increased spinal dynorphin levels (Cheng et al 2002). This study demonstrates the power of mobilizing endogenous receptor ligands to counteract pain, and points to future research directions.

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