Sensory hyperinnervation after neonatal skin wounding: effect of bupivacaine sciatic nerve block

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The response to tissue injury includes sensitization of peripheral nociceptors and central neuronal pathways leading to acute clinical and inflammatory pain. A further response is sprouting of sensory nerve terminals in the region of skin damage. This hyperinnervation response is particularly intense in neonates compared with adults. In this study, we tested the effect of regional nerve block at the time of injury on skin hyperinnervation. Anaesthetized newborn rat pups were treated with percutaneous sciatic nerve block injections of 0.25% bupivacaine 25 µl followed by a localized hindpaw skin wound. Cutaneous innervation was studied by image analysis of immunostained skin sections, 7 days after wounding, and sensory thresholds were assessed using von Frey hairs. The results showed that both hyperinnervation and hypersensitivity were not significantly altered by the application of a regional nerve block at the time of injury. This suggests that regional analgesia, used commonly in clinical practice, is unlikely to prevent the hyperinnervation that follows skin wounding.

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Inflammation in early life differs from that in the adult such that tissue injury in a critical period of early life may result in long-term effects on the developing nervous system that are not observed when the injury occurs in adult life. One striking consequence of neonatal inflammation and skin damage is an intense sprouting response in local sensory nerve terminals. This hyperinnervation is most marked and long lasting when skin loss occurs early in development and becomes a weak and transient response in more mature animals. In addition, hypersensitivity to mechanical stimuli is detected for a prolonged period after the wound has healed.

Local anaesthetic agents have a central role in the provision of clinical analgesia to human neonates in the surgical setting. We tested if the presence of a nerve block at the time of tissue injury alters the prolonged hyperinnervation and sensory changes that follow neonatal tissue injury in an animal model.

Methods and results

After obtaining approval for the study (Home Office licence No. 70/13878), we studied Sprague–Dawley rat pups from postnatal day 0. Pups were anaesthetized by hypothermia using an ice–water slurry. During anaesthesia, a percutaneous sciatic nerve block was performed unilaterally with 0.25% bupivacaine 25 µl (Marcaine) and epinephrine 1:200 000 (n=12). Ten pups had sciatic injections of sterile saline and were used as controls.

While still anaesthetized and directly after sciatic nerve block injection, standard wounds were made on the dorsal surface of both hindpaws. Percutaneous sciatic injections were repeated 6 and 24 h after wounding.

Sciatic injections of saline resulted in no observable motor deficit and sensory thresholds were unchanged after recovery from anaesthesia. Bupivacaine injection caused an immediate dense unilateral motor block of the appropriate hindlimb. Paralysis of the limb lasted 4–6 h. No permanent motor deficits were detected.

Pups were sacrificed 7 days after wounding using barbiturate injection and perfused with paraaldehyde. Both hindpaws were collected for sectioning. Tissue sections, 100-µm thick, were cut on a freezing microtome for immunohistochemical staining with protein gene product (PGP) 9.5. The degree of hyperinnervation was quantified as the percentage area of staining in a standardized frame (approximately 0.2 mm²) drawn on captured digital images (Leica Image analysis–Qwin).

Skin sections obtained 7 days after wounding showed vigorous axonal sprouting in the epidermis and subepidermal regions (Fig. 1). Subepidermal neural bundles were seen to be drawn up into the region of wound healing and to divide into a dense plexus of new terminals just below the wound surface. Quantitative analysis of the density and pattern of innervation showed that there was no change in the hyperinnervation response when wounds...
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Fig 1 A: Photomicrographs showing hyperinnervation of the hairy skin of the hindpaw. Cross-section (upper) and longitudinal section (lower) obtained from a 7-day-old pup after neonatal wounding and immunostained for protein gene product (PGP) 9.5. The scale bars (vertical 0.25 mm, horizontal 0.8 mm) also represent the measuring frame used to quantify the density of innervation. B: Development of hypersensitivity after skin wounding. Thresholds for the flexion withdrawal reflex were determined using von Frey hairs. The normal pattern of increasing threshold values (mean (SEM), n=12) in unwounded rat pups is shown. Wounded pups with bupivacaine and saline injections (n=6 in each group) showed equal reductions in threshold values for 6 weeks after wounding.

were made in the presence of sciatic nerve block with bupivacaine.

In a parallel experiment, mechanical sensory thresholds were documented in 24 rat pups using calibrated von Frey hairs. In this experiment, 12 pups had hindpaw wounds made under anaesthesia, as described above. Six of these were also treated with sciatic nerve block injections of bupivacaine while six had sciatic injections of sterile saline. Twelve pups acted as unwounded controls. These pups were followed until weaning, and sensory threshold testing was carried out at regular intervals.

Sensory thresholds for the flexion withdrawal reflex were documented over 6 weeks after neonatal paw wounds and compared with thresholds obtained in unwounded control littermates. Hindpaw wounds produced a significant decrease in sensory thresholds that became apparent from 1 week after wounding and remained below control values for the 6-week period of observation. This reduction in the flexion reflex threshold was not influenced by bupivacaine sciatic nerve blockade at the time of wounding. There was no significant difference between the reduction in thresholds that occurred in pups that received saline sciatic injections and in those that received local anaesthetic (Fig. 1).

Comment

Inflammatory lesions and skin wounds are well known to cause secondary changes in both peripheral and central sensory processing mechanisms. In the neonate, similar phenomena, such as hyperinnervation and hyperalgesia after injury, are observed and are frequently more exaggerated than in the adult.

Our results confirm an intense and long lasting peripheral neural response to surgical skin damage in neonatal rat pups. This hyperinnervation was accompanied by reduction in the mechanical sensory threshold over the wounded area that far outlasted the healing process. Furthermore, the growth of new nerve terminals and hypersensitivity were not influenced by blockade of nerve conduction at the time of wounding.

Compared with the adult pattern of sprouting, the neonatal pattern showed greater involvement of larger axon bundles and a striking deflection of deeper axon bundles into the wounded area. Overall terminal density is greater for neonatal wounds and hyperinnervation lasts for much longer.

The histological changes associated with skin wounds are paralleled closely by changes in skin sensory function. In human adults, touch thresholds, heat and cold thresholds and two-point discrimination are each altered by skin wounds, but by 4 weeks after injury these variables of sensory function have returned to pre-injury levels.

Changes in sensory function after tissue damage have also been documented in human neonates and infants. Using the flexion reflex threshold as a measure of nociceptive function, Fitzgerald, Millard and McIntosh showed that skin damage and minor inflammation induced by heel lancing produced hypersensitivity in babies. The use of a topical local anaesthetic cream (EMLA) during the period of study blocked the decrease in cutaneous mechanical thresholds that characterized the response of these neonates to skin wounds.

To what extent the degree of hypersensitivity is a function of changes in central neural processing or peripheral tissue-related events has yet to be determined. Peripheral sensitization is probably a result of the synergistic actions of several chemical mediators, including potassium, hydrogen ions, 5-HT, histamine and bradykinin. Central sensitization after inflammation appears to involve modulation of NMDA receptors with subsequent increases in glutamate sensitivity.
Tissue inflammation also leads to the release of growth factors and tropic cytokines from tissue macrophages and other immune cells. This is the most likely mechanism underlying hyperinnervation of the injured area. Of particular importance is nerve growth factor (NGF) which is markedly upregulated in inflamed tissues and more notably so in early development than in adults.7

Early sprouting of sensory nerve fibres into a wounded area would seem to be integral in returning the protective aspects of nociceptive processing to an insensitive area. It has also been postulated that nerve sprouts may act to deliver trophic factors to damaged tissue and hence facilitate rapid healing.8 This is supported by recent evidence that some neurotrophic factors can be transported anterogradely by peripheral neurones and released by nerve depolarization.9

Our results showed that sensory nerve sprouting in response to skin loss occurred in the absence of neuronal electrical activity or sensory transmission in the immediate phase of tissue injury (first 24 h). Similarly, the prolonged hypersensitivity that characterizes peripheral tissue inflammation in neonatal rat pups was not blocked by acute nociceptive afferent block. This suggests that regional analgesia, used commonly in clinical practice, is unlikely to prevent the hyperinnervation that follows skin wounding.

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References