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Noradrenergic receptor mRNA expression in adult rat superficial dorsal horn and dorsal root ganglion neurons

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Abstract

Noradrenaline (NAdr) has well documented analgesic actions at the level of the spinal cord. Released from bulbospinal projections onto superficial dorsal horn (SDH) neurons, NAdr modulates the excitability of these neurons through the activation of α_1, α_2 or β adrenoceptors. This study utilised in situ hybridisation to determine the specific expression of adrenoceptors within adult rat lumbar SDH and dorsal root ganglion (DRG) neurons, and reports the presence of α_{1A}, α_{1B}, α_{2B}, β_1 and β_2 adrenoceptor mRNA within SDH neurons, and the presence of α_{1A}, α_{1B} and α_{2C} adrenoceptor mRNA within DRG neurons. The present study provides an insight into the modulation of sensory processing at the level of the spinal cord following adrenoceptor activation.

Keywords: Sensory processing; In situ hybridisation; Pain; Substantia gelatinosa

Noradrenaline (NAdr) has been shown previously to modulate pain processing at the level of the spinal cord [25,29]. Such actions are believed to stem from Noradrenergic neurons present in the locus coeruleus which release NAdr into the superficial dorsal horn (SDH) via bulbospinal projections terminating in laminae I and II [4,5,21].

The actions of NAdr are mediated through activation of metabotropic adrenoceptors, classified into three subgroups; α_1 receptors (α_{1A}, α_{1B} and α_{1D}), α_2 receptors (α_{2A}, α_{2B} and α_{2C}) and β receptor subtypes: β_1, β_2, β_3 and β_4 (see [13,14] for reviews). These may be intrinsically present on SDH neurons in addition to being present presynaptically on the terminals of primary afferent fibres, and on the terminals of descending tracts where they may function as autoreceptors to control NAdr release [14].

The analgesic actions of NAdr are predominantly mediated by α_2 adrenoceptors [4,11]. However, there is also evidence in support of the modulation of sensory transmission by α_1 and β adrenoceptors [1,19]. Furthermore, disagreement exists over the precise subtype and pre- or post-synaptic location of each adrenoceptor. This study, therefore, investigated the expression of each adrenoceptor subtype within the SDH and dorsal root ganglion (DRG) neurons using in situ hybridisation in an attempt to resolve these inconsistencies.

All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Male Wistar rats (250–300 g) were killed by isoflurane overdose, and lumbar spinal cord and L4/L5 DRG were rapidly removed, snap frozen in isopentane, and stored at −80 °C prior to sectioning. 10 mm of sections were cut using a Leica CM3050 cryostat, thaw mounted onto poly-D-lysine coated slides, then fixed in 4% paraformaldehyde in sterile phosphate buffered saline and stored under 95% ethanol at 4 °C until hybridisation. cDNA anti-sense and sense oliogonucleotide probes were designed for each adrenoceptor using OLIGO (Plymouth, MN, USA, see Table 1). The specificity of each probe was controlled for by further hybridisations with an additional anti-sense probe (see Table 1) and corresponding sense probes, in conjunction with BLAST searching all available databases.

Probes were labelled using terminal deoxynucleotidyl transferase (Roche, East Sussex, UK) and [^{35}S]dATP at 37 °C for 1 h, and were hybridised to sections as previously described [16]. Following exposure to photo-emulsion (LM-1, Amersham, Bucks) for 8 weeks, slides were developed,

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Table 1
Anti-sense oligonucleotide probes designed to investigate NAdr receptor mRNA expression

Gene	Accession number	Probe 1 oligonucleotide sequence (5′–3′)	Nucleotides	Probe 2 oligonucleotide sequence (5′–3′)	Nucleotides
$NAdr_{\alpha 1A}$	NM017191	ggagctggtgggtgggtgcagttggagccttccgaagcattttca	50–94	gctggtgggtgggtgcagttggagccttccgaagcattttcagag	47–91
$NAdr_{\alpha 1B}$	X51585	tgaaactttgctcccctccgtggtcttctttccagaggcgtcctcc	122–166	tgcccagatgtcacagaagatgcgccccagcacccagtagccaag	562–606
$NAdr_{\alpha 1D}$	L31771	cgtaccggtccacagagatggtgcagaggctaaggatggaggcag	1003–10047	ggtgaggggaacatacagttaggagtgtggggaagagggcagtgg	2666–2710
$NAdr_{\alpha 2A}$	U79031	gagtgctaggggctgcatgcgtagacgcgccccctcgaagcccag	1400–1444	agtcccctccaaactgggtattacacagagcaggaaggtccaggg	1450–1494
$NAdr_{\alpha 2B}$	M32061	ccagcgtccctacagtctgtcccagagaagttttccaagttgtcc	127–171	ggaacaggttttgtggtgcacggagtgagcggctggtcaacacag	284–328
$NAdr_{\alpha 2C}$	X57659	gccggggcgcaggttgccgttcttccgggtccctgcaactttact	400–444	cccaggtccgcgttttggccgtcggcgccgcctcgccctccccgc	301–346
$NAdr_{\beta 1}$	NM012701	tcaccaacacgttgcccactacgatgagcagcacgatgagcgcca	194–238	cgatggccacgatcaccaacacgttgcccactacgatgagcagca	206–250
$NAdr_{\beta 2}$	L39264	tcgtcgtcgctgctgttggctagctgtgctatgtggtgcagga	1–45	agacttatgccgaaccacagccacagacaccgagacacacccgcg	3019–3063

stained with cresyl violet and mounted on coverslips. Analysis of in situ hybridisation sections was carried out using an MCID image analysis system (Model M4, Imageworks) to measure grain counts within areas containing neurons of the SDH (laminae I and II), and within small (<25 μm), medium (25–45 μm) and large (>45 μm) diameter DRG neurons. To ensure this represented selective neuronal expression, probe signals were considered positive only when silver grains were visually determined to be localised to neurons, in conjunction with the corresponding calculated grain count density being significantly greater than control background counts obtained from equivalent regions from sense hybridisations (Students unpaired *t*-test; $P < 0.01$).

Within the SDH, neuron-specific positive probe signals corresponding to mRNA expression were determined for α_{1A}, α_{1B}, α_{2B}, β_1 and β_2 adrenoceptors. Analysis of the corresponding grain counts indicated that these counts were significantly ($P < 0.01$) higher than background values obtained from sense hybridisations ($n = 4$, 50 measurements per data point per probe Fig. 1A–E). No neuronal-selective signal for α_{1D}, α_{2A} or α_{2C} adrenoceptor mRNA was determined (Fig. 1F–H).

Within DRG neurons, selective expression of α_{1A}, α_{1B} and α_{2C} adrenoceptor mRNA was identified (5–10 DRGs each obtained from four animals, 50–300 measurements per data point per probe Fig. 2A–C). Analysis of the corresponding grain counts indicated significantly higher values than background values obtained from sense hybridisations ($P < 0.01$). No selective signal for α_{1D}, α_{2A}, α_{2B}, β_1 or β_2 adrenoceptor mRNA was observed within DRG neurons (Fig. 2D–H).

Whilst there is little previous data regarding precise mRNA expression patterns for α_1 adrenoceptor subtypes; α_1 binding sites have previously been identified in the dorsal horn [24]. Furthermore, a pharmacological study has suggested the presence of both α_{1A} and α_{1B} adrenoceptors and the absence of α_{1D} in the dorsal horn [28]. The current findings, are therefore, in agreement with previous reports, and suggest that the excitatory effects of α_1 adrenoceptor activation on SDH neurons [8,10] may be mediated by α_{1A} and α_{1B} subtypes.

Facilitation of presynaptic transmitter release has also been suggested to contribute to the increase in SDH neuronal excitability following α_1 adrenoceptor activation [17]. Furthermore, a recent study reported that application of an α_1 agonist evoked a depolarisation of DRG neurons [20]. The current study indicates that such actions may be mediated by α_{1A} and α_{1B} adrenoceptors expressed by DRG neurons. Although this study cannot determine that the corresponding protein products are transported centrally, it does support the suggestion of a presynaptic α_1 adrenoceptor population within the SDH [17]. As far as we are aware, this is the first report to document the presence of α_1 adrenoceptor subtype mRNA in DRG neurons.

Dense α_2 receptor binding sites within the SDH have been reported by previous studies [15,27] although most of these binding sites are located on fibres and varicosities [21], and only a small number of dorsal horn cells actually contain α_2 adrenoceptor mRNA (see [14] for review). This study reports the presence of α_{2B} adrenoceptor mRNA within SDH neurons and the presence of α_{2C} adrenoceptor mRNA within DRG neurons.

The absence of α_{2C} adrenoceptor mRNA within SDH neurons is in agreement with previous studies [15,22]. Furthermore, the presence of α_{2C} adrenoceptor mRNA found distributed throughout small, medium and large diameter DRG neurons correlates with previous findings [14,15] and suggests that the high α_{2C} immunoreactivity previously reported in nerve terminals and varicosities within the SDH is of primary afferent origin [18,26].

The absence of α_{2A} adrenoceptor mRNA is surprising and contrasts with studies demonstrating α_{2A} binding sites and mRNA in the SDH [23,26]. However, α_{2A} mRNA expression within the SDH has been reported to be sparse [15], and α_{2A} binding sites may be of predominantly presynaptic origin as α_{2A} immunoreactivity dramatically decreases following dorsal rhizotomy and neonatal capsaicin treatment [26]. Whilst a presynaptic origin of α_{2A} would correlate with the absence of α_{2A} mRNA within SDH neurons, α_{2A} mRNA was also found to be absent within DRG neurons. Whilst mRNA for α_{2A} adrenoceptors has previously been identified in a small population of DRG neurons [15], it has been suggested that α_{2A} adrenoceptor mRNA is absent from control DRG neurons [7], and is up-regulated in sensory neurons following injury [2]. As α_{2A} mRNA expression was observed within the intermediolateral cell column (unpublished observations); an area responsive to α_2 pharmacological agents [9] and known