Selective action of noradrenaline and serotonin on neurones of the spinal superficial dorsal horn in the rat

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The superficial dorsal horn of the spinal cord (SDH; laminae I and II) receives strong input from thin primary afferent fibres and is involved in nociception, pain, temperature sensing and other experiences. The SDH also is the target of serotonergic and adrenergic projections from the brain stem. The interaction between descending pathways that utilize particular mediators and the neurone population of the SDH is poorly understood. To explore this issue, in rat spinal cord slices during whole-cell recordings from identified SDH neurones, noradrenaline (NA) or serotonin (5HT) were briefly applied in the superfusing artificial cerebrospinal fluid. The action of these agents proved specifically related to the type of SDH neurone and its dorsal-root afferent input. Vertical, radial and tonic central lamina II cells consistently expressed outward current to both NA and 5HT, but transient central and Substance P (SP)-insensitive lamina I cells were unaffected directly by either NA or 5HT. Extended islet cells responded with outward current to NA and inward current to 5HT. Lamina I SP-sensitive cells expressed an outward current regularly to NA. 5HT had inhibitory effects on Aδ and C fibre input to all types of SDH neurones. NA inhibited C fibre input to transient central neurones. The present results support the idea that descending systems may have multiple functions, including but not limited to nociceptive modulation.

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The superficial dorsal horn (SDH) of the spinal cord (laminae I and II) receives terminals from many thinly myelinated and unmyelinated primary afferent fibres. It is involved in nociception, pain, temperature sensing and other experiences (Light & Perl, 1979, 2003; Light et al. 1979; Perl, 1984, 1992; Sugiura et al. 1986; Andrew & Craig, 2001; Wilson et al. 2002; Ling et al. 2003; Mason, 2005). Neurones of the SDH are also the target of descending pathways from the brain stem that utilize monoamine mediators (Satoh et al. 1982; Miletic et al. 1984). These descending projections from the brain stem received special attention following the report by Reynolds (1969) that electrical stimulation of the periaqueductal grey produces analgesia sufficient to permit surgery. Pathways responsible for such stimulus-produced analgesia (SPA) utilize noradrenaline (NA) or serotonin (5HT) to mediate their spinal effects (Basbaum & Fields, 1978; Reddy & Yaksh, 1980; Aimone & Gebhart, 1987; Aimone et al. 1987; Mason, 2001).

Exogenous NA and 5HT are described to have a variety of actions on SDH neurones. NA is reported to produce hyperpolarization in the majority of spinal and trigeminal lamina II neurones by activation of adrenergic α2 receptors (North & Yoshimura, 1984; Grudt et al. 1995); induce depolarization in some lamina II neurones mediated by activation of adrenergic α1 receptors (North & Yoshimura, 1984; Grudt et al. 1995); increase the frequency of GABAergic and glycinergic spontaneous IPSPs in most lamina II neurones through the activation of presynaptic adrenergic α1 receptors (Baba et al. 2000a,b). Inhibition of dorsal root-evoked EPSCs in some lamina II neurones by activation of presynaptic adrenergic α2 receptors is also described (Li & Zhuo, 2001; Kawasaki et al. 2003).

Similarly, 5HT has been variously reported to hyperpolarize 70% of lamina II neurones by activating 5HT1A receptors (Grudt et al. 1995); to depolarize 9% of lamina II neurones (Grudt et al. 1995); to increase the frequency of spontaneous IPSPs in 20% of lamina II neurones (Grudt et al. 1995); to evoke biphasic excitatory and inhibitory activity (Hori et al. 1996). Further, 5HT has been found to suppress dorsal root-evoked EPSCs in some lamina II neurones (Ito et al. 2000; Li & Zhuo, 2001). The receptors mediating some 5HT effects remain unclear, in many instances due to lack of selective agonists
and antagonists (see review by Yoshimura & Furue, 2006).

The SDH is a complex region containing a number of different types of neurones distinguished by combinations of morphological and functional features (Grudt & Perl, 2002; Grudt et al. 2002; Lu & Perl, 2003, 2005a; for other criteria used to classify SDH neurones, see Ruscheweyh & Sandkühler, 2002; Graham et al. 2004, 2007). Variability in the reported effects of exogenous NA and 5HT could be due to differences among mixed samples of SDH neurones. Although both noradrenergic- and serotonergic-mediated connections have been implicated in descending modulation, how their actions interact remains murky. In other words, do the descending systems utilizing NA and 5HT affect different populations of neurones or are their actions overlapping? We undertook to test the hypothesis that NA and 5HT act differently on particular types of SDH neurones. Preliminary observations have been communicated at scientific meetings (Lu & Perl, 2002, 2005b).

Methods

The Institutional Animal Care and Use Committee of the University of North Carolina, Chapel Hill approved all procedures on living animals. Young adult Sprague–Dawley rats (6–10 weeks; Charles River) were deeply anaesthetized with urethane (1.5 g kg\(^{-1}\), i.p.). The lumbosacral spinal cord (L1–S3) was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O\(_2\) and 5% CO\(_2\). Harvesting of the spinal cord usually led to cessation of breathing and cardiac arrest but in the cases where this did not happen, animals were killed by an overdose of pentobarbital (100 mg kg\(^{-1}\), i.p.). The spinal cord was cut on a vibrating microtome into 600–800 \(\mu\)m transverse or 400–600 \(\mu\)m sagittal slices with attached segmental dorsal roots. The spinal slices were kept at room temperature (22–25\(^\circ\)C) in standard ACSF (mm: 125 NaCl, 2.5 KCl, 2 CaCl\(_2\), 1 MgCl\(_2\), 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), and 25 d-glucose) equilibrated with 95% O\(_2\) and 5% CO\(_2\).

Electrophysiological recording procedures, labelling and identification of neuronal types followed details described in previous publications (Grudt & Perl, 2002; Grudt et al. 2002; Lu & Perl, 2003, 2005a). Slices were viewed with visible light and infrared to locate cellular profiles targeted for recording. Tight-seal, whole-cell recordings were made with patch-type pipette electrodes containing biocytin (0.5%) in an internal solution consisting of (mm): 130 potassium gluconate, 5 KCl, 4 Mg-ATP, 10 phosphocreatine, 0.3 lithium-GTP, 10 Hepes, pH 7.3, 300 mosmol l\(^{-1}\).

Afferent volleys from the segmental dorsal root were initiated by graduated electrical pulses (0.1–0.5 ms) applied through a suction electrode. Conduction velocities (CV) of primary afferent fibres evoking monosynaptic EPSPs were estimated from the latency of the evoked response and the conduction distance, ignoring utilization time and synaptic delay. (The latter had minimal effect on CV calculations.) The primary afferent fibres evoking responses were judged to be C fibres if the calculated CV < 0.8 m s\(^{-1}\) and A\(\delta\) fibres if the CV was > 1.0 m s\(^{-1}\) (Lynn & Carpenter, 1982; Lawson et al. 1997; Lu & Perl, 2005a). A dorsal root (DR)-evoked response was judged to be monosynaptic if its latency varied little in repetitive trials (Yoshimura & Nishi, 1993; Li & Perl, 1994). The DR monosynaptic EPSPs were mediated by glutamate (Schneider & Perl, 1988; Yoshimura & Jessell, 1990).

The firing pattern of each neurone was determined in current clamp to 1 s depolarizing pulses (−60 to −20 mV) from a holding potential of −60 mV. After completion of the electrophysiological observations, the spinal slice was fixed by immersion in a cold solution of 4% paraformaldehyde–phosphate buffer for 3–8 days. Following cryoprotection, transverse slices were sectioned in a parasagittal plane at 60 \(\mu\)m in a cryostat. Sagittal slices were processed whole. The avidin–biotin complex reaction with a fluorescent label was used to visualize cells labelled with biocytin during whole-cell recordings. Marked neurones were viewed in a compound microscope fitted with a digital camera and in a confocal microscope.

Chemical agents were briefly applied in the superfused ACSF. The concentrations of NA and 5HT used in the present study are based on dose–response observations. NA at 20 \(\mu\)M and 5HT at 10 \(\mu\)M evoked nearly maximal responses. Application of chemical agents began 10 min after establishing whole-cell recording and compounds were applied at 15 min intervals to allow recovery. After the test of effects of NA and 5HT on DR-evoked responses, 1–2 \(\mu\)M tetrodotoxin (TTX) was added to ACSF to block action potentials so as to permit testing of direct effects on the recorded neurone. Biocytin, 5HT, NA, phenylephrine, prazosin, SP, TTX and yohimbine were obtained from Sigma (St Louis, MO, USA). UK14304 was obtained from Research Biochemicals International (Natick, MA, USA). Numerical data are presented as the mean ± s.e.m. Two-sample Student’s \(t\) test was used to estimate the possibility that differences in values could have occurred by chance (\(P < 0.05\) was considered significant).

Results

Tight-seal, whole-cell recordings were made from over 300 SDH neurones. The variety of cell categories in this sample are consistent with those found in previous studies on hamster and rat (Grudt & Perl, 2002; Grudt et al. 2002; Lu & Perl, 2003, 2005a). The criteria used to classify SDH neurones are summarized in Supplementary Table 1. Morphological and electrophysiological details were sufficient to allow identification of 52 recordings to be from neurones classified as vertical, 26 as radial, 48 as transient-central, 53 as islet, 31 as
Table 1. NA and 5HT direct effects on SDH neurones

<table>
<thead>
<tr>
<th>Cell type (cell number)</th>
<th>NA (20 μM)</th>
<th>SHT (10 μM)</th>
<th>Inward current (pA)</th>
<th>NA (20 μM)</th>
<th>SHT (10 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical (52)</td>
<td>43.6 ± 11.3 (n = 52)</td>
<td>39.2 ± 9.8 (n = 52)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Radial (26)</td>
<td>41.9 ± 8.9 (n = 26)</td>
<td>40.1 ± 12.3 (n = 26)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Transient central (48)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Islet (35)</td>
<td>39.2 ± 9.3 (n = 27)</td>
<td>54.6 ± 12.5 (n = 6)</td>
<td>38.6 ± 6.9 (n = 8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Extended islet (18)</td>
<td>32.2 ± 11.3 (n = 18)</td>
<td>0</td>
<td>0</td>
<td>55.1 ± 9.3 (n = 18)</td>
<td></td>
</tr>
<tr>
<td>Tonic central (31)</td>
<td>44.7 ± 8.6 (n = 31)</td>
<td>42.6 ± 9.5 (n = 31)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lamina I SP sensitive (18)</td>
<td>33.8 ± 13.2 (n = 15)</td>
<td>40.6 ± 6.5 (n = 3)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lamina I SP insensitive (12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Values given are the mean ± S.E.M. 0, no noticeable base line fluctuation for tests with concentration up to 50 μM NA; 30 μM SHT.

Some cell categories showed no effects, others exhibited outward currents, and still others inward currents (Figs 1C, 2C, 3C and 5C; Table 1). Regardless of neuron type, NA outward currents were completely blocked by the adrenergic α2 receptor antagonist yohimbine (10 μM, n = 11, not shown). The α2 receptor agonist UK14304 (5 μM), also induced outward current in cells showing tonic-central, 18 as lamina I SP-sensitive and 12 as lamina I SP-insensitive.

General actions of NA and 5HT

The direct actions of NA (20–50 μM in the presence of TTX) varied according to the category of SDH neurone.

Figure 1. Effects of NA and 5HT in SDH vertical cells

A, confocal image of the biocytin-stained vertical cell from which the electrophysiological recordings (B–D) were obtained. B, action potential firing pattern of the vertical neurone (A) in response to a 1 s duration depolarizing pulse. C, chart recordings from the neurone (A) illustrating NA- and SHT-induced outward currents in voltage clamp (holding potential: −60 mV). D, voltage-clamp, whole-cell recordings showing that the Aδ fibre EPSCs evoked by dorsal root stimulation in the neurone (A) were suppressed by SHT but not by NA. E, schematic summarizing the effects of exogenous NA and SHT on vertical neurones and their DR inputs. Vertical neurones are inhibited by both NA and SHT; their DR input is suppressed solely by SHT. DR, dorsal root; C, caudal; D, dorsal; R, rostral; V, ventral; −, inhibition; +, excitation.
outward NA current blocked by yohimbine (n = 11). In contrast, the directly produced NA inward currents were blocked by the adrenergic α1 receptor antagonist prazosin (1 μM, n = 6, not shown). The α1 receptor agonist phenylephrine (10 μM) initiated inward currents in neurons exhibiting block of inward currents by prazosin (n = 6, not shown).

NA at 20 μM suppressed the amplitude of DR monosynaptic C fibre EPSCs (eEPSCs) evoked in certain neurone categories (Fig. 2D, Table 2), but had no effect on Aδ fibre eEPSCs (Fig. 1D, Table 2) in vertical and radial cells. The NA action on eEPSCs was mimicked by the α2 receptor agonist UK14304 (5 μM, n = 12, not shown). The α1 receptor agonist phenylephrine (10 μM) had no effect on the eEPSCs (n = 7, not shown). These results suggest that the direct inhibitory effects of NA on SDH neurones and their C primary afferent inputs were mediated through the activation of α2 receptors. On the other hand, excitatory actions of NA on certain SDH neurones involved adrenergic α1 receptors. These results are consistent with previous studies (North & Yoshimura, 1984; Baba et al. 2000a,b; Yoshimura & Furue, 2006).

Serotonin (10–30 μM in the presence of TTX) also had variable effects depending upon the SDH neurone category: no direct action on SDH neurones (Figs 2C and 5C; Table 1); induced outward current or evoked inward currents (Figs 1C and 3C; Table 1). Serotonin at 10 μM suppressed the amplitude of Aδ (Fig. 1D) and C fibre (Figs 2D, 3D and 5D and Table 2) eEPSCs.

**Actions of NA and 5HT on identified categories of neurones**

**Vertical and radial neurones.** Both NA (20 μM) and 5HT (10 μM) evoked outward currents in all tested vertical (52/52, Fig. 1C, Table 1) and radial (26/26, Table 1) cells. Serotonin at 10 μM suppressed the amplitude of Aδ eEPSCs to all tested vertical (n = 18, Fig. 1D, Table 2).

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**Figure 2. Effects of NA and 5 HT in transient central cells**

A, confocal image of the biocytin-stained transient central cell from which the electrophysiological recordings (B–D) were obtained. B, action potential firing pattern of this transient central neurone in response to a 1 s duration depolarizing pulse. C, chart recordings showing lack of direct effect on the transient central cell by either NA or 5HT (holding potential: −60 mV). D, voltage-clamp, whole-cell recordings showing C fibre monosynaptic EPSCs evoked by dorsal root stimulation were suppressed by both NA and 5HT. E, schematic summarizing the effects of NA and 5HT on transient central neurones and their DR inputs. Transient central neurones do not show direct action by NA and 5HT; their DR input is inhibited by both NA and 5HT. DR, dorsal root; C, caudal; D, dorsal; R, rostral; V, ventral; −, inhibition; +, excitation.

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and to all tested radial neurones \( (n = 9, \text{Table 2}) \). NA at 20–50 μm had no effect on Aδ eEPSCs in vertical \( (n = 18, \text{Fig. 1D, Table 2}) \) and radial \( (n = 9, \text{Table 2}) \) cells.

**Transient central neurones.** Transient central neurones were unaffected directly by 10–50 μm NA or 10–30 μm 5HT (no noticeable base line fluctuation, 48/48, Fig. 2C; Table 1). On the other hand, NA at 20 μm and 5HT at 10 μm suppressed the amplitude of C fibre eEPSCs in all transient central neurones tested \( (n = 21, \text{Fig. 2D and Table 2}) \).

**Islet neurones.** Of the 35 islet neurones, 21 had outward current induced by NA at 20 μm, 6 had an outward current in response to both 20 μm NA and 10 μm 5HT, and 8 showed an inward current to 20 μm NA (Table 1). The NA-induced depolarization of ‘standard’ islet neurones evoked action potentials (not shown). Serotonin at 10 μm suppressed the C fibre eEPSCs of islet cells \( (12/13, \text{Table 2}) \).

**Extended islet neurones.** A subset \( (18) \) of islet-type neurones with cell geometry and electrophysiological features similar to the standard type had a dendritic tree that was notably more extensive in the rostrocaudal direction \( (768 ± 128 \mu m, \text{Fig. 3A}) \). These extended islet cells also differed from the standard islet category by expression of an outward current to 20 μm NA and an inward current to 10 μm 5HT \( (18/18, \text{Fig. 3C, Table 1}) \). Serotonin at 10 μm suppressed the C fibre eEPSCs to extended islet \( (\text{Fig. 3D, Table 2}) \).

Excitation of extended islet cells by 5HT could evoke action potentials \( (\text{Fig. 4C}) \). In three simultaneous recordings from an extended islet cell and a transient central neurone, 5HT-induced action potentials in the extended islet cells evoked GABA-mediated IPSPs (bicuculline-sensitive) in the transient central cells \( (\text{Fig. 4C and D}) \).

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*Figure 3. Effects of NA and 5 HT in extended islet neurones and their dorsal root inputs*

A, confocal image of a biocytin-stained extended islet neurone from which the electrophysiological recordings \( (B–D) \) were obtained. B, action potential firing pattern of this extended islet neurone in response to a 1 s depolarizing pulse. C, chart recordings showing NA-induced outward current and 5HT-induced inward current. D, voltage-clamp, whole-cell recordings demonstrating suppression of the C fibre EPSCs evoked by dorsal root stimulation by 5HT but not by NA. E, schematic summarizing the effects of NA and 5HT on extended islet neurones and their DR inputs. The extended islet neurones show inhibition by NA and excitation by 5HT; their DR input is inhibited by 5HT. DR, dorsal root; C, caudal; D, dorsal; R, rostral; V, ventral; −, inhibition; +, excitation.

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Table 2. NA and 5HT effects on DR-evoked EPSPs/EPSCs

<table>
<thead>
<tr>
<th>Cell type</th>
<th>5HT (10 μM)</th>
<th>NA (20 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical</td>
<td>16.8% ± 5.3%, <em>P</em> &lt; 0.05, <em>n</em> = 18</td>
<td>No effect, <em>n</em> = 18</td>
</tr>
<tr>
<td>Radial</td>
<td>15.6% ± 6.5%, <em>P</em> &lt; 0.05, <em>n</em> = 9</td>
<td>No effect, <em>n</em> = 9</td>
</tr>
<tr>
<td>Transient central</td>
<td>29.6% ± 11.2%, <em>P</em> &lt; 0.05, <em>n</em> = 21</td>
<td>32.3% ± 6.7%, <em>P</em> &lt; 0.05, <em>n</em> = 21</td>
</tr>
<tr>
<td>Islet</td>
<td>36.8% ± 9.3%, <em>P</em> &lt; 0.05, <em>n</em> = 12</td>
<td>No effect, <em>n</em> = 12</td>
</tr>
<tr>
<td>Extended islet</td>
<td>26.8% ± 14.3%, <em>P</em> &lt; 0.05, <em>n</em> = 5</td>
<td>No effect, <em>n</em> = 5</td>
</tr>
<tr>
<td>Tonic central</td>
<td>31.2% ± 13.2%, <em>P</em> &lt; 0.05, <em>n</em> = 8</td>
<td>No effect, <em>n</em> = 8</td>
</tr>
<tr>
<td>Lamina I SP sensitive</td>
<td>38.9% ± 8.6%, <em>P</em> &lt; 0.05, <em>n</em> = 7</td>
<td>No effect, <em>n</em> = 7</td>
</tr>
<tr>
<td>Lamina I SP insensitive</td>
<td>39.4% ± 12.5%, <em>P</em> &lt; 0.05, <em>n</em> = 9</td>
<td>42.3% ± 11.1%, <em>P</em> &lt; 0.05, <em>n</em> = 9</td>
</tr>
</tbody>
</table>

Values given are the mean% ± S.E.M. of control amplitude. In the cases of no effect, testing was done at concentrations up to 50 M NA and 30 μM 5HT.

**Tonic central neurones.** Both NA (20 μM) and 5HT (10 μM) evoked outward currents in tonic central neurons (29/31; Table 1). C fibre eEPSCs in tonic central cells were suppressed by 5HT (10 μM) (*n* = 8, Table 2).

**Lamina I neurones.** Lamina I neurones were divided into two categories, those excited by SP (2 μM) and those unresponsive to it. SP-sensitive lamina I neurones generally expressed an outward current to 20 μM NA

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**Figure 4.** 5HT-induced action potentials in extended islet cells evoke GABA-mediated inhibition of transient central cells (simultaneous recordings from two synaptically connected neurones)

*A,* confocal image of a connected pair of neurones. Presynaptic cell: extended islet cell. Postsynaptic cell: transient central cell. *B,* action potential firing patterns of the two neurones in response to a 1 s depolarizing pulse. *C,* current-clamp chart recording (bottom trace) showing 5HT-induced depolarization and action potentials. Middle trace expands the action potential trace. Upper trace shows postsynaptic IPSPs evoked by the 5HT-generated presynaptic action potentials. *D,* schematic summarizing the effects of NA and 5HT on a SDH circuit formed by an extended islet neurone and a transient central neurone with their DR inputs. Extended (dendrite) islet neurones are excited by descending 5HT-mediated connections, and in turn inhibit transient central neurones by a GABA-mediated connection. *A–C* are from same pair. APs, action potentials; DR, dorsal root; Pre, presynaptic; Post, postsynaptic; C, caudal; D, dorsal; R, rostral; V, ventral; −, inhibition; +, excitation.
(15/18, Fig. 5C), but rarely showed an outward current to 10 μm 5HT. Dorsal root C fibre eEPSCs in SP-sensitive lamina I neurones were suppressed by 10 μm 5HT (n = 7, Fig. 5D, Table 2). SP-insensitive lamina I neurones were not affected directly by either 20–50 μm NA or 10–30 μm 5HT (12/12, Table 1); however, both NA (20 μm) and 5HT (10 μm) suppressed DR C fibre-evoked EPSCs in SP-insensitive cells (9/11, Table 2).

In summary, NA and 5HT actions vary explicitly according to the type of neurone and the nature of its DR afferent input. (1) Both NA and 5HT act directly to hyperpolarize vertical, radial and tonic central cells. (2) Transient central cells and lamina I SP-insensitive cells do not respond directly to either NA or 5HT. Transient central cells do show indirect inhibition by 5HT through their linkage to GABAergic extended islet cells. (3) NA directly inhibits the majority of lamina I SP-responsive neurones. (4) NA inhibits or excites different subsets of the standard islet cell category. Both NA and 5HT inhibit other subsets of the standard islet cells. (5) NA inhibits extended islet neurones while 5HT excites them. (6) 5HT has inhibitory effects on Aδ- and C fibre input to all types of SDH neurones. NA inhibits C fibre input to transient central neurones only.

**Discussion**

The present results show NA and 5HT actions on SDH neurones to vary as a function of the type of neurone and the nature of its DR afferent input. Inhibitory postsynaptic current (outward) or presynaptic inhibition are common; however, both 5HT and NA produce direct excitatory (depolarizing) actions on certain inhibitory neurones. NA excites about 20% of the standard islet cell category; 5HT excites all of the extended islet cell group.

![Figure 5. Effects of NA and 5HT in lamina I, substance P (SP)-sensitive neurones](image)

**A**, confocal image of a biocytin-stained lamina I SP-sensitive, neurone from which the electrophysiological recordings (**B**–**D**) were obtained. **B**, action potential firing pattern of this lamina I neurone in response to a 1 s duration depolarizing pulse. **C**, chart recordings showing SP-induced inward current, NA-induced outward current, and absence of response to 5HT. **D**, voltage-clamp whole-cell recordings showing that C fibre monosynaptic EPSCs evoked by dorsal root stimulation are suppressed by 5HT but not by NA. **E**, schematic summarizing the effects of NA and 5HT on lamina I, SP-sensitive neurones and their DR inputs. Lamina I, SP-sensitive neurones are inhibited by NA; their DR input is inhibited by 5HT. DR, dorsal root; C, caudal; D, dorsal; R, rostral; V, ventral; −, inhibition; +, excitation.

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The SDH contains excitatory (vertical, radial, transient central) and inhibitory (islet and tonic central) interneurones as well as projection lamina I neurones (Lu & Perl, 2003, 2005a; Hantman et al. 2004). These neurones form several neuronal circuits (Lu & Perl, 2003, 2005a) capable of modulating primary afferent information. The differential effects of NA and 5HT suggest that descending systems can exert control over SDH circuits through the following mechanisms: (1) direct inhibition of excitatory interneurones and projection neurones; (2) direct excitation of GABAergic inhibitory interneurones which in turn inhibit excitatory neurones; (3) direct inhibition of certain primary afferent inputs to SDH neurones. Both NA and 5HT also produce inhibitory effects on certain inhibitory interneurones (tonic central and islet cells). The functional consequences of such inhibition of inhibitory cells need further investigation.

Some uncertainty exists in classification of islet neurones. The present results suggest that islet-type cells with particularly extended dendritic expansions (over 600 μm) differ systematically from otherwise similarly configured cells with smaller dendritic expansions. The extended islet type gave uniquely opposite responses to 5HT (excitatory; inward current) and NA (inhibitory; outward current). These distinctions suggest the islet-cell category as defined in earlier work (Grudt & Perl, 2002) may represent more than one functional neuronal type.

The pharmacological receptor subtype mediating the NA and 5HT actions deserves comment. The direct inhibitory effects of NA on SDH neurones and on their C primary afferent inputs are mediated through the activation of α2 receptors. In contrast, the excitatory effects of NA on certain SDH neurones occur through activation of adrenergic α1 receptors. The observations are consistent with conclusions of previous studies (North & Yoshimura, 1984; Baba et al. 2000a,b; Yoshimura & Furue, 2006). Serotonin receptors are represented by numerous subtypes. Selective agonists and antagonists are not yet available for many of the subtypes, limiting analysis of pharmacological testing. It is reported that 5HT-induced inhibition in SDH neurones involves activation of 5HT1A receptors (Grudt et al. 1995; Ito et al. 2000); however, the receptor responsible for the 5HT-induced inhibition in the region has not been identified (Yoshimura & Furue, 2006). Further analysis of 5HT-receptor subtype actions on SDH neurones awaits availability of more selective pharmaceutical agents.

Although the descending modulation of dorsal horn neurones often has been cast in the light of anti-nociception and analgesia (Reynolds, 1969; Basbaum et al. 1976; Basbaum & Fields, 1978; Yoshimura & Furue, 2006), Mason (2001, 2005) has proposed that this focus fails to account for the nature of descending effects on dorsal horn neurones under various physiological and behavioural conditions. Whereas projections descending from the brain stem can powerfully alter nociceptive transmission, they do not act exclusively on pain-related activity. Recent evidence indicates that primary afferents other than nociceptors project to the spinal dorsal horn (Andrew & Craig, 2001; Wilson et al. 2002; Light & Perl, 2003; Ling et al. 2003). Afferent neurones of visceral organs and skeletal muscle as well as cutaneous unmyelinated C afferent fibres terminate substantially in lamina II. At the very least, lamina II seems to participate in integration or modulation of afferent information from subcutaneous sources. Thus, ideas about the function of the superficial dorsal horn concentrating on nociception and pain appear too restricted (Light & Perl, 2003). The present results are consistent with the idea that descending systems from the brain stem serve several functions, not limited to nociceptive modulation (Mason, 2001, 2005).

References


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Supplemental material

Online supplemental material for this paper can be accessed at: http://jp.physoc.org/cgi/content/full/jphysiol.2007.131565/DC1 and http://www.blackwell-synergy.com/doi/suppl/10.1113/jphysiol.2007.131565