Enhanced analgesic effect of morphine-nimodipine combination after intraspinal administration as compared to systemic administration in mice[§]

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Calcium plays an important role in the pathophysiology of pain. A number of studies have investigated the effect of L-type calcium channel blockers on the analgesic response of morphine. However, the results are conflicting. In the present study, the antinociceptive effect of morphine (2·5 μg) and nimodipine (1 μg) co-administered intraspinally in mice was observed using the tail flick test. It was compared to the analgesic effect of these drugs (morphine – 250 μg subcutaneously; nimodipine – 100 μg intraperitoneally) after systemic administration. Nimodipine is highly lipophilic and readily crosses the blood brain barrier. Addition of nimodipine to morphine potentiated the analgesic response of the latter when administered through the intraspinal route but not when administered through systemic route. It may be due to direct inhibitory effect of morphine and nimodipine on neurons of superficial laminae of the spinal cord after binding to μ-opioid receptors and L-type calcium channels respectively.

[Verma D, Ray S B, Patro I and Wadhwa S 2005 Enhanced analgesic effect of morphine-nimodipine combination after intraspinal administration as compared to systemic administration in mice; *J. Biosci*. **30** 491–497]

1. Introduction

Calcium plays an important role in the transmission of pain signals in the central nervous system. At the presynaptic nerve terminal, voltage-gated calcium channels (VGCCs) open in response to action potentials to allow an influx of calcium ions. The influx is a graded process varying in a linear manner with the frequency of action potentials. The influx, in turn, leads to release of various neurotransmitters that diffuse across the synaptic cleft to the postsynaptic membrane and binds to their specific receptors. Morphine is the drug of choice for treatment of chronic pain (McCarberg and Barkin 2001). It binds to μ-opioid receptor (MOR) on the pre- and postsynaptic membranes. However, administration of morphine also produces serious side effects like tolerance and dependence, which limits its long-term use. The exact underlying reasons for tolerance and dependence are not definitively known (Ray and Wadhwa 2001).

Binding of morphine to MOR leads to inhibition of neurons concerned with transmission of pain. MOR does so by blocking VGCCs, opening inwardly rectifying potassium channels and inhibiting activity of adenylyl cyclase (North 1993). The release of pain producing neurotransmitters like substance P from the presynaptic terminals in the spinal cord is thereby decreased leading to relief from pain (Smith *et al* 2002).

Since their discovery, VGCCs have been the subject of intense investigation (Fatt and Katz 1953). Six varieties of calcium channels (L-, N-, P-, Q-, R- and T-types) have

Keywords. Intraspinal; morphine; nimodipine; tail flick latency

[§]Patent applied for.

Abbreviations used: CCBs, Calcium channel blockers; MOR, μ-opioid receptor; MPE, maximum permissible effect; VGCCs, voltage-gated calcium channels.

been demonstarted in neurons (Catterall 2000). Among these, the L- and N-types are responsible for neurotransmitter release from sensory neurons of the dorsal column of spinal cord (Nowycky *et al* 1985). A number of studies have shown an increase in analgesic response of opioids like morphine, when co-administered with L-type calcium channel blockers (CCBs) (Contreras *et al* 1988; Carta *et al* 1990; Dierssen *et al* 1990; Omote *et al* 1993; Neugebauer *et al* 1996; Santillan *et al* 1998). Contrary to this, other reports have found no beneficial effect (Roca *et al* 1996; Hasegawa and Zacny 1997; Diaz and Dickenson 1997; Sluka 1998). Thus, the present study was undertaken to observe the effect of nimodipine, a L-type CCB, on morphine-induced analgesia, both after intraspinal (intrathecal) and systemic administration in mice. The dose of morphine and nimodipine administered intraspinally was 2·5 µg and 1 µg. However, the doses of the same drugs were increased by 100 times for systemic administration to compensate for increased volume of distribution. Nimodipine is highly lipophilic and crosses the blood brain barrier in contrast to other CCBs. A potentiation of analgesic effect of morphine would help in lowering the dose of morphine. This would bring about a corresponding decrease of side effects. This is the first report on the interaction between morphine and nimodipine after intraspinal administration.

2. Methods

Male mice of Swiss strain (n = 46) weighing between 20–25 g were procured from the experimental animal facility of All India Institute of Medical Sciences. Prior approval of the Institutional Animal Ethics Committee of AIIMS was obtained. The animals were housed in well-ventilated cages with food and water given *ad libitum*. Twelve hour light and dark cycles were maintained.

2.1 *Drug administration*

The animals were divided into six groups – I to VI (G I-VI). G I (n = 6) received 2·5 µg of morphine intraspinally, G II (n = 8) received a combination of 2·5 µg of morphine and 1 µg of nimodipine intraspinally, G III (n = 9) received morphine (10 mg/kg) subcutaneously while G IV (n = 7) received morphine (10 mg/kg) subcutaneously and (10 mg/kg) nimodipine intraperitoneally. In G IV, nimodipine was administered 20 min before morphine. Only nimodipine (1 µg) was also injected intraspinally into a group (G V) of mice (n = 5). A separate group (G VI) of mice (n = 6) received nimodipine (10 mg/kg) intraperitoneally. Normal saline was also injected intraspinally (n = 5). The amount of morphine injected intrathecally was about 1 : 100 of that injected subcutaneously. Intrathecal injections were given into the spine in the midline (between L5 and L6 vertebrae) in unanaesthesized mice using previously standardized technique (Hylden and Wilcox 1980). The total volume injected intrathecally was 10 µl. Subcutaneous injections were given in one of the hind limbs.

2.2 *Assessment of sensitivity to noxious thermal stimuli*

The analgesic response was measured by the tail flick apparatus (UGO Basile). The animal was placed in a restrainer with its tail outside. The tail (distal 1/3rd) was exposed to an infrared source of radiation. The animal flicks its tail away from the source of heat on feeling pain. Baseline latency for the tail flick was recorded at the beginning of the experiment and was within 2–4·5 s. Analgesic drugs like morphine delay the response time in a dose-dependent manner. However, if there was no response within 10 s, the animal was removed to prevent damage to the tail (cut off time). The maximum permissible effect (MPE) was calculated from the values of tail flick test using the following formula: % MPE = [(observed latency-baseline latency)/(cut off time – baseline latency)] × 100.

The tail flick latency was measured after 15 and 30 min of morphine and/or nimodipine administration. Subsequently it was measured every 30 min till 5 h in G I and II and 3·5 h in G III and IV.

Statistical evaluation was done using ANOVA with post hoc multiple comparisons between groups I–IV only. $P < 0{\cdot}05$ was considered significant. Results of tail flick response from each group were calculated as mean ± standard error of mean.

2.3 *Drugs*

Morphine sulphate IP ampoules were purchased from Govt. Pharmacy after permission from Narcotic Commissioner. It was diluted in normal saline IP to obtain desired concentration. Nimodipine was obtained from Sigma, USA. It was dissolved in a solution containing polyethylene glycol, physiological saline and absolute alcohol in 2 : 2 : 1 ratio under subdued lighting, as nimodipine is light sensitive.

3. Results

3.1 *General observations*

The analgesic response was evident within 15 min of administration of morphine (figure 1). Addition of nimodipine produced 100% response as compared to morphine alone, after both intraspinal and systemic administration

at 15 min (figure 2). The rate of decrease of analgesia was also less, when nimodipine was co-administered (G II and IV). Even after 5 h of administration, the MPE in G II was 37·5% as compared to G I (12·29%).

3.2 *Between-group comparison*

Addition of nimodipine to morphine increased the analgesic effect of morphine when given intraspinally (G I and II; figure 1, table 1). The tail flick latency in G II showed a significant increase ($P < 0{\cdot}05$ at 2, 2·5 and 3 h). Maximum analgesia was seen as early as 15 min after administration. The analgesic response of subcutaneous morphine along with intraperitoneal nimodipine showed an increase but it was not statistically significant at any time point (G III and IV). Only nimodipine groups (G V and VI) did not show any analgesic response. None of the rats showed any signs of motor paralysis (intact stepping reflex and righting reflex). The saline group showed values close to the baseline.

4. Discussion

Previous reports indicate that intraspinal administration of morphine produces potent analgesia in the postoperative period (Cousins and Mather 1984; Domsky and Kwartowitz 1992). However, distressing side effects like pruritus, urinary incontinence, nausea and delayed respiratory depression may complicate its use, particularly in the elderly (Slappendel *et al* 2000; Goodarzi and Narasimhan 2001). In order to reduce the incidence of side effects, the amount of morphine must be kept to the minimum. One way to do this would be to combine it with other drugs so as to achieve a potentiation of the analgesic effect of morphine. Thus, in the present study, the analgesic effect of morphine-nimodipine combination was investigated.

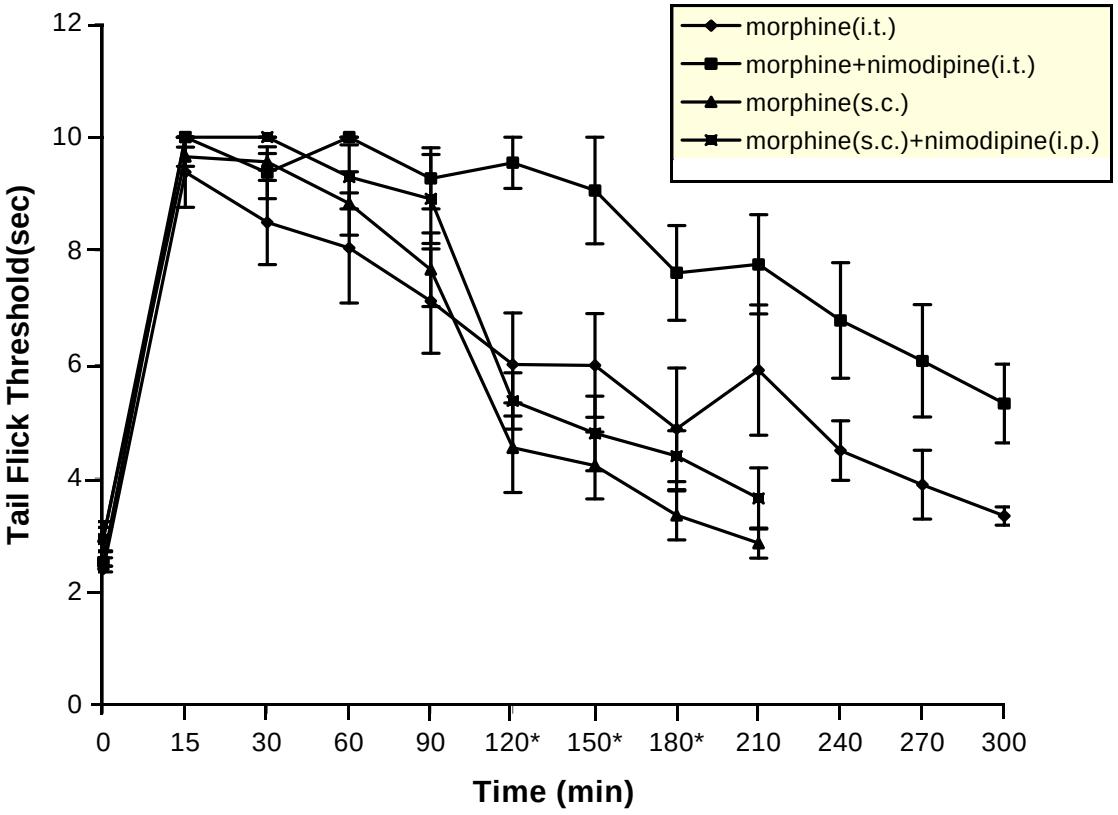

Figure 1. The time course of tail flick latency after intraspinal morphine (G I), intraspinal morphine + nimodipine (G II), subcutaneous morphine (G III) and subcutaneous morphine + intraperitoneal nimodipine (G IV) administration. Significantly higher threshold (marked with*) was noted in G II as compared all other groups at 2, 2·5 and 3 h ($P < 0{\cdot}05$).

Table 1. The mean, standard error, minimum and maximum values of tail flick latency for all the groups (G I–VII) have been shown. The time period for which values of tail flick latency were recorded was one hour for G V–VII.

Time	Groups	Number of animals	Mean	Standard error	Minimum values	Maximum values
0:00	G I	6	2·4	0·05	2·2	2·5
	G II	8	2·5	0·07	2·2	2·9
	G III	9	2·9	0·26	1·8	4·1
	G IV	7	2·9	0·21	2·2	3·8
	G V	5	2·8	0·17	2·3	3·1
	G VI	6	3·4	0·27	2·8	4·4
	G VII	5	3·1	0·14	2·8	3·3
0:15	G I	6	9·3	0·62	6·0	10·0
	G II	8	10·0	0·00	10·0	10·0
	G III	9	9·6	0·17	9·0	10·0
	G IV	7	10·0	0·00	10·0	10·0
	G V	5	2·6	0·19	2·2	3·1
	G VI	6	2·9	0·23	2·0	3·6
	G VII	5	3·3	0·26	2·8	3·7
0:30	G I	6	8·5	0·74	5·9	10·0
	G II	8	9·0	0·45	6·4	10·0
	G III	9	9·5	0·14	9·0	10·0
	G IV	7	10·0	0·00	10·0	10·0
	G V	5	3·3	0·27	2·5	3·8
	G VI	6	2·8	0·17	2·2	3·5
	G VII	5	3·4	0·46	2·6	4·2
1:00	G I	6	8·0	0·97	5·0	10·0
	G II	8	10·0	0·00	10·0	10·0
	G III	9	8·8	0·56	5·0	10·0
	G IV	7	9·3	0·56	6·0	10·0
	G V	5	2·6	0·46	1·6	3·8
	G VI	6	3·3	0·26	2·5	4·0
	G VII	5	3·7	0·24	3·4	4·2
1:30	G I	6	7·1	0·91	5·3	10·0
	G II	8	9·2	0·53	5·6	10·0
	G III	9	7·6	0·64	4·5	10·0
	G IV	7	8·9	0·78	4·6	10·0
2:00	G I	6	6·0	0·90	3·8	10·0
	G II	8	9·0	0·45	6·4	10·0
	G III	9	4·5	0·78	1·8	10·0
	G IV	7	5·3	0·49	3·8	7·4
2:30	G I	6	6·4	0·98	4·1	10·0
	G II	8	8·5	1·0	2·5	10·0
	G III	9	4·2	0·58	2·6	8·4
	G IV	7	4·7	0·66	2·8	7·8
3:00	G I	6	5·0	1·2	3·0	10·0
	G II	8	7·5	1·0	3·0	10·0
	G III	9	3·3	0·43	2·0	6·0
	G IV	7	4·3	0·44	2·0	6·0
3:30	G I	6	6·3	1·3	2·6	10·0
	G II	8	7·8	1·0	2·6	10·0
	G III	9	2·8	0·26	1·3	3·9
	G IV	7	3·6	0·53	2·2	5·7
4:00	G I	6	4·6	0·62	3·1	6·8
	G II	8	6·8	1·0	3·3	10·0
	G III	9	–	–	–	–
	G IV	7	–	–	–	–
4:30	G I	6	3·9	0·74	2·7	6·8
	G II	8	5·5	1·0	2·4	10·0
	G III	9	–	–	–	–
	G IV	7	–	–	–	–
5:00	G I	6	3·3	0·11	3·2	3·7
	G II	8	4·6	0·55	3·0	6·6
	G III	9	–	–	–	–
	G IV	7	–	–	–	–

The results of the present study indicate that nimodipine increases the analgesic effect of morphine. Moreover, the intraspinal route appears to be better than systemic administration presumably due to the absence of dilution within the blood. The dose required is also 1/100th less.

Considering the various types of calcium channels and their blockers, some degree of uncertainty persists regarding the efficacy of various CCBs, when given along with morphine (Venegas and Schaible 2000). The reason may be that presynaptic terminals from even the same axon possess different calcium channels mediating release of neurotransmitters, described as a functional patchwork (Reid *et al* 2003). Earlier studies have shown a superior analgesic effect resulting from combination of morphine with L-type CCBs in experimental animals when given intrathecally (Omote *et al* 1993; Dogrul *et al* 2001). Even analgesic effect has been noted with only CCBs without morphine on intrathecal administration, though it was of short duration (15 min) and sometimes associated with motor paralysis (Hara *et al* 1998). In the present study, no analgesic effect was observed with only nimodipine, probably due to its low dose. Nimodipine has been shown to facilitate pain relief in combination with morphine in patients suffering from cancer, when given orally (Santillan *et al* 1998). Further, nimodipine could prevent the escalation of morphine dosage in a statistically significant manner without any major side effects (dyspepsia being the commonest).

To the best of our knowledge, all of the experimental studies on the analgesic effect of morphine in combination with L-CCBs have recorded pain sensitivity up to 3 h. However, in the present study, a higher analgesic effect of morphine-nimodipine combination was observed even at 5 h after intraspinal administration as compared to morphine alone (figure 2). Remarkably, more than 1/3rd of the peak analgesic effect persisted in G II at 5 h. In a clinical scenario, this can make a difference between tolerable and intolerable pain. It was also persistently higher throughout the period of observation (15 min to 5 h). Thus the combination had an enhanced effect (synergistic response) while nimodipine by itself did not have an analgesic effect. Further, another dihydropyridine, amlodipine had a similar potentiative effect on morphine throughout the period of observation for 1 h (Dogrul *et al* 2001). But the dose of amlodipine injected into individual rats was 10 μg, which was comparatively much higher than in the present study. Nimodipine is used for treatment of hypertension. A previous study did not find any evidence of systemic hypotension after intrathecal administration of 50 μg of nimodipine nor any alteration in the blood flow within the spinal cord (Imamura and Tator 1998). Presumably, these side effects of nimodipine were absent in the present study as the dose of nimodipine injected intraspinally was 1 μg. It is possible that after systemic administration of morphine-nimodipine combination, reduced blood flow to the spinal

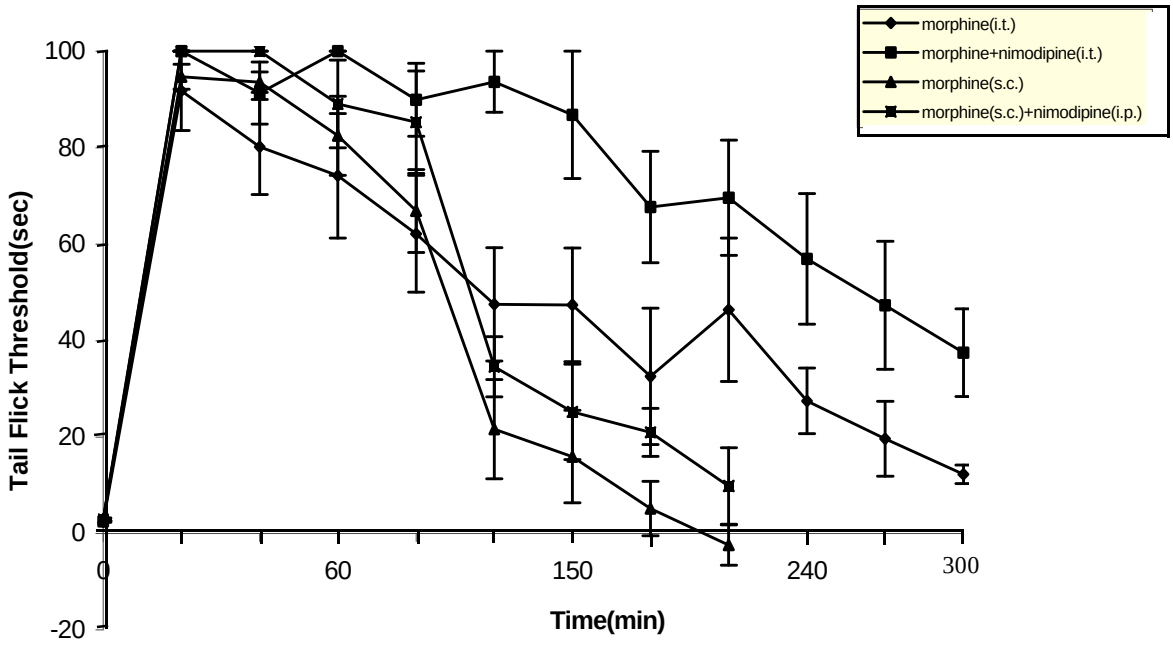

Figure 2. Maximum permissible effect (MPE) observed in G I–IV at different time points as in figure 1.

cord and brain may have decreased the analgesic efficacy of this combination.

Some studies have reported synergistic effect between morphine and N-type CCBs (Omote *et al* 1996; Wang *et al* 2000). Particularly, ziconotide – a synthetic analogue of *w*-conotoxin MVIIA – is being used for treatment of neuropathic pain. A related peptide CVID, also known as AM336, has been reported to have a larger therapeutic window as compared to ziconotide (Smith *et al* 2002). However, neurological side effects are common with N-type CCBs.

A recent report has shown the relative functional importance of different VDCCs in neurons of lamina I of the rat spinal cord (Heinke *et al* 2004). An inhibition of postsynaptic current was observed with L-type CCB (verapamil) after electrical stimulation of dorsal nerve root. However, maximum inhibition was observed with N-type CCB (*w*-conotoxin GVIA). It is possible that intraspinal nimodipine could have potentiated the action of morphine by a similar mechanism in the present study. Neurons of laminae I and II also show higher expression of MOR (Ray *et al* 2005). Thus, morphine could directly bind to MOR after intraspinal administration and decrease neuronal excitability. Apart from pharmacokinetic factors, pharmacodynamic factors also play a role in the synergistic interaction between morphine and nimodipine. A recent study has shown that prior administration of CCBs (diltiazem, nimodipine and verapamil belonging to benzothiazepine, dihydropyridine and phenylalkylamine classes respectively) to morphine significantly increased the concentration of the morphine in the serum after systemic administration (Shimizu *et al* 2004). The increased concentration of morphine in the serum produced a statistically significant increase in its analgesic effect. On systemic administration of morphine and nimodipine, we also observed a higher analgesic effect though it was not statistically significant. These may be explained by the different experimental conditions. Interestingly, some of the CCBs (diltiazem and verapamil) also increase morphine levels in the brain when co-administered together, as compared to morphine alone, after systemic administration (Shimizu *et al* 2004). Thus, combinations of morphine and CCBs represent exciting clinical entities in the near future.

In conclusion, the present study highlights the greater analgesic effect obtained with combination of morphine and nimodipine as compared to morphine alone after intraspinal administration.

Acknowledgement

This study was financially supported by an Intramural research grant from the All India Institute of Medical Sciences, New Delhi.

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MS received 23 November 2004; accepted 13 June 2005

*e*Publication: 28 July 2005

Corresponding editor: INDRANEEL MITTRA