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Activation of a bradykinin receptor in peripheral nerve and spinal cord in the neonatal rat *in vitro*

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In an *in vitro* preparation of the neonatal rat spinal cord with attached tail, administration of bradykinin (Bk) to the spinal cord or to the tail produced depolarization of a ventral root (L_3–L_5). The effect of Bk at each site was selectively and reversibly antagonized by D-Arg [Hyp^2, Thi^{5,8} D-Phe^7]-Bk but could not be mimicked or antagonized by the B_1-receptor ligands [des-Arg^9]-Bk or Leu^8[des-Arg^9]-Bk, respectively. Peripherally evoked noxious responses produced by capsaicin or heat, were unaffected by either antagonist administered to the spinal cord. These data suggest that Bk-evoked responses in the spinal cord and at peripheral nociceptors were mediated via a receptor which by definition is of the B_2-type. Additionally Bk is unlikely to be a physiological mediator of acute nociception in the spinal cord.

Introduction Bradykinin (Bk), a nonapeptide with algesic and proinflammatory properties, is known to depolarize sensory neurones and to activate peripheral nociceptors following its formation at sites of injury (Erdos, 1979; Lindsay & Rang, 1987). In addition Bk may have a physiological role in spinal nociception as both Bk-immunoreactivity, and receptor binding sites have been demonstrated in the spinal dorsal horn (see Steranka *et al.*, 1988). Evidence on the possible involvement of Bk in the spinal cord and whether the peripheral effects of Bk are mediated through specific receptors requires the use of selective pharmacological antagonists. Recently antagonists with improved activity have been reported (Schachter *et al.*, 1987) and a number of studies suggest that several receptor subtypes for Bk, (B_1 and B_2) may exist (e.g. Braas *et al.*, 1988). The B_1-receptor may be activated by [des-Arg^9]-Bk and antagonized by Leu^8[des-Arg^9]-Bk, whereas the B_2-receptor is defined by exclusion, being insensitive to the B_1 agonist and antagonist (Regoli & Barabe, 1980). In the present study we have explored the Bk receptor involved in the activation of peripheral nociceptive fibres and of spinal neurones in the neonatal rat.

Methods The spinal cord with attached tail was isolated from 1–2 day old rat pups. The surface of the skin was removed from the tail and both the spinal cord and tail were superfused separately with a physiological salt solution (composition in mM: NaCl 138.6, KCl 3.35, $CaCl_2$ 1.26, $MgCl_2$ 1.16, $NaHCO_3$ 21.0, $NaHPO_4$ 0.58, glucose 10) at 24°C, gassed with 95% O_2:5% CO_2. The activation of peripheral fibres or spinal neurones was recorded as the depolarization of a spinal ventral root (L_3–L_5). Peripheral nociceptors were activated by superfusion of the tail with solution heated to 48°C and with a submaximal dose of Bk (0.2–0.5 μM) or capsaicin (0.5 μM). Activation of spinal neurones was also produced by direct administration of Bk (20–50 nM) in the spinal cord superfusate. Stimuli were separated by a 15 min period but Bk doses were separated by at least 60 min to avoid tachyphylaxis. The Bk antagonist used was D-Arg [Hyp^2, Thi^{5,8} D-Phe^7]-Bk (Hyp = L-4-hydroxyproline: Thi = β-(2-thienyl)-L-alanine), one of the more potent Bk antagonists described by Vavrek & Stewart (1985) and two further Bk analogues, [des-Arg^9]-Bk and Leu^8[des-Arg^9]-Bk, considered to be an agonist and antagonist respectively at a B_1-receptor (Regoli & Barabe, 1980). All peptides were obtained commercially.

Results Reproducible ventral root responses were obtained over many hours following stimulation of peripheral fibres with noxious heat or a submaximal dose of Bk (ED_{50} = 0.2 μM) and capsaicin (ED_{50} = 0.5 μM) (Figure 1). However [des-Arg^9]-Bk was ineffective at concentrations up to 100 μM (n = 4). The response to Bk was selectively and reversibly antagonized by concomitant superfusion of the tail with D-Arg[Hyp^2, Thi^{5,8} D-Phe^7]-Bk (Figure 1) at concentrations of 1, 5 and 10 μM (n = 4 at each concentration). Superfusions with Leu^8 [des-Arg^9]-Bk (10–100 μM, n = 4) did not produce depolarization nor was the response to Bk altered.

Ventral root depolarizations, produced by submaximal doses of Bk and capsaicin administered in the spinal cord superfusate, were also reproducible. At this site [des-Arg^9]-Bk was also ineffective as an agonist (10–100 μM, n = 4) and Leu^8 [des-Arg^9]-Bk (10 μM, n = 3) did not antagonize the effect of Bk. However D-Arg [Hyp^2, Thi^{5,8} D-Phe^7]-Bk adminis-

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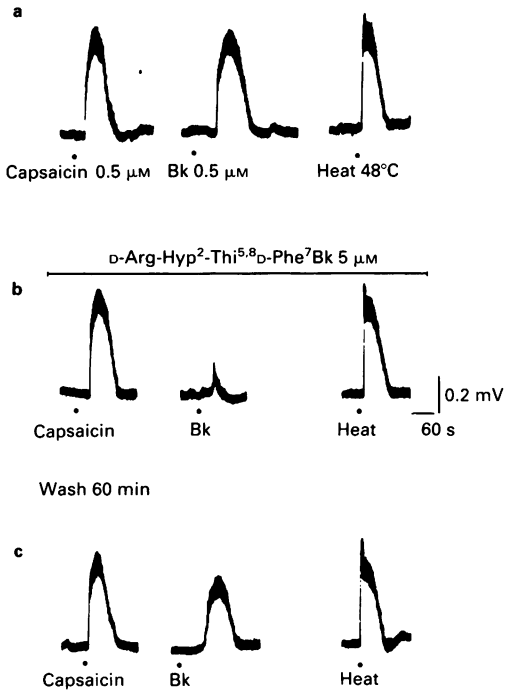

Figure 1 Selective antagonism of bradykinin-induced depolarization of peripheral fibres by D-Arg [Hyp², Thi⁵,⁸ D-Phe⁷]-Bk. Trace (a) shows control spinal ventral root depolarizations following peripheral fibre activation by a submaximal dose of capsaicin (0.5 μM) and bradykinin (Bk, 0.5 μM) administered to the tail. Depolarization was also evoked by noxious heat (48°C). Trace (b) shows selective antagonism of the Bk response by D-Arg [Hyp², Thi⁵,⁸ D-Phe⁷]-Bk (5 μM) administered in the tail superfusate 5 min prior to and throughout each test stimulus. Following a 60 min washout period, partial recovery of the Bk response was observed (c).

tered in the spinal cord superfusate (1 μM, $n = 5$) reversibly attenuated or abolished the effect of Bk without changing responsiveness to spinal or peripheral capsaicin. Higher concentrations (10 μM, $n = 4$) of this antagonist did not affect noxious responses evoked by heat or peripherally administered capsaicin.

Discussion In the present study, using a recently described Bk antagonist, we have demonstrated that Bk-induced activation in the spinal cord and that of peripheral nociceptors is mediated by a specific Bk-receptor. Two receptor subtypes B_1 and B_2 have been proposed for Bk (Regoli & Barabe, 1980). The fact that both [des-Arg⁹]-Bk and Leu⁸ [des-Arg⁹]-Bk were inactive at peripheral and central sites suggests that the B_1-receptor was not present and that the acute neural effects of Bk must have been mediated via a B_2-receptor interaction.

In the spinal cord Bk receptors are localized to the dorsal horn, afferent fibres and sensory ganglia (Steranka *et al.*, 1988). Direct spinal administration of Bk activates spinal neurones (Henry, 1976) and in unanesthetized animals, alters nociceptive reflex activity by an interaction with a B_2-receptor (Laneuville & Couture, 1987). It is unclear at present whether excitability changes also occur in primary afferent nerve terminals but these observations suggest that Bk may be a physiological mediator of nociception in the spinal cord. However, this is not supported by the present observations, since peripherally evoked chemical and noxious heat responses were unaffected by spinal administration of the antagonist at concentrations in excess of those that abolished the spinal effects of Bk. On the other hand the present demonstration of a Bk receptor on peripheral nociceptors is important since a number of Bk-antagonists have recently been shown to attenuate Bk-induced peripheral pain (Steranka *et al.*, 1988). From our results this receptor is clearly not of the B_1-type; it is therefore of the B_2-type.

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