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EXPRESSION OF α_1-ADRENOCEPTORS ON PERIPHERAL NOCICEPTIVE NEURONS

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Abstract—The purpose of this study was to determine whether α_1-adrenoceptors are expressed on primary nociceptive afferents that innervate healthy skin. Skin and dorsal root ganglia were collected from adult male Wistar rats and assessed using fluorescence immunohistochemistry with antibodies directed against α_1-adrenoceptors alone or in combination with specific labels including myelin basic protein and neurofilament 200 (markers of myelinated nerve fibres), protein gene product 9.5 (a pan-neuronal marker), tyrosine hydroxylase (sympathetic neurons), isolectin B_4 (IB_4: non-peptidergic sensory neurons), calcitonin gene related peptide (CGRP) and transient receptor potential vanilloid receptor 1 (TRPV1) (peptidergic sensory neurons). Double labelling in dorsal root ganglia confirmed the expression of α_1-adrenoceptors within sub-populations of CGRP, IB_4 and TRPV1 immunoreactive neurons. Myelinated and unmyelinated sensory nerve fibres in the skin expressed α_1-adrenoceptors whereas sympathetic nerve fibres did not. The expression of α_1-adrenoceptors on C- and A-delta nociceptive afferent fibres provides a histochemical substrate for direct excitation of these fibres by adrenergic agonists. This may help to explain the mechanism of sensory-sympathetic coupling that sometimes develops on surviving primary nociceptive afferents in neuropathic pain states.

Key words: alpha-adrenergic receptor, sensory afferent, skin, dorsal root ganglion, immunohistochemistry.

Following peripheral nerve injury, sensory neurons may acquire sensitivity to α-adrenergic agonists and electrical stimulation of the sympathetic chain (Devor and Janig, 1981; Devor, 1983; Habler et al., 1987). In particular, some uninjured C-fibre nociceptors develop abnormal α-adrenergic sensitivity and spontaneous ectopic firing (Ali et al., 1999). This aberrant coupling between sensory and sympathetic neurons arises at the nerve injury site and in the dorsal root ganglia (DRG) (Janig and Habler, 2000), such that activation of sympathetic postganglionic efferents excites sensory neurons in the DRG during the period shortly after nerve injury (Devor et al., 1994; Michaelis et al., 1996). This sensory-sympathetic coupling may contribute to allodynia and hyperalgesia in neuropathic pain syndromes such as post herpetic neuralgia and complex regional pain syndrome (Choi and Rowbotham, 1997; Ali et al., 2000; Baron et al., 2002).

Although sensory-sympathetic coupling is well-recognized, the mechanism is unclear. One possibility is that noradrenaline released from sympathetic nerve fibres acts upon adrenoceptors located on sensory nerve fibres (Janig and Baron, 2003). The primary adrenergic target after peripheral nerve injury is thought to be α_2-adrenoceptors (Sato and Perl, 1991; Abdulla and Smith, 1997), but effects may also be mediated by excitatory α_1-adrenoceptors (α_1-ARs) on adjacent nerve fibres that escape injury. Support for this hypothesis has been obtained both in animal models of neuropathic pain (Ali et al., 1999; Lee et al., 1999, 2000; Nam et al., 2000) and in clinical studies on patients (Davis et al., 1991; Drummond et al., 1996; Teasell and Arnold, 2004).

Whether α_1-ARs are expressed constitutively on peripheral nerve fibres or become expressed or functional only after nerve or tissue injury is uncertain. α_1-ARs have been found to augment nociceptive discharge in the normal state (Dogrul et al., 2006), during acute inflammation (Baik et al., 2003) and after nerve trauma (Ali et al., 1999; Lee et al., 1999; Nam et al., 2000). Moreover, mRNA for α_{1A}- and α_{1B}-AR was identified in nerve cell bodies in the DRG of uninjured rats (Nicholson et al., 2005), and mRNA for α_{1B}-AR increased in the DRG following peripheral nerve section or ligation of spinal nerves supplying those ganglia (Xie et al., 2001; Maruo et al., 2006). In addition, the proportion of dissociated DRG cells that responded to noradrenaline increased markedly after chronic nerve injury (Petersen et al., 1996). However, neither stimulation of the sympathetic chain nor close arterial injection of noradrenaline was found to influence nociceptive discharge in uninjured animals (Sato and Perl, 1991). Similarly, in healthy human volunteers, sympathetic nerve discharge did not alter the firing properties of polymodal C-fibre afferents either before or after sensitization of their cutaneous receptive field with mustard oil (Elam et al., 1999).

The purpose of this study was to examine α_1-AR expression on primary nociceptive afferents in the DRG and skin of rats under normal conditions. Immunohistochemistry using double labelling with antibodies directed against α_1-ARs and specific neuronal markers for sympathetic and nociceptive fibres was employed to ascertain receptor ex-

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Abbreviations: cAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene related peptide; DRG, dorsal root ganglia; IB_4, isolectin B_4; MBP, myelin basic protein; NF200, neurofilament 200; PGP9.5, protein gene product 9.5; TH, tyrosine hydroxylase; TPBS, TRIS phosphate buffer solution; TRPV1, transient receptor potential vanilloid receptor 1; α_1-ARs, α_1-adrenoceptors.

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pression and to identify the fibre type(s). The presence of α_1-ARs on primary nociceptive afferents would provide a molecular target for direct excitation of these fibres by adrenergic agonists, thereby supporting the hypothesis that these receptors have the capacity to contribute directly to peripheral nociceptive neurotransmission in neuropathic pain states.

EXPERIMENTAL PROCEDURES

General

Tissue samples were obtained from 17 male Wistar rats aged between 9 and 12 weeks. All experiments followed the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and the National Health and Medical Research Council Code of Practice for the Care and Use of Animals in Research in Australia, and with the approval of the Murdoch University Animal Ethics Committee.

Animal tissue preparation for immunohistochemistry

Rats were euthanized with a lethal dose of thiopentone sodium (Thiobarb, Jurox, Rutherford, Australia; 100 mg/kg i.p.) and perfused transcardially with 30 ml of ice-cold 0.9% saline solution containing heparin (Jurox, Rutherford, Australia; 1000 U/L), followed by perfusion with 180 ml Zamboni's solution (2% formaldehyde and 1% picric acid (v/v) in 0.9% saline). The hind paws were severed and post-fixed in the same fixative for 2 h at 4 °C, and the skin from the footpad was removed. Tissue was fixed for a further 2 h in the same fixative at 4 °C, then washed in 0.1 M phosphate buffer (pH 7.4), incubated in 50% ethanol for 30 min followed by a further two rinses in phosphate buffer and processed further for use as a fixed frozen sample. Similar procedures were used to fix the L5 DRG from seven of the animals. Samples were immersed in a cryoprotectant solution consisting of 0.01 M phosphate buffer containing 0.01 M TRIS (TRIS phosphate buffer solution; TPBS) and 30% sucrose for 2–3 days at 4 °C, embedded in Tissue-Tek (ProSciTech, Queensland Australia), then frozen on dry ice and stored at −80 °C. Ten micrometres tissue samples were sectioned by cryostat onto StarFrost® silane coated slides (ProSciTech), air-dried for 1 h at room temperature and rehydrated in 0.1 M phosphate buffer.

Immunohistochemistry

To identify which type of nerve fibre expresses the α_1-AR we undertook a series of experiments, dual or triple labelling tissues with antibodies directed against α_1-ARs and specific neuronal markers. In these experiments, tyrosine hydroxylase (TH) was used as a marker of sympathetic neurons; isolectin B_4 (IB_4) as a marker of non-peptidergic nociceptive C-fibres, calcitonin gene-related peptide (CGRP) and transient receptor potential vanilloid receptor 1 (TRPV1) as markers of peptidergic nociceptive fibres, and myelin basic protein (MBP) and neurofilament 200 (NF200) as markers of myelinated nerve fibres.

Prior to immunohistochemical procedures, sections were treated with 0.3% Triton X-100 diluted in TPBS for 10 min. When used in combination with the α_1-AR antiserum, the isolectin IB_4-FITC (Table 1) was diluted in TPBS containing 0.1% sodium azide and sections incubated for 12–18 h in a humidified chamber at 4 °C followed by three washes of TPBS for 15 min at room temperature prior to antibody labelling. For studies using guinea pig and sheep-derived primary antiserum, sections were pre-incubated for 1 h in a blocking solution containing TPBS, 0.1% sodium azide, 20% rat serum and 20% normal donkey serum (Sigma-Aldrich, NSW, Australia) at room temperature. For all other primary antisera, sections were pre-incubated in a second blocking solution containing TPBS, 0.1% sodium azide and 10% normal donkey serum. Primary antibody (Table 1) in various combinations was diluted in the second blocking solution and applied

Table 1. Primary and secondary antibodies: dilutions and source

Antigen and host species	Dilution		Product code and source
	Skin	DRG	
BS-I isolectin B_4 FITC conjugate	1:100	1:200	L2895: Sigma-Aldrich, MO, USA
Primary antibodies			
Anti PGP9.5: guinea pig polyclonal	1:300		AB5898: Millipore, MA, USA
Anti α_1-adrenergic receptor: rabbit polyclonal	1:100	1:600	A270: Sigma-Aldrich, MO, USA
Anti TH: sheep polyclonal	1:4000		Gift of Hon. Prof. Paul Pilowsky
Anti MBP: mouse monoclonal	1:250		Sc-66064: Santa Cruz, CA, USA
Anti CGRP: goat polyclonal	1:400	1:400	1720-9007: AbD Serotec, NC, USA
Anti TPRV: goat polyclonal	1:250	1:250	Sc-12498: Santa Cruz, CA, USA
Anti NF200: chicken polyclonal	1:5000	1:7500	AB5539: Millipore, MA, USA
Secondary antibodies			
Cy3: donkey anti-rabbit IgG	1:750		Jackson ImmunoResearch, PA, USA
DyLight-488: donkey anti-rabbit IgG	1:500		Jackson ImmunoResearch, PA, USA
DyLight-549: donkey anti-rabbit IgG	1:1000	1:1000	Jackson ImmunoResearch, PA, USA
Cy3: donkey anti-sheep IgG	1:750		Jackson ImmunoResearch, PA, USA
DyLight-488: donkey anti-sheep IgG	1:500		Jackson ImmunoResearch, PA, USA
DyLight-649: donkey anti-mouse IgG	1:200		Jackson ImmunoResearch, PA, USA
DyLight-488: donkey anti-goat IgG	1:500	1:500	Jackson ImmunoResearch, PA, USA
DyLight-549: donkey anti-goat IgG	1:500		Jackson ImmunoResearch, PA, USA
FITC: donkey anti-guinea pig IgG	1:200		Jackson ImmunoResearch, PA, USA
Cy3: donkey anti-chicken IgG	1:750	1:750	Jackson ImmunoResearch, PA, USA
Cy2: donkey anti-chicken IgG	1:200	1:200	Jackson ImmunoResearch, PA, USA

Abbreviations: PGP9.5, protein gene product-9.5; CGRP, calcitonin gene related peptide; MBP, myelin basic protein; TH, tyrosine hydroxylase; TRPV1, transient receptor potential vanilloid subfamily type 1; FITC, fluorescein isothiocyanate; Cy3, indocarbocyanine.