Spinal Allografts of Adrenal Medulla Block Nociceptive Facilitation in the Dorsal Horn

IAN D. HENTALL, 1,2 BRIAN R. NOGA, 1 AND JACQUELINE SAGEN1
1The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, Florida 33136; and 2Department of Biomedical Sciences, University of Illinois College of Medicine, Rockford, Illinois 61107

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Hentall, Ian D., Brian R. Noga, and Jacqueline Sagen. Spinal allografts of adrenal medulla block nociceptive facilitation in the dorsal horn. J Neurophysiol 85: 1788–1792, 2001. Transplantation of chromaffin cells into the lumbar subarachnoid space has been found to produce analgesia, most conspicuously against chronic neuropathic pain. To ascertain the neurophysiological mechanism, we recorded electrical activity from wide-dynamic-range dorsal horn neurons in vivo, measuring the short-lasting homosynaptic facilitatory effect known as windup, which is induced by repetitive C-fiber input. Rats were given adrenal medulla allografts, or, as controls, striated-muscle allografts. The adrenal-transplanted rats showed analgesia 3–4 wk after transplantation, measured as a reduction in flinching reflexes 30–55 min after subcutaneous formalin injection. Recordings were made under halothane anesthesia, 3–7 days following the behavioral testing. The average C-fiber response and subsequent afterdischarge were facilitated severalfold in control rats by 1-Hz cutaneous electrical stimulation. Such facilitation was essentially absent in adrenal-transplanted animals and also in the A-fiber response of both preparations. Extirpation of transplanted tissue several hours prior to recording did not significantly affect this difference. In conclusion, the adrenal transplants block short-term spinal nociceptive facilitation, probably by stimulating some persistent cellular process that may be an important determinant, but not the only one, of their analgesic effect.

INTRODUCTION

Persistent pain, induced experimentally in rats and inferred from their cutaneous withdrawal reflexes, is lowered by transplanting isolated adrenal chromaffin cells, or fragments of adrenal medulla tissue, into the spinal subarachnoid space. For example, such transplants depress the prolonged (1-h) period of flinching that follows subcutaneous formalin injection (Sagen and Sagen 1997). They also reverse chronic neuropathic hyperreflexia of central or peripheral origin, for example, that which follows chronic constriction injury of the sciatic nerve (Decosterd et al. 1998; Hama and Sagen 1994a; Yu et al. 1998) or excitotoxic spinal cord injury (Brewer and Yezierski 1998). In humans, implantation of chromaffin cells is reported to relieve cancer pain (Lazorthes et al. 2000; Winnie et al. 1993).

How chromaffin cells cause this persistent analgesia is unclear, although cytochemical and behavioral studies have identified several affected processes in the spinal dorsal horn. The continuous presence of inhibitory neurotransmitters released from chromaffin cells, in particular catecholamines and enkephalins, has been shown to be important (Sagen and Kemmler 1989; Sagen et al. 1991; Siegan and Sagen 1997). Indeed, catecholamine release is enhanced by a factor present in the cerebrospinal fluid of rats with chronic neuropathic pain (Hentall and Sagen 2000). In addition, the prolonged cytochemical changes seen in animal models of chronic pain are blocked. These include quantitative increases in c-fos protein (Sagen and Wang 1995), nitric oxide synthase (Hama and Sagen 1994b), and cyclic GMP (Siegan et al. 1996), all of which can be initiated by activation of excitatory N-methyl-D-aspartate (NMDA) subtype glutamate receptors independently of catecholamines or opioid peptides. The decrease in numbers of GABA-containing interneurons is also opposed (Ibuki et al. 1997).

Until now there have been no neurophysiological studies of cell-transplant analgesia, regardless of the donor cells or target tissue. We examine here the wide-dynamic-range (WDR) cells of the dorsal horn. WDR neurons, typically located in lamina 5, are thought to carry the principal ascending signal that leads to perceived pain (Price and Dubner 1977). Their mecanosensory input includes large-diameter, myelinated Aβ cutaneous afferents, which respond to nonnoxious mechanical stimulation. Their specifically nociceptive inputs arrive in slowly conducting, unmyelinated C-fibers and myelinated, small-diameter A-delta fibers. When C-fibers are repetitively stimulated at certain rates (around 0.5–2 Hz, but not as slow as 0.1 Hz), they evoke progressively increasing responses. This homosynaptic facilitation, known as windup, requires activation of NMDA-subtype glutamate receptors (Haley et al. 1990; Woolf and Thompson 1991) but outlasts the tetanic stimulus by only a few seconds. It thus offers an advantageous neurophysiological model in which repeated tests can be made on the same preparation.

METHODS

Experiments were carried out under a protocol approved by the Institutional Animal Care and Use Committee of the University of Miami School of Medicine. Donors and recipients were adult male Sprague-Dawley rats. Small pieces of tissue (largest dimension <0.1 mm) were taken from two adrenal medullae or from an equal volume of striated muscle serving as a control. The tissue was injected through...
a slit in the dura, exposed by a laminectomy over the L1 vertebra. The 27-gauge injection needle was pointed rostrally, and the cells usually implanted themselves on the pial surface of the cord under the T13 and L1 vertebrae, covering the region where the L3–S1 dorsal roots enter (i.e., spinal segments L3–S1). The immunosuppressant drug cyclosporine-A (10 mg/kg ip) was given for 4 days, beginning 1 day before transplantation.

Three to 4 wk later, nociception was tested once per animal by subcutaneously injecting 5% formalin into the intraplantar surface of a hind limb, and monitoring the consequent flinching movements. Three to 7 days after testing with formalin, the laminectomy was re-opened and extended rostrally for several segments. The two periods from implantation to either formalin testing or recording fell well within times when adrenal transplants have been found previously to produce hyperalgesia and allodynia. These effects can be measured within 2 wk of transplantation and last at least the 6-wk duration of the experimental neuropathic pain state (Sagen 1990). Furthermore, transplanted adrenal medulla cells have been found to be viable histologically and to produce catecholamines for at least 6 mo (Sagen and Kemmler 1989; Sagen et al. 1991).

All surgical procedures were performed with the rats under halothane anesthesia (1–1.5%, in air), given through a face mask for induction and then through a tracheal cannula, at a concentration set to prevent corneal reflexes. The rats breathed spontaneously. An intravenous (jugular) line delivered lactated Ringer solution for fluid replacement. Arterial (intracarotid) blood pressure and expired carbon dioxide were monitored; recording was abandoned if these two variables fell outside their normal ranges. Body temperature was main-
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FIG. 1. Flinching reflexes following subcutaneous injection of formalin into the plantar region of the hind paw, showing averages from the 2 treatment groups. Standard error bars are shown.

The formalin-evoked flinching occurred in two phases, roughly 1–5 min and 15–60 min after injection, as found previously (Siegan and Sagen 1997). The two preparations had significantly different mean responses ($P = 0.014$, ANOVA) over the entire 1-h measurement period (Fig. 1). When individual times were analyzed separately, significant differences occurred only at times between 30 and 55 min after injection (Fisher’s post hoc test, $P < 0.05$), with the adrenal-transplanted animals ($n = 5$) showing much less flinching than muscle-transplanted ones ($n = 5$).

A sample of 27 neurons was recorded, 12 from adrenal-transplanted rats and 15 from muscle-transplanted rats, including 6 cells rostral to intact adrenal transplants and 5 cells rostral to intact muscle transplants. In 18 neurons, recordings were sufficiently stable to test windup three times. Spontaneous firing rates were not significantly different (2-sided $t$-test), respectively, $3.5 \pm 1.2$ (mean $\pm$ SE) and $1.9 \pm 0.6$ spikes/s in adrenal-transplanted and muscle-transplanted rats. Also, the mean number of cells found per microelectrode trajectory (1.0 for adrenal transplants and 1.33 for muscle transplants) showed no significant difference.

Examples of windup tests from the two preparations are

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Examples of wide-dynamic-range (WDR) neurons in adrenal-transplanted (A) and muscle-transplanted (B) rats responding to cutaneous stimulation of C-fibers at 1 Hz. Each marker (dot) represents 1 action potential, plotted against time on both axes so that each column of dots is separated by roughly 1 s. The continuous line is the average number of dots (spikes) in consecutive 20-ms periods, plotted on the horizontal axis to make 1 spike scale the same as 1 s.
presented in Fig. 2, and the averages of all tests are shown in Fig. 3. The main numerical results appear in Table 1. Fast stimulation did not potentiate A-fiber responses in either preparation. The stimulus current, set to cause a fixed C-fiber response, did, however, cause a larger baseline A-fiber response in controls (Fig. 3A). Both C-fiber responses (average 328%) and afterdischarges (average 484%) were potentiated in controls, but in adrenal-transplanted rats neither was potentiated to any meaningful extent. The difference between preparations was significant, at $P = 0.009$ (C-fiber) and $P = 0.00004$ (afterdischarge). When all recordings made rostral to intact transplants were excluded from the analysis, the same significant contrasts emerged at $P = 0.031$ (C-fiber) and $P = 0.006$ (afterdischarge). When only the transplant-intact recordings were examined, the results appeared quantitatively similar, but there were too few cells for statistical comparison.

**DISCUSSION**

These results show that adrenal medulla transplants block short-term, homosynaptic facilitation of nociception in the dorsal horn (i.e., windup), but have no discernible action on nonnoxious (A-fiber) transmission there. The two facilitated measures, the C-fiber response and the afterdischarge, are probably two aspects (maximum response and duration) of the same excitatory synaptic mechanism (Dickenson and Aydar 1991), although the latter may have a larger component due to positive modulatory actions of co-released substance P on NMDA receptors (Rusin et al. 1992). The facilitation seen in rats with control transplants was essentially similar to that reported in normal animals and in psychophysical studies of humans (Gozariu et al. 1997; Haley et al. 1990; Price et al. 1994; Woolf and Thompson 1991).

NMDA receptors play an essential role both in windup, which disappears after a few seconds, and in the chronic hyperalgesia and allodynia induced by experimental peripheral neuropathy, which may last many weeks. Yet varied evidence, including the disparate time courses, suggests these phenomena are not equivalent (Herrero et al. 2000; Woolf 1996). Therefore the suppressant effect of adrenal transplants on chronic pain is not necessarily due to a blocking of the windup mechanism. Nevertheless, an established state of chronic hyperalgesia may augment the effect of windup, and conversely, windup may facilitate induction of such states, as recently suggested (Sandkühler 2000). This implies that adrenal trans-

**TABLE 1. Mean potentiation of sampled WDR cells by 1-Hz cutaneous stimulation**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>n</th>
<th>A-Response</th>
<th>C-Response</th>
<th>Afterdischarge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal transplant, combined</td>
<td>8</td>
<td>108 ± 19</td>
<td>127 ± 32*</td>
<td>127 ± 34*</td>
</tr>
<tr>
<td>Adrenal transplant, acutely extirpated</td>
<td>4</td>
<td>130 ± 37</td>
<td>124 ± 30*</td>
<td>128 ± 62*</td>
</tr>
<tr>
<td>Adrenal transplant, intact</td>
<td>4</td>
<td>86 ± 7</td>
<td>128 ± 56</td>
<td>127 ± 40</td>
</tr>
<tr>
<td>Muscle transplant, combined</td>
<td>10</td>
<td>95 ± 7</td>
<td>328 ± 50*</td>
<td>484 ± 161*</td>
</tr>
<tr>
<td>Muscle transplant, acutely extirpated</td>
<td>7</td>
<td>93 ± 10</td>
<td>310 ± 58*</td>
<td>504 ± 185*</td>
</tr>
<tr>
<td>Muscle transplant, intact</td>
<td>3</td>
<td>98 ± 7</td>
<td>380 ± 120</td>
<td>233 ± 170</td>
</tr>
</tbody>
</table>

Values are means ± SE in percent; n is number of cells. WDR, wide-dynamic-range. *Significant difference ($P < 0.05$) between cells in muscle-transplanted and adrenal-transplanted rats.

**FIG. 3.** Averaged responses of WDR neurons during a 1-Hz, high-amplitude tetanus, comparing samples from adrenal-transplanted and control animals. The A-fiber (A), C-fiber (B), and afterdischarge (C) responses are graphed along with their standard error bars. Note that the amplitude was set experimentally to give a fixed C-fiber response to the 1st stimulus, which is why the other 2 responses show different starting values.
plants, in their suppression of windup, can influence chronic pain states in two ways. First, they can reduce the likelihood that such a state will develop or survive. Second, they can lessen the pain produced by sustained stimuli during this state.

Windup, being expressible as a ratio, is fairly insensitive to experimental factors that are difficult either to control or replicate, such as the depth of anesthesia, the somatic position of the receptive field, and the location of the stimulus. Unfacilitated responses, in contrast, are not amenable to scale-free measurement and so are harder to compare across neurophysiological preparations. They may also be less susceptible to transplants, unless a chronic pain state has been induced. For example, only the later stages of the second phase of formalin-induced flinching was found to be blocked by adrenal transplants in the present experiments. This second phase, but not the first, depends on NMDA receptors (Haley et al. 1990) and hence presumably on prior activity. Also, previous work has shown that adrenal transplants do not reliably suppress reflexes caused by brief noxious stimulation of normal skin unless catecholamine release is enhanced by systemic administration of nicotine (Sagen et al. 1986). In rats with chronic painful neuropathies, catecholamine release by transplants appears to be greater (Hentall and Sagen 2000), which likely results in their continuous inhibitory action contributing more to the analgesia.

The nature of the mechanism (or mechanisms) responsible for the observed block of windup is at present unclear. Analgesic substances released from chromaffin cells include not only the catecholamines norepinephrine and epinephrine, but also the co-released peptides met-enkephalin and neuropeptide Y (Christton et al. 1997). All four compounds have spinal analgesic activity and inhibit dorsal horn neurons (Yaksh et al. 1999). Adrenal transplants have been found to release large amounts of the catecholamines and met-enkephalin for many months (Sagen and Kemmler 1989; Sagen et al. 1991). Yet the effect of these substances on windup seems to be modulatory, rather than one of direct interference with the critical, NMDA-initiated cascade (Herrero et al. 2000). Moreover, the persistence of the block following removal of transplants implies that the mechanism does not depend on the continuous presence of inhibitory substances. Thus it is worth considering the role of other neuroactive compounds found in chromaffin cells, some of which have neurotrophic or neuroprotective effects (Unsicker 1993). The only one known to have antagonistic influences on the NMDA receptor is the peptide histogranin (Rogers and Lemaire 1993). Recently, the stable analogue ser-histogranin, given intrathecally 15 min either before or after intrathecal NMDA, was reported to prevent the subsequent 1–2 h of hyperalgesia and allodynia normally produced in rats (Hama et al. 1999). The cause of this prolonged influence is unknown, but the unbound molecules are likely to have disappeared much earlier, due to diffusion, enzymatic degradation, blood flow, and cerebrospinal fluid flow. Perhaps histogranin binds persistently to NMDA receptors or causes an enduring reversal of some NMDA-mediated second-messenger effect.

We conclude that spinally transplanted chromaffin cells prevent the normal development of windup in WDR neurons, most likely by liberating molecules that persistently block either the NMDA receptors themselves or cellular events mediated by these receptors. Suppressed windup can partly account for the analgesia produced by adrenal transplants, although a reduction in resting (unfacilitated) transmission by continuously released inhibitory molecules may also contribute.

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REFERENCES


