

Activation of Central Terminal Vanilloid Receptor-1 Receptors and $\alpha\beta$ -Methylene-ATP-Sensitive P2X Receptors Reveals a Converged Synaptic Activity onto the Deep Dorsal Horn Neurons of the Spinal Cord

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Using a spinal cord slice preparation and patch-clamp recordings from spinal cord dorsal horn neurons, we examined excitatory and inhibitory circuits connecting to lamina V neurons after the activation of afferent central terminal vanilloid receptor-1 (VR1) receptors and P2X receptors. We found that single neurons in lamina V often received excitatory inputs from two chemically defined afferent pathways. One of these pathways was polysynaptic from capsaicin-sensitive afferent terminals. In this pathway the capsaicin-sensitive afferent input first activated interneurons in superficial laminae, and then the excitatory activity was transmitted onto lamina V neurons. The second excitatory input was monosynaptic from $\alpha\beta$ m-ATP-

sensitive/capsaicin-insensitive afferent terminals. Both capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathways also recruited polysynaptic inhibitory inputs to lamina V neurons. Furthermore, we demonstrated that simultaneous activation of both capsaicin-sensitive afferent pathways and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathways could generate a temporal summation of excitatory inputs onto single lamina V neurons. These convergent pathways may provide a mechanism of sensory integration for two chemically defined sensory inputs and may have implications in different sensory states.

Key words: capsaicin; VR1 receptors; ATP; α , β -methylene-ATP; P2X receptors; EPSCs; IPSCs; spinal cord slice preparation

The capsaicin vanilloid receptor-1 receptor (VR1) is a ligand-gated cation channel that functions as a sensor for heat, protons, and endogenous VR1 ligands (Caterina et al., 1997; Kress and Zeilhofer, 1999; Zygmunt et al., 1999; Hwang et al., 2000; Smart et al., 2000). VR1 is expressed on a subpopulation of primary afferent neurons that have small diameter and are potentially nociceptive sensory neurons (Jancso et al., 1977; Holzer, 1991; Caterina et al., 1997; Guo et al., 1999). At peripheral nerve endings VR1 activation may be associated with the generation of burning pain sensation (Szolcsanyi, 1977; Holzer, 1991; Tomimaga et al., 1998). VR1 plays an essential role in tissue injury- and inflammation-induced thermal hyperalgesia (Caterina et al., 2000; Davis et al., 2000). In addition to their expression at peripheral nerve endings, VR1 immunoreactivity (VR1-ir) also is found on the central terminals of nociceptive afferent fibers (Guo et al., 1999).

P2X receptors (P2X) are a family of cation channels gated by extracellular ATP (Jahr and Jessell, 1983; Krishtal et al., 1983). To date, seven P2X subunits (P2X₁ to P2X₇) have been cloned (North and Surprenant, 2000; Khakh et al., 2001), and six (P2X₁ to P2X₆) are expressed on primary sensory neurons (Collo et al., 1996; Vulchanova et al., 1996, 1997, 1998; Xiang et al., 1998; C. Li

et al., 1999; Novakovic et al., 1999). Activation of P2X at peripheral sensory nerve endings may initiate sensory impulses and may be associated with nociceptive and non-nociceptive sensory signals (Burnstock and Wood, 1996; Cook et al., 1997; Sawynok and Reid, 1997; Dowd et al., 1998; Cockayne et al., 2000; Souslova et al., 2000; Tsuda et al., 2000). Immunochemical studies have shown the presence of P2X subunits on the central terminals of primary afferents (Guo et al., 1999) and have shown that presynaptic P2X could modulate sensory synaptic transmission (Gu and MacDermott, 1997; Li et al., 1998).

Dorsal root ganglion (DRG) neurons expressing VR1 usually also express P2X₃ receptors (Guo et al., 1999; Ueno et al., 1999; Wood, 2000). Therefore, capsaicin-sensitive afferent fibers are usually ATP-sensitive as well (C. Li et al., 1999; Ueno et al., 1999; Petruska et al., 2000). Based on the immunoreactivity for VR1 and P2X₃ (Guo et al., 1999), sensory signals induced by the activation of VR1 or P2X₃ should be conveyed primarily to the superficial laminae of the spinal cord. However, many DRG neurons express P2X, but not VR1 (C. Li et al., 1999; Ueno et al., 1999; Petruska et al., 2000). These primary sensory neurons are ATP-sensitive/capsaicin-insensitive (Tsuda et al., 2000). For ATP-sensitive/capsaicin-insensitive afferents, many of their central terminals synapse directly on deep dorsal horn (DH) neurons in lamina V (Nakatsuka and Gu, 2001). Thus, capsaicin-sensitive and ATP-sensitive/capsaicin-insensitive afferents are two chemically distinct populations of sensory fibers.

Within the neuronal network of the DH, synaptic interactions of different sensory inputs may play a role in sensory phenomena such as hyperalgesia and allodynia. The sensory interactions could be involved in capsaicin-induced receptive field expansion

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and secondary hyperalgesia (Simone et al., 1989a; LaMotte et al., 1991; Pedersen et al., 1996). Here, we examined synaptic pathways of capsaicin-sensitive inputs and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive inputs and their interactions in the DH neuronal network.

MATERIALS AND METHODS

Tissue preparation. Transverse spinal cord slices (500 μ m in thickness) were prepared from L5 spinal cords of rats at the postnatal age of 11–21 d (Nakatsuka et al., 2000). Some slices were prepared from adult rats at ages between 7 and 9 weeks as described previously (Yoshimura and Jessell, 1990). Spontaneous EPSCs (sEPSCs), miniature EPSCs (mEPSCs), spontaneous IPSCs (sIPSCs), and miniature IPSCs (mIPSCs) were recorded from slices without dorsal roots attached. Evoked EPSCs (eEPSCs) were recorded from slices with L5 dorsal roots attached. The length of the dorsal roots was in the range of 4–15 mm. In each experiment a spinal cord slice was transferred to a recording chamber and placed on the stage of an upright microscope equipped with an infrared differential interference contrast (IR-DIC) system. The volume of the recording chamber was \sim 0.5 ml. Lamina regions were identified with a 10 \times objective. Recordings from spinal slices of postnatal rats were performed under visual guidance; individual neurons could be identified with a 40 \times objective. A blind-patch technique was used for recordings in spinal cord slice preparations from adult rats (Nakatsuka et al., 2000). The spinal cord slice was superfused with Krebs' solution flowing at 10 ml/min at room temperature (22°C). The Krebs' solution contained (in mM): 117 NaCl, 3.6 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃, and 11 glucose; the solution was equilibrated with 95% O₂/5% CO₂, and the pH of the saturated solution was 7.4.

Patch-clamp recordings. Whole-cell patch-clamp recordings were made from DH neurons with electrodes filled with an internal solution containing (in mM): 135 K⁺-gluconate, 5 KCl, 0.5 CaCl₂, 2 MgCl₂, 5 EGTA, and 5 HEPES; the pH of the solution was adjusted to 7.3 with NaOH, and the osmolarity was adjusted to 320 mOsm with sucrose. The resistances of the electrodes were \sim 5 M Ω when filled with the internal solution. The junction potentials of the electrodes were not corrected. The access resistance was below 20 M Ω and was not compensated. Signals were amplified and filtered at 2 kHz (Axopatch 200B) and sampled at 5 kHz. When EPSCs were recorded, the cells were held at -60 mV. At this holding potential the outward IPSCs were minimized and usually undetectable. sEPSCs were recorded in the absence of tetrodotoxin (TTX), and mEPSCs in the presence of 0.5 μ M TTX, 20 μ M bicuculline, and 2 μ M strychnine were present in the bath solution in some experiments. When IPSCs were recorded, the cells were held at -10 mV. This holding potential was close to the reversal potential for glutamate receptors. Therefore, the inward EPSCs were minimized and usually undetectable. sIPSCs were recorded in the absence of TTX and mIPSCs in the presence of 0.5 μ M TTX. In some experiments for the recordings of sIPSCs and mIPSCs, 20 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 50 μ M D-(+)-2-amino-5-phosphonopentanoic acid (D-APV) also were present in the bath solution. In experiments designed to record sEPSCs and sIPSCs simultaneously, the cells were held at -45 mV. At this holding potential the sEPSCs were inward currents and the sIPSCs were outward currents. α , β -Methylene-ATP ($\alpha\beta$ m-ATP; 100 μ M), capsaicin (2 μ M), and other testing compounds were applied via the bath solution. The intervals for multiple applications of testing compounds were 20 min. Analyses of sEPSCs, mEPSCs, sIPSCs, and mIPSCs were performed as described previously (Gu and MacDermott, 1997) with the commercial software Mini Analysis (Synaptosoft, Decatur, GA; <http://www.synaptosoft.com>). Cells were assigned to be responsive to the testing compounds when there were $>20\%$ increases in the frequency of EPSCs or IPSCs. To record eEPSCs, we applied stimuli (30–120 μ A, 0.1 msec for A δ fiber; 200–800 μ A, 0.1 msec for C-fiber) to a dorsal root with a suction electrode, and we judged monosynaptic connection by constant latency of eEPSCs (Nakatsuka et al., 2000). Conduction velocity was calculated on the basis of the latency of eEPSCs and the length of the dorsal root. In experiments to determine action potential firing on postsynaptic neurons, DH neurons in lamina V were under current-clamp configuration.

VR1 immunostaining. Spinal cord slice sections (250 μ m in thickness) were obtained in the same way as those sections for electrophysiology recordings. They were put in a 35 mm Petri dish and fixed with 4% paraformaldehyde (PFA; in PBS buffer solution) for 12 hr at 4°C. The slices were transferred into a solution containing 4% PFA and 0.4%

Triton X-100 and incubated at 4°C for 2 hr. They were washed three times with PBS and then mounted onto glass slides and allowed to air dry. Then they were encircled with hydrophobic resin (PAP Pen, The Binding Site). Slices were incubated for 1 hr in a solution of 1:30 normal goat serum in PBS with 0.4% Triton X-100 (GS-PBS-T) to block non-specific antibody binding. Slices were incubated with a polyclonal guinea pig anti-VR1 receptor antibody (1:2000; Neuromics, Minneapolis, MN) overnight at 4°C. After a rinse with 1% goat serum in PBS solution three times, for 20 min each time, the slices were incubated further for 3 hr at room temperature with a secondary antibody. The secondary antibody (1:100 in 1% goat serum PBS solution) was a goat anti-guinea pig IgG conjugated with AlexaFluor 594 (Molecular Probes, Eugene, OR). Slices were washed three more times with 1% goat serum in PBS solution. After a glycerol-based anti-photobleach medium was applied, the slices were covered with coverslips. Sections were viewed by a fluorescent microscope (Olympus IX-70), and images were captured with a digital camera.

α , β -Methylene-ATP, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), capsaicin, bicuculline, strychnine, and capsazepine were purchased from Sigma (St. Louis, MO). CNQX, D-APV, and TTX were purchased from Tocris Cookson (St. Louis, MO). Unless otherwise indicated, the data represent the mean \pm SEM. Paired Student's *t* tests were used for statistical comparison, and significance was considered at the *p* < 0.05 level.

RESULTS

Spreading excitatory activity of capsaicin-sensitive inputs to deep laminae

In the spinal cord slice preparation, the central terminals of primary afferent fibers remain in the slice sections and are functionally intact for synaptic transmission, although the dorsal roots are removed (P. Li et al., 1999; Nakatsuka et al., 2000). In this study synaptic activity in the lamina V neurons was examined. When capsaicin (2 μ M) was bath applied for 1 min, there was a large increase in the sEPSC frequency in all 18 neurons that were recorded (Fig. 1A). The sEPSC frequency was $382 \pm 55\%$ of control (*n* = 18; *p* < 0.05) after capsaicin application. The effects of capsaicin lasted for >2 min. Capsaicin-induced increases of sEPSC frequency ($495 \pm 81\%$ of control, *n* = 6) were abolished in the presence of the VR1 receptor antagonist 10 μ M capsazepine ($101 \pm 4\%$, *n* = 6; Fig. 1B). CNQX (10 μ M), a non-NMDA receptor antagonist, completely blocked sEPSCs (*n* = 6; Fig. 1C). When similar experiments were performed and recordings were made from neurons in lamina III–IV, four neurons of seven recordings also showed increases in sEPSC frequency. The remaining three cells showed few changes after capsaicin application (data not shown). Thus, capsaicin-sensitive inputs can transmit excitatory activity to deep laminae, especially to lamina V.

TTX has been used widely to block active interneuronal transmission; any polysynaptic transmission between DH neurons should be blocked by 0.5 μ M TTX (Gu et al., 1996; Gu and MacDermott, 1997). In sharp contrast to the above capsaicin effects in the absence of TTX, in the presence of 0.5 μ M TTX the capsaicin did not produce any increase in the frequency of mEPSCs in 20 of 21 cells that were recorded in lamina V (Fig. 1D). These results strongly suggest that most capsaicin-sensitive inputs to lamina V neurons are transmitted polysynaptically and that few capsaicin-sensitive terminals synapse monosynaptically on lamina V neurons.

To determine whether, in the absence of TTX, capsaicin-induced excitatory synaptic activity in lamina V was via interneurons in the superficial laminae, we removed superficial laminae (Fig. 2A, left) and then performed experiments in the same way as those shown in Figure 1A. Under this condition only a few neurons (3 of 15 cells; Fig. 2A, right) in lamina V showed increases of sEPSC frequency by 2 μ M capsaicin. This result is in sharp contrast to the recordings of sEPSCs from intact slices in

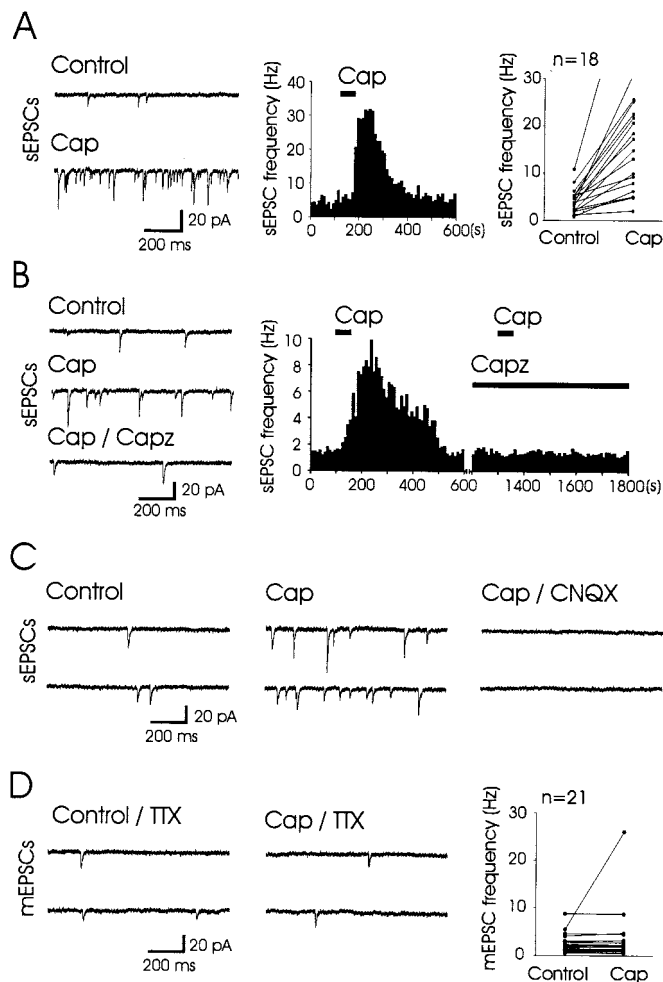


Figure 1. Capsaicin-induced excitatory synaptic activity in lamina V neurons. *A*, Capsaicin produced a large increase in sEPSC frequency in lamina V neurons. Two sample traces (left) show sEPSCs recorded from a lamina V neuron before (Control) and after the application of 2 μ M capsaicin for 1 min (Cap). The time course of the changes in sEPSC frequency is shown in the histogram (middle). The graph on the right shows the pooled results from 18 lamina V neurons. The capsaicin effect on each cell was obtained from a time course histogram of sEPSC frequency and is the averaged response in 30 sec around the peak value. *B*, VR1 receptor antagonist capsazepine (10 μ M) abolished the effects of capsaicin. Three sample traces (left) show sEPSCs before (Control) and after the application of 2 μ M capsaicin (Cap) and after the application of 2 μ M capsaicin in the presence of 10 μ M capsazepine (Cap/Capz). The time course of sEPSC frequency is shown in the histogram (right). Similar results were obtained in five other neurons. Capsazepine was preapplied for 10 min. *C*, The sEPSCs were blocked completely by 20 μ M CNQX ($n = 6$). *D*, Capsaicin produced little effect on mEPSC frequency in lamina V neurons. Experiments were performed in the presence of 0.5 μ M TTX. Two sets of sample traces show mEPSCs before (Control/TTX) and after the application of 2 μ M capsaicin (Cap/TTX). The graph on the right shows the pooled results from 21 cells. In all of the experiments capsaicin was applied for 1 min. The intervals for multiple applications of testing compounds were 20 min in all of the experiments.

the absence of TTX. Figure 2*B* is a summary comparing the percentage of lamina V neurons responsive to capsaicin applications in the three different experimental conditions that have been described above.

To determine whether, in the superficial laminae, capsaicin-sensitive central terminals and DH neurons were connected monosynaptically, we determined the effects of capsaicin on

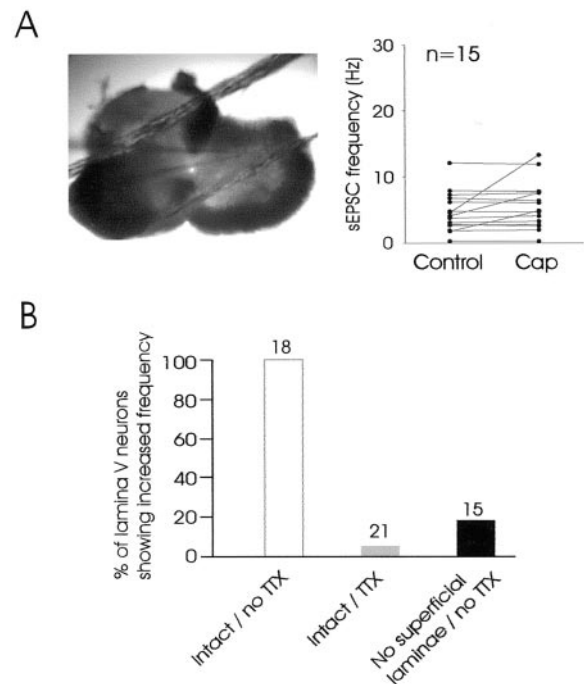


Figure 2. Removal of superficial laminae abolishes the capsaicin-induced activity in lamina V neurons. *A*, The photomicrograph (left) shows a spinal cord slice with one side of the superficial laminae removed. A part of a patch electrode is seen also, and the electrode tip is inside the tissue ~ 70 μ m from the surface. The location of the electrode tip is in lamina V. The graph on the right shows sEPSC frequency recorded from lamina V neurons in such preparations after a 1 min application of 2 μ M capsaicin ($n = 15$). *B*, A summary comparing the percentage of lamina V neurons that had increased sEPSCs by capsaicin under three conditions: the intact spinal cord slice in the absence of TTX (Intact/no TTX; $n = 18$), the intact spinal cord slice in the presence of TTX (Intact/TTX; $n = 21$), and the spinal cord slice with one side of the superficial laminae removed and in the absence of TTX (No superficial laminae/no TTX; $n = 15$). Data are from Figures 1*A*, *D*, 2*A*.

mEPSCs in lamina II neurons in the presence of 0.5 μ M TTX. After the application of 2 μ M capsaicin, 14 of 16 cells showed increases in the mEPSC frequency (Fig. 3*A*, *B*); the overall changes of mEPSC frequency were $460 \pm 60\%$ of control ($n = 16$; $p < 0.05$). This result provided electrophysiological evidence that capsaicin-sensitive central terminals synapse monosynaptically on superficial DH neurons. Consistent with this conclusion, immunostaining with an antibody against VR1 receptors showed that VR1-ir was distributed mainly in the superficial laminae (laminae I and II; Fig. 3*C*). The results from Figures 1 to 3*C* support a major capsaicin-sensitive pathway that relays excitatory activity to lamina V neurons (Fig. 3*D*).

$\alpha\beta$ -Methylene-ATP-sensitive/capsaicin-insensitive inputs to lamina V neurons

A previous study in our laboratory (Nakatsuka and Gu, 2001) has indicated that many afferent central terminals to lamina V neurons express $\alpha\beta$ m-ATP-sensitive P2X receptors and that these terminals appear to be capsaicin-insensitive. The monosynaptic connection of these terminals to lamina V neurons and their capsaicin sensitivity were confirmed in the present study. As shown in Figure 4, 100 μ M $\alpha\beta$ m-ATP increased mEPSC frequency in a lamina V neuron in the presence of 0.5 μ M TTX. The effect lasted for >3 min with a 1 min $\alpha\beta$ m-ATP application (Fig. 4*C*), suggesting that a nondesensitizing $\alpha\beta$ m-ATP-sensitive type of P2X receptor mediated the responses. Of 28 lamina V neurons

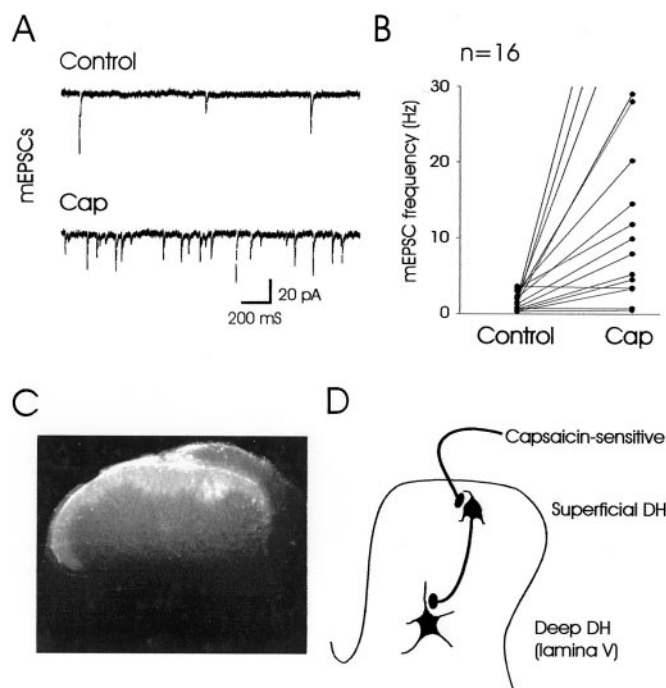


Figure 3. Monosynaptic connections between capsaicin-sensitive afferent terminals and superficial lamina neurons. *A*, Capsaicin produced a large increase in mEPSC frequency in lamina II neurons. Two sample traces show mEPSCs before (*Control*) and after the application of 2 μ M capsaicin in the presence of 0.5 μ M TTX. *B*, Pooled results show that 2 μ M capsaicin produced increases in mEPSC frequency in most lamina II neurons that were tested (14 of 16 cells). *C*, Fluorescent microscopic image of VR1 receptor immunoreactivity in a spinal cord section. Strong VR1-ir was observed in superficial laminae ($n = 8$). *D*, A summary of the proposed capsaicin-sensitive pathway based on the results from Figures 1–3C.

that were recorded, 27 cells showed such a response to 100 μ M $\alpha\beta$ m-ATP. The overall changes of mEPSC frequency were $391 \pm 56\%$ of control ($n = 28$; $p < 0.05$). The $\alpha\beta$ m-ATP-induced increases of mEPSC frequency were abolished ($n = 6$; Fig. 4*A*) in the presence of the P2X receptor antagonist PPADS (10 μ M). CNQX (20 μ M) completely blocked mEPSCs in the cells for which 100 μ M $\alpha\beta$ m-ATP increased mEPSC frequency (Fig. 4*B*; $n = 6$). Because few capsaicin-sensitive terminals monosynaptically connected with lamina V neurons (Fig. 1*D*), most $\alpha\beta$ m-ATP-sensitive terminals that synapse directly on lamina V neurons should be $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive terminals. This was confirmed by testing the effects of both capsaicin and $\alpha\beta$ m-ATP on mEPSC frequency in the same lamina V neurons in the presence of 0.5 μ M TTX (Fig. 4*C*). As demonstrated in Figure 4*C*, capsaicin did not produce any change in mEPSC frequency in a cell for which $\alpha\beta$ m-ATP produced a large increase in mEPSC frequency (see also Fig. 5*A,B*). Figure 4*D* schematically illustrates the major monosynaptic $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathway to lamina V neurons, based on the effects of $\alpha\beta$ m-ATP and capsaicin on mEPSC frequency recorded from lamina V neurons (see also Nakatsuka and Gu, 2001).

Synaptic convergence of excitatory inputs from capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathways

To determine whether excitatory inputs from capsaicin-sensitive terminals and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive terminals

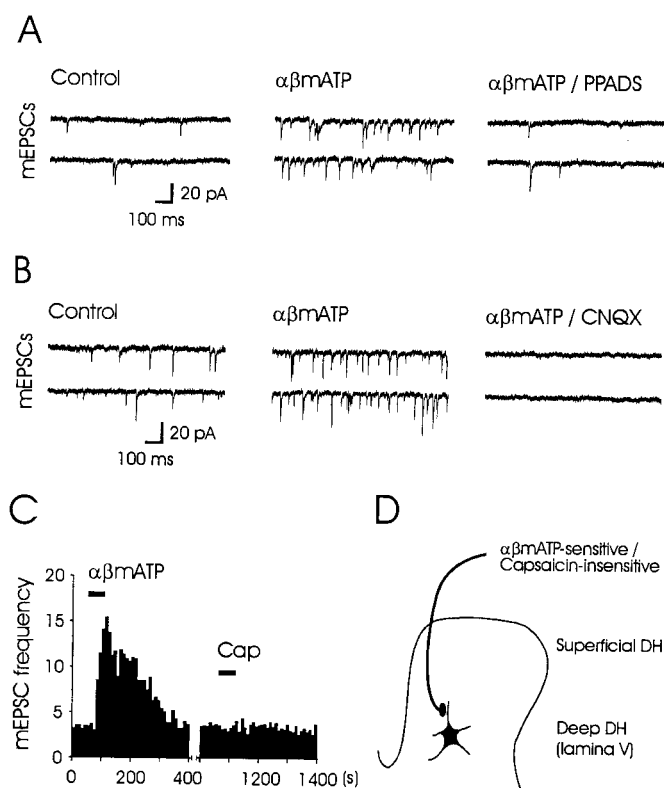


Figure 4. Monosynaptic connections between $\alpha\beta$ m-ATP-sensitive terminals and lamina V neurons. *A*, Activation of $\alpha\beta$ m-ATP-sensitive P2X receptors produced increases in mEPSC frequency in lamina V neurons. Three sets of traces show mEPSCs before (*Control*; left) and after the application of 100 μ M $\alpha\beta$ m-ATP (*middle*) and after the application of 100 μ M $\alpha\beta$ m-ATP in the presence of 10 μ M PPADS (*right*). The mEPSC frequency became $391 \pm 56\%$ of control ($n = 28$) after the application of 100 μ M $\alpha\beta$ m-ATP. The effects of $\alpha\beta$ m-ATP were abolished completely in the presence of 10 μ M PPADS ($n = 6$). *B*, The sEPSCs were blocked completely by 20 μ M CNQX ($n = 6$). *C*, The histogram shows that 100 μ M $\alpha\beta$ m-ATP produced an increase in mEPSC frequency in a lamina V neuron and that 2 μ M capsaicin had no effect in the same neuron. Similar results were observed in 15 other cells. *D*, The diagram illustrates the presence of an $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathway to lamina V neurons (see also Nakatsuka and Gu, 2001).

converged onto single lamina V neurons, we recorded mEPSCs and sEPSCs from the same neurons after the sequential applications of capsaicin and $\alpha\beta$ m-ATP. As shown in Figure 5*A*, $\alpha\beta$ m-ATP (100 μ M), but not capsaicin (2 μ M), produced increases of mEPSC frequency in the presence of 0.5 μ M TTX. However, in the same neuron in the absence of TTX, not only $\alpha\beta$ m-ATP but also capsaicin produced large increases of sEPSC frequency (Fig. 5*A*). Figure 5*B* summarizes the changes in the frequency of mEPSCs after the sequential applications of capsaicin and $\alpha\beta$ m-ATP in the same neurons in the presence of TTX. Capsaicin (2 μ M) did not have any effect on mEPSC frequency ($n = 16$). However, mEPSC frequency was increased to $447 \pm 106\%$ of control ($n = 16$; $p < 0.05$; Fig. 5*B*) by 100 μ M $\alpha\beta$ m-ATP in the same neurons. After the washout of TTX the effects of capsaicin (2 μ M) and $\alpha\beta$ m-ATP (100 μ M) were determined in 14 of the above 16 cells. The results are summarized in Figure 5*C*. In contrast to mEPSCs, after the washout of TTX the sEPSC frequency was increased substantially by capsaicin in all 14 cells that were tested. The overall changes were $465 \pm 81\%$ of control ($n = 14$; $p < 0.05$; Fig. 5*C*). Similar to the effects of $\alpha\beta$ m-ATP on

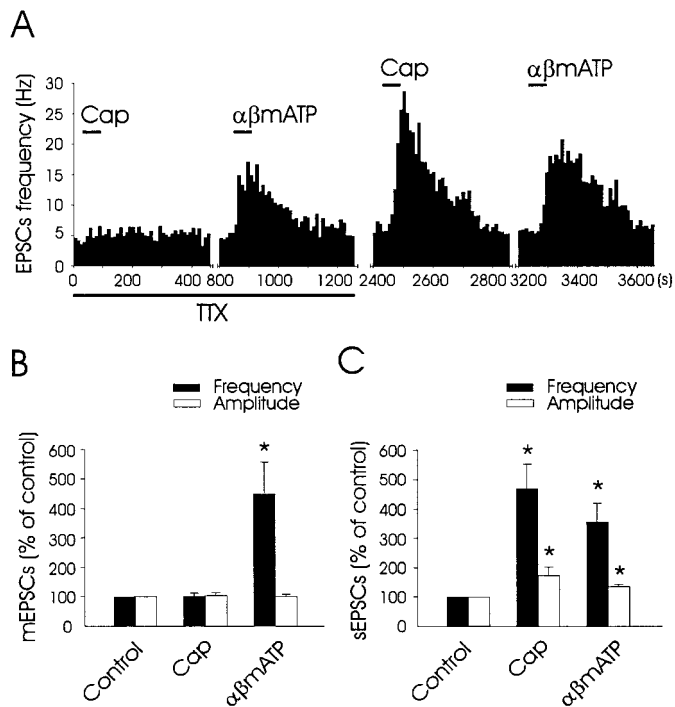


Figure 5. Synaptic convergence of excitatory inputs from capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathways. *A*, The histogram shows the effects of capsaicin and $\alpha\beta$ m-ATP on the frequency of mEPSCs and sEPSCs in the same neuron. Capsaicin ($2\ \mu\text{M}$) did not induce any change in mEPSC frequency. In the same neuron $\alpha\beta$ m-ATP ($100\ \mu\text{M}$) induced an increase in mEPSC frequency. After the washout of TTX both capsaicin and $\alpha\beta$ m-ATP increased the sEPSC frequency. *B*, Summary of the changes in the frequency and amplitude of mEPSCs in the experiments represented in *A* ($n = 16$). Only $\alpha\beta$ m-ATP induced increases of mEPSC frequency in lamina V neurons. *C*, Summary of the changes in the frequency and amplitude of sEPSCs in the experiments represented in *A* ($n = 14$). sEPSC frequency was increased by capsaicin and by $\alpha\beta$ m-ATP in the absence of TTX. The data represent the mean \pm SEM; * $p < 0.05$, paired Student's *t* tests.

mEPSCs, $\alpha\beta$ m-ATP also produced increases of sEPSC frequency ($408 \pm 68\%$ of control, $n = 14$; $p < 0.05$). In addition to the changes in sEPSC frequency, there was also a slight but significant increase in the sEPSC amplitude by capsaicin and $\alpha\beta$ m-ATP (Fig. 5C). These results indicate the convergence of two excitatory inputs from capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathways to lamina V neurons (see Fig. 10).

Young rats were used in the above experiments (ages between 11 and 21 d). We also performed experiments in spinal cord slices obtained from adult rats (ages between 7 and 9 weeks) to determine whether similar results could be obtained in mature animals. In six lamina V cells that were tested in the presence $0.5\ \mu\text{M}$ TTX, $100\ \mu\text{M}$ $\alpha\beta$ m-ATP increased mEPSC frequency in all six cells ($170 \pm 13\%$ of control; $p < 0.05$; data not shown). Of these cells, four cells were tested with $2\ \mu\text{M}$ capsaicin, and none of them showed increases of mEPSC frequency ($104 \pm 5\%$ of control). However, when TTX was not present, $2\ \mu\text{M}$ capsaicin increased sEPSC frequency ($283 \pm 102\%$ of control, $n = 4$; $p < 0.05$; data not shown). These results are similar to those obtained in young rats.

Recruitment of inhibitory pathways

Both capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive inputs also recruited inhibitory inputs onto lamina V

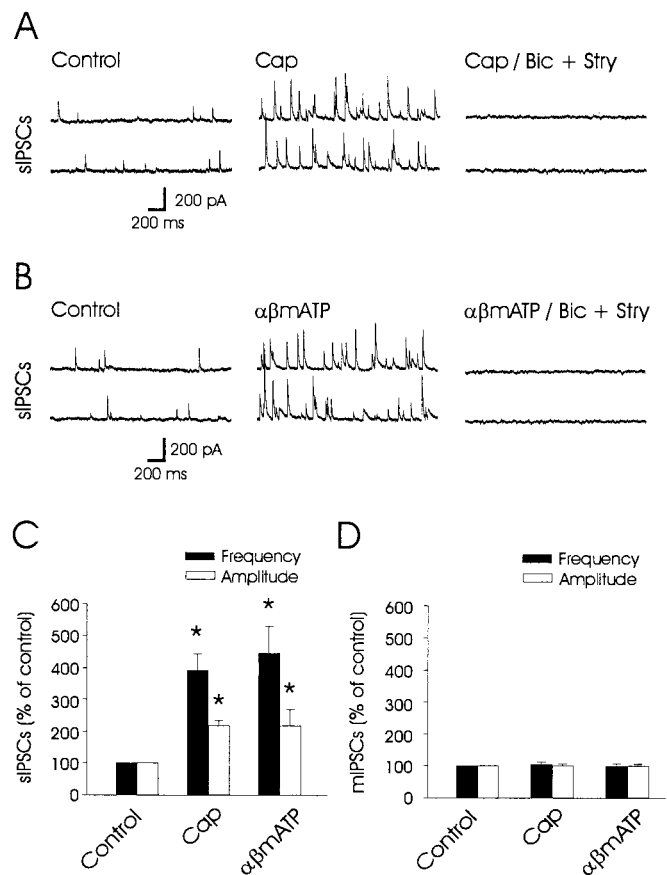


Figure 6. Recruitment of inhibitory circuitry after the activation of central terminal VR1 receptors or P2X receptors. *A*, Capsaicin induced an increase in sIPSC frequency. Three sets of sample traces show sIPSCs recorded from a lamina V neuron in control (left), after the application $2\ \mu\text{M}$ capsaicin alone (middle), and after the application of $2\ \mu\text{M}$ capsaicin in the presence of both $20\ \mu\text{M}$ bicuculline and $2\ \mu\text{M}$ strychnine (right). *B*, The $\alpha\beta$ m-ATP induced an increase of sIPSC frequency. Experiments were similar to those in *A* except that $100\ \mu\text{M}$ $\alpha\beta$ m-ATP was tested. The recordings in *A* and *B* were performed on the same neuron. *C*, Summary of the increases in sIPSC frequency induced by $2\ \mu\text{M}$ capsaicin or $100\ \mu\text{M}$ $\alpha\beta$ m-ATP in the same neurons (filled bars; $n = 12$). The changes of sIPSC amplitude are shown also (open bars; $n = 12$). The experiments were performed in the absence of TTX. *D*, Capsaicin and $\alpha\beta$ m-ATP had no effect on mIPSCs. Of the 12 cells tested in *C*, eight cells also were tested in the presence of $0.5\ \mu\text{M}$ TTX. The frequency (filled bars) and amplitude (open bars) of mEPSCs were not affected by $2\ \mu\text{M}$ capsaicin or $100\ \mu\text{M}$ $\alpha\beta$ m-ATP ($n = 8$). In all recordings the cells were held at $-10\ \text{mV}$. The data represent the mean \pm SEM; * $p < 0.05$; paired Student's *t* tests.

neurons (Fig. 6). The recruitment of inhibitory inputs was determined by measuring sIPSCs when lamina V neurons were held at $-10\ \text{mV}$. The inhibitory inputs produced outward postsynaptic currents under this condition (Fig. 6A). The frequency of sIPSCs was increased after the application of $2\ \mu\text{M}$ capsaicin in all 12 cells that were recorded (Fig. 6A,C). In the same neurons the frequency of sIPSCs also was increased after the application of $100\ \mu\text{M}$ $\alpha\beta$ m-ATP (Fig. 6B,C). The sIPSCs were blocked completely in the presence of $20\ \mu\text{M}$ bicuculline plus $2\ \mu\text{M}$ strychnine (Fig. 6A,B), indicating that the sIPSCs were mediated by GABAergic/glycinergic inputs from DH inhibitory interneurons. Figure 6C summarizes the changes in the frequency and amplitude of sIPSCs after the sequential applications of $2\ \mu\text{M}$ capsaicin and $100\ \mu\text{M}$ $\alpha\beta$ m-ATP in the same neurons. sIPSC frequency was $388 \pm 51\%$ of control ($n = 12$; $p < 0.05$) after capsaicin application and

441 \pm 83% of control ($n = 12$; $p < 0.05$) after $\alpha\beta\text{m-ATP}$ application. sIPSC amplitude also was increased slightly after the applications of $\alpha\beta\text{m-ATP}$ and capsaicin. When experiments were performed in the presence of 0.5 μM TTX to block active inter-neuronal transmission (Fig. 6D), both mIPSC frequency and amplitude were not changed after the application of 2 μM capsaicin ($n = 8$) or 100 μM $\alpha\beta\text{m-ATP}$ ($n = 8$). These results indicate that both capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive pathways recruit inhibitory synaptic circuitry and that the recruited inhibitory inputs can converge onto the same lamina V neurons.

Synaptic convergence of excitatory and inhibitory inputs to the same lamina V neurons after the activation of capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive pathways

To determine whether both excitatory and inhibitory inputs converge on the same lamina V neurons, we recorded sEPSCs and sIPSCs simultaneously, and we examined the effects of capsaicin as well as $\alpha\beta\text{m-ATP}$ in the same lamina V neurons. The simultaneous recordings of sEPSCs and sIPSCs were performed with cells held at -45 mV. At this holding potential the glutamatergic sEPSCs were inward currents and GABAergic/glycinergic sIPSCs were outward currents (Fig. 7A). As shown by the sample traces in Figure 7A, the increases in the frequency of both sEPSCs and sIPSCs were observed clearly under this simultaneous recording condition after the applications of $\alpha\beta\text{m-ATP}$ (100 μM) and capsaicin (2 μM). The sEPSC frequency increased to $347 \pm 97\%$ of control ($p < 0.05$; $n = 6$), and the sIPSC frequency increased to $447 \pm 186\%$ of control ($p < 0.05$; $n = 6$) after the application of 2 μM capsaicin (Fig. 7B). In the same neurons $\alpha\beta\text{m-ATP}$ (100 μM) increased the sEPSC frequency to $363 \pm 68\%$ of control ($p < 0.05$; $n = 6$) and increased the sIPSC frequency to $372 \pm 107\%$ of control ($p < 0.05$; $n = 6$; Fig. 7B). Based on the results from Figures 1 to 7, a schematic diagram in Figure 10 summarizes the major pathways involved in the excitatory and inhibitory convergence of capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive pathways.

Temporal summation in lamina V neurons

Because capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive pathways displayed convergent excitatory and inhibitory activities onto lamina V neurons, we determined whether the presence of two excitatory inputs could overcome the inhibitory influence, resulting in hyperactivity in lamina V neurons. Under current-clamp configuration the occurrence of action potential spikes was measured after the applications of 2 μM capsaicin alone, 100 μM $\alpha\beta\text{m-ATP}$ alone, or both of them together. Some action potential spikes were observed for brief periods when capsaicin or $\alpha\beta\text{m-ATP}$ was applied separately (Fig. 8A,B). However, when capsaicin and $\alpha\beta\text{m-ATP}$ were coapplied, a larger increase in spike frequency occurred, lasting up to 10 min after a 2 min application of capsaicin plus $\alpha\beta\text{m-ATP}$ ($n = 4$; Fig. 8A,B). These results suggest that the convergent excitatory inputs from capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive pathways may produce a nonlinear summation and long-lasting enhancement of lamina V activity.

Dorsal root stimulation-evoked synaptic convergence of sensory inputs from capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive afferent fibers

Dorsal root stimulation evoked monosynaptic A-fiber eEPSCs with or without polysynaptic eEPSCs in lamina V neurons. The

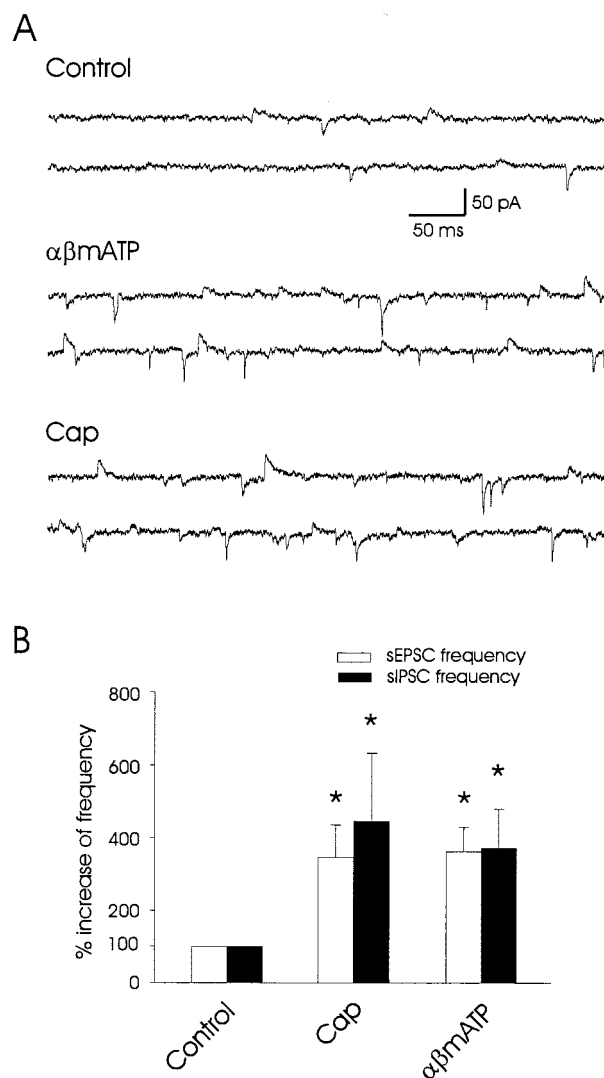


Figure 7. Synaptic convergence of both excitatory and inhibitory inputs on the same lamina V neurons. *A*, Sample traces show simultaneous recordings of both sEPSCs and sIPSCs in a lamina V neuron in normal bath solution (Control), after the application of 100 μM $\alpha\beta\text{m-ATP}$, and after the application of 2 μM capsaicin in a lamina V neuron. The cell was voltage clamped at -45 mV. At this holding potential both the sEPSCs (inward currents) and sIPSCs (outward currents) could be detected simultaneously. *B*, A summary shows the increases of both sEPSC frequency and sIPSC frequency in experiments ($n = 6$) as represented in *A*. The data represent the mean \pm SEM; * $p < 0.05$, paired Student's *t* test.

occurrence of polysynaptic eEPSCs often depended on stimulation intensity. As shown in Figure 9, at A δ -fiber stimulation intensity (100 μA , 0.1 msec) a lamina V neuron showed only monosynaptic A δ -like eEPSCs (Fig. 9A). When stimulation intensity increased to C-fiber intensity (500 μA , 0.1 msec) (Nakatsuka et al., 2000), dorsal root stimulation resulted in monosynaptic eEPSCs followed by polysynaptic eEPSCs in the same lamina V neuron (Fig. 9B). Similar results were obtained in 16 other cells. At C-fiber stimulation the conduction velocity for the initial monosynaptic eEPSCs was 2.8 ± 0.2 m/sec (2.0–5.1 m/sec; $n = 17$), within the A δ -fiber conduction range. The subsequent polysynaptic eEPSCs were separated either completely (Fig. 9B) or partially (Fig. 9C) from the monosynaptic A δ -like eEPSCs, depending on the length of the dorsal roots.

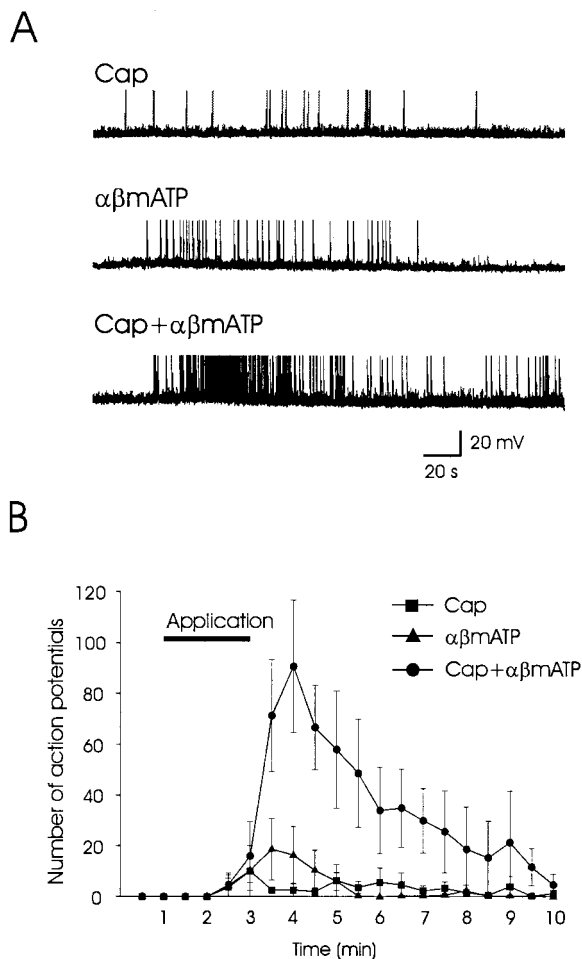


Figure 8. Temporal summation and action potential spikes after simultaneous activation of both capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive pathways. *A*, Three traces show the action potential spikes after the application of $2 \mu\text{M}$ capsaicin alone (top), $100 \mu\text{M}$ $\alpha\beta$ m-ATP alone (middle), and both of them together (bottom). *B*, Time course of action potential spikes after $\alpha\beta$ m-ATP alone, capsaicin alone, and both of them together ($n = 4$). Resting membrane potentials were -68 ± 7 mV. Action potential spikes are truncated to show some eEPSPs. Each data point in *B* represents the number of action potentials in a period of 30 sec.

The monosynaptic A δ -like inputs and the polysynaptic inputs to the same lamina V neurons were examined further for their sensitivity to capsaicin. Dorsal root stimulation was applied at C-fiber stimulus intensity to elicit monosynaptic A δ -like eEPSCs and polysynaptic eEPSCs first (Fig. 9C), and then the effects of $2 \mu\text{M}$ capsaicin on these eEPSCs were determined (Fig. 9D). In four lamina V neurons that were tested, the monosynaptic A δ -like eEPSCs were not affected significantly by capsaicin (224 ± 35 pA in control vs 229 ± 32 pA in the presence of capsaicin; Fig. 9C–E). However, the polysynaptic eEPSCs were sensitive to capsaicin in all four cells that were tested (Fig. 9C–E); the average amplitude of polysynaptic eEPSCs was decreased significantly from 156 ± 45 pA in the control condition to 24 ± 10 pA in the presence of $2 \mu\text{M}$ capsaicin. Together with our recent study that showed $\alpha\beta$ m-ATP sensitivity of monosynaptic A δ -like eEPSCs in almost all lamina V neurons that were recorded (Nakatsuka and Gu, 2001), these results suggest that sensory impulses elicited at high stimulation intensity may result in the convergence of synaptic

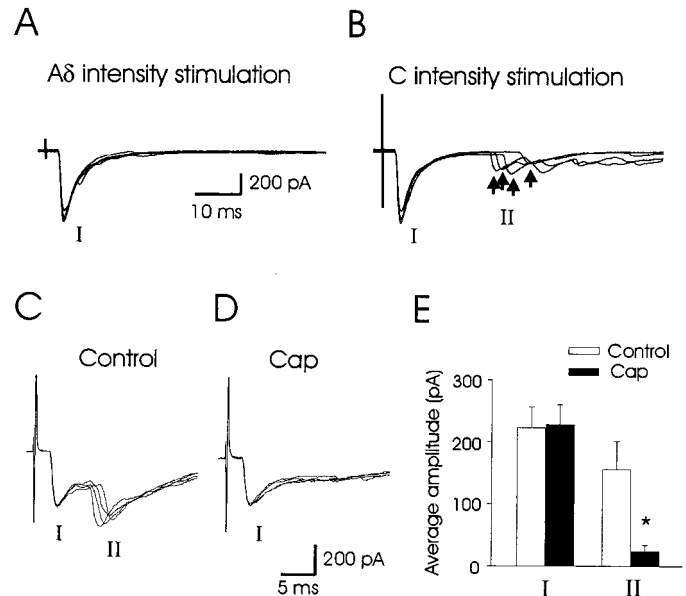


Figure 9. Evoked monosynaptic and polysynaptic excitatory inputs to lamina V neurons and their sensitivity to capsaicin. *A*, Four sample traces, aligned at the time of each stimulus, represent eEPSCs (indicated by I) obtained in a lamina V neuron after dorsal root stimulation (4 stimuli) at A δ -fiber stimulation intensity ($\sim 100 \mu\text{A}$, 0.1 msec). Note that there is no change in the latency of eEPSCs, indicating monosynaptic transmission. *B*, eEPSCs were elicited from the same neuron in *A* after dorsal root stimulation (4 stimuli) at C-fiber stimulus intensity ($\sim 500 \mu\text{A}$, 0.1 msec). Two groups of eEPSCs were observed. In the first group (indicated by I), eEPSCs occurred with the same latency as those shown in *A*, and the afferent conduction velocity was 5.3 m/sec. In the second group (indicated by II), eEPSCs had a longer latency. Arrows point to the changes in the latency of the group II eEPSCs, indicating polysynaptic transmission. The apparent conduction velocity for these polysynaptic inputs was ≤ 0.6 m/sec. Conduction velocity is calculated on the basis of eEPSC latency and the length of the attached dorsal root. In the spinal cord slice used in *A* and *B*, the attached root was extra long (15 mm). *C*, *D*, An example shows monosynaptic A δ -like eEPSCs (I) and polysynaptic eEPSCs (II) recorded from a lamina V neuron in normal bath solution (*C*), and after the bath application of $2 \mu\text{M}$ capsaicin for 10 min (*D*). The afferent conduction velocity was 2.1 m/sec for the monosynaptic inputs that generated monosynaptic A δ -like eEPSCs. The apparent conduction velocity was ≤ 0.46 m/sec for the polysynaptic inputs. The length of the dorsal root in this experiment was 4 mm. *E*, A summary of the results ($n = 4$) from experiments represented in *C* and *D*. The amplitude of polysynaptic eEPSCs was determined after subtracting the extrapolated decay component of the previous monosynaptic eEPSCs.

inputs from capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive afferent fibers to lamina V neurons.

DISCUSSION

We have studied putative synaptic circuits involved in the transmission of capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive inputs in the spinal cord by activation of central terminal VR1 receptors and $\alpha\beta$ m-ATP-sensitive P2X receptors. Capsaicin-sensitive inputs initially are transmitted from primary afferent terminals to the superficial laminae. The excitatory activity of capsaicin-sensitive inputs is transformed further to both excitatory and inhibitory signals in the dorsal horn and is spread into lamina V neurons. Furthermore, polysynaptic capsaicin-sensitive inputs converge with monosynaptic $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive inputs onto lamina V neurons (Fig. 10). This convergence produces temporal summation in a single lamina V neuron.

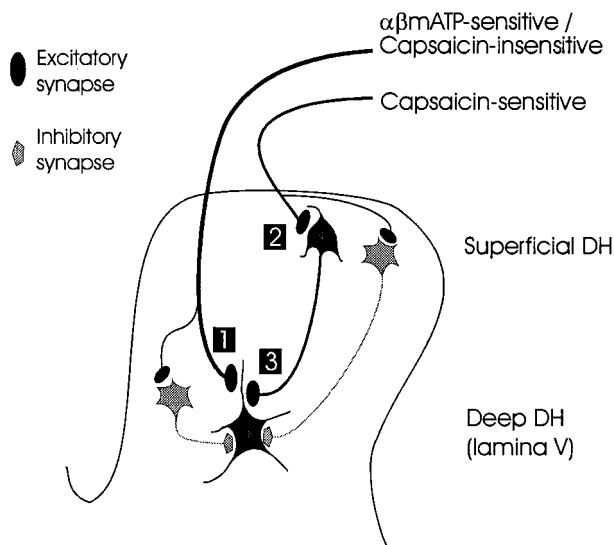


Figure 10. Schematic illustration of a proposed synaptic convergence of capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive sensory pathways onto lamina V neurons. The diagram shows two excitatory sensory pathways. One is from $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive afferent central terminals. These terminals make monosynaptic excitatory synapses onto lamina V neurons (1). The other pathway is from capsaicin-sensitive afferent central terminals. These terminals first synapse onto interneurons in superficial lamina (2). Then the excitatory inputs are relayed to lamina V neurons (3). The site 1 synapse is sensitive to $\alpha\beta$ m-ATP but insensitive to capsaicin. The site 2 synapse is sensitive to capsaicin. Because most capsaicin-sensitive afferent neurons express rapidly desensitizing P2X receptors (C. Li et al., 1999; Ueno et al., 1999; Nakatsuka and Gu, 2001), capsaicin-sensitive terminals also may be sensitive to $\alpha\beta$ m-ATP, i.e., capsaicin-sensitive/ $\alpha\beta$ m-ATP-sensitive terminals. The diagram also shows the recruitment of inhibitory pathways mediated by GABAergic/glycinergic interneurons (light gray). Other pathways may be present and are not shown in the diagram.

Spreading excitatory activity of capsaicin-sensitive inputs from the superficial laminae to lamina V neurons

VR1-ir has been observed mainly in the superficial laminae (see also Tominaga et al., 1998; Guo et al., 1999). In the present study the mEPSC frequency was increased when recordings were made from lamina II neurons. Because mEPSCs were measured in the presence of TTX, the active interneuronal transmission among DH neurons was prevented (Gu et al., 1996). Therefore, monosynaptic connections between capsaicin-sensitive fibers and lamina II neurons were revealed electrophysiologically. In contrast to the recordings in lamina II, few lamina V neurons showed increased mEPSC frequency by capsaicin. This indicates that few capsaicin-sensitive afferent fibers have monosynaptic connections with lamina V neurons. However, when sEPSCs were examined in the absence of TTX, capsaicin produced an increase in sEPSC frequency. This result suggests that capsaicin-sensitive inputs could be transmitted to lamina V polysynaptically. The spread of capsaicin-sensitive activity to deep laminae was mediated by interneurons in the superficial laminae. This is supported further by the results that capsaicin-induced increases of sEPSCs in lamina V neurons were abolished after the removal of the superficial laminae. These results indicate that there are extensive neuronal interactions between functionally distinct spinal cord laminar regions. It has been assumed that neurons in the superficial laminae interact with cells in the deep DH. Consistent with this assumption, anatomical evidence showed synaptic-like contacts

between superficial and deep DH neurons (Ritz and Greenspan, 1985; Light and Kavookjian, 1988). However, this assumption has been questioned because of the lack of strong electrophysiological evidence (Willis and Coggeshall, 1991). Our results indicate that superficial and deep laminae, the two functionally distinct sensory regions in the DH, have extensive signal transmission when VR1 receptors on primary afferent fibers are activated.

The VR1 receptor has been shown to be a sensor for nociceptive heat stimuli (Caterina et al., 1997, 2000; Davis et al., 2000). The widespread neuronal activity after the activation of VR1 receptors suggests that capsaicin-sensitive inputs have profound effects on spinal cord neuronal activity not only in superficial laminae but also in deep laminae. The widespread neuronal activity after the activation of VR1 receptors may be a mechanism for the development of secondary hyperalgesia after intradermal capsaicin injections or burning injury (Simone et al., 1989b; LaMotte et al., 1991; Pedersen et al., 1996) and for VR1 receptor-mediated thermal allodynia after tissue inflammation (Caterina et al., 2000; Davis et al., 2000).

$\alpha\beta$ -Methylene-ATP-sensitive/capsaicin-insensitive inputs to lamina V neurons

The increase of mEPSC frequency by $\alpha\beta$ m-ATP in lamina V neurons indicates the presence of $\alpha\beta$ m-ATP-sensitive P2X receptors on the presynaptic terminals to lamina V neurons. We have demonstrated previously that $\alpha\beta$ m-ATP-sensitive terminals on lamina V neurons were derived mainly from A δ fibers and that activation of P2X receptors on those terminals not only increased spontaneous glutamate release from those central terminals but also potentiated the evoked EPSCs (Nakatsuka and Gu, 2001). The present work, together with our previous results (Nakatsuka and Gu, 2001), also indicates that $\alpha\beta$ m-ATP-sensitive terminals to lamina V neurons are capsaicin-insensitive. The presence of ATP-sensitive/capsaicin-insensitive sensory neurons has been shown previously in acutely dissociated DRG neurons (C. Li et al., 1999; Ueno et al., 1999; Petruska et al., 2000; Tsuda et al., 2000). It remains to be shown whether $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive afferent fibers represent all or part of ATP-sensitive/capsaicin-insensitive afferent fibers. Functionally, it has been shown that P2X receptors on $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive neurons play a role in mechanical allodynia (Tsuda et al., 2000).

Synaptic convergence and summation of capsaicin-sensitive input and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive input onto lamina V neurons

We have found that the two chemically distinct sensory inputs, capsaicin-sensitive and $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive inputs, synaptically converge onto lamina V neurons. Lamina V neurons of the spinal cord DH are known to receive a variety of sensory inputs, including nociceptive and non-nociceptive inputs (Willis and Coggeshall, 1991; Woolf, 1994). Sensory convergence has been observed in many lamina V neurons after electrical or physical stimuli (Mendell, 1966; Wagman and Price, 1969; McMahon and Morrison, 1982; Takahashi and Yokota, 1983; Alarcon and Cervero, 1990). Our study shows that two chemically defined sensory inputs synaptically converge onto lamina V neurons. The demonstration of the synaptic convergence of capsaicin-sensitive input and the $\alpha\beta$ m-ATP-sensitive/capsaicin-insensitive input should help us to understand further the functional roles of VR1 receptors and a subtype of P2X receptors in sensory transmission.

The synaptic convergence shown here includes both excitatory

and inhibitory pathways. The recruitment of inhibitory inputs during the activation of capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive pathways may provide a negative feedback mechanism. The recruitment of inhibitory pathways after the application of $\alpha\beta\text{m-ATP}$ was less likely because of the direct activation of P2X receptors on DH interneurons. Previously, two studies showed that ATP increased the release of GABA and glycine from a subpopulation of DH neurons (Hugel and Schlichter, 2000; Rhee et al., 2000). However, in the same studies the effects were not produced by $\alpha\beta\text{m-ATP}$, indicating the absence of $\alpha\beta\text{m-ATP}$ -sensitive P2X receptors on inhibitory DH interneurons in postnatal rats. Consistently, we did not find significant changes of mIPSC frequency by $\alpha\beta\text{m-ATP}$. Thus, the increase of sIPSC frequency by $\alpha\beta\text{m-ATP}$ is attributable to the recruitment of inhibitory DH interneurons (Fig. 7).

Convergent excitatory inputs may overcome inhibitory controls and produce a nonlinear temporal summation, resulting in long-lasting increases of action potential firing in lamina V neurons (Fig. 8). Another possible contribution to the increased activity in lamina V during the coapplication of $\alpha\beta\text{m-ATP}$ and capsaicin (Fig. 8*A,B*) is the synergistic effect of $\alpha\beta\text{m-ATP}$ and capsaicin on capsaicin-sensitive terminals. However, capsaicin-sensitive DRG neurons usually only express the rapidly desensitizing P2X receptors (Guo et al., 1999; C. Li et al., 1999; Petruska et al., 2000; Nakatsuka and Gu, 2001), and these receptors only have transient synaptic effects when activated (Labrakakis et al., 2000). Therefore, it is less likely that $\alpha\beta\text{m-ATP}$ action on capsaicin-sensitive terminals plays a significant role in the long-lasting temporal summation. However, long-lasting synaptic effects of $\alpha\beta\text{m-ATP}$ have been observed for the $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive terminals to lamina V neurons (Fig. 4*C*; see also Nakatsuka and Gu, 2001). Thus the long-lasting increases of action potential firing in lamina V neurons after the coapplication of $\alpha\beta\text{m-ATP}$ and capsaicin (Fig. 8) should represent mainly the temporal summation of excitatory inputs from capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive inputs.

Dorsal root stimulation-evoked synaptic convergence of sensory inputs from capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive afferent fibers

We have shown that monosynaptic A δ -like eEPSCs and polysynaptic eEPSCs converge on the same lamina V neurons after dorsal root stimulation at C-fiber intensity. By testing their capsaicin sensitivity, we have shown that the monosynaptic A δ -like inputs are insensitive to capsaicin, but most polysynaptic afferent inputs are sensitive to capsaicin (Fig. 9). These results extend our findings on the basis of the recordings of sEPSCs and mEPSCs. Together with our previous findings that the evoked monosynaptic A δ -like eEPSCs were sensitive to $\alpha\beta\text{m-ATP}$ (Nakatsuka and Gu, 2001), our results suggest that sensory impulses carried by capsaicin-sensitive fibers and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive fibers can converge on the same lamina V neurons.

Capsaicin-sensitive polysynaptic inputs were evident by the suppression of polysynaptic eEPSCs in the presence of capsaicin. Consistently, monosynaptic capsaicin-sensitive eEPSCs in lamina II were blocked by capsaicin (Yang et al., 1999), potentially because of conduction block (Urban and Dray, 1993; Yang et al., 1999). We found that polysynaptic eEPSCs were not abolished completely by capsaicin. This may suggest that some capsaicin-insensitive inputs also converge polysynaptically on lamina V neurons. The lack of capsaicin sensitivity for monosynaptic A δ -like eEPSCs was observed in all four cells that were tested in

lamina V, suggesting that A δ -afferent fibers to these neurons were capsaicin-insensitive. However, our results do not exclude the presence of capsaicin-sensitive A δ -fibers (Urban and Dray, 1993; Ringkamp et al., 2001). Capsaicin-sensitive fibers appear to be rare in lamina V, based on VR1-ir and our mEPSC experiments. Thus, capsaicin-sensitive A δ -fibers, if present in a large number, may be located mainly in the superficial laminae.

Implications

Simultaneous activation of different sensory pathways by endogenous ligands for VR1 receptors and P2X receptors may be common during tissue injury, inflammation, and other pathological conditions because chemical mediators such as protons, ATP, and ADP may be released under these conditions. Thus the putative excitatory summation of capsaicin-sensitive and $\alpha\beta\text{m-ATP}$ -sensitive/capsaicin-insensitive inputs may occur, which may lead to the development of hyperactivity in deep DH neurons. However, convergent inhibitory inputs may play an important role in preventing such hyperactivity. The balance among these excitatory and inhibitory circuits may have important physiological function. A shift of the balance may represent state-dependent sensory processing.

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