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Mechanism of the dynorphin-induced dualistic effect on free intracellular Ca^{2+} concentration in cultured rat spinal neurons

Wen-Hui Hu [a,b,*], Cui-Hua Zhang [c], Hui-Fen Yang [b], Yong-Fang Zheng [c], Na Liu [b], Xiu-Jun Sun [b], Jun Jen [b], Min-Feng Jen [b]

[a] *Research Institute of Surgery, The Third Military Medical University, Daping, Chongqing 630042, PR China*

[b] *Department of Pharmacology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, 5 Dong Dan San Tiao, Beijing 100005, PR China*

[c] *Department of Physiology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, 5 Dong Dan San Tiao, Beijing 100005, PR China*

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Abstract

In order to study the different mechanisms of dynorphin spinal analgesia and neurotoxicity at low and high doses, the effects of various concentrations of dynorphin A-(1–17) on the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in the cultured rat spinal neurons were studied using single cell microspectrofluorimetry. While dynorphin A-(1–17) 0.1–100 μM had no significant effect on basal $[Ca^{2+}]_i$, dynorphin A-(1–17) 0.1 and 1 μM significantly decreased the high KCl-evoked peak $[Ca^{2+}]_i$ by 94% and 83% respectively. Dynorphin A-(1–17) 10 and 100 μM did not affect the peak $[Ca^{2+}]_i$ following K^+ depolarization, but in all these neurons there was a sustained and irreversible rise in $[Ca^{2+}]_i$ following high-K^+ challenge. Pretreatment with the specific κ-opioid receptor antagonist nor-binaltorphimine 10 μM, but not the competitive NMDA receptor antagonist, DL-2-amino-5-phosphonovalerate (APV) 10 μM, significantly blocked the inhibitory effect of dynorphin A-(1–17) 0.1 μM on peak $[Ca^{2+}]_i$. However, APV 10 μM and nor-binaltorphimine 10 μM significantly antagonized the sustained rise in $[Ca^{2+}]_i$ induced by a high concentration of dynorphin A-(1–17) 10 μM. Furthermore, in the presence, and following the addition, of increasing concentrations of dynorphin A-(1–17) (0.1, 1, 10 and 100 μM), the high concentrations of dynorphin A-(1–17) failed to produce a sustained rise in peak $[Ca^{2+}]_i$. These results suggested that dynorphin exerted a dualistic modulatory effect on $[Ca^{2+}]_i$ in cultured rat spinal neurons, inducing a sustained and irreversible intracellular Ca^{2+} overload via activation of both NMDA and κ-opioid receptors at higher concentrations, but inhibiting depolarization-evoked Ca^{2+} influx via κ-opioid but not NMDA receptors at lower concentrations. Serial addition of graded concentrations of dynorphin A-(1–17) prevented the effect of high concentrations of dynorphin A-(1–17) on $[Ca^{2+}]_i$. © 1998 Elsevier Science B.V.

Keywords: Dynorphin; Ca^{2+}; (Neuron); Spinal cord; Cell culture; Glutamate receptor; (Rat); Fura-2

1. Introduction

Dynorphin, an endogenous κ-opioid receptor agonist, produced extremely potent analgesia at low concentrations (Han and Xie, 1982; Herman and Goldstein, 1985; Spampinato and Candeletti, 1985; Millan, 1990; Shukla and Lemaire, 1994; Fiyibayashi and Iizuka, 1995) and induced a synergistic analgesia when co-administered with the *N*-methyl-D-aspartate (NMDA) receptor antagonist, DL-2-amino-5-phosphonovalerate (APV) (Li et al., 1994). Dynorphin and κ-opioid receptor agonists were remarkable for their low addiction liability; they did not induce respiratory depression, were not self-administered and, when given to morphine-tolerant rats, restored morphine-induced analgesia (Friedman et al., 1981; Millan, 1990, Pentel et al., 1995). The potential clinical application of dynorphin and κ-opioid receptor agonists as a new class of opioid analgesics has attracted much attention in recent years.

However, intrathecal administration of higher doses of dynorphin has been widely reported to produce dose-related paralysis of hindlimbs and tail, and cause serious neuropathological changes in the spinal cord (Faden, 1990; Long et al., 1994; Shukla and Lemaire, 1994; Tian et al., 1994). The mechanism(s) of dynorphin-induced spinal cord injury remains to be clarified. Several laboratories have reported that NMDA receptor activation contributes to dynorphin spinal neurotoxicity. The concentration of exci-

* Corresponding author. Tel.: +86-23-68808963; fax: +86-23-68810858; e-mail: jshaw@public.cq.sc.cn

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tatory amino acids in cerebrospinal fluid and extracellular fluid was significantly increased shortly after dynorphin-induced hindlimb paralysis (Skilling et al., 1992). A variety of competitive and non-competitive inhibitors of the NMDA receptor complex have been shown to significantly improve the functional recovery of hindlimbs after dynorphin spinal neurotoxicity (Long et al., 1989, 1994; Bakshi and Faden, 1990; Chen et al., 1995a). We have also demonstrated that the Ca^{2+} channel blocker, verapamil, protects the spinal cord from dynorphin neurotoxicity, indicating the involvement of Ca^{2+} in its mechanism (Chen et al., 1995a). While all these findings indicated a direct neurotoxic effect of dynorphin, recent evidence from Long's laboratory suggested that the neurological deficits and neuronal injury resulted predominantly from a vascular effect of dynorphin on the spinal cord rather than from a direct neurotoxic effect (Long et al., 1994).

To further explore the cellular mechanisms for dynorphin spinal pain modulation and neurotoxicity, the present study evaluated the effects of dynorphin A-(1–17) on the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) following high-K^+ depolarization and their receptor mechanisms in cultured single rat spinal neurons, using microspectrofluorimetry.

2. Materials and methods

2.1. Reagents and solutions

Dynorphin A-(1–17) (Lot number: 35234) was from Peninsula (Belmont, CA). nor-binaltorphimine was from RBI (Natick, MA). 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-2(2′-amino-5′-methyl-phenoxy)-ethane-N,N,N',N'-tetraacetic acid-pentaacetoxy-methylester (Fura-2/AM), Cremophor EL and Pluronic F-127 were from Molecular Probes (Eugen, OR). APV, Delbecco's modified Eagle's medium (DMEM), horse and fetal calf serum, L-glutamine, penicillin, poly-L-lysine (m.w. > 70,000) and cytosine arabinoside were from Sigma (St. Louis, MO).

HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid) buffered salt solution (HBSS) contained (in mM): NaCl 137, KCl 5, $MgSO_4$ 0.9, $CaCl_2$ 1.4, $NaHCO_3$ 3, Na_2HPO_4 0.6, KH_2PO_4 0.4, glucose 5.6 and HEPES 20 at pH 7.4. Dynorphin A-(1–17), APV and nor-binaltorphimine were freshly prepared with HBSS. Fura-2/AM was prepared as a 1 mM stock solution in dimethylsulfoxide (DMSO), Cremophor EL as 10% and Pluronic F-127 as 20% stock in DMSO.

2.2. Primary culture of spinal neurons

Primary cultures of spinal neurons were prepared from E14–15 fetal Wistar rats. The dorsal root ganglions were excluded during dissection. The dissected spinal cords were incubated for 30 min at 37°C in 0.05% trypsin in Dulbecco's phosphate buffered saline without Ca^{2+} and Mg^{2+} and then transferred to the plating DMEM containing 10% fetal calf serum, 10% horse serum, 21 mM glucose, 2 mM glutamine and 100 U/ml penicillin for trituration. The dissociated cells were seeded ($2–4 \times 10^5$ cells/ml, 4 ml per well) on to poly-L-lysine-coated glass coverslips (diameter 25 mm) in 35 mm diameter 6-well plates and then incubated at 37°C in a humidified 5% CO_2, 95% air atmosphere. After 1 d in vitro, cells were fed biweekly with the maintaining medium (plating DMEM minus 10% fetal calf serum). At 4–5 d, the cultures were treated with 10 μM cytosine arabinoside for 24 h to inhibit proliferation of non-neuronal cells. The presence of neurons and astrocytes in the culture was confirmed by immunocytochemical staining with monoclonal neurofilament 200 (NF_{200}) and polyclonal glial fibrillar-associated protein (GFAP) antibodies. Experiments were performed using mature neurons cultured for 12–14 d, with a neuronal proportion of more than 95%.

2.3. Determination of the cytoplasmic concentration of free intracellular Ca^{2+} ($[Ca^{2+}]_i$) by single-cell microspectrofluorimetry

The glass coverslips with attached cells were taken out from the 6-well plates and placed in a self-made bath trough. The cells were first washed twice with 1 ml of O_2-saturated HBSS and then loaded with the fluorescent Ca^{2+} indicator, Fura-2/AM, by incubating with 5 μM Fura-2/AM, 1 mg/ml Cremophor EL, 0.2 mg/ml Pluronic F-127 and 1 mg/ml bovine serum albumin in HBSS for 1 h at room temperature (20°C). After loading, the cells were rinsed twice and incubated with HBSS for 15 min at 37°C and washed again twice with HBSS. The cells were placed on the stage of an inverted fluorescence microscope (Nikon Diaphot-TMD) and visualized with a 40× objective. Changes in the fluorescence intensity (counts per second) at excitation wavelengths of 340 and 380 nm and emission wavelength of 505 nm were recorded in real time by the SPEX CM-X cation measurement system with a PC-compatible 386 Super computer. The ratio of fluorescence intensities at the two wavelengths (F_{340}/F_{380}) was counted by means of the SPEX DM3000 software V-2.5 microprocessing programs. The ratio reflects the dynamic and on-line changes of the intracellular free Ca^{2+} concentration and allows for comparison between treatments. The testing volume was 0.5 ml. Each sample including KCl challenge, drug treatments and KCl challenge was washed thoroughly with 1 ml HBSS about 10 times. Each coverslip was experimented with 3 times.

2.4. Statistical analysis

Changes in the difference of the peak $[Ca^{2+}]_i$ following high-K^+ challenge are expressed as the means ± standard deviation of the absolute ratio values or as percentages of the control for drug-treated samples. The control represents the net increase in $[Ca^{2+}]_i$ in the presence of KCl alone. Differences between treatments were evaluated using Stu-

Table 1
Dualistic modulatory effects of dynorphin A-(1–17) on the high K^+ evoked peak intracellular free Ca^{2+} concentration in cultured single spinal neurons

Dynorphin A-(1–17) (μM)	Basal ratio	Peak deviation		Percentage change
		before treatment	after treatment	
0.1 (6)	1.25 ± 0.07	0.58 ± 0.17	0.02 ± 0.07^{b}	-93.97 ± 9.76
1 (4)	1.10 ± 0.20	0.83 ± 0.41	0.25 ± 0.21^{a}	-83.28 ± 16.8
10 (7)	1.31 ± 0.11	0.43 ± 0.12	0.45 ± 0.12	17.83 ± 22.8
100 (4)	1.34 ± 0.10	0.62 ± 0.29	0.50 ± 0.18	-6.5 ± 9.95

Numbers in parentheses indicate times of determination viewing 2–5 neurons each. The peak responses showed no recovery and persisted throughout the observation time (20–30 min) after treatment with high concentrations of dynorphin A-(1–17).
[a]$P < 0.05$, [b]$P < 0.01$ indicate significant and very significant differences by Student's t-test as compared with the peak $[Ca^{2+}]_i$ before dynorphin A-(1–17) treatment.

dent's t-test (two-tailed). Values of $P < 0.05$ and $P < 0.01$ were accepted as significant and very significant for the number of determinations indicated (n).

3. Results

The spinal neurons cultured for 12–14 d exhibited extensive neurite formation and could easily be distinguished from surrounding cells by the presence of oval, phase-bright somata and by the morphology of their processes. Under inverted fluorescent microscopy, the neurons showed a homogeneous and moderate fluorescence in the cytoplasmic region and usually responded well to high-K^+ challenge. Cells with too intense and/or non-homogeneous fluorescence insensitive to KCl stimulation were excluded from measurements.

The mean ratio for resting $[Ca^{2+}]_i$ in all the neurons

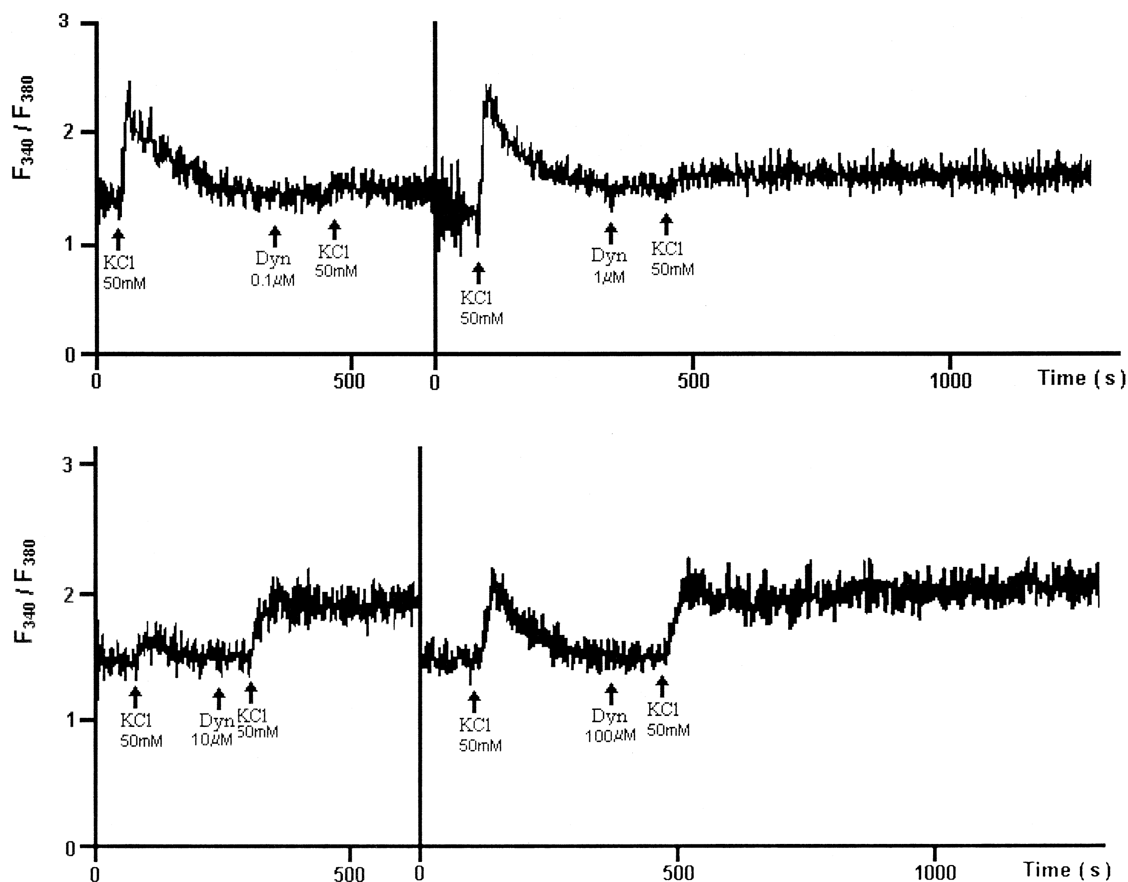

Fig. 1. Concentration–response effect of dynorphin A-(1–17) on depolarization-evoked intracellular free Ca^{2+} concentration in cultured rat single spinal neurons. The peak responses were significantly inhibited by low concentrations of dynorphin (upper) but persisted with no recovery at high concentrations of dynorphin throughout the observation period (lower). The ratio of fluorescence intensity (F_{340}/F_{380}) determined by microspectrofluorimetry reflected semi-quantitatively the dynamic and on-line changes in $[Ca^{2+}]_i$ and allowed for comparison between treatments.

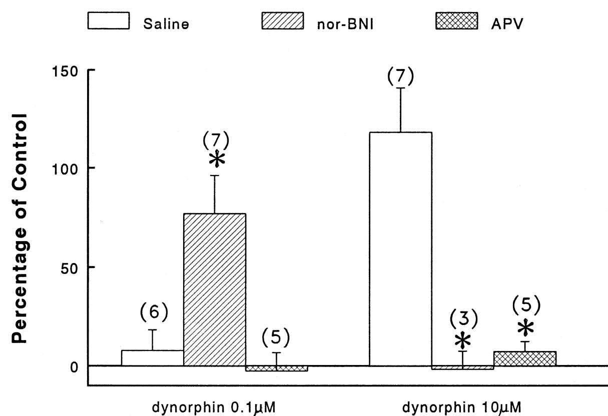

Fig. 2. Bar graphs demonstrating the receptor mechanism for the dualistic effect of dynorphin on the depolarization-evoked responses in free intracellular Ca^{2+} concentration in cultured single spinal neurons. Data are expressed as percentages of control, which represent the net increase in $[Ca^{2+}]_i$ in the presence of KCl 50 mM alone. Numbers in parentheses indicate the times of determination viewing 2–5 neurons each. $^*P<0.05$ indicates a significant difference by Student's *t*-test as compared with saline treatment.

tested was 1.34 ± 0.20 ($n=41$). After high-K^+ challenge, $[Ca^{2+}]_i$ in all the neurons tested rose rapidly to a peak value of 1.99 ± 0.49 ($n=41$) and recovered to its prestimulatory value within a few minutes, with a difference of 0.64 ± 0.48 ($n=41$) as compared to resting $[Ca^{2+}]_i$. No significant changes in either basal $[Ca^{2+}]_i$ or increase in peak $[Ca^{2+}]_i$ were observed before treatments among the neuronal cells studied (Table 1).

After the addition of different concentrations of dynorphin A-(1–17), no significant change in the resting $[Ca^{2+}]_i$ of cells was observed but high-K^+-evoked responses varied with the concentrations of dynorphin added. Low concentrations of dynorphin A-(1–17) 0.1 and 1 μM significantly reduced the KCl-stimulated Ca^{2+} influx, by 94 and 83% respectively (Table 1, Fig. 1). On the contrary, with high concentrations of dynorphin A-(1–17), 10 and 100 μM, the regular response of an increase in $[Ca^{2+}]_i$ following K^+ depolarization was sustained in most cells, and increased up to 1.5-fold in some neurons. Furthermore, the high-K^+-evoked peak response persisted and showed no recovery throughout the observation period (20–30 min), implying a sustained and irreversible rise in $[Ca^{2+}]_i$ following high-K^+ challenge, resembling the glutamate-evoked intracellular Ca^{2+} overload reported in the literature (Tymianski et al., 1993) (Table 1, Fig. 1).

The inhibitory effect of a low concentration of dynorphin A-(1–17) 0.1 μM on the KCl-evoked peak $[Ca^{2+}]_i$

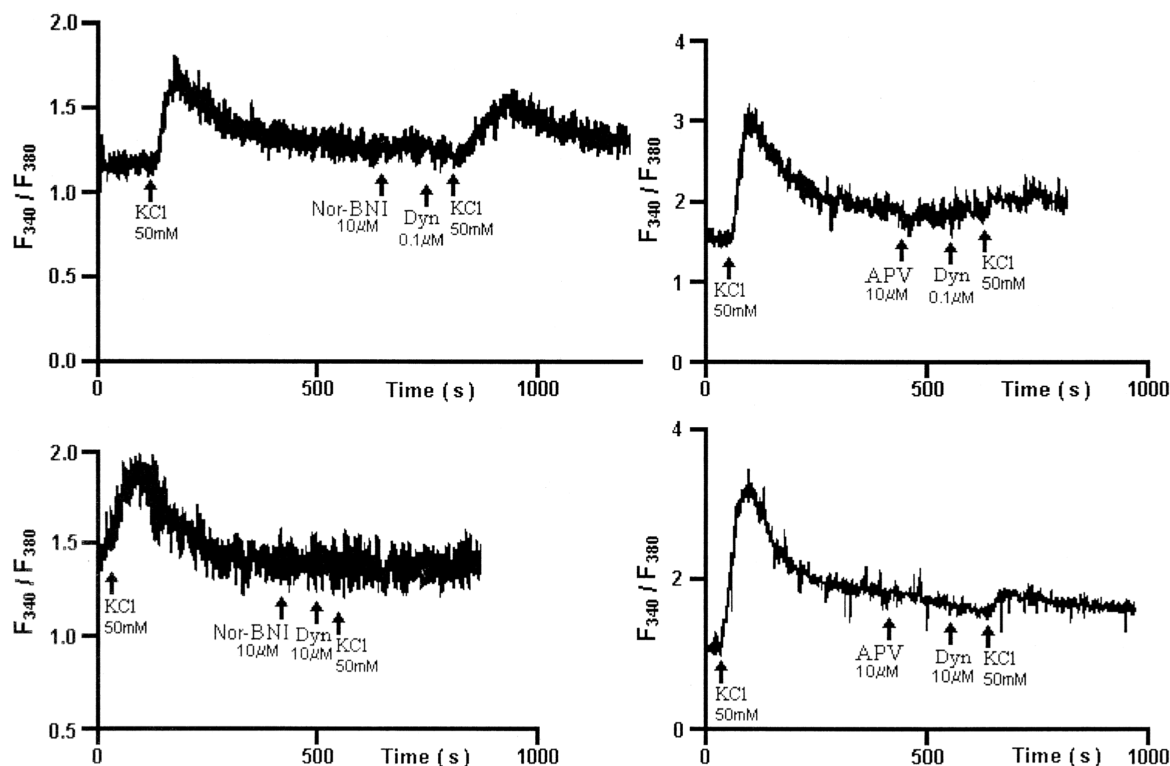

Fig. 3. Representative graphs demonstrating the mechanism for dynorphin-induced dualistic effect on free intracellular Ca^{2+} concentration in cultured single spinal neurons. The inhibitory effect of a low concentration of dynorphin was significantly antagonized by nor-binaltorphimine (nor-BNI) 10 μM, a κ-opioid receptor antagonist, but not DL-2-amino-5-phosphonovalerate (APV) 10 μM, a competitive NMDA receptor antagonist (upper). However, the enhancing effect of a high concentration of dynorphin was significantly blocked by both nor-BNI 10 μM and APV 10 μM (lower). The ratio of fluorescent intensity (F_{340}/F_{380}) determined by microspectrofluorimetry reflected semi-quantitatively the dynamic and on-line changes in $[Ca^{2+}]_i$ and allowed for comparison between treatments.

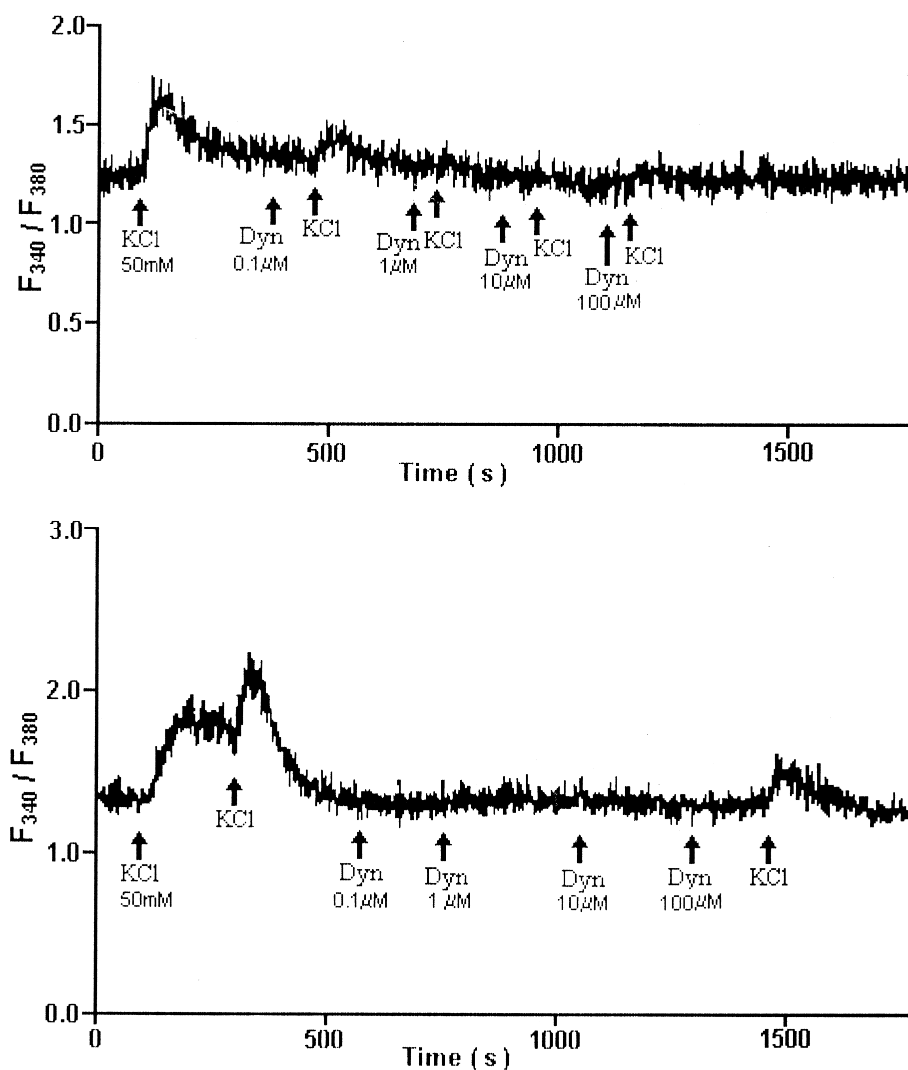

Fig. 4. Pretreatment with low concentration of dynorphin A-(1–17) eliminated the enhancing action on free intracellular Ca^{2+} concentration induced by a high concentration of dynorphin A-(1–17). Top panel: High-K^+ challenge at each concentration of dynorphin A-(1–17). Bottom panel: High-K^+ challenge only at the last and highest concentration of dynorphin A-(1–17). The ratio of fluorescence intensity (F_{340}/F_{380}) determined by microspectrofluorimetry reflected semi-quantitatively the dynamic and on-line changes in $[Ca^{2+}]_i$.

was blocked by the pretreatment with a specific κ-opioid receptor antagonist, nor-binaltorphimine 10 μM, but not by a competitive NMDA receptor antagonist, APV 10 μM (Figs. 2 and 3). Both APV 10 μM and nor-binaltorphimine 10 μM significantly antagonized the sustained and irreversible depolarization-evoked increase in $[Ca^{2+}]_i$ induced by a high concentration of dynorphin A-(1–17) (10 μM, Figs. 2 and 3). Alone, APV and nor-binaltorphimine had no effect on resting $[Ca^{2+}]_i$ (Fig. 3).

After the serial addition of increasing concentrations of dynorphin A-(1–17) 0.1, 1, 10 and 100 μM, high-K^+ challenge did not provoke any rise in $[Ca^{2+}]_i$ or in sustained Ca^{2+} overload (Fig. 4). When each of the stepwise additions of graded concentrations of dynorphin A-(1–17) was followed by high-K^+ challenge, the enhancing effect of a high concentration of dynorphin A-(1–17) was also suppressed or lost (Fig. 4). In both cases, low concentrations of dynorphin A-(1–17) apparently attenuated the increase in $[Ca^{2+}]_i$ induced by a high concentration of dynorphin A-(1–17).

4. Discussion

In present study, different concentrations of dynorphin A-(1–17) did not affect the basal $[Ca^{2+}]_i$ but produced significant inhibition of the high-K^+-evoked increase in $[Ca^{2+}]_i$ at low concentrations and an enhancing effect at high concentrations. The dualistic modulatory effect of dynorphin A-(1–17) is consistent with its analgesic effect on intrathecal injection at a low dose, in contrast to its neurotoxic effect at a high dose.

Early experimental studies had demonstrated that κ-opioid ligands have a direct effect on voltage-dependent

Ca^{2+} channels to inhibit Ca^{2+} influx (McFadzean, 1988; Attali et al., 1989). A patch clamp study indicated that dynorphin A-(1–17), an endogenous agonist for the κ-opioid receptor, significantly inhibited the Ca^{2+} current in cultured rat spinal cord neurons (Sah, 1990) and selectively reduced a large transient Ca^{2+} current of cultured mouse dorsal root ganglion neurons (Gross and Macdonald, 1987) at the low concentration of 1 μM. The present study demonstrated for the first time that dynorphin A-(1–17), at a low concentration, inhibited high-K^+-evoked Ca^{2+} influx in cultured single spinal neurons. Blocking of the depressive effect by the selective κ-opioid receptor antagonist, nor-binaltorphimine, but not by the NMDA receptor antagonist, APV, reflected the inhibitory effect of dynorphin A-(1–17) on spinal nociceptive inputs (Nahin et al., 1992; Chen et al., 1995b,c).

Another important finding of the present study was that a high dose of dynorphin facilitated Ca^{2+} influx via activation of both κ-opioid and NMDA receptors. It has been well documented that dynorphin at a high dose produced dose-dependent spinal cord injury in rats (Faden, 1990; Long et al., 1994; Tian et al., 1994), but underlying mechanisms are open to speculation. There are a number of reports concerning the κ-opioid mechanism in dynorphin-induced motor dysfunction, although there are also contradictory and controversial reports. The κ-opioid receptor is up-regulated after traumatic spinal cord injury (Faden et al., 1985; Krumins and Faden, 1986; Faden, 1990), and the neurotoxic effect of either dynorphin A-(1–17) or dynorphin A-(1–13) is always stronger than that of their non-opioid des-tyrosine analogues (Faden, 1990; Shukla and Lemaire, 1994). The relatively non-selective opiate antagonist naloxone (Spampinato and Candeletti, 1985), the selective κ-opioid receptor antagonist, (−)-*N*-(3-furylmethyl)α-noretazocine (MR2266), and the specific κ-opioid receptor antagonist, nor-binaltorphimine (Faden, 1990; Tian et al., 1994), have been reported to attenuate or block the paralytic effect of dynorphin A-(1–17) or dynorphin A-(1–13) but not of dynorphin A-(2–17). The present results indicated that nor-binaltorphimine antagonized the augmentation of depolarization-evoked intracellular Ca^{2+} influx induced by a high concentration of dynorphin A-(1–17), implying that the κ-opioid receptor is implicated in dynorphin neurotoxicity.

Recently, the non-opioid mechanisms of dynorphin action have attracted universal attention. The competitive NMDA receptor antagonists at the glutamate binding site, including APV (Chen et al., 1995a) and 4-[3-phosphonopropyl]-2-piperazine-carboxylic acid (CPP) (Bakshi and Faden, 1990), or the non-competitive antagonist, kynurenic acid (Chen et al., 1995a), and the partial agonist, 1-aminocyclopropanecarboxylic acid (ACPC) (Long and Skolnick, 1994), at the glycine modulatory site were shown to prevent dynorphin-induced motor dysfunction. A variety of non-competitive NMDA receptor antagonists such as (±)-5-methyl-10,11-dihydro-5H-dibeno[*a*,*d*] cyclohepten-5,10-imine maleate (MK801), ketamine, dextrorphin and dextrormethorphan were also demonstrated to attenuate dynorphin spinal neurotoxicity (Bakshi and Faden, 1990; Long et al., 1994; Chen et al., 1995a). Our previous studies indicated that the NMDA receptor antagonists, APV, kynurenic acid and MK801, significantly blocked the paralytic effect of dynorphin A-(1–17) and dynorphin A-(2–17) but the non-NMDA receptor antagonist, α-D-glutamylamino-methylsulphonate (GAMS), was not so effective (Tian et al., 1994; Chen et al., 1995a).

The mechanisms for NMDA receptor mediation in dynorphin neurotoxicity remain to be determined. It is possible that the local reduction in spinal cord blood flow subsequent to intrathecal injection of dynorphin induces spinal cord ischemia (Long et al., 1994) leading to release of excitatory amino acids (Faden, 1992; Skilling et al., 1992) and overstimulation of NMDA receptors. Dynorphin has been reported to reduce the regional cerebral and spinal cord blood flow and elevate the lactate content in cerebrospinal fluid (Long et al., 1994). Intrathecal co-administration of hydralazine, a potent vasodilator, completely antagonizes dynorphin-induced paralysis (Long et al., 1994).

It is also possible that dynorphin enhanced excitatory amino acids release through a presynaptic mechanism independent of ischemia (Faden, 1992). However, it has recently been indicated that dynorphin-induced motor dysfunction appears before any change in cerebrospinal fluid level of excitatory amino acids, suggesting a possible, direct, interaction between dynorphin and the NMDA receptor complex (Skilling et al., 1992). Furthermore, dynorphin A-(1–13) had been shown to first produce direct potentiation of NMDA-mediated C-fiber reflex followed by complete loss of C-fibre reflex owing to NMDA receptor-mediated neurotoxicity (Caudle and Isaac, 1988).

Results of receptor binding studies using rat brain membranes suggested that dynorphin could interact directly with the NMDA receptor at the glutamate (Massardier and Hunt, 1989), glycine (Dumont and Lemaire, 1994) and phencyclidine (Shukla et al., 1992) sites. The NMDA receptor is an ionotrophic Ca^{2+} channel that allows the entry of Ca^{2+} and Na^+ into cells and is subject to voltage-dependent Mg^{2+} blockade. The present study provided the first direct evidence that high concentration of dynorphin A-(1–17) modulates Ca^{2+} influx and leads to a sustained intracellular Ca^{2+} overload via both κ-opioid and NMDA receptor mechanisms.

The present study also demonstrated that a high concentration of dynorphin failed to induce a sustained and irreversible rise in $[Ca^{2+}]_i$ following the serial addition of graded concentrations of dynorphin A-(1–17). Attenuation of the neurotoxic effect upon serial administration of graded increasing doses of dynorphin has also been demonstrated in vivo (Tian et al., 1994). The underlying mechanism and its practical implications deserve further study. Dynorphin might produce its opioid effect predominantly at low con-

centrations but also non-opioid effects preferentially at high concentrations. While acute tolerance to dynorphin cannot be excluded, it is possible that the functional activities of the non-opioid sites might be reduced or abolished after the opioid site had been challenged in advance by a low concentration of dynorphin.

The important issue we addressed here is that of how dynorphin as a neuroregulator could have a dualistic neuromodulatory effect as illustrated in the present study at the cellular level in cultured single spinal neurons. The inhibitory effect of dynorphin at a low concentration of $[Ca^{2+}]_i$ was obviously in direct opposition to its enhancing effect at high concentrations. While it is difficult to elucidate its basic mechanism at this moment, it is clearly against the general rule of most drug dose–response relationships in classical pharmacology. There are several lines of electrophysiological evidence that dynorphin exerts both inhibitory and excitatory actions on nociceptive neurons (Dubner and Ruda, 1992; Nahin et al., 1992; Chen et al., 1995b,c). Randic et al. (1995) also reported that dynorphin A-(1–17) produces dual modulation of the peak amplitude of presumed monosynaptic non-NMDA receptor-mediated excitatory postsynaptic potentials, decreasing at nanomolar concentration and increasing at micromolar concentrations. Our recent study indicated that dynorphin enhances the release of ^{3}H-glutamate from spinal cord slices at high concentrations, while it inhibits its release at low concentrations (Li et al., 1997). However, dynorphin might not be the only neuroregulator that has a normal ‘physiological’ effect at low concentrations and an abnormal ‘pathophysiological’ effect at high dosages (e.g., somatostatin shares the effect of dynorphin at low and high dosages intrathecally) (Mollenholt et al., 1988).

Our finding that dynorphin as an analgesic or pain modulatory (protective) agent might be transformed into a neurotoxic (destructive) agent under stressful conditions has important clinical implications. It suggests the possibility that when the body protective mechanism is overstressed, the balance tilts with detrimental consequences. Our study on the interaction of dynorphin and excitatory amino acids receptor antagonists indicated that APV, 10 nmol, blocks dynorphin spinal neurotoxicity at 20 nmol, but produces dose-dependent synergetic spinal analgesia with subanalgesic doses of dynorphin (Li et al., 1994). Thus, the protective mechanism of dynorphin might be strengthened by another agent with therapeutic potential, which antagonizes its neurodestructive effect. Furthermore, it is interesting to note that low doses of dynorphin might also have a protective effect against the neurotoxic effect of a high dose of dynorphin given later, as illustrated by our cross-reactivity experiments.

In conclusion, dynorphin exerts a dualistic modulatory effect on high-K^+-evoked Ca^{2+} influx in cultured rat single spinal neurons, leading to a sustained increase in intracellular free Ca^{2+} via both NMDA and κ-opioid receptor activation at high concentrations, and producing significant inhibition of the high-K^+-evoked Ca^{2+} influx via κ-opioid receptor activation at low concentrations. These effects are in line with the analgesic effect at low doses and a neurotoxic effect at high doses upon intrathecal injection in intact animals.

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