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Cooperative mechanisms of neurotransmitter action in central nervous sensitization

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Introduction

During the past 5 years, our group has investigated the mechanisms of central sensitization of nociceptive pathways using 2 animal models: intradermal injection of capsaicin (Dougherty and Willis, 1992; Dougherty et al., 1992a; Simone et al., 1991; reviewed in Willis, 1994) and induction of acute arthritis (Dougherty et al., 1992b; Sluka et al., 1992; Sluka and Westlund, 1992, 1993a–d; Sorkin et al., 1992; Westlund et al., 1992; see review by Sluka et al., 1995b). We have also explored the possibility of provoking sensitization more directly by administering drugs into the dorsal horn, either by iontophoresis or by microdialysis (Dougherty and Willis, 1991; Dougherty et al., 1993, 1995; Palecek et al., 1993a,b). Agents administered have included neurotransmitter agonists and antagonists, as well as drugs affecting second messenger systems. Central sensitization is characterized in our experiments by the following: (1) changes in the responses of primate spinothalamic tract (STT) neurons to stimulation of the skin, including increased responses to innocuous and marginally noxious stimuli and an enlarged receptive field; (2) behavioral responses to stimulation of the skin in rats indicative of allodynia and/or hyperalgesia; (3) morphological alterations in the dorsal horn in rats and monkeys; (4) and release of neurotransmitters into the dorsal horn, as determined by microdialysis and high performance liquid chromatography (HPLC) in rats and monkeys.

Intradermal injection of capsaicin

When human subjects are given an intradermal injection of capsaicin (100 mg in 10 ml), severe pain occurs immediately, and primary hyperalgesia develops near the injection site. Secondary mechanical hyperalgesia and allodynia also develop over an extended area surrounding the site of primary hyperalgesia (Simone et al., 1989; LaMotte et al., 1991, 1992). A zone of analgesia just at the injection site is presumed to result from inactivation of nociceptive terminals where the capsaicin concentration is high (LaMotte et al., 1992). There is an area of primary heat hyperalgesia around the injection site in which the threshold for heat pain is reduced from 45 to 32°C. However, there is no area of secondary heat hyperalgesia. The pain caused by capsaicin injection lasts about 15–20 min. The time course of the secondary mechanical hyperalgesia and allodynia is much longer: the peak effect occurs within about 15–20 min, and these sensory changes last at least 1–2 h (Simone et al., 1989; LaMotte et al., 1991). A flare response also occurs, extending nearly to the limits reached by the secondary mechanical hyperalgesia.

In human subjects, microneurographic recordings from primary afferent C fibers supplying the area of secondary mechanical hyperalgesia and allodynia do not show enhanced responses following a capsaicin injection. A reduced excitability has been found for C

nociceptors whose receptive fields were in the analgesic zone; an increased excitability was seen in several units after topical application of capsaicin (LaMotte et al., 1992). An electrical stimulus applied through the microneurography electrode that initially caused a purely tactile sensation projected to an area away from the injection site became painful when the area of secondary mechanical hyperalgesia and allodynia expanded to include the projected receptive field (Torebjörk et al., 1992). The pain disappeared and only touch was felt when the area of secondary mechanical hyperalgesia and allodynia regressed.

In more recent human studies by Kilo et al. (1994), different types of stimuli were used to assess hyperalgesia and allodynia following topical capsaicin, and nerve blocks were used to decipher which fiber types mediated a particular response. Hypersensitivity to von Frey filaments (>400 mN bending force) applied to the receptive field, presumably reflecting mechanical hyperalgesia, was shown to be mediated by C-fiber afferents. Painful responses to innocuous brushing of the skin were mediated by low threshold mechano-sensitive A β fibers. Koltzenburg et al. (1994) further suggest that continuous C-fiber input is necessary to maintain A β fiber-mediated mechanical hyperalgesia, since stimulation of C-fibers results in both central sensitization and brush-evoked pain.

These studies suggest that activation of several peripheral fiber types is necessary for the perception of hyperalgesia and allodynia. C-fiber activation has a pivotal role both in establishing central sensitization and in allowing previously innocuous stimuli to produce pain. Activation of A β fibers results in allodynia when sensitization is present due to the effects of C nociceptor input to the spinal cord.

Parallel results have been obtained in animal experiments. Baumann et al. (1991) found in primates that only a few types of nociceptive fibers were activated sufficiently strongly by capsaicin injections to account for the pain that is produced by this procedure. Nociceptors supplying the skin surrounding a capsaicin injection site did not become sensitized to mechanical or heat stimulation after injection of capsaicin. Nociceptors innervating the injection site had reduced responses.

Our group has used the responses of primate spinothalamic tract (STT) cells as a model system to predict human sensory changes that would be produced by experimental manipulations of spinal cord function that can only be done in animal subjects. Intradermal injection of capsaicin (100 μ g in 10 μ l) into the skin in the receptive field of STT cells causes robust discharges that peak within seconds and then taper off over 15–20 min (Simone et al., 1991). The responsiveness of the neurons to mechanical and thermal stimulation of the skin changes in a characteristic manner. There may be an increase or a decrease in responses to mechanical stimuli applied at the injection site, and there is a lowered threshold for noxious heat pulses applied to the skin immediately around the injection site. However, the threshold for noxious heat does not change in the surrounding skin. Responses to weak tactile stimuli and to poking the skin with a von Frey filament that is painful on normal skin become considerably increased (Simone et al., 1991). Similar results are seen when a large dose of capsaicin (100 μ l of a 3% solution of capsaicin) is injected intradermally and the innocuous mechanical stimuli are brushing the skin (BRUSH) or application of a large arterial clip to a fold of skin (PRESS) (Fig. 1; Dougherty and Willis, 1992). In such experiments, the responses of STT cells to noxious stimuli, such as application of a small arterial clip (PINCH) to the skin, squeezing the skin or application of noxious heat, are often unchanged. The time course of the enhanced responses is similar to that of allodynia and secondary hyperalgesia in human skin, 15 min for a peak effect and 1.5 h duration; furthermore, the receptive fields of individual STT cells increase in size (Figs. 1 and 2A; Dougherty and Willis, 1992).

In experiments in which GLU receptor agonists were released in the vicinity of STT cells by iontophoresis (Dougherty and Willis, 1992), we showed that following intradermal injection of capsaicin, STT cells became more responsive to one or more of the following excitatory amino acids (EAAs): glutamate (GLU), aspartate (ASP), *N*-methyl-D-aspartate (NMDA), quisqualic acid (QUIS), α -amino-3-hydroxy-5-methyl-isoxazolepropionic acid (AMPA), and kainic acid (KAIN). The time course of the increased sensitivity to EAAs (Fig. 2B) parallels that of the alterations in sensitivity to mechanical stimulation of the skin (Fig. 2A). These observations indicate that a capsaicin injection causes a long-lasting increase in the excitability of STT neurons to locally applied EAAs, but it does not rule out the possibility that the enhanced excitability of these neurons depends upon a continued input from the periphery. However, recordings from peripheral nerve fibers have so far not revealed changes in the activity or excitability of sensory receptors supplying the skin in the area of secondary hyperalgesia (Baumann et al., 1991; cf., LaMotte et al., 1992).

Evidence that sensitization of central neurons can occur for periods of up to a few h without maintained peripheral input comes from both *in vivo* and *in vitro* experiments. Our group has found that local coadministration of an EAA, such as NMDA or QUIS, with substance P (SP) can result in the enhancement of the responses of STT cells to later applications of the EAA or to mechanical stimulation of the skin (Dougherty and Willis, 1991; Dougherty et al., 1993). Repeated applications of an EAA, such as NMDA, do not cause sensitization; instead, the responses tend to habituate (Dougherty and Willis, 1991). Nor does SP alone cause sensitization. The sensitization of STT cells produced by coadministration of NMDA or QUIS and SP may last hours (Fig. 3). Randic's group has found that neurons isolated from the dorsal horn and placed in an *in vitro* bath and then exposed to NMDA and SP show enhanced responses that persist for up to 50 min (Randic et al., 1990). Thus, dorsal horn neurons can be sensitized by exposure to the combination of an EAA and SP without any input from a peripheral nerve and without processing by dorsal horn circuits. However, this does not imply that sensitization is unaffected by an additional peripheral input.

These observations suggested to us that the sensitizing effect of an intradermal injection of capsaicin may depend on the co-release of EAAs and SP in the dorsal horn. A similar co-release of EAAs and SP may be responsible for the hyperalgesia seen in a number of animal models (see review by Urban et al., 1994). Therefore, we have investigated the effects of administering substances that antagonize EAA receptors and neurokinin receptors on the sensitization of STT cells by capsaicin injection (Dougherty et al., 1992a, 1994). As a control experiment, it was important to demonstrate that a second injection of capsaicin, given after the STT cell had recovered from the effects of the first injection, would produce a comparable sensitization. Since this was found to be the case (Dougherty et al., 1994), the design of the experiments was as follows. After testing the responses of an STT cell to mechanical stimulation of the skin, a first injection of capsaicin was given and the sensitized responses to the mechanical stimuli observed. A period of 1.5–2 h was allowed to elapse and then the responses were tested again to show that they had returned to the control level. Following this, an antagonist drug was administered by microdialysis, and the effects of the antagonist on the responses to mechanical stimuli were determined. Finally, a second dose of capsaicin was given (the injection was in a separate region of skin than the first injection) and the responses to mechanical stimulation again tested.

The antagonists that we have used in these experiments and the receptors that they block have included: CNQX, non-NMDA EAA receptors; AP7, NMDA EAA receptors; CP96345 and GR82334, NK1 receptors; and MEN 10376 and GR94800, NK2 receptors. The results of these experiments showed that both EAAs and SP are likely to be involved in the sensitization of STT cells that follows intradermal injection of capsaicin.

When non-NMDA receptors are blocked by CNQX, the responses of STT cells to BRUSH, PRESS, and PINCH are reduced to a minimum, as are the responses to capsaicin itself (Dougherty et al., 1992a). Although sensitization is prevented by CNQX, this is presumably not by a specific effect on sensitization but rather due to interference with synaptic transmission in the afferent pathways leading to the STT cells. On the other hand, AP7, which had no effect on the responses of STT cells to BRUSH or PRESS before capsaicin, prevents the enhancement of these responses following capsaicin (Dougherty et al., 1992a).

Similarly, the NK1 antagonists, CP96345 and GR82334, had no effect on the responses of STT cell to BRUSH, PRESS or PINCH stimuli, but prevented sensitization of these responses by capsaicin (Fig. 4; Dougherty et al., 1994; cf., Radhakrishnan and Henry, 1995). As a control, we also examined the effect of CP96344, which has a similar action to that of CP96345 on Ca^{2+} channels but none on NK1 receptors. CP96344 did not affect sensitization. On the other hand, the NK2 antagonists, MEN 10376 and GR94800, did not block sensitization, but instead appeared to enhance the responses of STT cells to BRUSH and PRESS stimuli before injection of capsaicin. We speculated that this may have resulted from a mixed agonist effect of these peptide antagonists (Dougherty et al., 1994).

These studies strongly suggest that sensitization of STT cells by intradermal injection of capsaicin depends on the release of EAAs and SP from the terminals of nociceptors in the dorsal horn. Others have shown that capsaicin causes the release of EAAs and SP in the dorsal horn (Gamse et al., 1979; Sorkin and McAdoo, 1993; Ueda et al., 1994).

Acute experimental arthritis

Acute joint inflammation is manifested in many arthritic conditions, including rheumatoid arthritis and osteoarthritis. Arthritis is most commonly accompanied by pain, which limits the range of motion and limb function. Animal models of inflammation are designed to mimic these human arthritic conditions. We have employed a model of acute joint inflammation, induced by intraarticular injection of kaolin and carrageenan, to study the role of dorsal horn neurons in the integration and transmission of arthritic pain in monkeys (Dougherty et al., 1992b; Sluka et al., 1992; Sorkin et al., 1992; Westlund et al., 1992). A similar model was developed in rats to explore the behavioral changes, as well as dorsal horn changes in neurotransmitters produced by acute arthritis (Sluka and Westlund, 1992, 1993a–d).

Several neurotransmitters and neuromodulators have been shown to be involved in the transmission of nociceptive information related to acute inflammation. For example, there is evidence that the EAAs, GLU and ASP, are released during the development of acute inflammation and mediate the accompanying heat hyperalgesia through both non-NMDA and NMDA EAA receptors (Sluka and Westlund, 1992, 1993d; Sorkin et al., 1992). SP and neurokinin A (NKA) and their receptors, the neurokinin 1 (NK1) and neurokinin 2 (NK2) receptors, are also involved in the integration of arthritic pain (Neugebauer et al., 1994b; Sluka and Westlund, 1993b,d; Sluka et al., 1995a).

After induction of acute inflammation, the primary afferent fibers become sensitized to mechanical stimulation applied to the peripheral receptive field and to joint movement (Schaible and Schmidt, 1985). In addition there is activation of previously silent neurons (Schaible and Schmidt, 1985). All joint afferent fiber types (Groups II, III and IV) become sensitized and develop an increase in background discharges and increased responses to innocuous and noxious joint movement. This increased activity in primary afferents results in central sensitization of dorsal horn neurons (Dougherty et al., 1992b; Neugebauer and Schaible, 1990; Schaible et al., 1987). Pretreatment with capsaicin to eliminate Group IV

(unmyelinated) fibers decreases the severity of the acute inflammatory response (Colpaert et al, 1983; Lam and Ferrell, 1989).

Spinothalamic cells and other dorsal horn neurons become sensitized to mechanical stimuli following induction of acute joint inflammation (Fig. 5; Schaible et al., 1987, 1991; Neugebauer et al., 1990; Dougherty et al., 1992b). Additionally, primate STT cells are more responsive to iontophoretically applied GLU and QUIS, a non-NMDA receptor agonist (Dougherty et al., 1992b). Schaible et al. (1990) demonstrated that rat dorsal horn neurons became more sensitive to iontophoretically applied NMDA, although Dougherty et al. (1992b) found a decrease in NMDA responses in primate STT cells. The differences between the two studies may be related to either the time following induction of inflammation or the type of cell tested. Dougherty et al. (1992b) tested the responses of identified spinothalamic cells within the first 3 h after induction of inflammation, whereas Schaible et al. (1991) tested the responses of unidentified dorsal horn neurons 4–8 h after development of inflammation. The sensitization of dorsal horn neurons to mechanical stimuli that occurs in rats with acutely inflamed knee joints is reversed by intravenous administration of either a non-NMDA (CNQX) or an NMDA (ketamine, AP5) receptor antagonist (Schaible et al., 1991; Neugebauer et al., 1993). Recently, we demonstrated that microdialysis administration of a NK1 receptor antagonist, CP99,994, can also reverse the sensitization of STT cells to brushing the peripheral receptive field and flexion of the knee joint (Rees et al., 1995a). Therefore, blockade of any of these three receptors can fully reverse the sensitization of dorsal horn neurons, including STT cells, to peripheral stimuli, indicating that multiple receptor activation is necessary for the sensitization of dorsal horn neurons.

Behaviorally, rats with inflamed knee joints guard the limb and are hyperalgesic to heat applied to the paw. The heat hyperalgesia, tested by applying a radiant heat source to the paw (see Hargreaves et al., 1988), becomes maximal at 4 h and lasts through 24 h (Sluka and Westlund, 1993b). The heat hyperalgesia can be prevented or reduced by pretreatment or posttreatment with the non-NMDA EAA receptor antagonist, CNQX, or the NMDA antagonist, AP7 (Sluka and Westlund, 1993d; Sluka et al., 1994a,b). On the other hand, pain-related behaviors, such as guarding of the limb, are reversed only by CNQX administered by microdialysis before or after induction of arthritis; AP7 had no effect on pain-related behaviors (Sluka and Westlund, 1993c; Sluka et al., 1994b). Neurokinin receptors in the spinal cord are also involved in the induction and maintenance of heat hyperalgesia (Sluka et al., 1995a). The NK2 receptor antagonist, SR48968, delivered by microdialysis prior to induction of inflammation can prevent the development of the heat hyperalgesia associated with acute inflammation; posttreatment with the NK2 antagonist had no effect on the hyperalgesia (Sluka et al., 1995a). In contrast, posttreatment with the NK1 receptor antagonist, CP99,994-1, by spinal cord microdialysis reversed the arthritis-induced heat hyperalgesia in a dose-dependent manner while pretreatment had no effect (Sluka et al., 1995a). Therefore, several receptors are involved in the development and maintenance of heat hyperalgesia induced by acute joint inflammation, including non-NMDA EAA, NMDA EAA, NK1 and NK2 receptors.

The release of neuropeptides has been monitored by introducing antibody microprobes into the spinal cord before and during acute joint inflammation (Schaible et al, 1990, 1992). The dorsal horn content of neurotransmitters and neuropeptides can also be monitored with computer quantification of immunohistochemically stained tissue (Sluka et al., 1992; Sluka and Westlund, 1993b). The antibody microprobe technique has been used to demonstrate the release of the neuropeptides, SP and NKA, into the dorsal horn during the development of acute inflammation and during flexion of the knee joint (Hope et al., 1990; Schaible et al., 1990). Immunohistochemical staining revealed that there is an initial decrease in staining

density for SP in the superficial dorsal horn (Sluka et al., 1992; Sluka and Westlund, 1993b) that is followed by an increase in SP content through 1 week following induction of inflammation (Sluka and Westlund, 1993b). In addition, the content of GLU is increased through the first 24 h of arthritis (Sluka et al., 1992; Sluka and Westlund, 1993b) and is significantly correlated with the decrease in Paw Withdrawal Latency to radiant heat (Sluka and Westlund, 1993b).

In studies using microdialysis and HPLC to measure substances in the extracellular fluid of the dorsal horn, there is a transient release of the EAAs, GLU and ASP, and of the IAAs, GLY and SER, at the time of injection (Sluka and Westlund, 1992; Sorkin et al., 1992). This is followed by a second, more prolonged phase of amino acid release, including ASP, GLU, and GLY in the anesthetized primate and ASP and GLU in the awake rat (Sluka and Westlund, 1992; Sorkin et al., 1992) (Fig. 6). In the awake rat, microdialysis can be used to look at longer time periods and release during the behavioral testing for paw withdrawal latency to radiant heat. An increased release of the IAAs, GLY and SER, is also observed during the PWL test for radiant heat (Sluka et al., 1994b). Thus, a multiplicity of neurochemical changes occur in the dorsal horn in response to the peripheral activation including an increase in both the content and release of glutamate and substance P.

Spinal cord administration of the non-NMDA receptor antagonist, CNQX, by microdialysis prior to the induction of arthritis prevented the release of ASP and GLU at the time of injection and during the prolonged release phase (Sluka and Westlund, 1993c), as well as the increased content of GLU and SP in the superficial dorsal horn (Sluka and Westlund, 1993d). Similarly, the increased release of ASP and GLU in the dorsal horn is prevented by pretreatment with an NMDA receptor antagonist (AP7), and the increased release of GLU is reversed by posttreatment with AP7 (Sluka et al., 1994b) (Fig. 7). The release of GLY and SER during the PWL test is blocked by posttreatment with either a non-NMDA (CNQX) or an NMDA (AP7) receptor antagonist (Sluka et al., 1994b). Further supporting a role of NK1 receptors in the development of acute inflammation, the increased release of the EAAs in the late phase is prevented by pretreatment with CP96.345 (NK1 antagonist) (Sluka and Westlund, 1993d).

Surprisingly, administration of the GABA_A receptor antagonist, bicuculline, into the dorsal horn by microdialysis prior to the induction of inflammation prevented the development of heat hyperalgesia (Sluka et al., 1993a). Furthermore, when administered prior to the induction of inflammation, bicuculline prevented the prolonged release of the EAAs and the increased release of the IAAs during the PWL test to radiant heat (Sluka et al., 1994d). Thus, blockade of non-NMDA EAA, NMDA EAA, NK1 and GABA_A receptors specifically alters the release of EAAs and IAAs induced by acute inflammation.

The most interesting finding in our investigation of the role of neurotransmitters in the spinal cord during the induction of acute arthritis is that either a non-NMDA EAA receptor antagonist (CNQX) or a GABA_A receptor antagonist (bicuculline) administered spinally prior to induction of inflammation can reduce the degree of joint swelling and the increase in skin temperature typical of the inflammation (Sluka and Westlund, 1993c; Sluka et al., 1993) (Fig. 8). In contrast, an NMDA receptor antagonist, AP7, or a GABA_B receptor antagonist, CGP35348, had no effect on the joint swelling and temperature (Fig. 8). Chemical and surgical sympathectomy also had no effect on the joint swelling or thermographic readings (Sluka et al., 1994c). On the other hand, sectioning the dorsal roots prevented the swelling and thermographic changes (Sluka et al., 1994c) (Fig. 8). These results led us to hypothesize that the spinal cord can influence the degree of joint inflammation, i.e. the neurogenic component of inflammation is made worse by the spinal dorsal horn through activation of the primary afferents.

The best explanation for the spinal control of inflammation is that depolarization of the primary afferents results in antidromic action potentials or dorsal root reflexes (Rees et al., 1994, 1995b; Sluka et al., 1995b). These dorsal root reflexes would then release neuropeptides into the knee joint, resulting in increased joint swelling and temperature. To test this hypothesis we have recorded from the central end of a cut medial articular nerve in rats, cats and monkeys and from the central end of cut dorsal root filaments in cats and monkeys. Since the portion of the nerve recorded was disconnected from the peripheral receptive field, any activity recorded would have to be generated centrally. Sympathetic discharges were ruled out by surgical and chemical sympathectomy (Rees et al., 1994, 1995b). Therefore, antidromic action potentials or dorsal root reflexes could be recorded in both articular afferents and in dorsal roots following induction of inflammation (Rees et al., 1994, 1995b; Sluka et al., 1995b). Most of the dorsal root reflex activity was evoked by mechanical stimulation of the peripheral receptive field (Rees et al., 1994). The dorsal root reflexes were found to occur in C-fibers, A δ fibers and larger A β fibers in the joint afferents and dorsal roots (Sluka et al., 1995b). Similar to our previous behavioral studies demonstrating a spinal involvement of non-NMDA EAA and GABA_A receptors, dorsal root reflexes could be eliminated with either CNQX or bicuculline (Rees et al., 1995b).

Future directions in research on inflammatory pain and central sensitization

Second messenger systems

As already mentioned, the sensitization produced by intradermal capsaicin injections or by acute experimental arthritis depends on the activation of a number of neurotransmitter receptors (e.g., non-NMDA and NMDA EAA receptors, NK1 and NK2 receptors, GABA_A receptors). Activation of a number of neurotransmitter receptors triggers a cascade of intracellular events that involve second messengers, protein kinases and protein phosphatases. Evidence is beginning to accumulate that implicates such signal transduction events in the sensitization of dorsal horn neurons. For example, microdialysis administration of the metabotropic EAA receptor agonist, trans-ACPD, increases the responsiveness of primate STT cells to innocuous mechanical stimulation (Palecek et al., 1993b), suggesting that metabotropic EAA receptors play a role in central sensitization. In support of this, blockade of metabotropic GLU receptors by L-AP3 reduces the arthritis-induced sensitization of dorsal horn neurons (Neugebauer et al., 1994a). Furthermore, activation of metabotropic GLU receptors by trans-ACPD also enhances the non-NMDA and NMDA responses of dorsal horn neurons (Bleakman et al. 1992; Cerne and Randic, 1992), indicating an intricate interaction between receptors that could contribute to central sensitization. In addition to these observations concerning metabotropic EAA receptors, there is evidence that the sensitization process depends on the release of NO in the spinal cord (Palecek et al., 1993c; cf., Meller and Gebhart, 1993; Coderre and Yashpal, 1994; Meller et al., 1994).

To explore the possibility that protein kinases, such as protein kinase C (PKC), are involved, we have introduced phorbol esters into the dorsal horn by microdialysis and have found that an active phorbol ester can increase the responses of primate STT cells to innocuous mechanical stimuli, whereas an inactive phorbol ester has no effect (Palecek et al., 1993a).

Activation of PKC by phorbol esters or intracellular injection of PKC enhances both non-NMDA and NMDA currents in dorsal horn neurons (Gerber et al., 1989; Chen and Huang, 1992) and increases the release of EAAs from dorsal horn slices (Gerber et al., 1989).

Immediate, early genes

The activation of second messenger systems can result in long-term changes in the central nervous system by activation of immediate early genes, which in turn causes increases or decreases in the expression of other genes (Mentrety et al., 1989; Herdegen et al., 1991; Abbadie and Besson, 1992). The ultimate changes in gene products have the potential to result in morphological and consequent functional alterations in the circuitry of the dorsal horn. Inflammatory pain is associated with the expression of immediate early genes (Mentrety et al., 1989; Abbadie and Besson, 1992). Possibly prolonged inflammatory pain states would result in a rewiring of dorsal horn circuitry.

Therapeutic potential

It is too early to forecast what new therapies for pain might arise from experimental work of the sort discussed here. However, it does seem likely that antagonists of neurotransmitter receptors will continue to receive close attention as candidate therapeutic agents. Whether interventions at the level of second messenger systems will prove feasible is more speculative (Zimmerman and Herdegen, this volume) since second messenger systems have many and diverse effects, and therefore blocking second messengers is likely to result in unexpected side effects. A thorough understanding of the cascade of events involving immediate early genes will be important, since it may be possible to intervene at a molecular level to prevent long-term alterations that may underlie chronic pain states.

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Fig. 1. Responses of a primate STT neuron to mechanical stimulation of the skin before and after intradermal injection of capsaicin. (A) shows the background activity before capsaicin and (B) the response to the injection (time indicated by the arrow). (C), (E) and (G) are the responses to mechanical stimulation of the skin at the 5 sites indicated in the drawing at the bottom, and (D), (F) and (H) are the responses to the same stimuli after the injection. The enlargement of the receptive field is indicated by the singly hatched area on the drawing. (From Dougherty and Willis, 1992)

Fig. 2.

(A) shows the time course of the increased responsiveness of a primate STT neuron to mechanical stimulation of the skin (BGD, background; BR, brush; PR, pressure; PI, pinch) and (B) to iontophoretically released EAAs (GLUT, glutamate; QUIS, quisqualate; ASP, aspartate; NMDA, *N*-methyl-D-aspartate) following an intradermal injection of capsaicin. (From Dougherty and Willis, 1992)

Fig. 3.

Prolonged increase in the responsiveness of a primate STT neuron to iontophoretically released NMDA following co-application of NMDA and SP by iontophoresis. (A) shows the control responses to graded iontophoretic currents. In (B) are shown the responses to NMDA pulses during the administration of SP. (C–F) show the responses to NMDA pulses at the times indicated following termination of the SP current. (From Dougherty and Willis, 1991)

Fig. 4.

Effects of NK1 receptor antagonists on the sensitization of primate STT cells. (A) shows the responses of an STT cell to intradermal capsaicin injections before and after administration of the NK1 antagonist, GR82334, by microdialysis. The bar graphs in B-E show the mean responses of populations of STT cells ($\pm$ S.E.) before and after capsaicin and before and after treatment with an NK1 antagonist. Two NK1 antagonists were used, CP96345 and GR82334. (B) shows the response to capsaicin and the sustained discharge that follows intradermal injection. The NK1 antagonists reduced the responses, especially the sustained component of the discharge, to capsaicin. (C) and (D) show increased responses to brush and press stimuli following the first dose of capsaicin. However, during administration of NK1 antagonists, a second dose of capsaicin failed to increase the responses; i.e., sensitization was blocked. The pinch responses were unaffected by capsaicin injection. (From Dougherty et al., 1994)

Fig. 5. Background activity and responses to flexion (FLEX), brushing (BRUSH) and pinching (PINCH) of a wide dynamic range spinothalamic tract cell before (left column) and 3 h after (right column) induction of arthritic inflammation. Mechanical stimuli were given at the points labeled on the leg. (From Dougherty et al., 1992)

Fig. 6.

EAA concentration (percent change $\pm$ SEM) was measured using fluorescent high performance liquid chromatography in (A) (control arthritic) and (B) (arthritic animals) pretreated with CNQX and in (C) (arthritic animals pretreated with AP7). Antagonists were delivered for 1 h after baseline and followed by a 1 h (CNQX) or 30 min (AP7) washout. The knee joint was injected with 3% kaolin and carrageenan (arrow, Inj). (From Sluka and Westlund, 1993a)

Fig. 7.

Differences in paw withdrawal latency before and after induction of inflammation in the paw ipsilateral to the inflamed knee. Animals were either pretreated (top graph) or posttreated (bottom graph) with the drugs (thick bar). The knee joint was injected after control testing (arrow). The drugs were delivered through the microdialysis fiber, and the doses were as follows: CNQX pretreatment (1.2 mM), CNQX posttreatment (1.0 mM), AP7 pretreatment (2 mM), AP7 posttreatment (2 mM), CP99994 pre- and posttreatment (10 mM), SR48968 pre- and posttreatment (1 mM), bicuculline pretreatment (0.01 mM), CGP35348 pretreatment (3.0 mM).

Fig. 8.

Lower body thermographic readings (abdomen and both knees) with the inflamed (right) and the contralateral (left) knee visualized 8 h after injection of kaolin and carrageenan into the knee joint cavity for control arthritic animals (A), bicuculline treated arthritic animals (B), sympathectomized arthritic animals (C) and dorsal rhizotomized animals (D). Bar represents a continuous heat scale from 29.5 (red) to 33.4°C (dark blue).