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INTERACTION OF PENTAZOCINE WITH CALCIUM CHANNEL BLOCKING DRUGS DURING CHEMICAL AND THERMAL PAIN IN MICE

VEENA VERMA, PRAMOD KUMARI MEDIRATTA AND K. K. SHARMA*

Department of Pharmacology,
University College of Medical Sciences & GTB Hospital,
Delhi – 110 095

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Abstract : The present study was designed to investigate the antinociceptive interaction of a clinically used opioid, pentazocine which produces its analgesic effect mainly through kappa receptors, with some calcium channel blockers (CCBs, viz. Diltiazem, flunarizine, nimodipine and verapamil-each representing one chemical class) in formalin and tail flick tests in mice. All the CCBs, except verapamil, significantly inhibited the formalin-induced pain response in a dose-dependent manner. However, none of these drugs affected tail flick latency at any of the studied doses. Pentazocine showed a significant antinociceptive response in both pain models, although a high dose was required to increase the tail flick latency. Pretreatment with all CCBs, individually enhanced the analgesic effect of pentazocine in both formalin and tail flick tests. In the latter test of nociception, a per se ineffective dose of pentazocine, showed a significant analgesic response in presence of CCB dose which itself was not effective in the test. Chronic concomitant administration of diltiazem with pentazocine didnot prevent the development of tolerance to the opioid compound. However, diltiazem when given in combination with pentazocine to pentazocine-tolerant animals, it effectively reversed the tolerance. Results of the study thus suggest that concomitant treatment with CCBs, irrespective of their chemical nature, not only potentiate the antinociceptive effect of pentazocine in opioid naive animals in both tonic and acute nociceptive tests but also reverse the pentazocine tolerance.

Key words : pentazocine interaction antinociceptive calcium channel blockers formalin test tail flick test

INTRODUCTION

Clinically used opioid analgesics which relieve pain by mu receptor action possess a number of adverse effects, like respiratory depression, high abuse potential and development of tolerance. Further, in situations of increased intensity of pain and development of opioid tolerance, mu receptor agonist drugs would no longer be able to

*Corresponding Author

provide adequate pain relief without unacceptable side effects. In addition, administration of high doses of opioids can lead to escalation of pain (1). All these problems necessitate the need for a search of novel and safe analgesics which are free of abuse potential and devoid of other side effects. Since, most of these adverse effects are mediated through mu receptors, efforts have been made to characterize analgesia mediated by non-mu sites, such as kappa opioid agonists are clinically weak analgesics and rarely show antinociceptive effect in experimentally induced thermal pain. One way to over come this problem is to coadminister a non-opioid augmenting agent that has minimal side effects by itself but potentiates the analgesic effect of kappa opioid analgesic compound.

Opioids have been suggested to mediate their action by either decrease in Ca^{2+} conductance or increase in specific membrane K^+-conductance causing hyperpolarization of nerve cell and shortening of the Ca^{2+} component of action potential (2, 3). Hence, agents which reduce intracellular calcium, like channel blockers (CCBs) may be expected to influence nonciception by themselves or may modulate opioid-induced analgesia. In fact some studies have demonistrated that mu-receptor agonist, morphine induced analgesia can be potentiated by L-type CCBs (4–7), and some of them may have antinociceptive effect of their own in certain nociceptive tests (5, 7). However, interaction of CCBs with kappa receptor agonist drugs has not been studied. Therefore, the present work was carried out to study the antinoceceptive interaction of CCBs with a clinically used opioid analgesic, pentazocine which is known to produce its antinociceptive effect mainly through kappa receptors (8). Four CCBs, each belonging to the available different chemical class, i.e., diltiazem (benzothiazepine), verapamil (phenylalkylamine), flunarizine (diphenylpiperazine) and nimodipine (dihydropyridine) were selected for studying the drug interaction on two nociceptive paradigms, namely formalin and tail flick tests in mice. These two nociceptive tests were included with a purpose, i.e. formalin test is a model of tonic (continuous) pain (9), whereas tail flick test is considered to be a model of acute pain (10). Since, L-type Ca^{2+} channels open in depolarized state (11), the likelihood of their being open during continuous pain is more, and hence the chance of CCBs showing an antinociceptive effect in formalin test would be greater than in tail flick response. Further, effect of a CCB on the development of tolerance to pentazocine was also investigated.

METHODS

Animals

The study was carried out on Swiss albino mice of either sex, weighing 25–30 g. The animals were procured from the local animal dealer and housed in controlled temperature (22 ± 2°C) and light (12 h light–12 h dark cycle) conditions. Pellet diet (Brooke Bond Lipton India Ltd.) and water were available *ad libitum*, except 1 h before and during the experiments. The animals were maintained as per the "Guidelines for the Care and Use of Laboratory Animals", prepared by the Indian National Science Academy, New Delhi (12).

Drugs and chemicals

Pentazocine lactate (Fortwin, Ranbaxy), Diltiazem (Sun Pharmaceutical Industries Ltd.), Verapamil (Isoptin, German Remedies), Nimodipine (Cipla Ltd.), Flunarizine (FDC) and MR-2266 (Boehringer Ingelheim) were dissolved in 0.9% saline, except the nimodipine which was dissolved in 100% ethanol. Formalin (1%) (v/v) solution was prepared by adding normal saline to the stock solution of 4% formaldehyde in water (10% formalin).

Treatment schedule

Animals were randomly allocated into different groups, each group comprising of 8–10 mice. The drugs/vehicle were injected intraperitoneally (ip) in a volume of 10 ml/kg, 30 min–1 h before the nociceptive assay. The doses of drugs were selected on the basis of the result of pilot experiments. For interaction studies minimum effective doses of CCBs were combined with a subeffective dose of pentazocine.

Formalin test (FT): 0.2 ml formalin (1%) was injected under the plantar surface of right hind paw of mouse (13). Left paw was injected with 0.2 ml of 0.9% saline which acted as control. The mouse was than kept in an open mouse perspex cage and the time spent by the animal licking the injected paw or leg was recorded. Two distinct periods of intensive licking and biting activity, i.e. an early phase (0–5 min) and late phase (25–30 min) were observed (Fig. 1) and scored separately for the animals of different groups. Simultaneously oedema of formalin-injected paw was measured at 1 and 24 h after the injection by plethysmography for studying the inflammatory response.

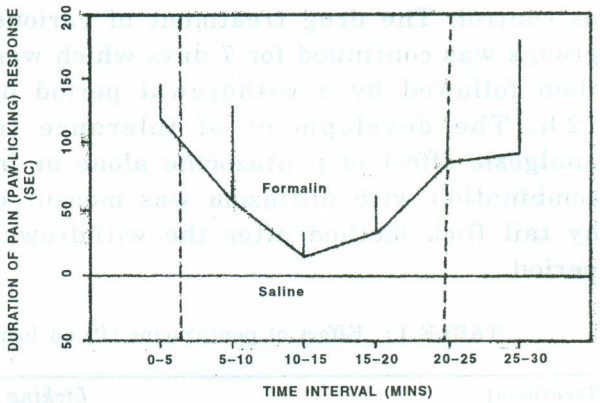

Fig. 1: Time course of paw licking response induced by 1% formalin injection. Each point represent the amount of time (sec) the animal spent in licking the injected paw during a block of 5 min observation period. (Values are Mean ± SEM, n = 8–10 mice/group).

Tail flick test (TFT): The tail of the animal was placed in the slot of water circulated jacket of analgesiometer (TECHNO) which had a tungsten wire 1 mm below the tail surface (14). The heat was adjusted daily to provide normal reaction time of 2–4 sec. A cut-off time of 10 sec was used for drug studies, i.e. if the mouse did not respond in 10 sec, it was removed and given a score of 10 sec.

Tolerance studies

The animals were randomly divided into different groups and were administered ip with appropriate vehicle or the drug. One group was treated with pentazocine 30 mg/kg twice daily at 0900 and 1500 h.

Second group was administered diltiazem in the dose of 40 mg/kg twice daily 10 to 20 min before pentazocine injection. The third group was injected with 0.9% saline in place of diltiazem and acted as control. The drug treatment of various groups was continued for 7 days which was then followed by a withdrawal period of 72 h. The development of tolerance to analgesic effect of pentazocine alone or in combination with diltiazem was measured by tail flick method after the withdrawal period.

TABLE I: Effect of pentazocine (P) on formalin-induced pain response and tail flick latency.

Treatment (*mg/kg*)	*Licking response (sec)*		*Tail flick*
	*Early phase** (*0–5 min*)	*Late phase** (*25–30 min*)	*latency** (*sec*)
Saline	116.45±12.01	90.48±13.75	2.60±0.27
P (15)	96.45±9.08	79.44±10.02	2.81±0.33
P (30)	59.54±9.08[a]	53.44±10.02[a]	3.85±0.50[a]
P (60)	38.72±6.53[a]	41.33±9.22[a]	4.35±0.70[a]

*Values are Mean ± SE (n = 8)
[a]P<0.05 compared to saline-treated (control) group.

Statistical analysis

The data obtained from FT and TFT were analysed by Mann Whitney 'U' test and Unpaired 't' test, respectively.

RESULTS

Formalin test

Effect of pentazocine: Pentazocine in a dose of 15 mg/kg, did not produce significant antinociceptive effect. However, when the dose was increased to 30 or 60 mg/kg, it produced a dose-dependent inhibition (P<0.05) of both phases of licking response (Table I).

Effect of CCBs: All the CCBs, except verapamil, significantly inhibited the formalin-induced pain response in a dose dependent manner, diltiazem being the most effective (Table II). Higher doses of verapamil (≥ 40 mg/kg) could not be tested as these doses produced significant mortality.

Interaction of CCBs with pentazocine: The pentazocine in a subeffective dose (15 mg/kg), when administered 30 min after treating the animals with minimum effective dose of CCBs, produced a significant (P<0.05; P<0.01) inhibition as compared to control animals in formalin-induced biphasic licking response (Table III).

Effect of MR2266 on the analgesic effect of pentazocine or CCBs: Prior administration of MR2266, a kappa receptor antgonist to pentazocine- or diltiazem-treated animals produced marked inhibition of the analgesic effect of pentazocine but not that of diltiazem (Table IV).

TABLE II: Effect of CCBs on formalin-induced pain response and tail flick latency.

Treatment (*mg/kg*)@	*Licking response (sec)*		*Tail flick latency (sec)**
	Early phase (0–5 min)*	*Late phase* (25–30 min)*	
Vehicle	116.45±12.01	90.48±13.75	2.60±0.34
D (20)	113.11±14.36	66.44±14.02	3.48±0.34
D (40)	58.16±21.58[b]	14.11±5.07[b]	2.91±0.22
D (80)	28.23±11.34[b]	1.16±1.16[b]	3.64±0.36
F (20)	94.18±11.07	33.46±10.21[a]	2.23±0.15
F (40)	61.16±9.95[b]	22.66±4.35[b]	2.12±0.19
F (80)	45.81±6.73[b]	13.83±4.14[b]	2.74±0.13
N (20)	72.06±11.06[a]	39.36±13.55[a]	2.93±0.12
N (4)	55.15±5.68[b]	30.33±6.75[b]	2.79±0.35
N (80)	50.16±3.18[b]	14.58±2.53[b]	2.97±0.29
V (10)*	88.61±12.70	50.63±19.62	3.33±0.33
V (20)*	90.16±11.05	48.51±25.47	3.65±0.19

@D = Diltiazem; F = Flunarizine; N = Nimodipine, V = Verapamil
*Values are Mean ± SE (n = 8)
a. P<0.05; b. P<0.01 compared to saline-treated (control) group
*Verapamil could not be tested in a dose of 40 mg/kg and above because of high mortality.

TABLE III: Effect of combination of CCBs with pentazocine (P) on formalin-induced pain response.

Treatment (*mg/kg*)@	*Licking response (sec) Mean ± SE*	
	Early phase (0–5 min)	*Late phase (25–30 min)*
Saline	116.45±12.01	90.48±13.75
P (15)	96.45±9.08	79.44±10.02
P (15) + D (20)	58.90±12.82[a+]	10.75±4.28[b+]
P (15) + F (20)	60.18±10.28[a+]	37.38±13.16[a+]
P (15) + N (20)	44.71±12.53[b+]	24.16±8.52[a+]
P (15) + V (20)	64.00±12.33[a+]	32.65±12.61[a+]

@D = Diltiazem; F = Flunarizine; N = Nimodipine, V = Verapamil
n = 8
[a]P<0.05; b. P<0.01 compared to saline-treated (control) group.
+P<0.05 compared to pentazocine-treated group.

Effect of CCBs on formalin-induced inflammatory response: None of the CCBs studied produced significant change in formalin induced paw oedema (Result not shown).

TABLE IV: Effect of MR 2266 on the analgesic effect of pentazocine (P) on and CCBs.

Treatment (mg/kg)	*Licking response (sec) Mean ± SE*	
	Early phase (0–5 min)	*Late phase (25–30 min)*
Saline	116.45±12.01	90.48±13.75
P (30)	59.54±9.08[a]	53.44±10.02[a]
P (30) + MR 2266 (0.3)	98.53±10.78[+]	89.11±7.42[+]
Diltiazem (80)	28.23±11.34[b]	1.16±1.16[b]
Diltiazem (80) + MR 2266 (0.3)	33.23±7.51[b]	5.10±2.11[b]

n = 8
[a]P<0.05; b. P<0.01 compared to saline-treated (control) group.
[+]P<0.05 compared to pentazocine-treated group.

Tail flick test

Pentazocine in a dose of 30 or 60 mg/kg, ip produced a small but significant increase in the tail flick latency (TFL) (Table I). None of the CCBs per se produced an effect on TFL at any of the doses used (Table II). However, when CCBs were administered 30 min–1 h prior to an ineffective dose of pentazocine (15 mg/kg), the combination produced a significant increase in the TFL (P<0.05; P<0.001) response which was found to be far more than the response observed with the highest dose of pentazocine (60 mg/kg) alone (Table V).

TABLE V: Effect of combination of CCBs with pentazocine (P) on tail flick latency.

Treatment (mg/kg)[@]	*Latency (sec) Mean ± SEM*
Saline	2.60±0.27
P (15)	2.81±0.33
P (30)	3.85±0.50[a]
P (60)	4.35±0.70[a]
P (15) + D (40)	5.31±0.32[b*]
P (15) + F (40)	5.63±0.31[b*]
P (15) + N (40)	9.96±0.33[c**]
P (15) + V (20)	6.66±1.29[b*]

n = 8
[@]D = Diltiazem; F = Flunarizine; N = Nimodipine, V = Verapamil
a. P<0.05; b. P<0.01 compared to saline-treated (control) group.
*P<0.05; **P<0.001 compared to pentazocine-treated group.

Tolerance studies

The chronic treatment with pentazocine produced only a minimal decrease in the TFL, when tested with 30 mg/kg of pentazocine after a withdrawal period of 72 h. The effect was found to be decreased by 14% as compared to nontolerant animals. However, when pentazocine-tolerant animals were tested with a combination of diltiazem and pentazocine, the analgesic response was observed to be increased by 47% (Table VI). In the animals, which were chronically treated with combination of diltiazem and pentazocine daily for 7 days, when tested with 30 mg/kg of pentazocine after 72 h of withdrawal period, the analgesic response was observed not to be statistically different as compared to pentazocine-treated tolerant animals.

TABLE VI: Effect of pentazocine (P) alone and in combination with diltiazem (D) on tail flick latency in pentazocine-tolerant animals.

Treatment (mg/kg)	*Chronic saline*	*Chronic pentazocine (P)*	*Chronic pentazocine (P) and diltiazem (D)*
Saline	2.82±0.14	3.32±1.12	3.18±0.56
P (30)	4.25±0.50[a]	3.31±0.89	3.52±0.73
P (30) + D (40)	6.40±0.98[b,c]	5.68±1.16[a,c]	4.97±0.94

N = 8
[@]D = Diltiazem; F = Flunarizine; N = Nimodipine, V = Verapamil
[a]P<0.05; b. P<0.01 compared to saline-treated animals.
[c]P<0.05 compared to corresponding pentazocine value obtained in chronic pentazocine-treated animals.

DISCUSSION

The administration of 1% formalin in the mouse paw produced a biphasic pain response, an early phase lasting from 0–5 min and a late phase occurring during the period of 25–30 min, after the injection. Similar biphasic response has also ben reported by other workers (9, 13, 15–17). However, the time interval of peak licking activity in the late phase was found to be different in different studies. This variation in time slots for exhibiting the latter phase of intense pain reactivity could be due to the differences in species and strain of animals and the strength of formalin solution used in different studies (17).

In the present study, various CCBs, like diltiazem, flunarizine and nimodipine produced marked inhibition of formalin-induced pain response in both phases; diltiazem being the most effective and consistent in its effects. Verapamil, however did not produce any significant effect on either of the phases of pain sensitivity in low doses and higher doses could not be tested in view of the high mortality produced by these doses.

The observed antinociceptive activity of CCBs could be attributed to their nonspecific effects such as local anesthesia or sedation. However, several observations suggest that these are not the underlying contributing factors. Local anesthesia would not be an acceptable explanation because of the different routes of administration of CCBs (ip) and the nociceptive agent, formalin (sc under plantar surface). Furthermore, in a previous report a CCB, nicardipine which lacks local anesthetic activity has been reported to produce analgesia (18). In addition, the observed analgesic activity of diltiazem has been reported to be stereospecific, since d-cis-diltiazem was found to be more potent than l-cis-diltiazem as analgesic, whereas both possessed equal local anaesthetic activity (5, 19). Sedation does not seem to be a likely explanation, since in the present study none of the CCBs in any of studied doses produced a sedative effect. It could be possible that these drugs produced the analgesic response by causing inhibition of calcium-dependent mechanism responsible for inflammation. Generally anti-inflammatory agents inhibit only late phase of formalin-induced pain response (20),

whereas in the present study the effective CCBs were observed to inhibit both phases of licking response. Besides, none of the CCBs produced a significant effect on formalin-induced paw oedema in the present study. Hence, analgesic effect of CCBs observed in the study does not appear to be related to effect on inflammatory mediators. The pretreatment with MR 2266, a specific kappa receptor antagonist (3) did not alter the analgesic response of CCBs, suggesting thereby that their antinociceptive effect is not mediated through kappa opioid receptors. Thus interference with the calcium influx at a cellular level seems to be a more likely explanation for the analgesic effect of these drugs.

Pentazocine preferentially acts as kappa receptor agonist (8). Although, it also has μ receptor antagonistic activity, this property becomes evident only when it is given in combination with a specific μ receptor agonist drug. Conforming to its kappa agonistic property, pentazocine produced a dose-dependent analgesic response in formalin test but displayed only a weak antinociceptive effect in the tail flick test, that too only in high doses (3).

Although CCBs exerted a significant effect on formalin-induced pain response per se, they were virtually ineffective in test of tail flick latency. However, in the interaction studies, when CCBs were coadministered with a low dose of pentazocine, all the studied compounds were observed to enhance the antinociceptive effect of pentazocine in both formalin-and thermally-induced tail flick tests. Similar observations were also made by other workers who failed to observe an antinociceptive effect of diltiazem (4, 6), verapamil (4, 6, 21) and nimodipine (22) in hot plate test and of nifedipine in tail flick test (23) when administered alone but when coadministered with an opioid, morphine, they enhanced the analgesic activity of the latter (4, 6, 21, 23). In the present study all the CCBs, irrespective of their chemical class not only demonstrated an additive property with pentazocine in formalin test but showed real potentiation of this opioid in tail flick test where a sub-analgesic dose of pentazocine in presence of CCBs displayed a powerful analgesic response not even seen with the highest dose of the opioid compound. As reported earlier that CCBs potentiate mu agonist, morphine analgesia (4–7), this paper reports for the first time that such potentiating interaction of CCBs can also occur with kappa opioid agonist for the analgesic response.

Results of the present study thus indicate that CCBs not only have analgesic activity of their own in formalin test, but they also enhance the analgesic effect of pentazocine in both the formalin and tail flick tests. The activation of kappa receptors causes decreases in calcium influx (3) and CCBs directly prevent the influx of this cation through voltage-dependent calcium channels. The enhancement of analgesic response to pentazocine with prior administration of CCBs could thus be attributed to the additive effect of these agents, acting through different mechanisms of Ca^{2+} influx to achieve the same end result. The sensory neurons are known to possess five distinct types of voltage-dependent calcium channels designated as L, T, N, P and Q (24–27). The P and Q type channels have been identified in dorsal horn neurons

and they are inhibited by activation of the mu, delta or kappa opioid receptors (28–29), whereas L-type Ca^{2+} channels have been shown to be coupled to kappa-opioid receptors in rat brain regions (30). The L-, N- and P/Q-type calcium channels have been implicated to play a pivotal role in mediating calcium influx that triggers depolarization evoked neurotransmitter release in nerve terminals (26, 27). Studies have shown that both mu and kappa receptor activation depresses neuronal calcium conductance and inhibits transmitter release through the opioid receptors located on presynaptic terminals (31). Thus it is possible that both the opioid drugs and CCBs may interact to achieve a common goal, i.e., inhibition of the release of neurotransmitters involved in the regulation of nociception by affecting Ca^{2+} conductance. This explains the mutual facilitation of the analgesic effect of two groups of drugs in test of nociception, where one group per se, i.e. CCBs had no effect of their own, i.e. in tail flick response in the present study.

Although opioids are the drugs of choice for the relief of moderate to severe pain one of the major problems encountered with their use is the development of tolerance on repeated administration. In the present study, therefore the effect of CCBs on pentazocine-induced tolerance was also investigated. It was observed that chronic concomitant administration of diltiazem, which produced most consistent effect in both the studied nociceptive tests, with pentazocine did not prevent the development of tolerance to pentazocine. However, when diltiazem was given in combination with pentazocine to pentazocine-tolerant animals, a marked increase in TFL was observed, indicating thereby that a combination of CCBs with pentazocine will still be effective in the tolerant animals. It appears that an acute administration of CCBs with pentazocine may be effective in reversing the tolerance of k-opioid drugs. In a previous study Contreras et al., also reported an antagonism of morphine tolerance by a number of CCBs, including diltiazem (6). Thus it is possible that like tolerance to mu-agonist morphine, CCBs can also overcome tolerance to kappa-agonists drugs, since pentazocine is known to produce its antinociceptive effect via this opioid receptor (8).

In conclusion, results of the present study suggest that CCBs have a potential to be used as analgesic agents either alone in situation of continuous mild pain or in combination with an opioid drug, where they could be expected not only to reduce the required dose of the opioid but in addition may decrease the frequency of side effects, including the tolerance associated with its use.

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