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Calcium channel modulation by dihydropyridines modifies sufentanil-induced antinociception in acute and tolerant conditions*

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Summary. The study was aimed at elucidating the possible participation of L-type Ca^{2+} channel in the acute analgesic effect of an opiate and the development of tolerance to this action. Sufentanil, a selective μ agonist, and two dihydropyridines, the Ca^{2+} antagonist nimodipine and the Ca^{2+} agonist Bay K 8644, were selected. The tail-flick test was used to assess the nociceptive threshold. In naive rats, nimodipine (200 μg/kg) potentiated the analgesic effect of sufentanil reducing the ED_{50} from 0.26 to 0.08 μg/kg. Similar results were observed with its (−)-enantiomer Bay N 5248, while the (+) enantiomer Bay N 5247 was ineffective. Tolerance to the opiate was induced by chronic subcutaneous administration of sufentanil with minipumps (2 μg/h, 7 days). In these conditions the dose-response curve to sufentanil was displaced to the right and the ED_{50} was increased to 1.49 μg/kg. In tolerant rats, nimodipine preserved its potentiating ability and prevented the displacement to the right of the sufentanil dose response-curve ($ED_{50} = 0.48$ μg/kg). When nimodipine was pumped (1 μg/h, 7 days) concurrently with sufentanil, the development of tolerance to the opioid was not disturbed. However, the expression of tolerance was abolished and even the effect of acutely administered sufentanil was markedly potentiated ($ED_{50} = 0.03$ μg/kg). Similar experiments were performed with Bay K 8644. In naive rats, Bay K 8644 at a low dose (20 μg/kg) that behaves as a calcium agonist, antagonized the analgesic effect of sufentanil ($ED_{50} = 0.58$ μg/kg), whereas at a high dose (200 μg/kg) it potentiated this action ($ED_{50} = 0.15$ μg/kg). In tolerant rats, Bay K 8644 (20 μg/kg) preserved its antagonizing ability inducing a displacement to the right of the sufentanil dose-response curve ($ED_{50} = 4.2$ μg/kg). When Bay K 8644 was pumped (1 μg/h, 7 days) concurrently with sufentanil, it enhanced the expression of tolerance to the opiate ($ED_{50} = 3.8$ μg/kg). These results suggest that the calcium fluxes through the L-type channel in neurones are functionally linked to the activation of the μ opiate receptor: the blockade of the channel increased the potency of sufentanil, whereas its activation reduced the potency of the opiate. In chronic experiments, DHPs concurrently administered with sufentanil did not affect the development of tolerance to the opiate. However, nimodipine prevented the expression of this phenomenon. Even more, the animals became hypersensitive to the opiate suggesting that the adaptative mechanisms induced by chronic opiate could be affected by chronic nimodipine.

Key words: Voltage sensitive calcium channel – Dihydropyridines – Nimodipine – Bay K 8644 – μ Opiate receptor – Sufentanil – Opiate analgesia – Opiate tolerance

Introduction

Several Ca^{2+}-related events are known to participate in the inhibitory actions of opioids on the neuronal function. The activation of opioid receptors induces membrane hyperpolarization that results from a Ca^{2+}-dependent increase in potassium conductance. In turn, the opening of the potassium channels leads to a shortening of the action potential duration and to a depression of Ca^{2+} spikes (Werz and MacDonald 1983; North 1984; North and Williams 1985). In addition, the activation of opioid receptors may inhibit some voltage-sensitive calcium channels (VSCC), either directly or after activation of a G-protein (Tsunoo et al. 1986; Attali et al. 1989; McFadzeam and Docherty 1989). A direct action of opioids on intraneuronal Ca^{2+} is also known to occur. Acute treatment with opiates inhibits Ca^{2+} accumulation

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in synaptosomal preparations (Cardenas and Ross 1976; Guerrero-Muñoz et al. 1979a, b; Kamikubo et al. 1983; Toru et al. 1989), in parallel with the production of analgesia (Harris et al. 1977). On the contrary, chronic administration of opioids increased Ca^{2+} vesicular content (Harris et al. 1977) and Ca^{2+} uptake by stimulated synaptosomes (Yamamoto et al. 1978; Guerrero-Muñoz et al. 1979a; Chapman and Way 1980).

Parallel to the electrophysiological and biochemical actions, the modulation of Ca^{2+} concentration at the brain level modificated the acute effects of opioids. Thus, the elevation of brain Ca^{2+} levels antagonized the antinociceptive effects of opiate agonists (Kakunaga et al. 1966; Harris et al. 1975; Iwamoto et al. 1978; Chapman and Way 1980; Lux et al. 1988), whereas Ca^{2+} chelators and Ca^{2+} channel blockers (CCB), inorganic and organic, potentiated some acute central effects (Harris et al. 1975; Chapman and Way 1980; Guerrero-Muñoz et al. 1981; Guerrero-Muñoz and Fearon 1982; Ben-Sreti et al. 1983; Benedek and Szikszay 1984; Del Pozo et al. 1987; Hoffmeister and Tettenborn 1986; Lux et al. 1988). This dependence of the opioid activity on the external Ca^{2+} has been confirmed by Dougall and Leff (1987), who showed that the reduction of external Ca^{2+} increased the efficacy of opiates at the myenteric plexus.

Mechanisms related to Ca^{2+} conductance and/or mobilization are also involved in the chronic actions of opioids. Chronic administration of opiates increases the number of brain sites labelled by radioactive dihydropyridines (DHP) in animals rendered tolerant to morphine (Ramkumar and El-Fakahany 1984, 1988; Inoki et al. 1989). Some preliminary results also suggest that CCB may reduce the intensity of opiate tolerance (Contreras et al. 1988) and abstinence (Bongianni et al. 1986; Baeyens et al. 1987).

The present study was aimed at elucidating the involvement of Ca^{2+} movements through the L-type channels in the antinociceptive effect of sufentanil, a highly selective μ-opiate agonist, under naive and tolerant conditions. Two DHPs were selected: the calcium antagonist nimodipine and the calcium agonist Bay K 8644. Because nimodipine is a racemic mixture of the (−)-enantiomer (Bay N 5248) and the (+)-enantiomer (Bay N 5247) that show different effectiveness in binding to DHP receptors, they were also tested. The interaction was quantified by administering the DHPs under the following conditions: (a) acutely, in naive or tolerant rats; and (b) chronically, in association with sufentanil during the development of tolerance.

Methods

Experiments were performed on male albino Wistar rats weighing 250−350 g. Each animal was used in one experiment and received a single dose of the opiate.

Drugs. Sufentanil was gift from Janssen, Beerse, Belgium. Dihydropyridines (DHPs; gifts from Bayer, Wuppertal, FRG): nimodipine (isopropyl-(2-methoxyethyl)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate), Bay N 5248 [(−)-enantiomer of nimodipine], Bay N 5247 [(+)-enantiomer of nimodipine], Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate). All the DHPs were diluted in a solution of 10% ethanol, 20% propylene glycol and 70% water and manipulations were carried out under sodium light. When administered acutely sufentanil was injected subcutaneously and DHPs intraperitoneally. The chronic delivery was carried out with minipumps (Alzet 2001) implanted under light ether anesthesia. These pumps deliver the drug solution at a constant rate of 1 μl/h.

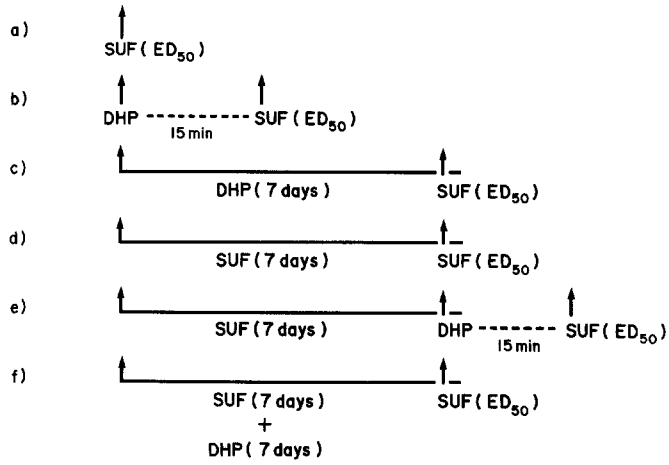

Fig. 1. Experimental protocols used in the study. SUF: sufentanil; DHP: dihydropyridines

Analgesic assay. The tail-flick technique was used to assess nociceptive threshold (D'Amour and Smith 1941). Tail-flick latency was automatically recorded with an analgesiometer (Letica, Barcelona, Spain) and was defined as the elapsed time between onset of a high intensity light beam focused on the tail and the reflex withdrawal response. The intensity of heat was adjusted so that the control latency was 3−5 s; animals with longer control latencies were excluded. A cut-off time of 12 s was used to prevent blistering. The tail-flick latencies were determined before and 1, 5, 15 and 30 min after the drug injection. Analgesic end-point was defined as an increase of 100% in the individual reaction time in relation to the predrug reaction latency. The analgesic effect was expressed as the percentage of animals reaching the analgesic end-point.

Experimental protocols. The different protocols used in the present study are summarized in Fig. 1. To test the analgesic effect of sufentanil the dose-response curve was performed, each animal receiving a single dose of the opiate (protocol a). In the acute interaction studies, DHPs were administered 15 min before each dose of sufentanil (protocol b). To test the influence of chronic administration of DHPs on the acute effects of sufentanil, either nimodipine or Bay K 8644 were pumped for 7 days and the dose-response curves of sufentanil were performed at the 7th day of infusion (protocol c). On the basis of previous studies (Ayesta and Flórez 1989), rats were rendered tolerant by subcutaneous administration of sufentanil for 7 days. Development of tolerance was evidenced by the recovery of the pain threshold to the baseline value. In these conditions, the expression of tolerance was quantified by performing a dose-response curve of sufentanil at the 7th day of infusion, while the minipump remained in place (protocol d). To test the influence of the acute administration of DHPs on the expression of tolerance, DHPs were injected in tolerant animals 15 min prior to the challenging doses of sufentanil (protocol e). In the chronic interaction studies, DHPs were infused for 7 days to rats that were simultaneously treated with sufentanil. The dose-response curves of sufentanil were performed at the 7th day of infusion (protocol f).

Evaluation of results and statistical analysis. The results are expressed as percentage of animals that achieved the analgesic endpoint. The

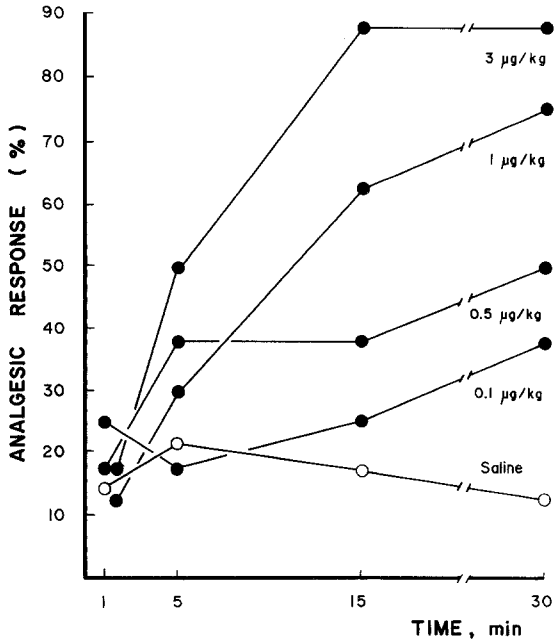

Fig. 2. Time-courses of the analgesic effects induced by saline (○) ($n = 40$) and the different doses of sufentanil (●) ($n = 40$). The results are expressed as percentage of animals showing the analgesic endpoint, defined as an increase of 100% in the individual reaction time in relation to the predrug control value

Table 1. Effect of pretreatment with dihydropyridines on the analgesic activity of sufentanil in naive rats

Pretreatment	n	ED_{50} (µg/kg)	Limits (95%)
Saline 1 ml/kg	40	0.26	(0.09–0.75)
Nimodipine 20 µg/kg	32	0.24	(0.04–0.47)
Nimodipine 200 µg/kg	30	0.08	(0.036–0.17)
Bay N 5247 200 µg/kg	19	0.21	(0.05–0.84)
Bay N 5248 200 µg/kg	19	0.10	(0.033–0.3)
Bay K 8644 200 µg/kg	20	0.15	(0.03–0.8)
Bay K 8644 20 µg/kg	22	0.58	(0.14–2.32)
Saline 1 µl/h/7 days	14	0.22	(0.04–0.84)
Solvent 1 µl/h/7 days	14	0.25	(0.06–0.9)
Nimodipine 1 µg/h/7 days	20	0.23	(0.06–0.64)
Bay K 8644 1 µg/h/7 days	18	0.25	(0.07–0.88)

percent values were transformed in probits before performing statistical assay (Tallarida and Murray 1987). The quantal dose-response curves were performed from the maximal effect obtained at any time after injection of the drug. The ED_{50} and confidence limits determinations were done on the basis of the method of Litchfield and Wilcoxon (1949). The dose-response regression, the comparison between curves and the parallelism assays were performed following the procedures of Litchfield and Wilcoxon (1949) and Finney (1964). The significance level for hypothesis was set at 0.05. Tolerance to sufentanil was evaluated by the tolerance index, defined as the ratio between the ED_{50} of sufentanil in tolerant and naive rats. Similarly, potentiation and antagonism induced by DHPs were assessed by the potentiation and antagonism indexes, defined as the ratio between the ED_{50} of sufentanil in animals pretreated and non-pretreated with DHPs.

Plasma levels of drugs. Blood samples were collected from four animals of each experimental group. Plasma concentrations of sufentanil were determined by radioimmunoassay (Michiels et al. 1983), the lowest detection limit being 0.02 ng/ml. Plasma concentrations of nimodipine were determined in a Hewlett Packard HP 5840 A gas chromatograph (Rämsch et al. 1986).

Results

Analgesic effects of acute sufentanil and DHPs. Saline solution ($n = 40$) (Fig. 2) and DHP solvent ($n = 32$) (1 ml/kg, s.c.) did not modify the nociceptive threshold. Subcutaneous sufentanil (0.1 to 3 µg/kg) induced a dose-dependent analgesia which reached the peak at 15–30 min (Fig. 2). The threshold dose was 0.1 µg/kg and the ED_{50} 0.26 µg/kg (Table 1). Neither intraperitoneal nimodipine at the doses of 20 and 200 µg/kg ($n = 17$) nor its enantiomers (−) Bay N 5248 ($n = 8$) and (+) Bay N 5247 ($n = 8$) at the dose of 200 µg/kg, altered significantly the tail-flick reaction times compared to the saline or DHP solvent control groups during the 30 min of the experiment. Similarly, Bay K 8644 at the doses of 20 and 200 µg/kg intraperitoneally ($n = 16$) did not show any antinociceptive effect. Plasma levels of nimodipine 15 min after intraperitoneal injection of 20 and 200 µg/kg were 2.9 ± 0.7 and 4.5 ± 0.8 ng/ml, respectively. The plasma level of sufentanil 15 min after subcutaneous injection of 0.5 µg/kg was 0.31 ± 0.08 ng/ml.

Influence of acute DHPs on the analgesic effect of acute sufentanil. All DHPs were injected intraperitoneally 15 min prior to subcutaneous sufentanil. Nimodipine at the dose of 20 µg/kg failed to modify significantly the analgesic response to sufentanil (Table 1). However, pretreatment with 200 µg/kg enhanced the analgesic response of sufentanil from the first minute after the injection of the opiate and shifted the dose-response curve significantly to the left (Fig. 3 A). The ED_{50} of sufentanil was reduced to 0.08 µg/kg and the potentiation index was 3.25 (Table 1). In these conditions the plasma level of sufentanil attained at the dose of 0.5 µg/kg was 0.24 ± 0.12 ng/ml.

The (−)-enantiomer Bay N 5248 (200 µg/kg) also potentiated the analgesic effect of sufentanil: the dose-response curve was shifted to the left ($P < 0.05$), the ED_{50} was reduced to 0.10 µg/kg and the potentiation index was 2.6 (Table 1). On the other hand, the same dose of the (+)-enantiomer Bay N 5247 did not modify the effect of sufentanil.

The influence of Bay K 8644 was dualistic and dose dependent. At 20 µg/kg, it partially antagonized the analgesic effect of sufentanil, as shown by the significant displacement to the right of the dose-response curve

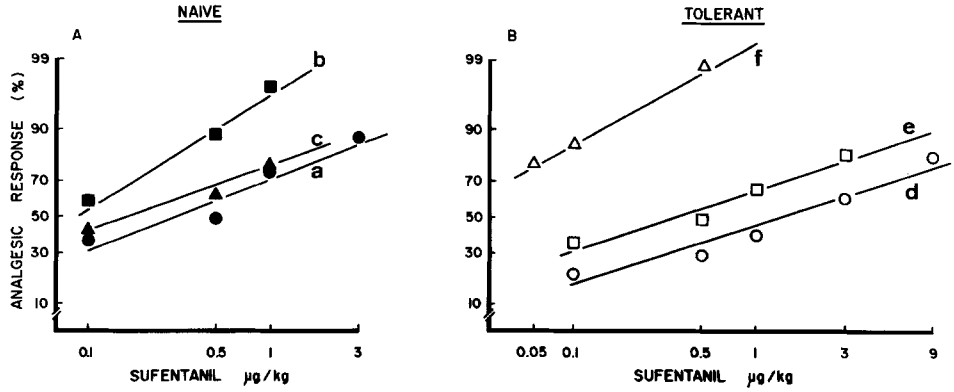

Fig. 3. Influence of pretreatment with acute and chronic nimodipine on the analgesic response to sufentanil in naive (**A**) and tolerant (**B**) conditions. **A**: Dose-response curves of sufentanil in naive animals pretreated with saline (protocol a), acute nimodipine 200 µg/kg (protocol b) and chronic nimodipine 1 µg/h, 7 days (protocol c). **B** Dose-response curves of sufentanil in tolerant animals pretreated with saline (protocol d), acute nimodipine (protocol e) and chronic nimodipine (protocol f). The results are expressed as percentage of animals showing analgesic response. When comparison between regression lines were performed (Finney 1964), significant differences were observed between: b vs a ($P < 0.01$); a vs d ($P < 0.01$); e vs d ($P < 0.001$); and f vs d ($P < 0.001$). No significant difference between c and a was found

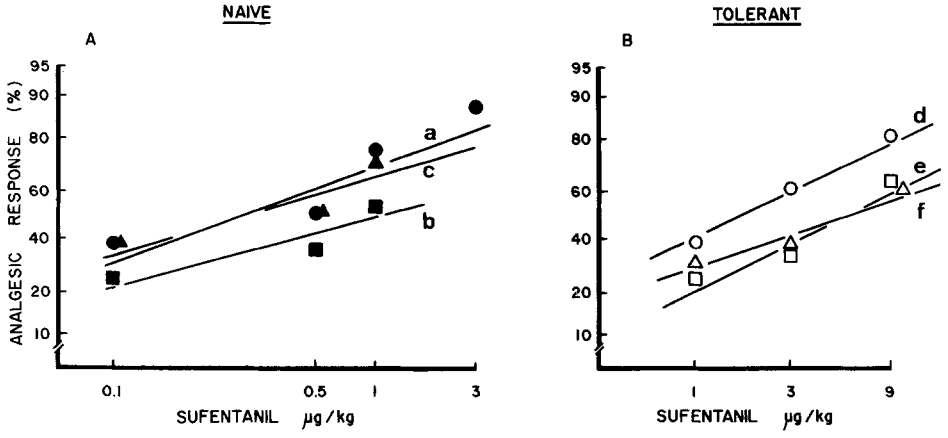

Fig. 4. Influence of pretreatment with acute and chronic Bay K 8644 on the analgesic response to sufentanil in naive (**A**) and tolerant (**B**) conditions. **A** Dose-response curves of sufentanil in naive animals pretreated with saline (protocol a), acute Bay K 8644 20 µg/kg (protocol b) and chronic Bay K 8644 1 µg/h, 7 days (protocol c). **B**: Dose-response curves of sufentanil in tolerant animals pretreated with saline (protocol d), acute Bay K 8644 (protocol e) and chronic Bay K 8644 (protocol f). The results are expressed as percentage of animals showing analgesic response. When comparison between regression lines were performed (Finney 1964), significant differences were observed between: b vs a ($P < 0.05$); e vs d ($P < 0.001$); and f vs d ($P < 0.01$). No significant difference between c and a was found

(Fig. 4A). The ED_{50} was increased to 0.58 µg/kg and the antagonism index was 2.2 (Table 1). On the other hand, at the dose of 200 µg/kg, it potentiated the sufentanil analgesia slightly: the dose response curve of sufentanil was displaced to the left, the ED_{50} was reduced to 0.15 µg/kg and the potentiation index was 1.7 (Table 1). The plasma levels of sufentanil attained after administration of 0.5 µg/kg were 0.27 ± 0.04 and 0.22 ± 0.02 ng/ml after pretreatment with 20 and 200 µg/kg of Bay K 8644, respectively.

Influence of chronic DHPs on the analgesic effect of acute sufentanil. Chronic administration for 7 days of either solvent (1 µl/h; $n = 4$), nimodipine (1 µg/h; $n = 10$) or Bay K 8644 (1 µg/h; $n = 10$) did not modify the nociceptive threshold. In addition, none of these pretreatments modified the dose-response curves of sufentanil performed at the 7th day (Figs. 3A and 4A). The ED_{50} were 0.23 and 0.25 µg/kg in nimodipine and Bay K 8644 pretreated rats, respectively (Table 1). The plasma level of sufentanil obtained with the dose of 0.5 µg/kg in the nimodipine infused rats was 0.14 ± 0.03 ng/ml, significantly lower than that of the control group ($P < 0.02$). The plasma level of sufentanil (0.5 µg/kg) attained after infusion of Bay K 8644 was 0.36 ± 0.14 ng/ml.

Development of tolerance to sufentanil. When sufentanil was chronically administered at the dose of 2 µg/h for 7 days, 100% of animals showed analgesic response on the first day. However, on the 6th day of the infusion the basal nociceptive threshold was again similar to the predrug control value, indicating tolerance (Fig. 5). This phenomenon was quantified by performing the dose-response curve of sufentanil (0.1 to 9 µg/kg) on the 7th

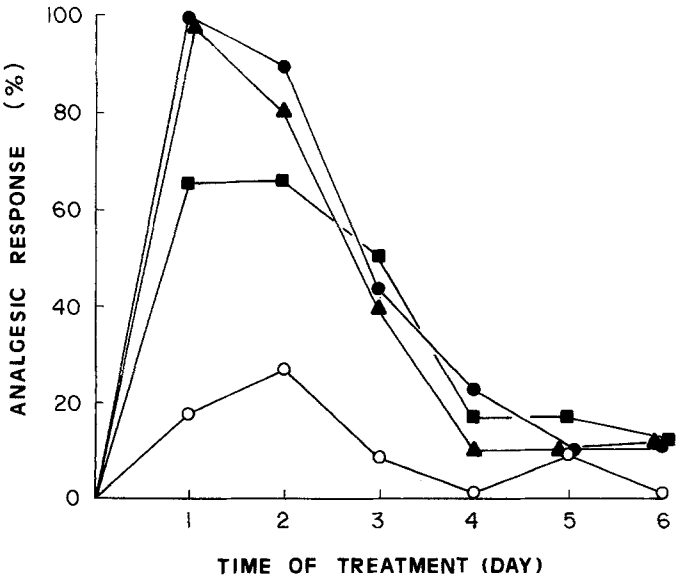

Fig. 5. Time course for the modifications induced by the chronic treatment with nimodipine (1 μg/h) and Bay K 8644 (1 μg/h) on the development of tolerance to sufentanil (2 μg/h). *Ordinate* indicates the percent of animals reaching the analgesic endpoint. ○, saline ($n = 15$); ●, sufentanil ($n = 16$); ▲, sufentanil plus nimodipine ($n = 29$); ■, sufentanil plus Bay K 8644 ($n = 18$)

Table 2. Effect of pretreatment with dihydropyridines on the analgesic activity of sufentanil in tolerant rats

Pretreatment	*n*	ED_{50} (μg/kg)	Limits (95%)
None	49	1.49	(0.68–3.2)
Nimodipine 200 μg/kg	25	0.48	(0.16–1.3)
Bay K 8644 20 μg/kg	18	4.2	(3.0–5.8)
Nimodipine 1 μg/h/7 days	29	0.03	(0.01–0.07)
Bay K 8644 1 μg/h/7 days	18	3.8	(0.76–9)

day, while the minipumps remained implanted to avoid withdrawal. The dose-response curve was displaced to the right, the ED_{50} of sufentanil was raised to 1.49 μg/kg, and the tolerance index was 5.7 (Table 2). The baseline plasma concentration on the 7th day of pumping was 0.42 ± 0.12 ng/ml.

Influence of acute DHPs on the analgesic effect of sufentanil in tolerant rats. In the rats made tolerant to sufentanil, acute nimodipine (200 μg/kg) injected 15 min prior to each challenging dose of sufentanil potentiated the analgesic effect in a way similar to that observed in naive rats: the dose-response curve was shifted to the left (Fig. 3B) and the ED_{50} was reduced to 0.48 μg/kg with a potentiation index of 3.1 (Table 2). This value was similar to that observed in naive rats. On the other hand, the acute administration of the low dose of Bay K 8644 (20 μg/kg) in tolerant rats antagonized the analgesic effect of sufentanil: the dose-response curve was displaced to the right (Fig. 4B), the ED_{50} was increased to 4.2 μg/kg and the antagonism index was 2.8 (Table 2).

Influence of chronic DHPs on the analgesic effect of sufentanil in tolerant rats. When nimodipine or Bay K 8644 were pumped concurrently with sufentanil for 7 days, 100% and 65% of animals showed analgesic response on the first day, respectively, while on the 6th day of infusion, the basal nociceptive threshold was again similar to the predrug control value, indicating tolerance (Fig. 5). In the nimodipine group, the dose response curve to sufentanil (0.05 to 0.5 μg/kg) performed on the 7th day, showed a strong potentiating effect (Fig. 3B): the dose-response curve was displaced to the left and the ED_{50} was reduced to 0.03 μg/kg. The potentiation index was 8.7 in relation to the naive ED_{50} value and 49.7 when compared to the ED_{50} of the tolerant control group (Table 2).

The plasma concentration of nimodipine was 3.0 ± 0.2 ng/ml, similar to the value obtained after acute administration of 20 μg/kg, a dose that had not induced potentiation in acute studies. The baseline plasma level of sufentanil at the 7th day of infusion was 0.48 ± 0.12 ng/ml, a concentration not significantly different from that observed in the group that received sufentanil alone.

In contrast, in the group that received chronic Bay K 8644 the antinociceptive effect induced by the challenging doses of sufentanil (1 to 9 μg/kg) was lower than that observed in the control tolerant rats (Fig. 4B). The dose-response curve was displaced to the right, the ED_{50} was increased to 3.8 μg/kg and the antagonism index was 2.6 (Table 2). The baseline plasma level of sufentanil obtained in the 7th day of infusion was 0.53 ± 0.26 ng/ml.

Discussion

The present results indicate that DHPs, at doses that did not modify by themselves the nociceptive threshold in rats, were able to modulate the antinociceptive activity of the opiate sufentanil. The blockade of the Ca^{2+} channel by nimodipine, its (−)-enantiomer Bay N 5248 and high doses of Bay K 8644 increased the potency of sufentanil. On the contrary, a low dose of Bay K 8644 that behaves as a calcium agonist (Bechem et al. 1988), reduced the potency of the opiate. These results confirm and extend those of Hoffmeister and Tettenborn (1986), including the biphasic activity of Bay K 8644, and support those reported by other investigators who used different VSCC blockers in acute studies, such as verapamil, diltiazem and cinnarizine (Benedek and Szikszay 1984; del Pozo et al. 1987; Lux et al. 1988). At the doses used in the present study, neither the blockade of the Ca^{2+} channel by nimodipine, nor the opening by Bay K 8644 were able, by themselves, to modify the basal nociceptive threshold. Although an increase in tail-flick latency by Bay K 8644 has been reported (Bourson et al. 1989), the doses were 5–20-fold higher than those used in the present study.

Since most of the pharmacological activity of DHPs is linked to their action on the L-type VSCC (Miller 1987),

it is tempting to correlate their modulatory activity on the opiate analgesia with their influence on the Ca^{2+} movement. This hypothesis is supported by several facts: (1) the activity of nimodipine was stereospecific, the (+) enantiomer Bay N 5247 being ineffective; (2) the direction of the modulation was consistent with the way in which the Ca^{2+} channel was affected; and (3) Bay K 8644 showed its characteristic dual activity depending on the dose tested (Bechem et al. 1988).

As indicated in the Introduction, the acute activation of μ-receptors induces complex ionic responses at the membrane and intraneuronal level, including Ca^{2+} mobilization from intracellular compartments. A functional coupling of opioid receptors to VSCC in some neuronal systems has also been suggested on the basis of electrophysiological (Tsunoo et al. 1986; Attali et al. 1989; McFadzeam and Docherty 1989) and binding studies (Gandhi and Ross 1988), although the nature of the opioid receptor subtype remains to be determined. In any case, the μ-opioid receptor-mediated agonism is known to be highly dependent on extracellular Ca^{2+} concentration, so that the opiate ligands exhibit a higher agonism when extracellular Ca^{2+} is reduced, implying a change in efficacy (Dougall and Leff 1987). The interaction between the DHPs and sufentanil persisted in the tolerant state because the potentiation and antagonism indexes after acute pretreatment with nimodipine and Bay K 8644, respectively, were altered in the same direction in naive and tolerant conditions. The results imply that the mechanisms related to Ca^{2+} influx through VSCC involved in the opiate action were not abolished in the tolerant state.

An important finding in our study was the ability of nimodipine, when administered concurrently with sufentanil for 7 days, to prevent the expression of tolerance to sufentanil without affecting the development of this phenomenon. Indeed, at the first day of infusion 100% of animals attained the analgesic endpoint, but the nociceptive threshold progressively returned to the predrug control value throughout the 7 days of infusion, indicating that nimodipine did not modify the development of tolerance. However, under these conditions the antinociceptive activity of the challenging doses of sufentanil was strongly potentiated as evidenced by the shift of the dose-response curve performed at the 7th day of infusion (PI = 49.7), indicating that the expression of tolerance was abolished. These results imply that while tolerance had been developed by the chronic opiate administration, the neural system involved in the acute reaction to the challenging dose and responsible for the expression of tolerance became hypersensitive to the opiate. Again, the low dose of Bay K 8644 displayed an opposite action. Preliminary results of Contreras et al. (1988) also suggest that calcium blockers may reduce the intensity of the expression of opiate tolerance. Important alterations in calcium disposition are known to occur during the chronic administration of opiates (see Introduction), including the up-regulation of DHP binding sites (Ramkumar and El-Fakahany 1984, 1988; Inoki et al. 1989). However, the simultaneous infusion of nimodipine with sufentanil for 7 days may offset this up-regulatory mechanism, because Gengo et al. (1988) demonstrated that chronic administration of nifedipine produced down-regulation of the neuronal Ca^{2+} channels. It is, therefore, possible that the chronic administration of nimodipine may impair the adaptative mechanisms induced by the chronic opiate and, like in acute studies, increase the sensitivity to the opiate. This would lead to a marked reduction in Ca^{2+} entrance and, consequently, to the potentiation of the opiate effect as well as to a reduction in tolerance expression. On the other hand, the chronic administration of Bay K 8644 would facilitate both Ca^{2+} entrance and the expression of tolerance.

In acute interaction studies, no significant differences between the plasma concentration attained by the same dose of sufentanil were observed in any experimental group. The possibility of an interaction based on pharmacokinetic mechanisms is therefore excluded. In chronic studies, the baseline plasma levels of sufentanil at the 7th day of infusion were similar in all groups, so that the dose-response curves of the opiate were performed under the same conditions. The plasma level of sufentanil attained in chronically pretreated animals with nimodipine was lower than that of the control group. This might explain the lack of potentiation observed in the chronic nimodipine group.

Finally, it must be pointed out that the challenging doses of sufentanil in the tolerant rats were administered while the infusion minipump remained implanted. This means that tolerance was analyzed in the absence of abstinence. Therefore, the potentiation induced by chronic administration of nimodipine should be considered as a direct consequence of the influence of the drug, and not as an expression of the reported capacity to inhibit some of the withdrawal manifestations (Bongianni et al. 1986; Baeyens et al. 1987).

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