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Involvement of calcium in pain and antinociception

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Abstract

Calcium ions are widely recognized to play a fundamental role in the regulation of several biological processes. Transient changes in cytoplasmic calcium ion concentration represent a key step for neurotransmitter release and the modulation of cell membrane excitability. Evidence has accumulated for the involvement of calcium ions also in nociception and antinociception, including the analgesic effects produced by opioids. The combination of opioids with drugs able to interfere with calcium ion functions in neurons has been pointed out as a useful alternative for safer clinical pain management. Alternatively, drugs that reduce the flux of calcium ions into neurons have been indicated as analgesic alternatives to opioids. This article reviews the manners by which calcium ions penetrate cell membranes and the changes in these mechanisms caused by opioids and calcium antagonists regarding nociceptive and antinociceptive events.

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Introduction

During the past few years evidence has accumulated about the property of opioid agonists to modify membrane excitability and intracellular signaling by direct or indirect modification of the transmembrane flux of calcium ions (Ca^{2+}). Among other alternatives presently under investigation, the combination of opioids with drugs able to interfere with Ca^{2+} function in neurons has been pointed out as a useful procedure to obtain safer clinical pain management. The author reviews here how Ca^{2+} enters cells and the changes in this process caused by opioids and Ca^{2+} antagonists regarding nociceptive and antinociceptive events.

Ca^{2+} and Ca^{2+}-channels

Calcium is widely recognized to play a fundamental role in the regulation of several biological processes. A transient increase in cytoplasmic Ca^{2+} concentration represents a key step for neurotransmitter release and the modulation of cell membrane excitability, and depends on the passage of Ca^{2+} through membrane channels, transport by ion pumps, or release of Ca^{2+} from internal stores (for a review, see Ref. 1).

Ca^{2+} influx occurs via three main pathways (for a review, see Ref. 2): the voltage-operated calcium channels (VOCC), which are opened by membrane depolarization, the ligand-gated nonspecific calcium channels, and the receptor-activated calcium channels (RACC). Two main types of RACC have been described: the store-operated, or capacitative, calcium channels and the intracellular messenger-activated nonselective channels. The VOCC give rapid but brief Ca^{2+} pulses, whereas RACC produce rapid

but sustained elevation in intracellular Ca^{2+}. Also, the mobilization of Ca^{2+} from internal stores, a mechanism known as Ca^{2+}-induced Ca^{2+} release, may amplify the Ca^{2+} signal initiated by the opening of VOCC.

The calcium channels consist of hetero-oligomeric complexes containing at least ß and $\alpha_2\delta$ subunits functionally stabilized by a central α_1 subunit, which forms the ion pore. Molecular cloning has identified nine calcium channel α_1 subunit genes (α_{1A} to α_{1E}, α_{1S}), four ß subunit genes ($ß_1$-$ß_4$), and a single gene encoding an $\alpha_2\delta$ subunit. The central α_1 subunit carries the channel's Ca^{2+} selectivity filter, controls its voltage-dependent opening and closing via its voltage sensors, and also determines its distinct pharmacological properties. The VOCC were classified into low-threshold (or T-type) and high-threshold activated channels. At least four types of high-threshold activated channels have been defined: the L-, N-, P/Q-, and R-types. The localization, possible functions, and sensitivity of VOCC to drugs are summarized in Table 1. More recent studies have provided evidence for the involvement of a membrane-delimited G protein ($G_{ß\gamma}$ subunit)-dependent pathway in the modulation of N-type and P/Q-type channels.

The cations Ce^{3+}, La^{3+}, Nd^{3+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+} and Mn^{2+} block the Ca^{2+}-channel pore in a nonselective manner and prevent Ca^{2+} from entering the cells (see Ref. 2). More selective agents include antagonists of L-type VOCC, which are classified as dihydropyridines, benzothiazepines, phenylalkylamines and diphenylalkylamines; antagonists of N-type VOCC, such as ω-conotoxin GVIA (ω-CgTX), obtained from the marine snail *Conus geographus* and its synthetic equivalents, and antagonists of P-type VOCC, represented by the funnel web spider toxin, ω-agatoxin GIVA (ω-AgaTX). Aminoglycoside antibiotics (such as streptomycin, kanamycin, neomycin, gentamicin, and amikacin) have been described as N-type (for a review, see Ref. 3) and P/Q-type (4) antagonists.

Ca^{2+}-channels and nociception

The L-, N- and P/Q-type Ca^{2+}-channels were demonstrated in the dorsal horn of the spinal cord (for a review, see Ref. 5). The L-type Ca^{2+}-channels were found in proximal

Table 1 - Classification of voltage-operated Ca^{2+}-channels.

Type	α_1 Subunit	Location	Activation threshold	Antagonist
L	C	Cardiac/smooth muscles	> -30 mV	Dihydropyridines: nifedipine, nimodipine, felodipine, nitrendipine, nisoldipine, nicardipine
	D	Neuroendocrine		Benzothiazepines: diltiazem
	S	Skeletal muscle		Phenylalkylamine: verapamil Diphenylalkylamines: flunarizine, cinnarizine
P/Q	A	Neuron	> -30 mV	ω-agatoxin GIVA
N	B	Neuron	> -30 mV	ω-conotoxin GVIA, SNX-111, SNX-159, SNX-239
R	E	Neuron	> -30 mV	Not available
T	G H	Widespread	> -70 mV	Amiloride, octanol, nonanol, decanol

dendrites and cellular bodies of neurons in the CNS, and in the subsynaptic membrane of some glutamatergic synapses. The N-type Ca^{2+}-channels are concentrated in presynaptic nerve terminals at the level of the more superficial laminae I and II of the dorsal horn of the spinal cord, a strategic location for a key role of these channels in neurotransmitter release from primary afferents. The L-type Ca^{2+}-channels seem to be more important for the regulation of cellular calcium-dependent events than for the neurotransmitter itself. They participate in the excitation-transcription coupling but are not necessary for fast synaptic transmission. Antagonists of the N-type Ca^{2+}-channels block the release of sensory neuropeptides from primary sensory neurons in culture. Also, N- and P/Q-type channels mediate fast synaptic transmission at virtually all chemical synapses. Thus, N-type channels and probably P-type channels can play a fundamental role in the modulation of nociceptive information, whereas the involvement of L-type channels in the process seems to be very restricted.

Nociception and Ca^{2+} availability

There are several lines of evidence for the involvement of Ca^{2+} in nociception (for references, see Table 2). The intracerebroventricular (*icv*) administration of calcium chloride produces hyperalgesia or has no effect in mouse models of pain. In contrast, intrathecal (*it*) calcium chloride produced

Table 2 - Effects of Ca^{2+}, a Ca^{2+} agonist, Ca^{2+} ionophores or Ca^{2+} chelators on nociception and opioid-induced antinociception.

icv = Intracerebroventricular; it = intrathecal; sc = subcutaneous; vo = oral; ip = intraperitoneal; FT = formalin test; HPT = hot-plate test; TCT = tail-clamp test; TFT = tail-flick test; TIT = tail-immersion test; VT = vocalization test; WT = writhing test; MPA = morphine-induced analgesia. ↑ = Increase; ↓ = decrease.

Drug	Animal	Route	Dose	Test	Effect	Reference
$CaCl_2$	Mouse	icv	0.1-0.5 µmol	TIT	Hyperalgesia	9
		icv	50-200 nmol	TFT	No effect (alone); ↓ MPA	10
		icv	15 µmol/kg	TFT	No effect (alone); ↓ MPA	11
		it	600 nmol	TFT	Antinociception ↓ by naloxone or naltrindole	6
		it	0.02-1 µmol	WT	Antinociception	12
BAY K 8644	Mouse	sc	0.05-5 mg/kg	WT	Antinociception	13
		sc	0.2 mg/kg	WT/HPT/TCT	↑ MPA	14
		vo	0.5-2.0 mg/kg	HPT	Antinociception	15
	Rat	ip	20-200 µg/kg	TFT	No effect (alone); low dose ↓ and high dose ↑ analgesia by sufentanil	16
		ip	100 µg/kg	VT	No effect (alone)	17
		it	1 µg	FT	↑ 1st and 2nd phases	7
A23187	Rat	it	1 µg	FT	↑ 1st and 2nd phases	7
X537A	Mouse	icv	1 µmol/kg	TFT	No effect alone (↑ Ca^{2+}-induced ↓ MPA)	11
EDTA	Mouse	icv	4 µmol/kg	TFT	No effect on MPA	11
EGTA	Mouse	icv	0.5-1 µmol/kg	TIT	No effect (alone); ↑ analgesia by κ-agonists	9
		icv	0.025-0.1 µmol	TFT	Antinociception	17
		icv	2 µmol/kg	TFT	↑ MPA	11
Quin-2	Rat	it	1 µg	FT	↓ 2nd phase	7

naloxone- or naltrindole-sensitive antinociception in the mouse tail-flick or writhing test, an effect imputed to a Ca^{2+}-induced spinal release of met-enkephalin (for a review, see Ref. 6).

Some experiments were conducted using drugs that increase the level of intracellular Ca^{2+}, such as Ca^{2+} ionophores (X537A and A23187) or Ca^{2+} agonists (BAY K 8644), yielding conflicting results. The *icv* administration of X537A did not change the nociceptive response of mice to thermal noxious stimuli, whereas the *it* administration of A23187 significantly elevated both phases of the response to formalin in rats. BAY K 8644 was ineffective in the rat tail-flick test following intraperitoneal (*ip*) administration, but produced antinociception in the mouse hot-plate or writhing test following subcutaneous (*sc*) or intravenous (*iv*) administration, respectively. In contrast, *it* BAY K 8644 increased the response of rats to formalin, thus indicating a critical role of intracellular Ca^{2+} level for the development of persistent pain in response to formalin (7). The dose of BAY K 8644 used in each case accounts for the differences (see ahead).

Other studies were conducted using Ca^{2+} chelators such as EDTA, EGTA or Quin-2. EGTA alone administered *icv* had no effect or produced dose-dependent antinociception in mice. EDTA alone had a weak or no antinociceptive effect in mice. Quin-2 administered *it* reduced the second, but not the first phase of the formalin test in rodents, thus indicating a critical involvement of Ca^{2+} influx in mediating central sensitization following tissue injury, but not in the transmission of inputs in response to brief noxious stimuli (7).

Evidence also exists for the involvement of Ca^{2+} in peripheral mechanisms mediated at the nociceptor level. The intraplantar administration of A23187 evokes hyperalgesia in rats that is potentiated by methylxanthines and antagonized by verapamil, La^{3+} or morphine, thus indicating that the hyperalgesic effect of the Ca^{2+} ionophore depends on the activity of adenylate cyclase on peripheral nociceptors (8).

Ca^{2+}-channel antagonists and antinociception

Several Ca^{2+}-channel antagonists have been used for the study of the effects of Ca^{2+} on nociception. Trivalent cations such as La^{3+} and Ce^{3+} produce antinociception in both the tail-flick and hot-plate tests following *icv* administration to mice (11,17). Intrathecal La^{3+} or Nd^{3+} also produces antinociception in the rat tail-flick and hot-plate tests and blocks both phases of the response to formalin in rats (18). Since the development of the 2nd phase (persistent pain) of the response to formalin depends on the occurrence of the 1st phase (phasic pain), the effects of the inorganic cations against both phases of the response indicate that VOCC are involved in both the induction and maintenance of the response to formalin (18). Intrathecal Ni^{2+}, which preferentially blocks T-type VOCC, was ineffective in the mouse writhing test, thus indicating that T-type channels are not implicated in the spinal processing of nociceptive information (12).

The effects of L-type Ca^{2+}-channel antagonists on nociception differ depending on the drug, dosage, and route of administration and algesimetric test used (for references, see Table 3). In general, the antinociception induced by the L-type Ca^{2+}-channel antagonists was demonstrated in rodents mainly when models of persistent pain, such as the writhing and formalin tests, were used. However, *it* diltiazem or verapamil has failed to reduce the persistent hyperalgesia induced by chronic sciatic ligature in rats, also a model of persistent pain. The remaining data on the effects of L-type Ca^{2+}-channel antagonists indicate that the drugs, whatever the route of administration, had little or no effect in models of phasic pain. More recently, Weissman and colleagues (19) have

Table 3 - Effects of L-type Ca^{2+}-channel antagonists on nociception and opioid-induced antinociception.

CSL = Chronic sciatic ligature; DHC = dorsal horn cell electrical activity. For other abbreviations see legend to Table 2.

Drug	Animal	Route	Dose	Test	Effect	Reference
Diltiazem	Mouse	icv	0.5-400 µg/kg	WT	Antinociception (alone)	20
		icv	60-120 µg	HPT	Antinociception (alone); ↑ MPA	21
		sc	60-120 µg	HPT	No effect (alone); ↑ MPA	21
		sc	15 mg/kg	HPT	No effect (alone); ↑ MPA	22
		sc	10-40 mg/kg	WT	Antinociception (alone)	13
		sc	10-30 mg/kg	WT	Antinociception (alone)	23
		ip	1-30 mg/kg	WT/HPT	No effect (alone)	24
		it	0.5-80 µg	WT	Antinociception (alone)	12
	Rat	sc	20 mg/kg	HPT	No effect (alone); ↑ MPA	25
		ip	1-30 mg/kg	FT	Antinociception (alone)	24
		it	3 µg	CSL	No effect (alone)	26
		it	100 µg	TFT	No effect (alone); ↑ MPA	27
		it	100 µg	FT	Minimal antinociception (alone)	18
Verapamil	Mouse	icv	0.5-400 µg/kg	WT	Antinociception (alone)	20
		icv	15-120 µg	HPT	Antinociception (alone); ↑ MPA	21
		icv	25-200 µg	HPT	Antinociception (alone)	19
		sc	20 µg	HPT	No effect (alone); ↑ MPA	12
		sc	10-80 mg/kg	HPT	Weak antinociception	19
		sc	5-20 mg/kg	WT	Antinociception (alone)	22
		sc	2-30 mg/kg	WT	Antinociception (alone)	24
		ip	1-30 mg/kg	WT/HPT	No effect (alone)	13
		it	0.5-80 µg	WT	Antinociception (alone)	23
		it	25-200 µg	HPT	Antinociception (alone)	19
	Rat	icv	20 nmol	TFT/HPT	↑ analgesia of DAMGO; ↓ analgesia of DPDPE	28
		sc	10 mg/kg	HPT	No effect (alone); ↑ MPA	25
		ip	1-30 mg/kg	FT	Antinociception (alone)	24
		it	5 and 50 µg	FT/DHC	No effect (alone)	29
		it	100 µg	FT	Minimal antinociception (alone)	18
		it	10 µg	FT	↓ 2nd phase (alone)	7
		it	250 µg	CSL	No effect (alone)	26
		it	50 µg	TFT	No effect (alone); ↑ MPA	27
Nifedipine	Mouse	icv	0.5-400 µg/kg	WT	Antinociception (alone)	20
		sc	15 mg/kg	HPT	No effect (alone); no change in MPA	22
		sc	5-20 mg/kg	WT	Antinociception (alone)	13
		sc	2-20 mg/kg	WT	Antinociception (alone)	23
		ip	1-30 mg/kg	WT/HPT	No effect (alone)	24
	Rat	ip	1-30 mg/kg	FT	Antinociception (alone)	24
		ip	2 mg/kg	TFT	↑ MPA	30
		it	24 nmol	FT	Minimal antinociception (alone)	18
		it	10 µg	FT	↓ 2nd phase (alone)	7
		it	0.8-7.0 µg	TFT/HPT	Antinociception (alone)	31
		it	50 and 100 µg	TFT	No effect (alone)	32

Continued on the next page.

Table 3 - Continued.

Drug	Animal	Route	Dose	Test	Effect	Reference
Nimodipine	Mouse	icv	0.5-400 µg/kg	WT	Antinociception (alone)	20
		icv	5 µg	TFT	No effect (alone); ↓ MPA	10
		vo	<100 mg/kg	HPT	No effect (alone); ↑ fentanyl-induced analgesia	15
		sc	20 mg/kg	WT/HPT	↑ MPA	14
		ip	1-30 mg/kg	WT/HPT	Antinociception (alone)	24
	Rat	iv	<100 mg/kg	VT	No effect (alone)	15
		ip	200 µg/kg	TFT	No effect (alone); ↑ sufentanil-induced analgesia	16
		ip	200 µg/kg	TFT	No effect (alone); ↓ U69593-induced analgesia	33
		ip	1-30 mg/kg	FT	Antinociception (alone)	24
		it	50-100 µg/kg	TFT	No effect (alone)	32
		it	60-240 pmol	TFT	No effect (alone); ↓ antinociception by DAMGO	34
Nicardipine	Mouse	sc	15-20 mg/kg	HPT	No effect (alone); ↑ MPA	22
		it	0.5-80 µg	WT	Antinociception (alone)	12
	Rat	it	20 µg	TFT	No effect (alone); ↑ MPA	27
Flunarizine	Mouse	icv	0.5-400 µg/kg	WT	Antinociception (alone)	20
		icv	1-200 µg	HPT	Antinociception (alone)	19
		sc	20 mg/kg	HPT	No effect (alone); ↑ MPA	22
		sc	5-80 mg/kg	HPT	Antinociception (alone)	19
		it	1-200 µg	HPT	Antinociception (alone)	19
Cinnarizine	Rat	ip	6-480 µg/kg	TFT	Antinociception (alone)	32
		it	3-9 µg	TFT	Antinociception (alone)	32

demonstrated that verapamil and flunarizine evoke antinociception in the mouse hot-plate test. Using specific opioid antagonists, they showed that these effects might be due to the agonistic activity of verapamil at µ-, δ- and κ_3-receptor subtypes. Flunarizine had a mixed opioid activity, acting as an agonist on µ-receptors and as an antagonist on δ- and κ-receptor subtypes. In a comparative study, *it* verapamil or nifedipine was less effective than *it* Quin-2 in reducing the 2nd phase of the rat response to formalin, results that were interpreted as evidence that Ca^{2+} influx through channels other than phenylalkylamine- and dihydropyridine-sensitive VOCC may be involved in the process (7).

The effects of N-type Ca^{2+}-channel antagonists on nociception may also differ depending on the route of administration and pain model used (for references, see Table 4). In general, the conopeptides ω-CgTX and SNX-111 produce weak antinociception in rodent models of phasic pain, but are usually very effective in models of persistent pain. Other conopeptides, SNX-239 and SNX-159, were very effective following *it* administration in the rat hot-plate or formalin tests and significantly reduced the allodynia evoked by chronic sciatic ligature. Differently from opiates, the continuous infusion of SNX-111 or SNX-239 reduces both phases of the formalin test in rats accompanied by no signs of tolerance (35). The site of the antinociceptive effect of N-type antagonists seems to be within the CNS since they were effective following *it*, but not systemic or topical application (26).

Aminoglycoside antibiotics have been demonstrated to interact competitively with Ca^{2+} in several processes including neurotransmitter release in peripheral synapses (for a review, see Ref. 36). We have exam-

Table 4 - Effects of N- or P-type Ca^{2+}-channel antagonists on nociception and opioid-induced antinociception.

IT = Incapacitation test; PIP = postincisional pain. For other abbreviations see legends to Tables 2 and 3.

Drug	Animal	Route	Dose	Test	Effect	Reference
ω-Conotoxin	Mouse	icv	10-100 ng	TFT	No effect (alone); ↑ MPA	10
	Rat	icv	24 ng	TFT	↑ Analgesia by DAMGO; ↓ analgesia by DPDPE	28
		icv	20 ng	TFT	Weak antinociception; ↑ MPA	38
		ip	5-10 µg/kg	TFT	Weak antinociception; ↑ MPA	38
		it	0.1-0.4 µg	FT/DHC	↓ 1st and 2nd phases	29
		it	16-64 pmol	TFT	Antinociception; ↑ DADLE-induced antinociception	34
SNX-111	Rat	iv	3 mg/kg	PIP	No effect	39
		it	0.82-14 ng/h	FT	Low dose ↓ 2nd phase; high dose ↓ both phases	40
		it	100 ng	FT	↓ 2nd phase	40
		it	30-300 ng	CSL	Antinociception; dose-dependent	40
		it	30-300 ng	PIP	Antinociception	39
		it	3-300 µmol/h	FT/HPT	Antinociception	35
		it	0.1-1.0 µg	FT/HPT	↓ 2nd phase; no effect in the HPT	41
		it	3.0 µg	CSL	↓ Allodynia	26
SNX-239	Rat	it	0.29 nmol/h	FT/HPT	Antinociception	35
		it	3.3 µg	CSL	↓ Allodynia	26
SNX-159	Rat	it	4.0 µg	CSL	↓ Allodynia	26
Gentamicin	Mouse	icv	40-60 µg	HPT/TFT	Antinociception	42
		ip	40-640 µg	HPT	Antinociception (bell-shaped dose-response curve)	37
		ip	0.5-4.0 µg	WT	Antinociception	12
	Rat	icv	20-80 µg	TFT	Antinociception	3
		ip	200-800 µg	IT	Antinociception	37
		ip	10-320 µg	TFT	Antinociception (bell-shaped dose-response curve)	37
		it	1.25-25 µg	TFT	Antinociception; ↑ MPA	3
Neomycin	Mouse	it	0.5-4.0 µg	WT	Antinociception	12
Amikacin	Mouse	sc/ip	30 mg/kg	WT	Antinociception	43
ω-Agatoxin	Rat	iv	30 µg/kg	CSL	No effect	26
		it	0.1 µg	CSL	No effect	26
		it	0.125-0.5 µg	FT/DHC	↓ 2nd phase	29
		it	0.2-6 pmol	FT	↓ 1st phase; block 2nd phase	18
		it	0.2-6 pmol	HPT	No effect	18

ined the effects of gentamicin on the response of rodents to thermal noxious stimuli (tail-flick and hot-plate tests) and to a hyperalgesic stimulus (carrageenan-induced knee incapacitation) (3,37). Gentamicin was antinociceptive in all tests when injected by the *icv*, *ip* or *it* route. Marked and dose-dependent antinociception was obtained after *it* administration, whereas weaker effects and bell-shaped dose-response curves were obtained following *ip* or *icv* administration. The effect of *it* gentamicin was transitory and dose-dependently reversed by *it* calcium chloride. Gentamicin, neomycin and kanamycin were also effective in the mouse writhing, hot-plate and tail-flick tests following *icv* or *it* administration, and *sc* kanamycin and amikacin were effective in the rat hot-plate test.

More recently, *sc* or *ip* amikacin was found to be antinociceptive also in the mouse writhing test. The effects of gentamicin and neomycin were not changed by naloxone, thus indicating that an opioid mechanism is unlikely to be involved in the effects of the antibiotics (12).

The P-type Ca^{2+}-channel antagonist ω-AgaTX was ineffective in the hot-plate test or against the hyperalgesia evoked by chronic sciatic ligature following *iv* or *it* administration to rats. However, the *it* toxin was highly effective in suppressing the 2nd phase of the rat response to formalin, but had no effect or a very modest suppressive effect against the 1st phase of the response to the same test. These results led to the notion that N-type, and possibly P-type, but not L-type VOCC antagonists exert a selective inhibitory effect on nociceptive transmission at the spinal cord level (18). In addition, N-type antagonists, but not ω-AgaTX, were still effective against the 2nd phase of the response to formalin when injected after the 1st phase, thus indicating that N- and P-type VOCC play different roles in nociception (18).

Opioid agonists and Ca^{2+}

Opioid agonists reduce the intracellular Ca^{2+} concentration either indirectly, through a μ- or δ-receptor-mediated increase in Ca^{2+}-dependent K^+ conductance that leads to nerve cell hyperpolarization and shortening of the action potential duration, or directly through a κ-receptor-mediated shortening of Ca^{2+} action potentials without any change in the resting membrane potential (for a review, see Ref. 19). All the opioid receptors seem to share common mechanisms involving activation of G protein ßγ or α subunit-mediated effects (for a review, see Ref. 44). Activation of cloned μ-, δ-, and κ-opioid receptors inhibits adenylyl cyclase activity via activation of inhibitory G proteins, thus reducing the Ca^{2+} influx, inhibits N-type and L-type VOCC via G_o protein, and stimulates phosphatidylinositol turnover, thus causing a transient increase in intracellular Ca^{2+} level. The μ- and δ-receptor inhibition of adenylyl cyclase is mediated by G_o and G_{i2} proteins, respectively, while the same effect via κ-receptor activation occurs without selectivity toward G_i/G_o proteins. The activation of G proteins selectively modulates α_{1A} and α_{1B} subunits in a manner identical to that for native P/Q- and N-type currents, respectively, whereas α_{1C} L-type currents do not exhibit G-protein sensitivity (for a review, see Ref. 1). Thus, reduction of the intracellular free Ca^{2+} concentration is the final result of stimulating different opioid receptors, but the interaction of Ca^{2+} antagonists with μ- or δ-agonists may be expected to differ from the interaction with κ-agonists (27).

The electrical stimulation of certain supraspinal regions produces antinociception in a variety of laboratory animals and reduces chronic pain in human patients (for a review, see Ref. 45). The regions most extensively studied include the mesencephalic periaqueductal gray matter, locus coeruleus, parabrachial area, and the ventromedullar raphe magnus and reticularis gigantocellularis nuclei. The electrical stimulation of, or microinjection of morphine into these regions produces antinociception by activating centrifugal pathways that descend through the dorsolateral funiculus to inhibit the responses of dorsal horn neurons to peripheral noxious stimuli. In order to exert an inhibitory influence on spinal neurons, the descending pathways utilize at least serotonin and noradrenaline as neurotransmitters. The serotonergic mediation seems to depend on a further activation of spinal intrinsic enkephalin- or dynorphin-containing neurons. In contrast, noradrenergic mediation can inhibit spinal neurons either directly or indirectly via cholinergic intrinsic spinal neurons. We may therefore suspect that opioid agonists can interfere with nociceptive processing acting either supraspinally to activate descending pain-inhibitoring pathways, or spi-

nally, to inhibit noxious inputs to dorsal horn neurons. On the other hand, pharmacological manipulations that interfere with Ca^{2+} availability can change nociceptive processing either supraspinally or spinally. Most of the studies reviewed here emphasize the spinal effects of calcium antagonists. To our knowledge, there is only one report showing that administration of Ca^{2+} into the periaqueductal gray produces a nonsignificant hyperalgesia in the rat tail-flick test but antagonizes the antinociceptive effect induced by *iv* morphine. The same study also demonstrates that administration of EGTA into the same region produces a dose-dependent antinociception in the same test (46).

Opioid-induced antinociception and Ca^{2+} availability

Acute administration of opioid agonists to rodents reduces the Ca^{2+} content in synaptic vesicles, synaptosomes and several rat brain areas. In contrast, the vesicular content of Ca^{2+} in synaptosomes, the Ca^{2+} uptake, and mainly the K^+-stimulated Ca^{2+} uptake by synaptosomes are increased during the development of opiate tolerance in rats. Parallel to these findings, opiate tolerance was correlated with an increase in the density of dihydropyridine-binding sites and higher basal free intracellular Ca^{2+} levels in the rat brain (for a review, see Ref. 47). Nimodipine, nifedipine and verapamil prevented the development of naloxone-precipitated withdrawal syndrome in rats (48). Previous, but not concurrent administration of nimodipine, prevented the development of tolerance to sufentanil in rats (16).

The literature has also provided several reports of changes produced by substances that interfere with Ca^{2+} influx or availability in opioid-induced antinociception (for references, see Tables 2 to 4). Intracerebroventricular calcium chloride reduced opioid-induced antinociception in mice. Subcutaneous BAY K 8644 increased morphine-induced antinociception in the mouse writhing, hot-plate and tail-clip tests. Low doses of BAY K 8644 reduced, whereas high doses enhanced, the antinociception induced by *sc* opioids in rodents (14,16). The dualistic effect of BAY K 8644 may derive from its Ca^{2+} agonistic property, which is prominent at low concentrations but declines at higher concentrations (15). Intracerebroventricular EGTA, but not EDTA increases morphine- and κ-agonist-induced antinociception in the mouse tail-flick test.

In general, L-type Ca^{2+}-channel antagonists potentiate opioid-induced antinociception in several rodent pain models (for references, see Table 3). However, there are reports showing antagonistic effects of nimodipine against the antinociception evoked by morphine in mice (10) or by the κ-agonist U69593 in rats (33).

The morphine-induced antinociception was potentiated by ω-CgTX following *icv* administration in mice, and *icv* or *ip* injection in rats (38). The *icv* administration of ω-CgTX to rats potentiates the antinociception produced by the μ-agonist DAMGO, and reduces the antinociception evoked by the δ-agonist DADLE (28). We found that the antinociceptive effect of *it* gentamicin was only additive to morphine antinociception in rats (3). The *icv* Ca^{2+}-induced inhibition of opiate antinociception seems to result from a Ca^{2+} influx through N-type VOCC since its effect was prevented by *icv* ω-CgTX but not by *icv* nimodipine (10).

We have recently shown (34) that *it* ω-CgTX GVIA, but not nimodipine, increased the latency for the rat tail-flick reflex. Nimodipine reduced the antinociception evoked by *it* DAMGO but did not change the effects of DADLE or bremazocine, δ- and κ-opioid agonists, respectively. In contrast, *it* ω-CgTX GVIA potentiated the antinociceptive effects of DADLE but did not change the effects of DAMGO or bremazocine, thus indicating that the combination of an N-type Ca^{2+}-channel blocker with a δ-opioid agonist would be

most effective for the management of pain in that model of phasic pain.

Ca^{2+} influx is considered to be critical in the transmission of persistent but not brief noxious inputs (for a review, see Ref. 7). Taddese and colleagues (49) have shown that the μ-opioid agonist DAMGO inhibits calcium channels in almost all small nociceptors, which mediate persistent pain, but has minimal effects on large nociceptors known to mediate phasic pain. Somatostatin has the opposite specificity, but the effects of both agonists were eliminated by ω-CgTX GVIA, thus indicating that the differences between DAMGO and somatostatin are not due to different Ca^{2+}-channels. However, the antinociceptive effect of N-type Ca^{2+}-channel antagonists has been shown in both phasic and persistent pain models. Therefore, the interactions between opioid agonists and Ca^{2+}-channel antagonists may in fact differ according to the type of noxious stimuli.

Clinical studies

The usefulness of Ca^{2+}-channel antagonists in the management of clinical pain has also been somewhat controversial, especially regarding the effects of L-type antagonists. An earlier study on this subject indicated that fentanyl-induced analgesia in patients undergoing thoracic surgery was potentiated by the intraoperatory infusion of nimodipine (50). However, the on-demand *iv* infusion of fentanyl for the control of pain after elective hysterectomy was not significantly different in groups of patients double-blindly assigned to the administration of an additional infusion of either placebo or nimodipine (51). Oral administration of slow-release tablets of nifedipine given 18, 9 and 1 h before the beginning of surgery significantly potentiated the analgesic effect of morphine slowly infused by the *iv* route in patients submitted to elective hysterectomy or orthopedic surgery (30). However, slow-release nifedipine given orally 12 and 1 h before surgery did not change significantly the postoperative pain relief produced by epidural fentanyl (52). In our experience, the postoperative pain relief provided by epidural morphine in patients undergoing elective gynecological surgery was significantly enhanced by the sublingual administration of nifedipine (53). In none of these studies did the Ca^{2+}-channel antagonists have an analgesic effect by themselves.

Few reports are presently available regarding the usefulness of Ca^{2+}-channel antagonists in the control of chronic pain. Oral nifedipine at doses increased weekly from 10 mg twice a day to 30 mg twice a day produced complete (7 cases), partial (2 cases) or no relief (1 case) of pain symptoms in patients suffering from reflex sympathetic dystrophy (54). Nimodipine given orally at a dose of 30 mg every 8 h for 3 days did not change the analgesia produced by the concomitant use of morphine in the earlier phase of treatment for cancer pain (55). In contrast, oral slow release nimodipine reduced the daily dose of oral morphine required for pain management in cancer patients. In this case, the dose of nimodipine was 60 mg on the first day, and was then increased up to 120 mg/day divided into four doses. In addition, the antagonist was introduced only when the patients met the criteria for tolerance to morphine (56). A similar design used oral slow-release nifedipine (15 mg twice a day) to show that the antagonist significantly reduced the daily intake of oral morphine for the adequate management of cancer pain (57), but nifedipine was effective only 3 to 5 days after the beginning of treatment. Altogether, these data point to the dependency of the improvement of opioid analgesia on an effective plasma concentration of the Ca^{2+}-channel antagonist. Moreover, the efficacy of L-type Ca^{2+}-channel antagonists in the management of chronic pain indicates that pharmacological interference with Ca^{2+}-related events may modify the chronic effects

of opioid analgesics (56).

Based on the effectiveness of N-type Ca^{2+}-channel antagonists in animal models of pain, recent clinical trials have confirmed that these drugs can provide relief of chronic pain also in human subjects. A dose-dependent analgesic effect of continuous *it* infusion of SNX-111 was demonstrated in one case of intractable deafferentation and phantom limb pain secondary to brachial plexus avulsion and subsequent amputation (58). Thirty patients undergoing elective abdominal hysterectomy, radical prostatectomy or total hip replacement were treated with an *it* infusion of SNX-111 (ziconotide, 0.7 to 7.0 µg/h), which was started before the surgical incision and continued for 48 to 72 h postoperatively. It was shown that the daily patient-controlled administration of morphine was significantly lower in ziconotide-treated than in placebo-treated patients (59). However, dose-dependent adverse effects such as dizziness, blurred vision, nystagmus and sedation were noticed in both studies. Continuous *it* infusion of ziconotide (0.4 to 5.3 µg/h) in 3 patients suffering from chronic pain, in addition to the aforementioned side effects, also produced potentially serious outcomes, including bradycardia, orthostatic hypotension, nausea and vomiting, coma, ataxia, dysmetria, agitation, hallucination, rash, hypoglycemia, diarrhea, nasal congestion and urinary retention (60).

Conclusions

The results reviewed here indicate that Ca^{2+} plays an important role in regulating endogenous pain systems. In addition, they indicate a close relationship between the analgesic effects of opioids and Ca^{2+} availability in the CNS. Thus, elevation of extracellular free Ca^{2+} concentration or facilitation of its transmembrane flux reduces the opioid antinociception. On the other hand, reduction of extracellular free Ca^{2+} concentration or of its transmembrane flux increases opioid antinociception or promotes antinociception by itself.

The experiments with VOCC antagonists revealed that L-, N-, and P/Q-, but not T-type channels are involved in nociception. Antinociception is more frequently obtained with N- or P/Q-type antagonists than with L-type antagonists. Potentiation of opioid-induced antinociception was more frequently seen with L-type antagonists, while N-type antagonists were only additive to opioid antinociception. In contrast, L-type antagonists prevented the development of opioid tolerance and were more effective than N-type antagonists in reducing symptoms of opioid withdrawal. The experiments also indicated that the interactions between opioid agonists and Ca^{2+}-channel antagonists differ according to the type of noxious stimuli used in each case.

Few clinical studies on the efficacy of Ca^{2+}-channel antagonists for the management of acute or chronic pain are presently available. However, in agreement with the studies performed on laboratory animal models of pain, they indicate the usefulness of N-type antagonists as analgesics and of L-type antagonists as an alternative to improve opioid analgesia. The analgesic effect of chronic administration of N-type antagonists lacks tolerance, which is an advantage of opioid analgesia. However, the analgesic effect of N-type antagonists was confirmed in patients only when continuous *it* drug infusion was provided, a procedure that somewhat restricts its usefulness in clinical practice. In addition, more recent studies have reported various potentially dangerous side effects, which certainly limit the clinical use of these drugs. The use of L-type antagonists combined with opioids seems to be an alternative to reduce the intake of opioids for acute and mainly chronic pain management. At present there is no explanation for the mechanism of such a combination but the possibility remains that the use of selective L-type antagonists reduces or prevents the

development of tolerance to opioid analgesia. Finally, the observation that N- and P/Q-type channels can participate in different phases of the response to persistent noxious stimulation points to the possible clinical usefulness of aminoglycoside antibiotics for the management of persistent pain since this drug class is the only one presently known to act as an antagonist of these types of channels.

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