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Dynorphin A increases substance P release from trigeminal primary afferent C-fibers

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Abstract

Dynorphin A-(1–17) has been found to produce spinal antianalgesia and allodynia. Thus, we studied whether dynorphin A-(1–17) modulates substance P release evoked by the C-fiber-selective stimulant capsaicin (1 μM) from trigeminal nucleus caudalis slices. Very low concentrations of dynorphin A-(1–17) (0.01–0.1 nM) strongly facilitated capsaicin-evoked substance P release. This dynorphin A-(1–17) effect was not blocked by the opioid receptor antagonists naloxone (100 nM), β-funaltrexamine (20 nM), naloxonazine (1 nM), nor-binaltorphimine (3 nM) and ICI 174,864 (*N*,*N*-dialyl-Tyr-Aib-Phe-Leu; 0.3 μM). Yet, the effect of dynorphin A-(1–17) was blocked by the NMDA receptor antagonist MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d] cyclohepten-5-10-imine maleate; 0.3 μM). Neonatal treatment with capsaicin (50 mg/kg s.c.), which destroys substance P-containing primary afferents, abolished the excitatory effect of dynorphin A-(1–17) on K^+-evoked substance P release. In conclusion, dynorphin A-(1–17) increases substance P release from C-fibers by the activation of NMDA receptors which supports the involvement of presynaptic mechanisms in dynorphin-induced antianalgesia and allodynia.

Keywords: Substance P; Dynorphin; Opioid receptor antagonist; NMDA receptor antagonist; Primary afferent

1. Introduction

Dynorphins, known as κ-selective endogenous opioid peptides, do not show analgesic activity in either thermal or chemical nociception assays unless higher doses, which produce motor impairment, are used (Stevens and Yaksh, 1986). In contrast, intrathecal dynorphin A-(1–17) or des-Tyr-dynorphin fragments have been found to produce a long-lasting allodynia that is not mediated by opioid receptors but instead by NMDA receptors (Vanderah et al., 1996). Moreover, dynorphin A-(1–17) has been proposed as a mediator of an antianalgesic system since exogenously administered or endogenously released dynorphin A-(1–17) antagonizes the analgesia produced by the intrathecal administration of morphine in rats (Schmauss and Hertz, 1987; Fujimoto and Holmes, 1990; Rady et al., 1991). This antianalgesic effect of dynorphins has been implicated in the development of the persistent pain produced by chronic nerve constriction injury or axotomy (Draisci et al., 1991), peripheral inflammatory conditions like arthritis (Iadarola et al., 1988), and spinal cord trauma (Cox et al., 1985; Yakovlev and Faden, 1994), where increased levels of dynorphin A peptide and preprodynorphin mRNA in the dorsal spinal cord have been measured. Post-synaptic non-opioid mechanisms have been proposed for many of these effects of dynorphins (Vanderah et al., 1996). Yet, some findings suggest that presynaptic mechanisms may also be involved. For example, dynorphin A-(1–13) facilitates cutaneous C-fiber-evoked responses of spinal dorsal horn neurons by a mechanism not sensitive to opioid-receptor blockade with naloxone (Knox and Dickenson, 1987). In addition, 1 nM dynorphin A-(1–13) prolongs the action potential duration in dorsal root ganglia sensory neurons in culture by decreasing opioid receptor-gated K^+ conductance (Shen and Crain, 1990). Finally, dynorphin A-(1–17)-containing cell bodies and fibers are present in superfi-

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cial layers of the spinal dorsal horn and the trigeminal nucleus caudalis (Cruz and Basbaum, 1985) and make synaptic contacts with C-type primary afferent fibers (Takahashi et al., 1988).

In this study, we examined the possibility that presynaptic mechanisms contribute to dynorphin-mediated antianalgesia, hyperalgesia and allodynia by facilitating the stimulation-evoked release of substance P from C-type primary afferents. Thus, we evaluated the effects of dynorphin A-(1–17) on the release of substance P selectively evoked by capsaicin from C-type fibers. We found that very low concentrations of dynorphin A-(1–17) facilitate the stimulation-evoked release of substance P by activation of glutamate NMDA receptors, which supports the involvement of presynaptic non-opioid mechanisms in the modulation of nociception by dynorphins.

2. Material and methods

2.1. Superfusion of trigeminal nucleus slices

Male Sprague–Dawley rats weighing 150–250 g were killed by decapitation. The trigeminal nucleus caudalis was immediately dissected on a cold plate (4°C) under cold oxygenated superfusion medium with the aid of a stereo microscope. The rostral and caudalis landmarks for dissection were 2 mm above and below the obex, respectively, and medially, by a line drawn from the cuneatus fasciculus to the dorsal spinocerebellar tract. Isolated trigeminal nuclei were chopped into 400-μm coronal slices with the trigeminal tract lying on the plate. About 70–130 slices from 3–4 rats were selected and pooled in continuously oxygenated chilled superfusion buffer. Approximately 12 to 16 slices were placed in 0.1-ml volume polypropylene chambers. These chambers were perfused with superfusion buffer. Six to eight chambers were run simultaneously and were placed in a water bath maintained at 37°C and superfused at a rate of 0.35 ml/min with medium that contained (in mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 10, ascorbic acid 0.57, as well as 20 μM bacitracin and 0.1% bovine serum albumin. The medium was gassed continuously with 95% O_2/5% CO_2 to maintain a pH of 7.4.

Collection of superfusate was initiated after 45 min of superfusion with either drug-free medium or selective antagonists. To evaluate the effect of irreversible opioid antagonists (β-funaltrexamine or naloxonazine), these compounds were superfused for 30 min followed by an additional 15-min period of superfusion in the absence of the antagonist. When reversible antagonists (naloxone, nor-binaltorphimine, ICI 174,864 (*N*,*N*-dialyl-Tyr-Aib-Phe-Leu) and MK-801((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d] cyclohepten-5-10-imine maleate) were used, they were superfused 15 min before the start of sample collection and throughout the experiment. Superfusate fractions (0.7 ml) were collected in polyethylene tubes kept at 4°C on ice. The collected samples were frozen at −20°C until they were used for substance P radioimmunoassay. In each experiment, slices were exposed to two 2-min periods of depolarization (*S*1 and *S*2). *S*1 was achieved by raising the K^+ concentration in the superfusate from 6 to 50 mM. To maintain the isotonicity of the superfusion medium, the concentration of NaCl was reduced to 74.8 mM. *S*2 was produced with either 1 μM of the C-fiber stimulant capsaicin (Nagy et al., 1981) or 50 mM K^+ 30 min after the *S*1 depolarization period. Capsaicin is a neurotoxin that releases neurotransmitters selectively from sensory neuron terminals (Jancso and Kiraly, 1980; Gamse et al., 1981). Eight chambers were superfused in parallel in each experiment. Two chambers served as controls (i.e., no dynorphin or antagonists in the superfusate during *S*1 and *S*2). To evaluate the action of dynorphin on capsaicin or K^+-evoked substance P release, a given concentration of this peptide was superfused 16 min before *S*2 (*S*1 serving as an internal control). The effect of dynorphin A-(1–17) was also evaluated during *S*2 in the presence of antagonists.

At the completion of the experiment, substance P was extracted from the slices by homogenization with a mixture of acetone:H_2O:HCl (40:5:1) and centrifugation at $7800 \times g$ for 30 min at 4°C. Aliquots of 20 μl of supernatant solution were dried under nitrogen and reconstituted in 0.3 ml of superfusion medium. The amount of substance P in each superfusate fraction and in tissue extracts was determined with a substance P radioimmunoassay.

2.2. Substance P radioimmunoassay

Substance P antibody was generously donated by Dr. Peter Petrus, Department of Anatomy and Cellular Biology, University of North Carolina at Chapel Hill, NC, USA. This substance P antiserum is highly specific, since it requires at least seven amino acids of the substance P carboxylic terminal and that this terminal be amidated. Substance P antiserum does not cross-react with fragments of the substance P terminal amino, the substance P-free acid, or with related tachykinins, such as Substance K and Neuromedin K, or with other non-related peptides such as bradykinin and dynorphin A-(1–17). The substance P tracer was prepared by radioiodination of Tyr^8-substance P by the chloramine-T method (Hunter and Greenwood, 1962). The substance P radioimmunoassay was carried out as previously described (Suarez-Roca and Maixner, 1992). Briefly, antiserum (100 μl, 1:600,000 final dilution) and substance P standards or superfusate samples (100 μl) were incubated at 4°C for 24 h before the addition of tracer (100 μl/10,000 cpm). After an additional 24-h incubation period, free and antibody-bound peptide was separated by the addition of 1 ml of 0.5% dextran-coated charcoal in 0.05 M sodium phosphate buffer, pH 7.5, with 10% heat inactivated horse serum. After centrifugation at $2300 \times g$ for 20 min at 4°C, the charcoal pellets with free tracer

were counted. The amount of substance P tracer bound to the antiserum was determined by subtracting the amount of tracer bound to the charcoal in the presence and in the absence of antiserum. The amount of substance P-like immunoreactivity was analyzed on a smoothed spline-fitted standard curve. Assay sensitivity, as determined with one-tailed Student's *t*-test ($P < 0.05$), was 0.25 pg/100 ml (12% of displacement from maximal binding) whereas half displacement (50%) occurred at 3 pg/100 μl. The intra-assay and inter-assay variations were 3% and 11%, respectively. The drugs used in this study, at the concentrations tested, did not alter the binding of substance P peptide to its antibody or the displacement of the peptide by the tracer.

2.3. Neonatal treatment with capsaicin

One group of rats was treated neonatally with capsaicin as described by Nagy et al. (1981). Briefly, 2- to 3-day-old animals were injected subcutaneously, while under ether anesthesia, with capsaicin (50 mg/kg; 30–50 μl). The second group (control) was treated with vehicle (10% Tween 80 and 10% ethanol v/v in 0.9% NaCl). Animals were placed under a tungsten lamp and after a 10 min recovery period, they were returned to their mothers. They were weaned at 4 weeks and allowed to grow over a period of 2 to 3 months to 200–300 g of body weight. Food and water were available ad libitum for both groups.

2.4. Data analysis

The amount of substance P released by depolarization is expressed as a percentage of substance P content in the tissue at the beginning of each stimulation ($S1$ and $S2$). The amount of substance P at the beginning of stimulation was determined by summing the amount of substance P in the tissue extract and the amount of substance P in the superfusion buffer from the beginning of the stimulation (i.e., either $S1$ and $S2$) to the termination of superfusion. The effects of the drugs on the evoked release of substance P are expressed in terms of $S2/S1$ ratios. This ratio reflects the evoked release in the presence of drugs ($S2$) over the evoked release in the absence of drugs ($S1$). Results are shown as means ± S.E.M. An independent observation was defined as the recording of a value obtained from a single chamber or a mean obtained from several chambers (usually duplicates) during a single experiment.

Statistical evaluation of multigroup data was performed by analysis of variance (ANOVA) followed by Duncan's multiple range test to measure the statistical significance of the mean difference between controls and drug-treated groups. Statistical evaluation of two sample data was done by Wilcoxon's rank sum test. One-tailed significance tests were used when a priori hypotheses were available, such as 'an antagonist blocks an agonist-mediated effect'. Otherwise, two-tailed tests were applied. Significance was assumed at $P = 0.05$.

3. Results

3.1. Characteristics of capsaicin-evoked substance P release

In our preparation, spontaneous substance P release was below the detection limit of the RIA. Fig. 1 shows that superfusion with either 50 mM K^+ or 1 μM capsaicin evoked a reliable release of substance P. In control chambers, $S1$ (50 mM K^+) released 0.89 ± 0.15% of the tissue substance P content, while $S2$ (1 μM capsaicin) released 1.27 ± 0.26% of the tissue content of the peptide, giving a $S2/S1$ ratio of 2.49 ± 0.39 ($n = 60$). Capsaicin-evoked substance P release was reduced to 9 ± 1% of control values by 25 μM capsazepine, a selective antagonist of the capsaicin receptor in sensory neurons (capsaicin $S2/S1 = 2.96 \pm 0.92$; capsaicin + capsazepine $S2/S1 = 0.25 \pm 0.05$; $n = 4$; Wilcoxon test; $P < 0.05$).

3.2. Modulatory action of dynorphin on capsaicin-evoked substance P release

Dynorphin A-(1–17), at the concentrations used in this study (0.01 nM–1 μM), did not evoke by itself the release of substance P from trigeminal slices. Yet, there was a strong enhancement of capsaicin-evoked substance P release by dynorphin A-(1–17) (ANOVA, $F(6,94) = 6.51$;

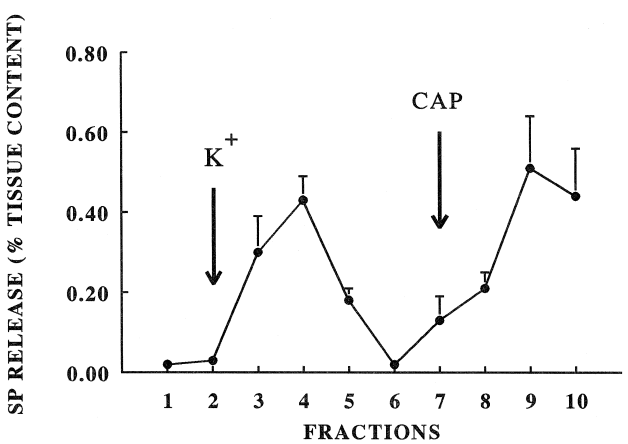

Fig. 1. Stimulation-evoked substance P release from trigeminal nucleus slices. Slices were subjected to two stimulation periods, indicated by the vertical arrows. The first stimulation (K^+) was carried out with 50 mM K^+. The second stimulation (CAP) was done with 1 μM capsaicin. The ordinate shows substance P release as percent of tissue content. The abscissa shows ten 2-min fractions collected at a flow rate of 0.35 ml/min. There was a 18-min void period between fractions 5 and 6. Each point represents the mean ± S.E.M. of 60 independent observations.

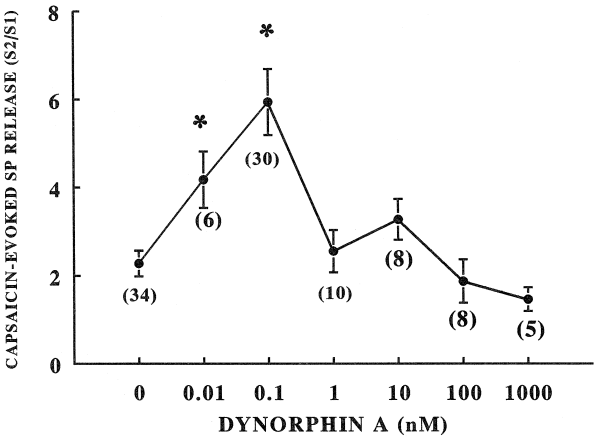

Fig. 2. Dynorphin A-(1–17) concentration-response curve for capsaicin-evoked substance P release. A given concentration of dynorphin A-(1–17) was present during *S*2 (1 μM capsaicin). The ordinate shows capsaicin-evoked substance P release as *S*2/*S*1 ratios. The abscissa shows dynorphin A-(1–17) concentration (nM). Each point represents the mean ± S.E.M. The number of independent observations for each point is shown in parentheses. * denotes significant difference with respect to control release ($P < 0.0001$; one-way ANOVA followed by Duncan's multiple range test).

$P < 0.0001$) (Fig. 2). The maximal effect of dynorphin A-(1–17), about a 3-fold increase with respect to control, was observed at 0.1 nM. A further increase in dynorphin A-(1–17) concentration produced a decline in this facilitatory effect.

To determine if opioid receptors were involved in the enhancement by dynorphin A-(1–17) of substance P release, we attempted to block the facilitatory actions of 0.1 nM dynorphin A-(1–17) by using selective opioid receptor antagonists. As shown in Fig. 3, the stimulatory action of Dyn A-(1–17) on capsaicin-evoked substance P release was not blocked by either the μ-opioid receptor antagonists, β-funaltrexamine (20 nM) and naloxonazine (1 nM), the δ-opioid receptor antagonist ICI 174,864 (0.3 μM), the κ-opioid receptor antagonist nor-binaltorphimine (3 nM) or the non-selective opioid receptor antagonist naloxone (100 nM).

To evaluate if the effect of dynorphin A-(1–17) on substance P release was mediated by the activation of NMDA glutamate receptors, we assessed the effect of the NMDA receptor antagonist MK-801 on the facilitatory action of dynorphin A-(1–17) on substance P release evoked by capsaicin. Fig. 3 shows that the facilitatory action of dynorphin A-(1–17) was completely blocked by 0.3 μM MK-801 ($P < 0.05$; Wilcoxon test).

3.3. Effect of neonatal capsaicin on the facilitation of substance P release by dynorphin A

To determine whether the facilitatory effect of dynorphin A-(1–17) on substance P release was exerted via C-type primary afferents, neonatal rats were treated with capsaicin (50 mg/kg, s.c.). The effectiveness of 50 mg/kg neonatal capsaicin in destroying primary afferent C-fibers was supported by: (1) 1 μM capsaicin did not elicit substance P release from trigeminal slices from rats neonatally treated with capsaicin (see Fig. 4A); (2) we have previously found that this dose of neonatal capsaicin pro-

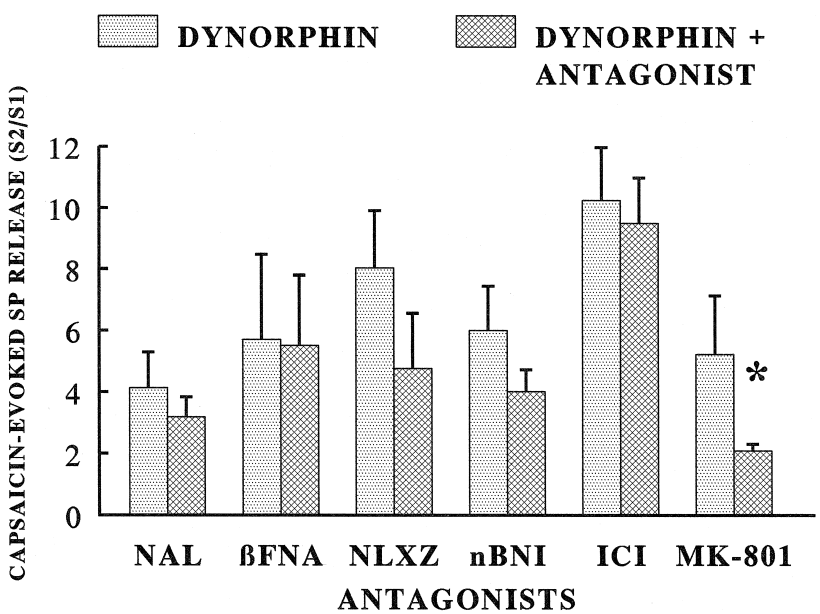

Fig. 3. Action of subtype-selective opioid and glutamate receptor antagonists on the facilitatory effect of dynorphin A-(1–17) on capsaicin-evoked substance P release. Responses to dynorphin A-(1–17) (0.1 nM) were obtained from slices treated or not with the following antagonists: naloxone (NAL, 100 nM), β-funaltrexamine (β-FNA, 20 nM), naloxonazine (NLXZ, 1 nM), norbinaltorphimine (*n*-BNI, 3 nM), ICI 174,864 (ICI, 0.3 μM), or MK-801 (0.3 μM). The ordinate shows capsaicin-evoked substance P release as *S*2/*S*1 ratios. Each bar represents the mean ± S.E.M. of 4–7 independent observations. * denotes significant difference in agonist effect between the presence and absence of the antagonist ($P < 0.05$, Wilcoxon test).

duces a robust thermal analgesia and a clear lack of protective eye wiping movements after the topical application of capsaicin (Suarez-Roca et al., 1996). Yet, since 50 mg/kg capsaicin can destroy some lightly myelinated primary afferents (Nagy et al., 1983), the abolishment of the dynorphin A-(1–17)-induced enhancement of substance P release by neonatal capsaicin could be in part related to the decrease in the number of these sensory fibers.

Neonatal capsaicin did not significantly alter the substance P release evoked by 50 mM K^+ (Fig. 4B). Fig. 5 shows that 0.1 nM dynorphin A-(1–17) did not increase K^+-evoked substance P release from trigeminal slices obtained from rats neonatally treated with capsaicin. In contrast, 0.1 nM dynorphin A-(1–17) produced a 3-fold increase in K^+-evoked substance P release in control rats

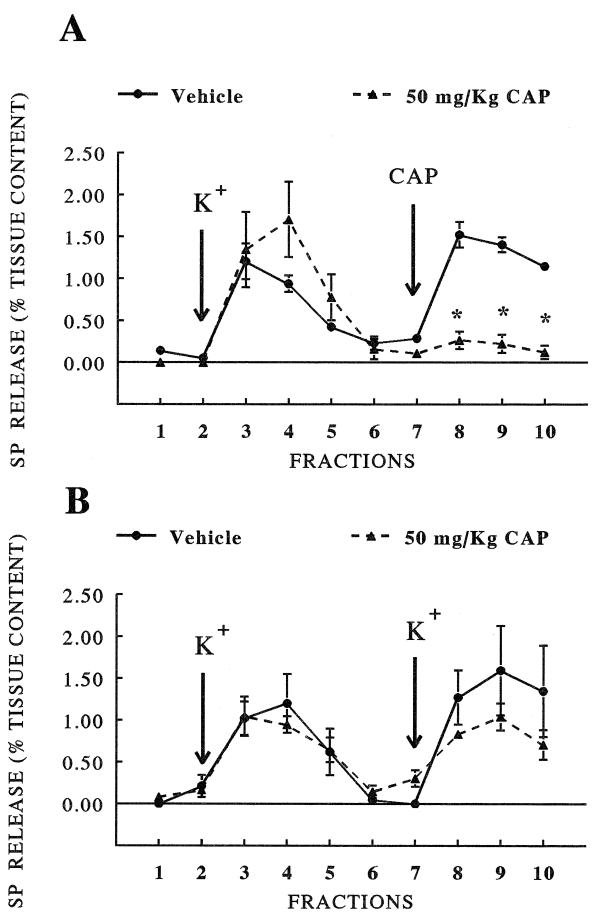

Fig. 4. Stimulation-evoked substance P release from trigeminal nucleus slices obtained from rats neonatally treated with capsaicin. Slices were subjected to two stimulation periods, indicated by the vertical arrows. The first stimulation (K^+) was carried out with 50 mM K^+. The second stimulation was done with either 1 μM capsaicin (CAP, panel A) or 50 mM K^+ (K^+, panel B). The ordinate shows substance P release as percent of tissue content. The abscissa shows ten 2-min fractions collected at a flow rate of 0.35 ml/min. There was a 18-min void period between fractions 5 and 6. Each point represents the mean ± S.E.M. of 10 independent observations. * denotes significant difference with respect to vehicle ($P < 0.05$, Wilcoxon test).

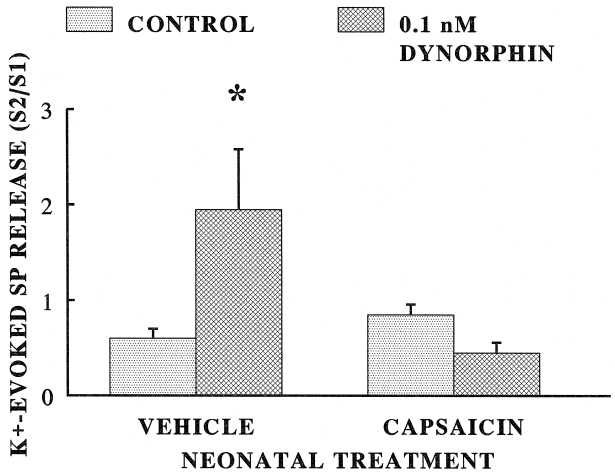

Fig. 5. 5yEffect of neonatal treatment with capsaicin on the facilitation by dynorphin A-(1–17) of K^+-evoked substance P release from trigeminal slices. The ordinate shows capsaicin-evoked substance P release as $S2/S1$ ratios. The abscissa indicates the neonatal treatment with capsaicin (50 mg/kg) or vehicle. Each bar represents the mean ± S.E.M. from four independent observations. * denotes significant difference with respect to control release ($P < 0.05$; Wilcoxon test).

neonatally treated with vehicle ($F(3,22) = 4.35$, $P < 0.015$).

4. Discussion

4.1. Excitatory effects of dynorphin

Dynorphin A-(1–17) exhibited a biphasic concentration-response curve for capsaicin-evoked substance P release, i.e., an excitatory effect at picomolar concentrations and no effect at higher nanomolar concentrations. In agreement, the action potential duration of dorsal root ganglia neurons is prolonged and shortened by low nanomolar and micromolar concentrations of dynorphin A-(1–13), respectively (Shen and Crain, 1990). The receptor mechanism involved in the biphasic effect of dynorphin A-(1–13) on dorsal root ganglion neurons is not clear since it was blocked by diprenorphine (1 nM) but not by naloxone (30 nM), both opioid receptor antagonists (Shen and Crain, 1990). We have previously reported that κ_1-opioid receptors (Suarez-Roca and Maixner, 1993) and the μ/δ-opioid receptor complex (Suarez-Roca and Maixner, 1992) are involved in the facilitation of K^+-evoked substance P release from rat trigeminal slices. Thus, we tried to determine the involvement of opioid receptors in the facilitation by dynorphin A-(1–17) of stimulation-evoked substance P release, using subtype-selective opioid receptor antagonists. Yet, the effect of dynorphin A-(1–17) on capsaicin-evoked substance P release was not blocked by either the putative antagonist of the μ/δ-opioid receptor complex β-funaltrexamine (Rothman et al., 1991) or the κ_1-opioid receptor antagonist nor-binaltorphimine (Rothman et al., 1990). Moreover, other opioid receptor subtypes did not seem to be involved either since the δ-selective opioid

receptor antagonist ICI 174,864, the μ_1-selective opioid receptor antagonist naloxonazine and naloxone did not significantly alter the effect of dynorphin A-(1–17) on capsaicin-evoked substance P release.

In contrast, the effect of dynorphin A-(1–17) on capsaicin-evoked substance P release was blocked by MK-801. MK-801, at the concentration used in this study (0.3 μM), likely antagonized the dynorphin A-(1–17)-induced enhancement of capsaicin-evoked substance P release by the selective blockade of NMDA glutamate receptors. This assumption is based on the following findings: (1) we used a relatively low concentration of MK-801, about 10-fold (one log unit) higher than its K_d (30 nM) in rat medulla and pons membranes (Wong et al., 1986); and (2) MK-801, at a concentration of 0.3 μM, has been found to block completely the 3-fold enhancement of capsaicin-evoked substance P release from trigeminal slices produced by a low concentration (1 μM) of L-CCG-IV (carboxycyclopropyl-glycine), a very potent and selective NMDA receptor agonist (Cuesta et al., 1996). Yet, MK-801 could block binding sites not related to NMDA receptors. In addition, we did not try another selective NMDA receptor antagonist in our study. Thus, we cannot rule out the possibility that the effect of dynorphin A-(1–17) is not mediated by NMDA glutamate receptors.

Interestingly, dynorphins have been shown to produce some behavioral effects by stimulating non-opioid receptors (Vanderah et al., 1996). Dynorphins could exert biological actions by directly stimulating NMDA receptors since: (1) dynorphin A-(1–13), but not other κ-opioid receptor ligands, displaces L-[^{3}H]glutamate from NMDA receptor binding sites (Massardier and Hunt, 1989); (2) dynorphins bind to a dithiothreitol-sensitive site of the NMDA receptor complex in isolated trigeminal neurons (Chen et al., 1995); (3) dynorphin A-(1–13) binds to the phencyclidine (PCP) site in the NMDA receptor complex (Urban and Dray, 1992); and (4) in low concentrations dynorphins can bind to NMDA receptors as agonists since they enhance NMDA-mediated synaptic currents in guinea pig hippocampal slices (Caudle et al., 1994). The NMDA-mediated modulation by dynorphin A-(1–17) of substance P release may be directly exerted on primary afferents because PCP sites have been detected in laminae I and II of the dorsal spinal cord (Aanonsen and Seybold, 1989), and NMDA receptors have been located in the release zone of the central synapses of these fibers (Liu et al., 1994).

4.2. Possible anatomical substrate mediating functional dynorphin-substance P interactions

Endogenously released dynorphin A-(1–17) could physiologically modulate the release of substance P from primary afferents since dynorphin A-(1–17)-containing neurons (Cruz and Basbaum, 1985) and substance P-containing primary afferents (Hökfelt et al., 1977) are co-localized in laminae I, IIa, and V of the spinal cord and trigeminal nucleus. In the spinal dorsal horn and the trigeminal nucleus, most of the dynorphin-positive cells are intrinsic neurons (Standaert et al., 1986) and they could release dynorphin A-(1–17) to stimulate substance P-containing primary afferents either by direct axo-axonic interactions or indirectly by volume-transmission (diffusion) (Fuxe et al., 1988). Indeed, compression of an inflamed ankle, but not a normal ankle, has been found to induce the ipsilateral release of dynorphin A-(1–8) in the laminae II–V of the dorsal horn (Riley et al., 1996). Moreover, the synaptic contacts of dynorphin A-(1–8)-containing neurons with both unmyelinated calcitonin gene-related peptide-containing primary afferents (Takahashi et al., 1988) and projection neurons in the superficial dorsal horn (Nahin et al., 1992) increase ipsilaterally in experimentally induced peripheral inflammation. Thus, dynorphin A-(1–17)-containing neurons and unmyelinated substance P-containing fibers may especially interact following peripheral inflammation.

4.3. Functional relevance of these in vitro findings for nociception

In the present study, dynorphin A-(1–17) likely modulated substance P release from C-type primary afferents since: (1) capsaicin acts selectively on C-fibers (Nagy et al., 1983); (2) capsaicin-evoked substance P release was abolished by capsazepine, which blocks a capsaicin receptor located only on C-type primary afferents (Urban and Dray, 1992); and (3) the effect of dynorphin A-(1–17) was abolished by neonatal capsaicin treatment, which destroys C-type primary afferents (Nagy et al., 1983). Thus, the enhancement of substance P release by dynorphin A-(1–17) could imply that dynorphins may presynaptically potentiate nociceptive transmission within the trigeminal nucleus and spinal cord by non-opioid mechanisms. A pro-nociceptive role for dynorphins is supported by the results of several studies. For example, dynorphin A-(1–13) produces a naloxone-insensitive facilitation of cutaneous C-fiber-evoked responses of nociceptive neurons in the spinal dorsal horn (Knox and Dickenson, 1987). Interestingly, intrathecal administration of dynorphin A-(1–17) antagonizes the spinal analgesia induced by morphine in rats (Schmauss and Hertz, 1987) and mice (Fujimoto and Holmes, 1990). Moreover, intracerebroventricular administration of various opioid and non-opioid compounds produces a blockade of intrathecal morphine analgesia (Fujimoto et al., 1990). This antianalgesic effect is thought to be mediated by the spinal release of endogenous dynorphin A-(1–13) since it is blocked by intrathecal administration of dynorphin A-(1–13) antiserum (Fujimoto et al., 1990). Accordingly, a dynorphin-dependent hyperalgesic system seems to be tonically active in the brainstem because microinjection of dynorphin A-(1–13) antiserum into the dorsal posterior mesencephalic tegmentum of in-

tact conscious rats produces an analgesic effect (Shukla and Lemaire, 1994).

The increase in the levels of dynorphin A peptide and preprodynorphin mRNA in the dorsal spinal cord during conditions of nerve tissue damage (Cox et al., 1985; Draisci et al., 1991) and peripheral inflammation (Iadarola et al., 1988) suggests a possible role for dynorphins in the maintenance of persistent pain states. In support of this view, the intrathecal administration of a single dose of dynorphin A-(1–17), dynorphin A-(1–13) or its des-Tyr-fragments produces a long-lasting allodynia that is mediated by NMDA receptors (Vanderah et al., 1996).

The pronociceptive actions of dynorphin A-(1–17) at the spinal level could be mediated by both pre- and/or post-synaptic mechanisms. Our results support the hypothesis that the analgesia and allodynia produced by dynorphin A-(1–17) may be presynaptically mediated by an increased release of substance P from primary afferents and, as a result, enhanced nociception. Most of evidence described above indicates that non-opioid receptor mechanisms seem to be mainly responsible for these properties of dynorphins. Dynorphins, as well as other opioid agonists, may stimulate non-opioid receptors that could functionally antagonize, and mask, their opioid receptor-mediated effects such as analgesia. Indeed, heroin, an opioid agonist, produces hyperalgesia that is sensitive to MK-801 during opioid receptor blockade with naloxone (Laulin et al., 1996). Moreover, Rady et al. (1991) provided indirect evidence that dynorphins and des-Tyr dynorphins bind to the same non-opioid receptor in the spinal cord. These dynamic receptor mechanisms could be involved not only in hyperalgesia and allodynia but also in opioid tolerance and withdrawal.

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