Substance P Enhances NMDA Channel Function in Hippocampal Dentate Gyrus Granule Cells

DAVID N. LIEBERMAN1 AND ISTVAN MODY 2
1Neuroscience Graduate Program, Stanford University School of Medicine, Stanford 94305; and
2Departments of Neurology and Physiology, UCLA School of Medicine, Los Angeles, California 90095-1769

Lieberman, David N. and Istvan Mody. Substance P enhances NMDA channel function in hippocampal dentate gyrus granule cells. J. Neurophysiol. 80: 113–119, 1998. Substance P (SP)–containing afferents and the NK-1 tachykinin receptor to which SP binds are present in the dentate gyrus of the rat; however, direct actions of SP on principal cells have not been demonstrated in this brain region. We have examined the effect of SP on N-methyl-D-aspartate (NMDA) channels from acutely isolated dentate gyrus granule cells of adult rat hippocampus to assess the ability of SP to regulate glutamatergic input. SP produces a robust enhancement of single NMDA channel function that is mimicked by the NK-1-selective agonist Sar9, Met(O2)11-SP. The SP-induced prolongation of NMDA channel openings is prevented by the selective NK-1 receptor antagonist (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994). Calcium influx or activation of protein kinase C were not required for the SP-induced increase in NMDA channel open durations. The dramatic enhancement of excitatory amino acid–mediated excitability by SP places this neuropeptide in a key position to gate activation of hippocampal network activity.

INTRODUCTION

Substance P (SP) is a neuroactive peptide, belonging to the tachykinin family, which produces slow excitation in many neurons (Nicoll et al. 1980). For example, SP has been shown to excite basal forebrain cholinergic neurons by suppressing an inwardly rectifying potassium current (Stanfield et al. 1985). SP also excites sympathetic neurons by suppressing the M current (Jones 1985) and N-type calcium currents (Bley and Tsien 1990; Shapiro and Hille 1993), which can lead to a reduction of Ca2+-activated potassium currents and of the resulting afterhyperpolarization (Adams et al. 1986). In addition, SP regulates nociceptive inputs to the spinal dorsal horn. Primary nociceptive afferents release SP along with excitatory amino acids. The two transmitter systems are thought to interact in the processing of nociceptive information in the spinal cord. Indeed, in acutely isolated spinal dorsal horn neurons, SP has been found to enhance glutamate currents mediated by N-methyl-D-aspartate (NMDA) receptors (Randic et al. 1990; Rusin et al. 1993a,b).

Little information is available regarding the role of SP in the hippocampal formation where NMDA receptors play an important role in lasting physiological and pathological plastic alterations. SP-containing fibers mainly originating from the supramammillary area innervate the hippocampal formation of the guinea pig (Gall and Selawski 1984), cat (Ito et al. 1988), monkey (Nitsch and Leranth 1994), and human (Del Fiacco et al. 1987), where the bulk of extrinsic SP axons appear to form asymmetric synapses with dentate granule cells. In contrast, studies in rats have demonstrated a slightly different innervation pattern of the hippocampal formation (Maglóczy et al. 1994), including the divergence of the calretinin and SP-containing neurons that innervate the CA2 subfield (Borhegyi and Leranth 1997a). Moreover, most of the intrinsic SP-containing fibers form symmetric synapses on rat hippocampal GABAergic interneurons (Ac-sády et al. 1997). To determine the possible modulatory effect of SP on the excitability of the rat hippocampus, we have examined the effect of SP on NMDA channels from acutely isolated dentate gyrus granule cells. We have observed that SP produces a robust enhancement of single NMDA channel function through a readily diffusible, but yet unidentified intracellular message.

METHODS

Preparation of cells

Dentate granule cells from adult (250–400 g) male Wistar rats were acutely dissociated and maintained in vitro as previously described (Köhr et al. 1993). In brief, coronal and horizontal brain slices (400–μm thick) were prepared on a vibratome and were maintained at 32°C in an artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 126 NaCl, 2.5 KCl, 2 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, and 1 pyruvic acid, pH 7.3. The ACSF was constantly bubbled with an 95% O2–5% CO2 gas mixture. For enzymatic digestion, single slices were incubated for 25–30 min (32°C) in 2 ml of oxygenated ACSF with 1.75 mg/ml pronase E (protease type XIV, Sigma). The dentate gyrus was microdissected and subsequently triturated using a fire-polished Pasteur pipette in a test tube containing 2 ml of cold (4°C) N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)–buffered ACSF (NaHCO3 was replaced by equimolar HEPES). The neuronal suspension was then transferred into a tissue culture dish. Before recordings, the neurons were thoroughly washed with a nominally Mg2+-free solution (see next section). Experiments were performed between 15 min and 2 h after isolation of the neurons.

Cell-attached patch recordings

Steady-state cell-attached recordings were performed using an Axopatch 200A integrating patch-clamp amplifier (Axon Instruments) at 22–25°C. Extracellular solution contained (in mM) 110 NaSO4, 5 CsSO4, 1.8 CaCl2, 10 HEPES, 10 glucose, 1 pyruvic acid, and 0.001 tetrodotoxin (TTX; Calbiochem). The pH was

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adjusted to 7.25 with NaOH, and the osmolarity varied between 285 and 305 mosM. Thin-walled borosilicate glass (1.50 mm OD, 1.12 mm ID with filament; Garner Glass) pulled in two stages on a Narishige PP-83 electrode puller was used for patch electrodes. Fire-polished, silicone elastomer (Sylgard)–coated pipettes (8–15 MΩ) were filled with 200–500 nM L-aspartic acid and 8 μM glycine dissolved in the bath solution. Stock solutions of L-aspartic acid (1 mM) and glycine (1 mM) were prepared fresh and used at proper dilutions. SP (Peninsula Labs) and Sar9, (MetO)11-SP (Bachem) were stored frozen in a buffer of 0.1 N acetic acid at 10 mM and were diluted in the extracellular recording medium to the final concentration right before use. The specific NK-1 antagonist (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994) was a gift from Pfizer Pharmaceutical. With the exceptions noted, all chemicals were purchased from Fluka.

Data analysis

Recordings were filtered through the 10-kHz Bessel filter (4-pole, −3 dB) of the amplifier at a gain of 100 mV/pA and were stored in a pulse-code modulated digitized form (44 kHz; Neurodata) on videotapes. Recordings were analyzed off-line with the PAT program of the Strathclyde Electrophysiology Software (courtesy of J. Dempster) using a 50% threshold crossing algorithm for event detection. Any event that surpassed this level of detection was considered a full opening. Likewise, any closure to a level below threshold was considered complete. Thus openings either to or from another state and closures to or from substates were not noted. However, >90% of the channel record comprised single openings with substates making up <5% of all openings.

The continuous records were filtered at 2 kHz (8-pole Bessel, −3 dB; Frequency Devices model 902LF) and continuously sampled at 20 kHz (DT 2821 A/D board, Data Translation using an Intel 80486-based computer). The analysis of dwell time distributions was done using software written and developed by Y. DeKon- inck and I. Mody. Each exponential component of the closed time distribution of NMDA channels recorded with low agonist concentrations corresponds to a shut interval that separates groupings of NMDA channel openings. Shut time distributions were calculated by fitting multiple exponential distributions with a Simplex-based maximum likelihood method to log binwidth histograms at 9 bins/decade plotted on a square-root ordinate (Sigworth and Sine 1987). Critical closed times (Tc) for determination of burst, cluster, and supercluster durations were calculated from respective time constants of the shut time distributions according to Colquhoun and Sakmann (1985) by solving the following equation for Tc:

\[ T_c = \frac{1}{\sum \frac{1}{\tau_i}} \]

where \( \tau_i \) is the ith time constant from the distribution of shut times, and \( i = 1, 2, 3, \text{or} 4 \). Calculating \( T_c \) in this manner equalizes the percentage of gaps belonging to a long gap distribution misclassified as short and the percentage of gaps belonging to a short distribution misclassified as long (Colquhoun and Sakmann 1985; Edmonds and Colquhoun 1992). The first two shut periods describe the intraburst closed times. Bursts were thus defined as groups of openings separated by a gap longer than the \( T_c \) between the second and third components of the shut time distribution. Similarly, clusters were defined as groups of bursts separated by a closed time longer than the \( T_c \) between the third and fourth components of the shut time distribution. Finally, groups of clusters separated by a gap longer than the \( T_c \) between the fourth and fifth components of the shut time distribution defined superclusters. We used this method to classify the groupings of openings to describe the kinetics of NMDA channel openings (Edmonds et al. 1995).

RESULTS

SP modulates NMDA channel activity in dissociated hippocampal granule cells

We examined the effect of an acute application of SP on NMDA channel function recorded in granule cells acutely dissociated from the adult rat hippocampal dentate gyrus. Several minutes of steady-state baseline channel activity were recorded to provide a sufficient number of openings to perform a detailed analysis. Within 2–5 min after application of 200–500 nM SP onto the granule cells (n = 10), NMDA channel openings were dramatically prolonged (Fig. 1). Virtually all open channel parameters were affected, including the characteristic complex groupings into bursts, clusters, and superclusters (Edmonds et al. 1995; Gibb and Colquhoun 1992; Köhr et al. 1993; Lieberman and Mody 1994). Specifically, the mean open time for all openings increased to 180 ± 22% (mean ± SE) of control (e.g., Fig. 2), and the burst, cluster, and supercluster lengths increased to 175 ± 17%, 277 ± 60%, and 267 ± 59% of control, respectively, without any change in channel conductance. Furthermore, the total time spent in the open state during complex openings increased significantly to 255 ± 65%, 355 ± 117%, and to 434 ± 153% of control during bursts (e.g., Fig. 2), clusters, and superclusters, respectively. The increases in total open time during the complex openings resulted from a combination of more channel openings and longer durations of each opening. The mean values are summarized in Table 1, where the results obtained for 200–500 nM SP were pooled together.

SP modulation of NMDA channels is NK-1 receptor dependent

Although neurokinin-1 (NK-1) receptors that bind SP with the greatest affinity have not been found immunohistochemically on rat dentate granule cells, tritiated high-affinity NK-1 ligands have been localized in the granule and molecular layers of the rat dentate gyrus (Dam et al. 1990). To test whether functional NK-1 receptor activation was respon-
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The mean open time was 105 ± 9% of control, whereas the burst, cluster, and supercluster durations were 97 ± 9%, 111 ± 12%, and 114 ± 16% of control, respectively. The summary of the data is shown in Table 1, where the effects of 1 or 10 μM CP-99,994 were pooled.

Nonpeptide NK-1 receptor antagonists have been shown to block voltage-dependent calcium channels (Lombet and Spedding 1994; Schmidt et al. 1992). Although it is unlikely that calcium channels in the membrane exposed to the nonpeptide inhibitor are active at rest, we blocked any residual voltage-dependent calcium channels by applying 200 μM CdCl₂ (n = 4) to the cells after the cell-attached recording configuration had been achieved. This treatment had no effect on NMDA channel openings (data not shown).

If SP activates an endogenous NK-1 receptor located on the preserved dentate granule cell somata or dendrites, then a selective NK-1 receptor agonist should reproduce the effects of SP on NMDA channels. Indeed, 200–500 nM Sar₉Met(O₂)¹¹-SP caused a robust increase in the duration of simple and complex NMDA channel openings (Fig. 4). A 2- to 5-min exposure of dentate granule cells to this selective NK-1 agonist lengthened all openings to an average of 145 ± 14% of control and increased the duration of bursts, clusters, and superclusters by 176 ± 19%, 218 ± 23%, and 196 ± 31% of control, respectively. The total open time in these complex openings increased by comparable amounts (Table 1). Furthermore, the average number of openings within complex groupings of openings also increased significantly.

SP modulation of NMDA channel activity is independent of calcium influx and activation of PKC

Because cellular excitability is frequently regulated by intracellular calcium levels and SP has been found to regulate NMDA channels in spinal dorsal horn neurons via calcium-dependent protein kinase C and A (PKC and PKA, respectively) (Rusin et al. 1993a), we examined the calcium dependence of the effects on NMDA channel openings following NK-1 receptor activation. In three of three cells examined, Sar₉Met(O₂)¹¹-SP still produced a dramatic increase in NMDA channel activity when calcium was removed from the extracellular medium (Fig. 5). Although the possible effects of calcium release from intracellular stores were not investigated, our results are not consistent with calcium influx being required for the enhancement of NMDA channel function by SP. Furthermore, this experiment argues against the possibility that a NK-1 receptor

![Graph](image)

**FIG. 2.** Cumulative distributions of open time and total open time/burst for the cell shown in Fig. 1. The mean open time during the control period was 1.32 ms and increased to 4.03 ms during SP. The total open time in bursts increased from 2.46 to 7.02 ms after perfusion of SP.

### Table 1. The effects of SP, its agonists, and antagonists on NMDA channel openings recorded in cell-attached patches

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SP</th>
<th>Sar₉</th>
<th>Control</th>
<th>Sar₉</th>
<th>Control</th>
<th>SP + CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open duration, ms</td>
<td>2.36 ± 0.20</td>
<td>3.91 ± 0.33*</td>
<td>2.30 ± 0.20</td>
<td>3.41 ± 0.32*</td>
<td>1.88 ± 0.22</td>
<td>1.95 ± 0.25</td>
<td></td>
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<tr>
<td>Burst length, ms</td>
<td>5.95 ± 0.90</td>
<td>10.2 ± 1.9*</td>
<td>3.74 ± 0.38</td>
<td>6.32 ± 0.56*</td>
<td>3.29 ± 0.65</td>
<td>2.96 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Total open/burst, ms</td>
<td>3.88 ± 0.52</td>
<td>8.70 ± 1.85*</td>
<td>3.50 ± 0.36</td>
<td>5.69 ± 0.56*</td>
<td>2.83 ± 0.55</td>
<td>2.68 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Cluster length, ms</td>
<td>12.3 ± 1.9</td>
<td>31.2 ± 6.6*</td>
<td>9.89 ± 1.18</td>
<td>19.3 ± 3.2*</td>
<td>10.8 ± 2.4</td>
<td>12.2 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Total open/cluster, ms</td>
<td>6.28 ± 1.00</td>
<td>18.0 ± 4.8*</td>
<td>5.57 ± 0.69</td>
<td>10.4 ± 1.5*</td>
<td>4.97 ± 1.49</td>
<td>5.24 ± 1.37</td>
<td></td>
</tr>
<tr>
<td>Supercluster length, ms</td>
<td>8.23 ± 19.7</td>
<td>213.8 ± 71.5*</td>
<td>67.0 ± 13.7</td>
<td>96.8 ± 19.6</td>
<td>130.8 ± 62.2</td>
<td>107.8 ± 32.0</td>
<td></td>
</tr>
<tr>
<td>Total open/S-cluster, ms</td>
<td>11.5 ± 1.9</td>
<td>40.8 ± 12.0*</td>
<td>10.3 ± 1.5</td>
<td>20.3 ± 4.2*</td>
<td>10.2 ± 3.4</td>
<td>11.3 ± 4.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; number of cells for SP and its Control was 10, for Sar₉ and its Control was 11, and for SP + CP and its Control was 10. Control values were measured during the 5–10 min preceding the perfusion of the drug. SP is 200–500 nM substance P. Sar₉ is 200–500 nM Sar₉Met(O₂)¹¹-SP; CP is 1–10 μM CP-99,994. * Significant difference from values measured during the control periods (P < 0.05; 2-tailed paired t-tests).
antagonist could prevent SP-induced increases in NMDA channel open times by preventing calcium influx through voltage-dependent calcium channels.

In cell-attached recordings, channels inside the recording pipette are protected from the direct action of agents perfused onto the rest of the cell. Thus the increases in NMDA channel open durations produced by both SP and Sar\(^9\),Met(O\(_2\))\(^{11}\)-SP described above did not result from binding of SP to the NMDA receptor itself, but from an indirect action of SP through an intracellular pathway. Furthermore, the delay in the action of SP after its application is consistent with the production of a diffusible second messenger. SP is classically associated with hydrolysis of phosphatidylinositol-4,5-bisphosphate by phospholipase C (Hanley et al. 1980; Taylor et al. 1986; Watson and Downes 1983), resulting in the activation of PKC (Takano et al. 1995). Therefore we attempted to block the SP effect on NMDA channels with the broad spectrum kinase inhibitor H-7, which is particularly effective on PKC. In three of three experiments, SP still prolonged the NMDA channel open times in the presence of H-7.

![Figure 3](http://jn.physiology.org/lookup/suppl/doi:10.1152/jn.1998.280.1.165/-/DC1/F3fig3.png)

**FIG. 3.** SP effects on single NMDA channel open times are blocked by 1–10 \(\mu\)M of the NK-1 receptor antagonist CP-99,994. **Top panel:** mean open time is plotted against recording duration binned in 10-s intervals. The mean open time for this recording was 1.55 ms in control and 1.65 ms after addition of 300 nM SP in the presence of 10 \(\mu\)M CP-99,994. Continuous line represents the 30-point running average. **Bottom panels:** cumulative distributions of open time and total open time/burst in the same cell. The total open time/bursts was 2.06 ms in control and 2.19 ms in SP + CP-99,994.

![Figure 4](http://jn.physiology.org/lookup/suppl/doi:10.1152/jn.1998.280.1.165/-/DC1/F4fig4.png)

**FIG. 4.** SP agonist specific for NK-1 receptors reproduces the effects of SP on NMDA channel openings. **Top panels:** continuous raw traces of cell-attached NMDA channel activity before and after perfusion of 300 nM Sar\(^9\),Met(O\(_2\))\(^{11}\)-SP. Mean open time during the control period was 2.03 ms and increased to 4.33 ms after addition of the SP analogue to the bath. The mean total open time in bursts increased from 3.48 ms during the control period to 10.12 ms after SP analog. Scale bars: 10 ms/2 pA. **Bottom panel:** mean open time is plotted against recording duration in 10-s bins. The continuous lines indicate 5- and 30-point running averages.

![Figure 5](http://jn.physiology.org/lookup/suppl/doi:10.1152/jn.1998.280.1.165/-/DC1/F5fig5.png)

**FIG. 5.** Effectiveness of the specific NK-1 receptor activating SP agonist in prolonging NMDA channel openings is unaltered when extracellular Ca\(^{2+}\) is removed from the bathing solution. The mean open time increased from 1.61 to 3.55 ms, whereas the total open time in bursts increased from 2.08 to 6.00 ms after addition of Sar\(^9\),Met(O\(_2\))\(^{11}\)-SP in the absence of extracellular Ca\(^{2+}\).
of H-7, which by itself had no effect on NMDA channel openings (data not shown).

**Discussion**

*Physiological effects of SP on hippocampal dentate granule cells*

Augmentation of NMDA receptor channel function by SP in spinal cord neurons is a well-established mechanism of modulating cellular excitability involved in the gating of nociceptive information. In the brain, the hippocampal dentate gyrus, and in particular the granule cells of this region, are thought to gate the amount of excitatory input to the hippocampus. Our results demonstrate that SP provides an excitatory influence on the granule cells of rat dentate gyrus. Specifically, SP altered NMDA channel gating by prolonging the time the channel spends in the open state, which in turn should increase the contribution of NMDA receptors to excitatory synaptic transmission.

Enhancement of NMDA channel activity in cell-attached patches after SP application to the outside of the cells suggests that some diffusible second-messenger pathway must have been activated by SP after its binding to NK-1 receptors. Although a G protein has been reported to couple SP receptors to phospholipase C in some tissues (Hanley et al. 1980; Taylor et al. 1986; Watson and Downes 1983), it is unlikely that PKC or PKA mediate SP effects on NMDA channels in dentate granule cells. In these cells, phorbol-12,13-dibutyrate (1–10 μM), the broad spectrum protein kinase inhibitor H-7 (10–20 μM), or the selective cell-permeable activators or inhibitors of PKA (e.g., Sp-8-CPT-cAMPS and Rp-8-CPT-cAMPS, 50–100 μM) have no effect on the mean open duration of NMDA channels (unpublished observations). Furthermore, in our present study H-7 failed to block the SP-induced prolongation of single NMDA channel openings. Although the NK-1 receptor found in dorsal horn neurons appears to be coupled to phospholipase C hydrolysis and activation of PKC, the NK-1 receptor present on dentate granule cells may be coupled to a different second-messenger system. It is also possible that activation of the SP receptor provides a direct, kinase-independent, G-protein action on the NMDA channel as has been reported for SP modulation of calcium and potassium channels in sympathetic neurons (Bley and Tsien 1990), although a membrane-delimited pathway should have been hampered by the cell-attached recording configuration.

*Localization of SP and NK-1 receptors in the hippocampus*

Extrahippocampal SP-containing fibers originate from the hypothalamic supramammillary nucleus and arborize in the granule and molecular layers of the dentate gyrus of the hippocampus in the human (Del Fiacco et al. 1987), monkey (Nitsch and Leranth 1994; Seress and Leranth 1996a), cat (Ino et al. 1988), and guinea pig (Gall and Selawski 1984). In the rat, these subcortical SP-containing fibers terminate predominantly on principal cells in CA2 subfield of the hippocampus (Borhegyi and Leranth 1997a), whereas the SP-containing afferents terminating on dentate gyrus granule cells in rat hippocampus appear to be sparse (Acsády et al. 1997; Borhegyi and Leranth 1997b). However, stimulation of SP-containing supramamillary fibers enhances perforant path–elicited population spikes recorded in the dentate gyrus (Mizumori et al. 1989).

In contrast, the highest density of immunoreactive SP receptors in the rat hippocampus is present on interneurons of the dentate hilar region (Acsády et al. 1997). In monkeys, a subset of these interneurons send SP-containing fibers to the outer two-thirds of the molecular layer where they would provide an inhibitory input onto dentate granule cells (Seress and Leranth 1996). Thus SP has been thought to exclusively modulate inhibition in the hippocampus. Furthermore, electrophysiological experiments have demonstrated that SP excites only the nonprincipal cells of the hippocampus (Dreifuss and Raggenbass 1986).

One contributing factor to the disputed role of SP in the brain is a general mismatch between the extent of SP-containing fibers and the quantity of SP/NK-1 receptors (Liu et al. 1994; Nakaya et al. 1994), which is particularly apparent in the hippocampal dentate gyrus. There is a very low density of SP-immunopositive fibers in the dentate gyrus and a very high density of SP receptors in this area, particularly in the hilus (Acsády et al. 1997; Borhegyi and Leranth 1997b; Nakaya et al. 1994). Perhaps in the hippocampus as in other CNS areas, SP may be reaching its target receptors via nonsynaptic diffusion (Mantyh et al. 1995), because no more than 15% of identified SP receptor sites ever appose SP-containing synaptic terminals (Liu et al. 1994). Furthermore, SP receptors appear to be associated with postsynaptic structures in addition to being homogeneously distributed on neuronal cell bodies and dendrites (Acsády et al. 1997; Borhegyi and Leranth 1997b; Liu et al. 1994). Thus diffusion of SP from its release sites onto granule cells of the dentate gyrus could modulate excitatory neurotransmission in this brain region.

A second confounding problem in the central actions of SP is that many SP-containing boutons were found to contact neurons purportedly devoid of SP/NK-1 receptors (Liu et al. 1994). Demonstration of both tachykinin receptor localization and activation is important because the presence of a particular neuropeptide transmitter in a nerve does not necessarily imply that it has an action on the innervated cell type. Nevertheless, the absence of NK-1 receptors in particular structures is difficult to assess, especially in light of recent pharmacological and biochemical studies suggesting that there are at least two molecular forms of the NK-1 receptor (Mantyh et al. 1996). The NK-1 receptor has a long and short isoform differing in the length of the cytoplasmic carboxyl-terminal tail extending beyond the seventh transmembrane domain (Kage et al. 1993), whereas two cDNA clones encoding two isoforms of the NK-1 receptor have been described, differing again in the length of the carboxyl-terminal tail (Fong et al. 1992). Because NK-1 receptor localization has generally been determined with an antibody raised against a portion of the cytoplasmic tail present in the long but not the short isoform (Vigna et al. 1994), it is possible that the discrepancy between SP peptide and NK-1 receptor localization can be ascribed to this difference in molecular forms of NK-1 receptor. This possibility would also suggest that a truncated NK-1 receptor might be coupled to a PKC-independent second-messenger system, because the C-terminal region of the NK-1 receptor may determine...
the specificity of G-protein activation (Fong et al. 1992; Kage et al. 1993; Mantyh et al. 1996).

Physiological and pathological significance of SP effects on hippocampal dentate granule cells

The dentate gyrus is a critical relay through which cortical projections reach the hippocampal formation. Modulation of the synaptic input to the dentate gyrus would thus have important functional consequences for cortical-hippocampal transmission. Because the dentate gyrus is also engaged during pathological states such as epilepsy and Alzheimer’s disease (AD), the robust effects of SP in the dentate gyrus might play a role in the pathogenesis of these or other neurological disorders. The number of immunopositive SP-containing cells is decreased in patients suffering from temporal lobe epilepsy while the levels of mRNA for preprotachykinin-A (SP precursor) are increased in the kainic acid model of temporal lobe epilepsy. Interestingly, perforant path stimulation has recently been shown to increase the immunoreactivity of dentate gyrus granule cells for SP (Borhegyi and Leranth 1997b), raising the possibility that SP may be released from the granule cells themselves in a manner similar to opioid peptides (Drake et al. 1994). Furthermore, SP-containing neurons are reduced in number in AD patients (Beal and Mzurek 1987). In the dentate gyrus of AD patients, the dense terminal-like staining that defines the outer two-thirds of the molecular layer as well as forming infragranular and supragranular plexuses typically found in normal brain persist, but with reduced intensity (Quigley and Kowall 1991).

Although the mechanism underlying the SP-induced enhancement of NMDA channel activity has yet to be determined, the presence of SP-containing fibers in the dentate gyrus and the robust sensitivity of dentate granule cells to NK-1 receptor activation implies that principal cells of the hippocampus can be modulated by this neuroactive peptide. Because peptides may be preferentially released from synaptic terminals at high stimulus frequencies, the possibility exists for SP to modulate physiologically or pathologically relevant plastic alterations in synaptic transmission in the hippocampus, such as long-term potentiation and epilepsy. An increase in hippocampal SP levels during epilepsy would significantly augment NMDA receptor-dependent excitability, whereas the loss of SP bearing cells in AD would tend to diminish NMDA receptor activation and consequently the ability of the hippocampus to process and store information.

We thank B. Oyama for technical assistance and Dr. Norbert Hajo for comments on the anatomy of SP neurons.

This research was supported by a Howard Hughes Predoctoral Fellowship, an American Federation for Aging Research Fellowship to D. N. Lieberman and by National Institute of Neurological Disorders and Stroke Grant NS-36142 to I. Mody.

Address for reprint requests: I. Mody, Dept. of Neurology, NNRC 3-131, UCLA School of Medicine, 710 Westwood Plaza, Los Angeles, CA 90095-1769.

Received 26 January 1998; accepted in final form 30 March 1998.

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