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Activation of the cAMP transduction cascade contributes to the mechanical hyperalgesia and allodynia induced by intradermal injection of capsaicin

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1 The spinal role of the cAMP transduction cascade in nociceptive processing was investigated in awake behaving rats (male, Sprague-Dawley) by activating or inhibiting this pathway spinally. Microdialysis fibres were implanted into the dorsal horn to infuse drugs directly to the spinal cord.

2 Animals, without peripheral tissue injury, were tested for responses to repeated applications (10 trials) of von Frey filaments and threshold to mechanical stimulation before and after infusion of 8-bromo-cAMP. In this group of animals treated spinally with 8-br-cAMP (1–10 mM) a dose-dependent hyperalgesia and allodynia were produced. This was manifested as an increased number of responses to 10 trials of von Frey filaments (10, 50, 150, 250 mN) and a decrease in mechanical threshold.

3 A second series of experiments studied the manipulation of the cAMP pathway spinally in a model of tissue injury induced by intradermal injection of capsaicin. Animals were either pre- or post-treated spinally with the adenylate cyclase inhibitor, tetrahydrofuryl adenine (THFA) or the protein kinase A inhibitor, myrosilated protein kinase (14–22) amide (PKI). Injection of capsaicin resulted in an increased number of responses to repeated applications of von Frey filaments and a decrease in threshold to mechanical stimuli outside the site of injection, secondary mechanical hyperalgesia and allodynia.

4 Pre-treatment with either THFA (1 mM) or PKI (5 mM) had no effect on the capsaicin-evoked secondary hyperalgesia and allodynia.

5 In contrast, post-treatment spinally with THFA (0.01–1 mM) or PKI (0.05–50 mM) dose-dependently reduced the mechanical hyperalgesia and allodynia produced by capsaicin injection. Furthermore, the mechanical hyperalgesia and allodynia blocked by the adenylate cyclase inhibitor, THFA (1 mM), was reversed by infusion of 8-bromo-cAMP (0.01–10 mM) in a dose-dependent manner.

6 Thus, this study demonstrates that activation of the cAMP transduction cascade at the spinal cord level results in mechanical hyperalgesia and allodynia and that the secondary mechanical hyperalgesia and allodynia following intradermal injection of capsaicin is mediated by this same transduction cascade.

Keywords: Adenylate cyclase; cAMP; protein kinase A; second messengers; capsaicin; hyperalgesia; pain; inflammation; microdialysis; spinal cord

Introduction

The cAMP transduction cascade is associated with several G-protein receptors found in the spinal cord such as the postaglandin receptors (Hingtgen *et al*., 1995), calcitonin gene-related peptide (Bushfield *et al*., 1993; Santicioli *et al*., 1995; Sun & Benhishin, 1995), substance P (Satoh *et al*., 1992; Smith *et al*., 1992), opioid (Comb *et al*., 1992; Aantaa *et al*., 1995), serotonin (Hen, 1993), adrenergic (Aantaa *et al*., 1995) and metabotropic glutamate (Schoepp *et al*., 1992; Schoepp & Johnson, 1993) receptors. Activation of these receptors would result in binding of adenylate cyclase to the G-protein linked receptor. If the receptors are associated with a stimulatory G-protein, adenylate cyclase converts ATP to cAMP, resulting in an increase in formation of cAMP in the cell (Alberts *et al*., 1989). The increase in cAMP then activates protein kinase A (PKA), resulting in phosphorylation of proteins (Alberts *et al*., 1989) involved in neurotransmitter release (Hell *et al*., 1995) or ion channels such as excitatory amino acids or calcium channels (Blackstone *et al*., 1995; Hell *et al*., 1995; Sculptoreanu *et al*., 1995; Smith *et al*., 1995). Thus, activation of cAMP could result in increased excitability of neurons and sensitization through increased effectiveness of ion channels or increased release of neurotransmitters.

The role of cAMP in nociception has been studied in the periphery by several investigators. For example, peripheral injection of forskolin to activate adenylate cyclase or analogues of cAMP can produce mechanical hyperalgesia (Taiwo & Levine, 1991). The mechanical hyperalgesia found in diabetic rats is blocked by local subcutaneous administration of an inhibitor of cAMP (Ahlgren & Levine, 1993). Additionally, inhibitors of protein kinase A (PKA) applied peripherally can reduce the mechanical hyperalgesia induced by local prostaglandin or serotonin injection (Taiwo *et al*., 1992; Ouseph *et al*., 1995). Centrally, the involvement of the PKC and cGMP transduction cascades has been investigated (Coderre, 1992; Mao *et al*., 1992; Meller & Gebhart, 1993; Coderre & Yashpal, 1994; Igwe & Ning, 1994). However, the role of cAMP in the spinal nociceptive processing is less clear. Several studies have measured cAMP content in the dorsal horn with radioimmunoassay with conflicting results (Przewlocka *et al*., 1991; Garry *et al*., 1994; Igwe & Ning, 1994). Therefore, this study investigated the role of the cAMP transduction cascade in the spinal cord in the transmission of nociceptive information in the normal and sensitized state.

Intradermal capsaicin injection in humans results in primary hyperalgesia to heat and mechanical stimuli applied near the injection site, as well as secondary mechanical hyperalgesia (increased pain from noxious stimuli) and mechanical allodynia (pain from innocuous stimuli) in an area surrounding the site of primary hyperalgesia (Simone *et al*., 1989; Lamotte *et al*., 1991). Similar changes are observed after capsaicin injection in rats; there is an area of secondary mechanical hyperalgesia without secondary heat hyperalgesia (Sluka & Willis, 1997). Recordings in human and primate subjects demonstrate that C-fibres supplying the area of secondary hyperalgesia and allodynia do not sensitize after capsaicin injection (Baumann *et al*., 1991; Lamotte *et al*., 1992). Electrophysiological studies

in primates demonstrate that spinothalamic tract neurons become sensitized to innocuous mechanical stimuli following capsaicin injection (Simone *et al*., 1991; Dougherty *et al*., 1992a). Thus, central sensitization of dorsal horn neurons does occur after capsaicin injection and may contribute to secondary mechanical hyperalgesia observed behaviourally.

This study, therefore, tested the hypotheses that: (1) mechanical hyperalgesia and allodynia can be induced by activation of the cAMP transduction cascade in the spinal cord, and (2) secondary hyperalgesia and allodynia induced by tissue injury (capsaicin injection) can be reduced by blockade of the cAMP transduction cascade. This data has appeared in abstract form (Sluka, 1996).

Methods

All experiments were approved by the Animal Care and Use Committee at the University of Iowa and are in accordance with the guidelines for Care and Use of Laboratory Animals.

Placement of the microdialysis fibre

Male Sprague-Dawley rats (250–350 g, Harlan) had a microdialysis fibre implanted across the dorsal horn of the spinal cord according to previously published procedures (Skilling *et al*., 1988; Sluka & Westlund, 1992). The animals were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.). Two holes were drilled in the lateral aspects of the T13 vertebra to expose a portion of the lumbar spinal cord (L5/L6). An epoxy coated microdialysis fibre (200 μm o.d., 45 000 MW cut-off, Hospal AN69) was passed transversely through the holes into the dorsal horn and stabilized with dental cement applied to the bone. The microdialysis fibre was implanted the day prior to behavioural testing, allowing the animals to recover for 12–24 h before the experiment. Awake animals implanted with microdialysis fibres eat, drink and groom normally, and have no motor or sensory impairment. No weight loss is observed following implantation of microdialysis fibres.

The microdialysis fibre was coated with epoxy except for a 2 mm gap which was positioned in the grey matter of the spinal cord. Artificial cerebrospinal fluid (ACSF; 151.1 mM Na^+, 2.6 mM K^+, 0.9 mM Mg^{2+}, 1.3 mM Ca^{2+}, 122.7 mM Cl^-, 21 mM HCO_3, 2.5 mM HPO_4^{2-}, 3.87 mM glucose, bubbled with 95% CO_2, 5% O_2, pH 7.2–7.4) was infused (5 μl min^{-1}) through the microdialysis fibre instead of drug as a control. All drugs were dissolved in ACSF and delivered through the microdialysis fibre.

The placement of the microdialysis fibre was established histologically in all animals. At the end of the experiment the animals were euthanized with an overdose of sodium pentobarbital. The spinal cord was removed and postfixed in 10% formalin. All sites were in the L5 or L6 spinal segments and within laminae II–VI. The majority of fibre sites were in the L5 spinal segment and in the deep dorsal horn (lamina IV or V).

Administration of drugs

All drugs were administered through the microdialysis fibre at a rate of 5 μl min^{-1} for 1 h per dose. The drugs were dissolved in ACSF and the pH was corrected to 7.2–7.4. The following drugs were used: (1) tetra-hydro-furyl adenine (Calbiochem, 0.01–10 mM; adenylate cyclase inhibitor), (2) myrosilated protein kinase inhibitor (12–22) amide (Biomol, 0.05–50 mM; protein kinase A inhibitor), and (3) 8-bromo-cAMP (Sigma, 0.1–10 mM; cAMP analogue). The concentrations of drugs in the dialysate are presumed to be approximately two to three orders of magnitude higher than the concentrations that reach neurons in the dorsal horn. In the past we have studied the diffusion across the microdialysis fibre *in vitro* of several drugs with different chemical properties and different sizes. The concentration ratio across the microdialysis fibre for all of these drugs was between 1 and 8% (Sluka *et al*., 1994, 1997a; Sluka & Westlund, 1993). It is expected that degradation of the drug and barriers to diffusion would further reduce the effective concentration.

Injection of capsaicin

Capsaicin (0.1%, 100 μl, Fluka) was injected intradermally into the plantar surface of the proximal portion of the foot in awake rats. Capsaicin was dissolved in Tween 80 (7%), alcohol (20%) and saline. Previously, (Sluka, 1997) I demonstrated that injection of the vehicle does not produce hyperalgesia and allodynia.

Behavioural testing

Animals were tested for reponses to von Frey filaments applied to the plantar surface of the foot as a measure of mechanical allodynia and hyperalgesia. Animals were placed in clear plastic cages on an elevated screen. Filaments of four bending forces (10 mN, 50 mN, 150 mN and 250 mN) were applied to the distal portion of the plantar surface of the foot 20 mm outside the area of injection and thus a measurement of secondary hyperalgesia and allodynia. The 10 and 50 mN forces chosen did not cause significant numbers of withdrawals in normal animals (>50% of animals responding to the von Frey filament) and were therefore considered innocuous. A small number of withdrawals to the 150 mN and the 250 mN bending forces occurred in normal animals and were therefore considered noxious. The number of responses out of ten trials of each von Frey filament was recorded for each animal before and after capsaicin, and after drug and at varying intervals after capsaicin (Sluka, 1997; Sluka & Willis, 1997).

Animals were also tested for threshold to mechanical stimuli applied to the plantar surface of the paw before and after injection of capsaicin. Increasing forces of von Frey filaments were applied to the distal portion of the hindpaw 20 mm outside the site of injection. Two trials per filament were used and the lowest force that caused a withdrawal was recorded for each animal (Sluka, 1997). Von Frey filaments with the following bending forces were tested in ascending order (2 trials/filament): 1 mN, 5 mN, 10 mN, 50 mN, 90 mN, 150 mN, 250 mN and 600 mN.

Experimental design

Four separate experiments were performed:

1. 1 h infusion of 8-br-cAMP (1 mM, $n=4$; 5 mM, $n=5$; 10 mM, $n=5$) in normal animals,
2. 1 h infusion of THFA (1 mM, $n=5$) or PKI (5 mM, $n=5$) prior to injection of capsaicin (pre-treatment; doses based on most effective concentration in post-treatment studies),
3. 1 h infusion of THFA (0.01 mM, $n=3$; 0.1, $n=3$; 1 mM, $n=6$, 10 mM, $n=3$;) or PKI (0.05 mM, $n=3$; 0.5 mM, $n=3$, 5 mM, $n=6$; 50 mM, $n=3$) beginning 60 min after injection of capsaicin (post-treatment from 60 to 120 min after injection of capsaicin),
4. 1 h infusion of THFA (1 mM) beginning 30 min after capsaicin injection followed by 30 min infusion of THFA (1 mM) + 8-Br-cAMP (0.1 mM, $n=3$; 1 mM, $n=6$; 10 mM, $n=3$).

In group 1, animals were tested before and 1 h after infusion of 8-br-cAMP for responses to repeated application of von Frey filaments and for threshold to mechanical stimulation. In group 2 (pre-treatment), animals were tested before and 1 h after infusion of drug (THFA or PKI) and at varying times after injection of capsaicin (15, 30, 60, 90, 120 and 150 min). In group 3 (post-treatment), animals were tested before and 1 h after injection of capsaicin and after administration of THFA or PKI (1 h and 1.5 h postdrug). In group 4, animals were tested before and 30 min after injection of capsaicin, 1 h after

administration of THFA (1 h postdrug, 90 min postcapsaicin) and 30 min after infusion of 1 mM THFA + 8-Br-cAMP (30 min postinfusion, 120 min postcapsaicin). Dose–response curves were prepared for each drug. Control animals received ACSF through the microdialysis fibre instead of drug ($n=6$).

Statistical analysis

Responses to von Frey filaments (10 mN, 50 mN, 150 mN and 250 mN) did not have a normal distribution, and so a Friedman's ANOVA was performed to evaluate changes. If overall significance was obtained, signed rank tests ($P<0.05$) were used to test within animals differences between time points against the null hypothesis of no change. Differences between groups were assessed with a sign rank test ($P<0.05$). Values for repeated applications of von Frey filaments are expressed as the mean ± s.e.mean. Values for threshold to mechanical stimuli are presented as the median.

Results

Infusion of 8-bromo-cAMP

Infusion of 8-bromo-cAMP produced a dose-dependent increase in the number of responses to repeated applications of von Frey filaments. Significant increases in the number of responses to repeated application (10 trials) of von Frey filaments occurred following infusion of 5 mM 8-br-cAMP for 50, 150 and 250 mN bending forces; and following infusion of 10 mM 8-br-cAMP for all bending forces (Figure 1a). No change from baseline occurred following infusion of 1 mM concentration of 8-br-cAMP. There was a significant overall effect for the number of responses to repeated application of von Frey filaments for the 10 mN ($\chi^2=8.3$, $P=0.01$), 50 mN ($\chi^2=11.3$ $P=0.004$), 150 mN ($\chi^2=10.6$, $P=0.005$) and 250 mN ($\chi^2=10.9$, $P=0.004$) bending forces.

There was also a significant decrease in the threshold to mechanical stimuli from 600 mN (range 250–600 mN) to 50 mN (range 50–90 mN) after infusion of 5 mM 8-br-cAMP and to 5 mN (range 1–50 mN) following infusion of a 10 mM concentration of 8-br-cAMP ($\chi^2=11.4$, $P=0.01$; Figure 1b). Spinal infusion of the 1 mM dose of 8-br-cAMP had no effect on reducing the threshold to mechanical stimuli. The threshold to mechanical stimuli following 1 h infusion of 1 mM 8-br-cAMP was 250 mN (range 150–600 mN) and was not significantly different from values taken prior to drug infusion (600 mN, range 250–600 mN).

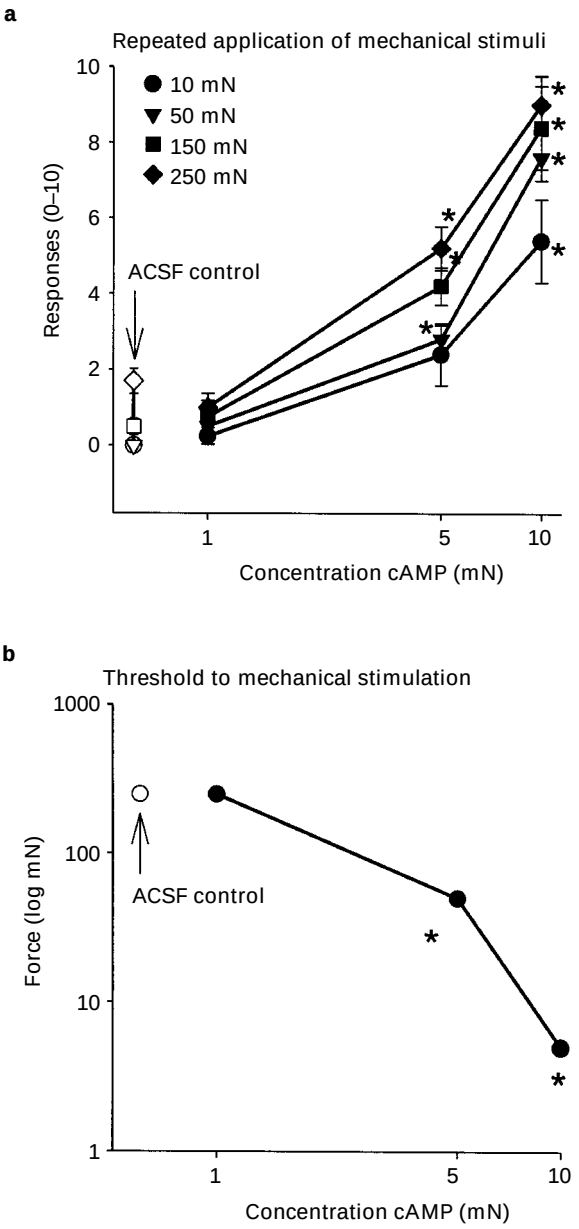

Figure 1 (a) Dose-dependent effects of 8-bromo-cAMP infused for 1 h into the spinal cord on responses to repeated applications (10 trials) of 10 (●), 50 (▼), 150 (■) and 250 (◆) mN bending forces. ACSF control animals were added for comparison (open symbols). (b) The threshold to mechanical stimulation following 1 h infusion of 1, 5 and 10 mM concentrations of 8-bromo-cAMP was dose-dependently reduced. *Significantly different from baseline.

Control capsaicin animals

Intradermal injection of capsaicin results in a significantly increased number of responses to mechanical stimulation with von Frey filaments of 10 ($\chi^2=28.5$, $P=0.0001$), 50 ($\chi^2=29.1$, $P=0.0001$), 150 ($\chi^2=25.1$, $P=0.0003$) and 250 mN ($\chi^2=22.4$, $P=0.001$) bending forces. The increased number of responses to repeated application (10 trials) of mechanical stimuli was significant by 5 min for the 50, 150 and 250 mN forces and by 10 min for the 10 mN bending forces. This increased number of responses remained significant through 2 h after injection of capsaicin (Figure 2a). The threshold to mechanical stimuli decreased significantly from 250 mN (range 600–100 mN) to 10 mN (range 1–10 mN) 30 min after injection of capsaicin ($\chi^2=20$, $P=0.002$). This decrease in threshold was maintained through at least 2 h (Figure 2b). There were no changes in the number of responses to von Frey filaments contralaterally or the threshold to mechanical stimuli contralaterally after capsaicin injection.

Blockade of adenylate cyclase or protein kinase A

Pre-treatment Pre-treatment with either THFA or PKI had no effect on the increased number of responses to repeated application of von Frey filaments induced by capsaicin injection (Figure 3a, b). There were, thus, significant increases in number of responses to repeated application of 10 mN ($\chi^2=21.8$, $P=0.001$, THFA; $\chi^2=23.6$, $P=0.001$, PKI), 50 mN ($\chi^2=25.5$, $P=0.0006$, THFA; $\chi^2=25.8$, $P=0.0005$, PKI), 150 mN ($\chi^2=24.3$, $P=0.001$, THFA; $\chi^2=26.2$, $P=0.0005$, PKI) and 250 mN ($\chi^2=24.4$, $P=0.001$, THFA; $\chi^2=25.2$, $P=0.0007$, PKI) bending forces. The threshold to mechanical stimuli in animals pre-treated with THFA significantly decreased from 600 mN (range 150–600 mN) to 10 mN (range 10–50 mN) 30 min after capsaicin injection and remained significantly decreased through 2.5 h. Similarly, in animals pre-treated with PKI, the threshold to mechanical stimuli decreased from 250 mN (range 150–600 mN) to 10 mN (range 5–10 mN) 1 h after capsaicin injection and remained decreased throughout the 2.5 h testing period. There were no

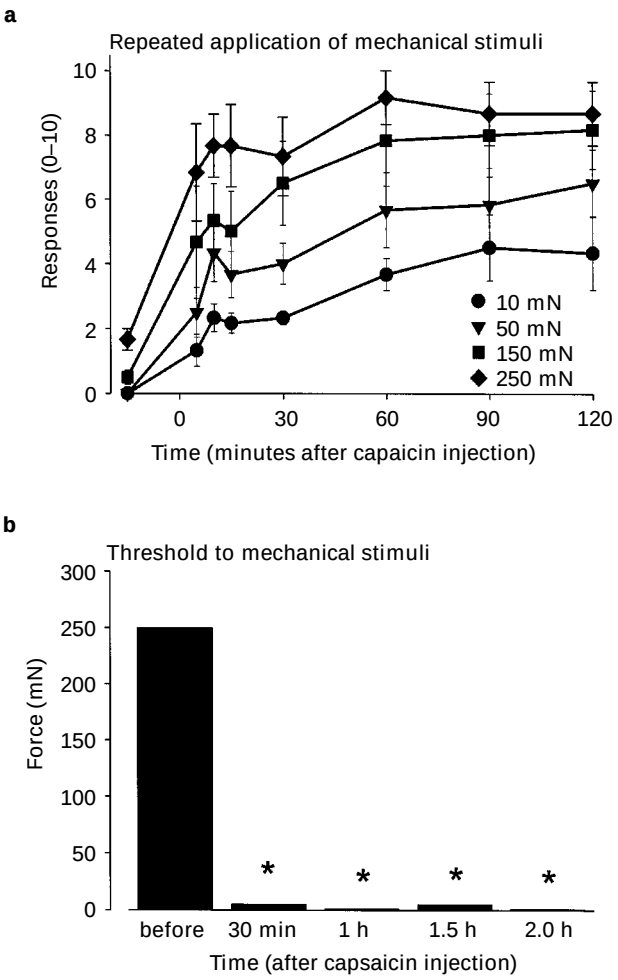

Figure 2 (a) Time course of responses to repeated application of von Frey filaments following capsaicin injection (time 0) with bending forces of 10 (◆), 50 (▼), 150 (■) and 250 (●) mN. Significant increases occurred through 2 h. (b) Bar graphs representing the threshold to mechanical stimulation before and after capsaicin injection. Significant decreases occurred through 2 h after capsaicin injection. *Significantly different from baseline.

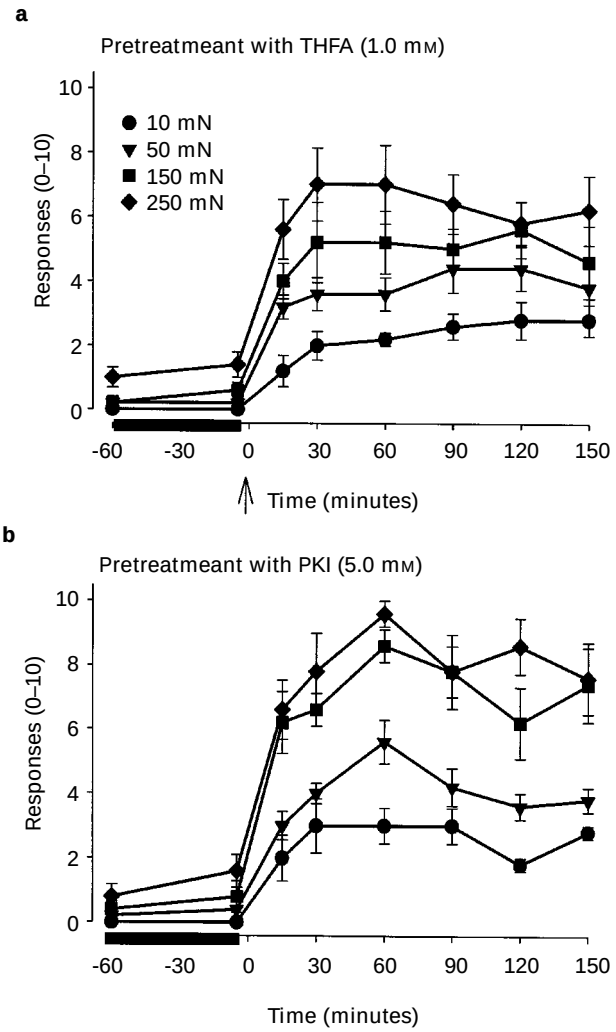

Figure 3 Graphs represent the number of responses to repeated application of von Frey filaments in the group of animals pretreated spinally (1 h infusion, thick bar) with 1 mM THFA (a) or 5 mM PKI (b). Bending forces of 10 (●), 50 (▼), 150 (■) and 250 (◆) mN were tested. Pre-treatment with THFA or PKI has no effect on the capsaicin-induced increase in the number of responses to repeated application of von Frey filaments. Arrow (time 0) = time of capsaicin injection.

significant differences between animals pre-treated with THFA or PKI and those treated with ACSF at any time after capsaicin injection for responses to repeated application of von Frey filaments or for threshold to mechanical stimuli.

Post-treatment Infusion (for 1 h from 60 to 120 min post-capsaicin) of the adenylate cyclase inhibitor, THFA or the protein kinase A inhibitor, PKI, dose-dependently reduced the increased responses to mechanical stimulation with repeated applications (10 trials) of von Frey filaments (Figure 4b). Figure 4a represents the number of responses to von Frey filament before capsaicin, 1 h after capsaicin and 1 h after administration of either 1 mM THFA, 5 mM PKI or ACSF (as a control). In the group of animals treated with PKI there was a significant change in the number of responses to 10 mN ($\chi^2=11.3$, $P=0.004$), 50 mN ($\chi^2=10.6$, $P=0.005$), 150 mN ($\chi^2=10.4$, $P=0.006$) and 250 mN ($\chi^2=10.4$, $P=0.006$) bending forces. Similarly in the group of animals treated with THFA there was a significant change in the number of responses to 10 mN ($\chi^2=12$, $P=0.002$), 50 mN ($\chi^2=11.6$, $P=0.003$), 150 mN ($\chi^2=10.2$, $P=0.006$) and 250 mN ($\chi^2=11.1$, $P=0.004$) bending forces. Blockade of adenylate cyclase or PKA significantly reduced the increased number of responses to repeated application of von Frey filaments induced by capsaicin injection for all bending forces. Infusion of ACSF as a control for 1 h had no effect on the increased responses to von Frey filaments induced by intradermal injection of capsaicin (Figure 4a). The effects of spinal infusion of THFA or PKI lasted for at least 30 min after removal of the inhibitor (data not shown). Figure 4b shows the dose-dependent effects of administration of THFA (0.01–10 mM) and PKI (0.05–50 mM).

A significant change in the threshold occurred in the groups of animals treated with THFA ($\chi^2=10.9$, $P=0.01$), PKI ($\chi^2=16.8$, $P=0.0008$) or ACSF ($\chi^2=20$, $P=0.002$). The threshold to mechanical stimulation decreased significantly 30 min after injection of capsaicin in all three groups: ACSF, THFA or PKI (Figure 5a, b). Spinal infusion of either 1 mM THFA or 5 mM PKI significantly increased the threshold to mechanical stimuli to 200 mN (range 150–250 mN) and 250 mN (range 90–600 mN), respectively, when compared to values after injection of capsaicin or ACSF control animals (Figure 5). The increase in threshold produced by THFA or PKI was dose-dependent (Figure 5b).

To further demonstrate that the cAMP pathway was involved, 8-br-cAMP was added back after blockade of adenylate cyclase with THFA. Blockade of adenylate cyclase with 1 mM THFA significantly reduced the capsaicin-induced increase in the number of responses to mechanical stimuli. Infusion of 8-br-cAMP (1 mM + 1 mM THFA) increased the

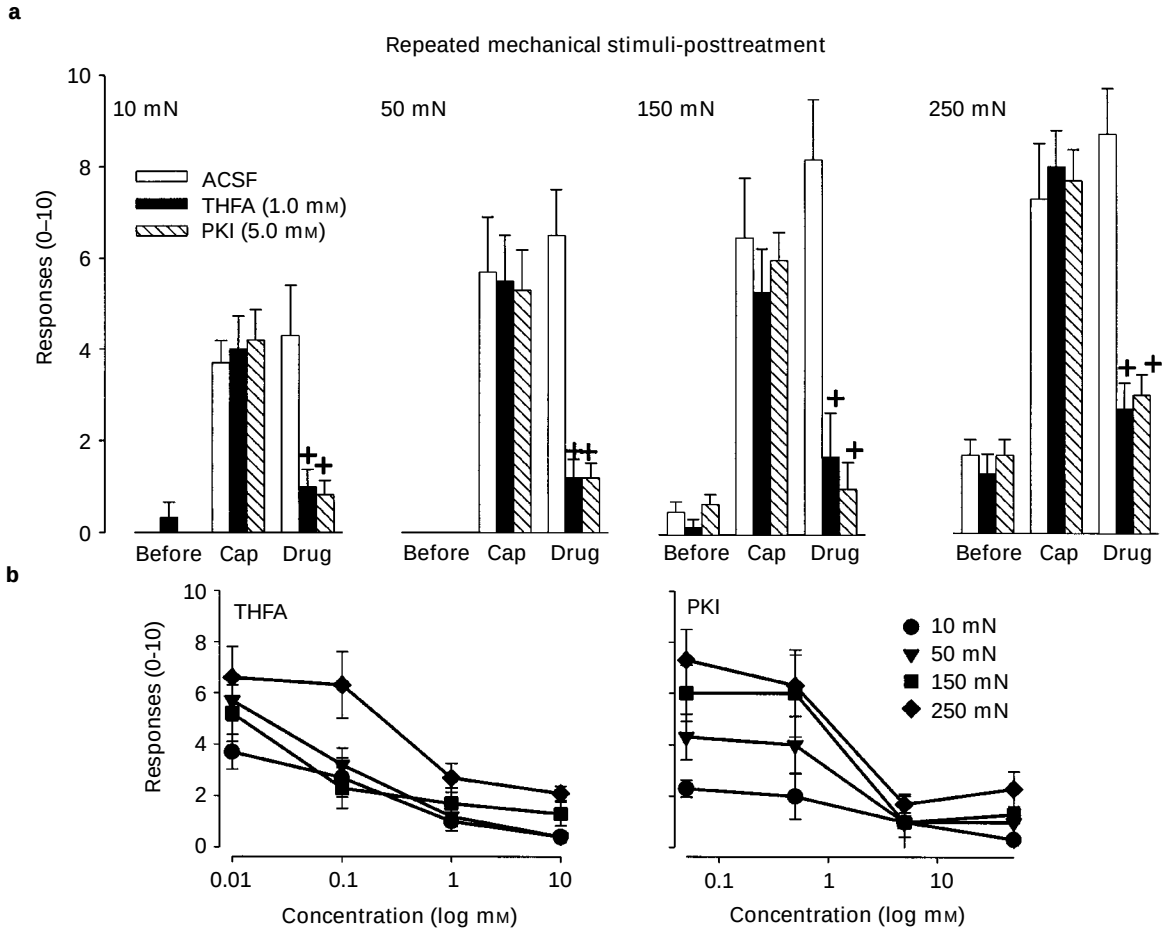

Figure 4 (a) Bar graphs representing the responses to repeated application (10 trials) of von Frey filaments in animals treated with ACSF (control, □), 1 mM THFA (adenylate cyclase inhibitor, ■) or 5 mM PKI (protein kinase A inhibitor, ▨) 60 min after injection of capsaicin. Responses after capsaicin injection (Cap) were significantly increased for all groups when compared to responses before capsaicin injection (Before). After infusion of ACSF the responses to von Frey filaments remained significantly increased when compared to responses before capsaicin injection. Infusion of THFA or PKI (Drug) significantly decreased the responses back toward those observed before capsaicin injection. $^{+}$Significantly decreased from responses after capsaicin injection or control animals treated with ACSF. (b) Dose–response curves for animals post-treated with THFA (left) or PKI (right) for the number of responses to repeated application of 10 (●), 50 (■), 150 (▼) and 250 (◆) mN bending forces.

number of responses to repeated applications (10 trials) of von Frey filaments. The return of the behaviours was comparable to that observed after injection of capsaicin (Figure 6a) or control animals treated with ACSF. There was a dose-dependent increase in the number of responses to von Frey filaments. The 1 mM dose of 8-br-cAMP increased the number of responses ipsilateral to the capsaicin injection with no change on the contralateral side; and responses were comparable to that observed after capsaicin injection (Figure 6b). There were significant changes in the number of responses to repeated application of von Frey filaments to 10 mN ($\chi^2 = 25.58$, $P = 0.0001$), 50 mN ($\chi^2 = 26.7$, $P = 0.0001$), 150 mN ($\chi^2 = 22.9$, $P = 0.0001$) and 250 mN ($\chi^2 = 25.7$, $P = 0.0001$) bending forces.

The decrease in threshold to mechanical stimuli induced by capsaicin injection (5 mN range 1–10 mN) was significantly increased following spinal infusion of 1 mM concentration of THFA (200 mN, range 50–600 mN). Following infusion of 8-br-cAMP (1 mM) with THFA (1 mM) the threshold to mechanical stimulation was significantly decreased (7.5 mN, 1–50 mN) to levels comparable to that observed 30 min after injection of capsaicin (Figure 7) or ACSF control animals. There was an overall significant effect for changes in the threshold to mechanical stimulation over time in the group of animals treated with THFA and 8-Br-cAMP ($\chi^2 = 15.4$, $P = 0.0015$). The 1 mM concentration of cAMP had no effect on the contralateral side, similar to that observed in normal animals. However, after infusion of the 10 mM dose of 8-br-cAMP the threshold to mechanical stimulation decreased contralaterally from 600 mN (range 250–600 mN) to 50 mN (range 10–50 mN).

Discussion

This study demonstrates that activation of the cAMP transduction cascade at the spinal cord level results in mechanical hyperalgesia and allodynia and that the secondary mechanical hyperalgesia and allodynia following intradermal injection of capsaicin is mediated by this same transduction cascade. Capsaicin was applied to the proximal portion of the hindpaw while the stimuli were applied to the distal portion of the paw outside the area encompassed by the injection of capsaicin. Thus, secondary mechanical hyperalgesia and allodynia associated with capsaicin injection was significantly reduced by blockade of the cAMP transduction cascade spinally after the development of secondary hyperalgesia and allodynia. In contrast, spinal blockade of adenylate cyclase or PKA prior to injection of capsaicin has no effect on the development of secondary mechanical hyperalgesia and allodynia. Concentrations of 8-br-cAMP that have no effect in normal animals returns the secondary mechanical hyperalgesia and allodynia blocked by THFA back to levels induced by capsaicin injection. Thus, this study documents a spinal role for the cAMP

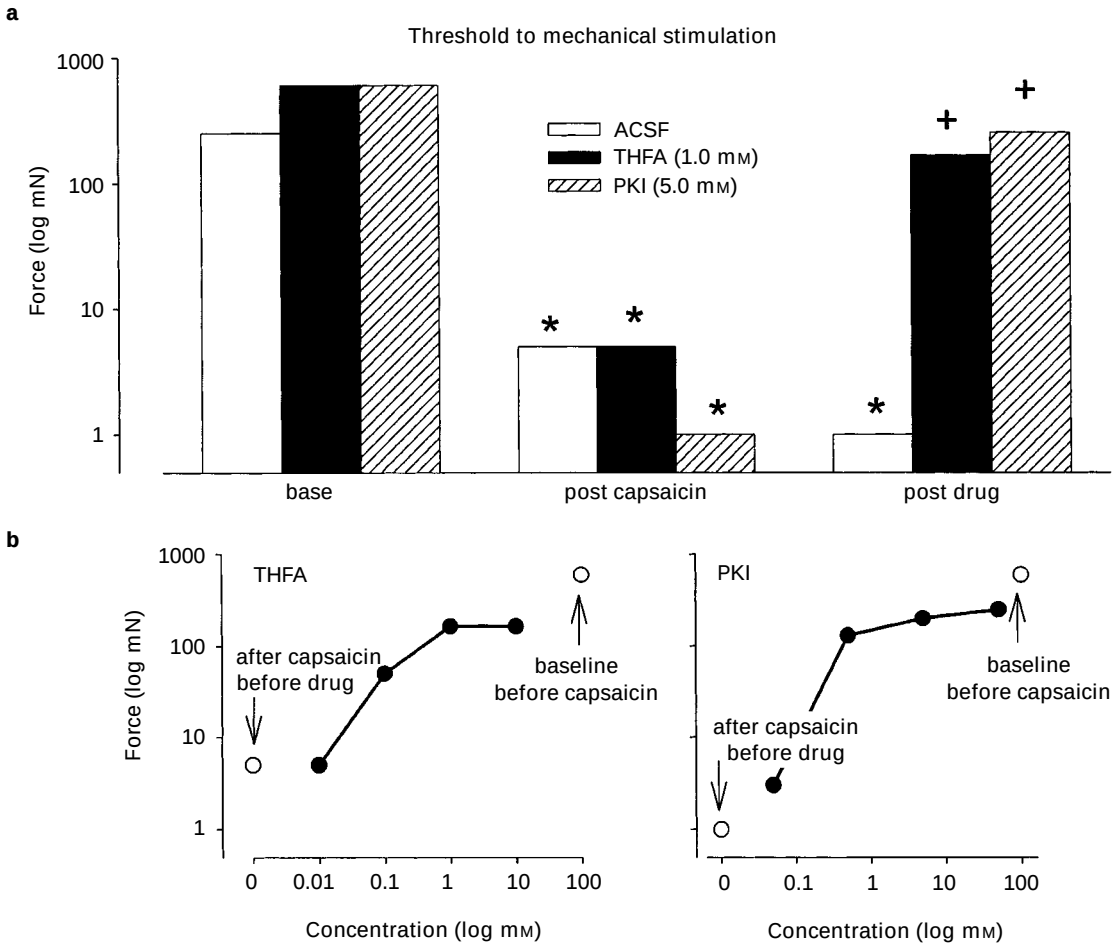

Figure 5 (a) Bar graphs representing the threshold to mechanical stimulation in animals posttreated with ACSF (control, □), THFA (adenylate cyclase inhibitor, ■) or PKI (protein kinase A inhibitor, ▨). *Significantly decreased from baseline, $^{+}$significantly increased from responses after capsaicin injection. (b) Dose-dependent effects (●) on threshold for animals post-treated with THFA or PKI. For comparison, the responses before and after capsaicin injection are illustrated (○).

transduction cascade in mechanical hyperalgesia and allodynia. It appears that the cAMP transduction cascade is involved in maintaining secondary hyperalgesia and allodynia (a measure of central sensitization), but not in the induction of central sensitization. Therefore, activation of the cAMP pathway must occur in response to mechanisms responsible for inducing central sensitization. A variety of receptors and channels have been implicated in the initiation of central sensitization. These include non-NMDA glutamate receptors (Na^+ channels) (Dougherty *et al.*, 1992a; Neugebauer *et al.*, 1993; Sluka & Westlund, 1993), NMDA glutamate receptors (Ca^{2+} channel) (Dickenson & Sullivan, 1987; Coderre & Melzack, 1992; Dougherty *et al.*, 1992a; Ren & Dubner, 1993), neurokinin 1 receptors (G-protein linked channel) (Fleetwood-Walker *et al.*, 1990; Radhakrishnan & Henry, 1991; Dougherty *et al.*, 1994; Sluka *et al.*, 1997a), metabotropic glutamate receptors (G-protein linked channel) (Young *et al.*, 1994; Neugebauer *et al.*, 1995) and high voltage calcium channels (Malmberg & Yaksh, 1994; Chaplan *et al.*, 1995; Sluka *et al.*, 1997b). Activation of the calcium channels or the G-protein linked channels could activate second messenger systems within the cell, thus starting a cascade of events that would lead to increased accumulation of cAMP intracellularly and activation of protein kinase A. The end result could be increased effectiveness of ionotropic channels (Blackstone *et al.*, 1995; Hell *et al.*, 1995; Sculptoreanu *et al.*, 1995; Smith *et al.*, 1995) and/or increased neurotransmitter release (Hell *et al.*, 1995) which would contribute to the central sensitization observed after injection of capsaicin. In fact, Dougherty *et al.* (1992b) have shown an increased effectiveness of the quisqualate channel after tissue injury. Furthermore, following capsaicin injection there is an increase in release of the excitatory amino acids, aspartate and glutamate, in the dorsal horn (Sorkin & McAdoo, 1993).

Injection of capsaicin in humans produces an area of primary hyperalgesia to heat and mechanical stimulation at the site of injection and an area of secondary hyperalgesia to mechanical stimuli outside the area of injection (Simone *et al.*, 1989; Lamotte *et al.*, 1991). Capsaicin has been shown to activate C-mechanoheat receptors in the periphery that contain neuropeptides such as substance P and calcitonin gene-related peptide (Senso & Dray, 1993). Spinothalamic tract (STT) neurons sensitize to mechanical stimuli outside the site of injection (Simone *et al.*, 1991; Dougherty *et al.*, 1992a; Sluka *et al.*, 1997b). This sensitization of STT cells to capsaicin injection can be prevented by spinal blockade of: (1) non-NMDA glutamate receptors (Dougherty *et al.*, 1992a), (2) NMDA glutamate receptors (Dougherty *et al.*, 1992a), or (3) neurokinin 1 receptors (Dougherty *et al.*, 1994). Furthermore, the sensitization of STT cells is reduced by post-treatment with inhibitors of G-proteins, protein kinase A or protein kinase C (Sluka *et al.*, 1997b).

From these studies it is not possible to determine if the effects of the drugs are presynaptic on primary afferent terminals and/or postsynaptic on dorsal horn neurons. It is quite probable based on previous literature that there is a contribution of the cAMP pathway from the central terminals of primary afferent fibres. Hingtgen *et al.* (1995) have shown that prostaglandin-

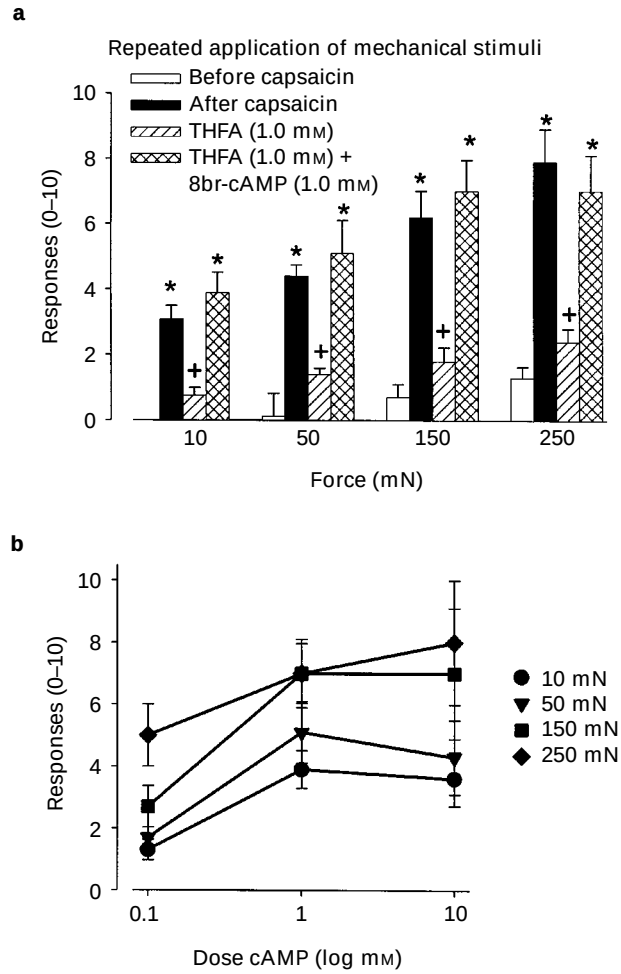

Figure 6 (a) Bar graphs represent the number of responses before capsaicin injection (□), after capsaicin injection (■), after infusion of THFA (▨, 1 mM) and after infusion of THFA (1 mM) + 8-bromo-cAMP (1 mM) (▩). THFA significantly reduced the increased number of responses to repeated application of von Frey filaments (10–250 mN bending forces) induced by capsaicin. At a dose that had no effect in normal animals, 1 mM 8-br-cAMP increased the number of responses back to levels observed after capsaicin injection. *Significantly increased from baseline, $^{+}$significantly reduced from responses after capsaicin injection. (b) Dose-dependent effect of 8-bromo-cAMP on the increased number of responses to repeated application of von Frey filaments with bending forces of 10 (●), 50 (▼), 150 (■) and 250 (◆) mN.

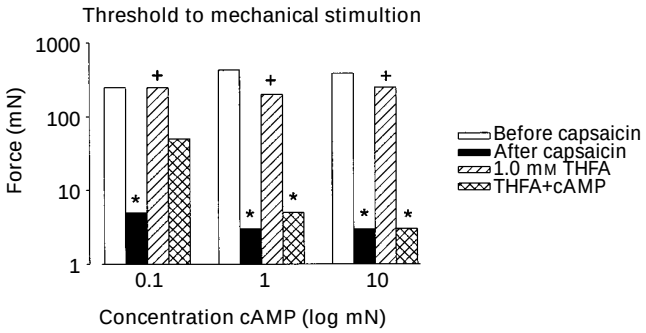

Figure 7 Bar graph representing the dose-dependent effects on the threshold to mechanical stimuli for animals treated with 8-bromo-cAMP + THFA after the injection of capsaicin. Significant decreases in threshold occurred after capsaicin injection (■) when compared to responses before injection of capsaicin (□). Spinal infusion of 1 mM THFA significantly reversed the decreased threshold to mechanical stimulation (▨). Spinal infusion of 8-bromo-cAMP + THFA (1 mM) (▩) dose-dependently decreased the threshold to levels observed after capsaicin injection.* Significantly decreased from baseline, $^{+}$significantly increased from responses after capsaicin injection.

induced release of neuropeptides (substance P and calcitonin gene-related peptide) from cultured dorsal root ganglion neurons is cAMP-dependent. Kress *et al.* (1996) demonstrated that cAMP analogues sensitize unmyelinated primary afferents in rat skin to heat stimulation and thus may contribute to heat hyperalgesia. Peripheral injections of analogues of cAMP or an adenylate cyclase activator produces mechanical hyperalgesia (Taiwo & Levine, 1991; Duarte *et al.*, 1992).

Spinally, changes in cAMP are unclear. Igwe & Ning, (1994) measured cAMP content in the spinal cord after unilateral injection of complete Freund's adjuvant (FCA) over a 42 day time course. This study demonstrated no change in cAMP content after inflammation at any time. In contrast, another study demonstrated decreased content of cAMP after a more acute inflammatory stimulus, carrageenan, 3 h after induction of arthritis (Garry *et al.*, 1994). Previously we were able to demonstrate that blockade of protein kinase A with H89 by microdialysis infusion in the dorsal horn reduced the sensitization of STT cells to capsaicin injection (Sluka *et al.*, 1997b). In addition we demonstrated that H89 delivered by microdialysis also reduced capsaicin induced mechanical hyperalgesia in rats (Sluka & Willis, 1997). The present study further establishes that mechanical hyperalgesia is reduced by blockade of adenylate cyclase or PKA when given after the development of central sensitization and that infusion of cell permeable analogues of cAMP can produce mechanical hyperalgesia.

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