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EFFECT OF NIMODIPINE ON NOCICEPTIVE PAIN AND ITS INTERACTION WITH OPIOID ANALGESICS IN RATS

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ABSTRACT: Objective: To explore the effect of nimodipine on nociception and on opioid-induced antinociception in rats by using the tail-flick method. **Method:** Sixty albino rats were used for the study. The tail-flick method was used to induce nociception in rats. Analgesic effect of nimodipine was evaluated by using three graded doses (200 mcg/kg, 400 mcg/kg, and 800 mcg/kg). To study the effect of nimodipine on opioid-induced antinociception, nimodipine (400 mcg/kg) was administered in combination with sub-effective doses of fentanyl and pethidine. Drugs were administered by intraperitoneal route. **Results:** Nimodipine at doses 200 mcg/kg, 400 mcg/kg, and 800 mcg/kg alone showed no significant difference in reaction times after 30 and 60 min as compared to baseline and control group. Similarly, effective sub doses fentanyl 15 mcg/kg and pethidine 10 mg/kg produced a non-significant increase in reaction times after 30 and 60 min as compared to baseline and control group. But the combination of nimodipine 400 mcg/kg and fentanyl 15 mcg/kg and the combination of nimodipine 400 mcg/kg and pethidine 10 mg/kg induced highly significant analgesia as compared to individual effect of the drugs. **Conclusion:** In the present study, no analgesic effect was observed with nimodipine alone, but the combination of nimodipine with opioids (fentanyl or pethidine) produced significantly greater analgesia suggesting a synergistic interaction between these drugs.

INTRODUCTION: Opioids are widely used in the management of pain [1]. Opioids exert their analgesic effect by binding to opioid receptors, which lead to inhibition of neurons concerned with the transmission of pain. It does so by blocking voltage-gated calcium channels (VGCCs), opening inwardly rectifying potassium channels and by inhibiting the activity of adenylyl cyclize [2].

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However, administration of opioids also produces adverse effects ranging from nausea, vomiting, dizziness, pruritus, urinary retention to respiratory depression [3]. Particularly on chronic administration, it may lead to the development of tolerance, physical dependence, and addiction [4]. These opioid-related adverse effects pose a great challenge in pain management [5]. Calcium plays an important role in the transmission of pain signals in the central nervous system. At the presynaptic nerve terminal, VGCCs open in response to action potentials to allow an influx of calcium ions. The influx, in turn, leads to the release of various neurotransmitters that diffuse across the synaptic cleft to the postsynaptic membrane and binds to their specific receptors [6].

There are evidence suggesting the involvement of calcium ions in nociception [7, 8, 9] and also involvement VGCCs in pain transmission [10]. Nimodipine is an L-type calcium channel blocker and is more lipophilic than other L-type calcium channel blockers, thus capable of crossing the blood-brain barrier [11].

So presumably, a combination of opioids with drugs able to interfere with calcium ion conductance in neurons could be a useful alternative for safer clinical pain management by limiting the opioid-related dose-dependent adverse effects to some extent.

This current study was designed to study the effect of nimodipine, an L-type calcium channel blocker on nociceptive pain, and its interaction with opioid analgesics in rats.

AIMS AND OBJECTIVE: To study the effect of nimodipine on nociceptive pain and its effect on opioid-induced antinociception in rats by using the tail-flick method.

MATERIAL AND METHODS: After obtaining the permission from Institutional Animal Ethics Committee (IAEC) and Committee for Control and Supervision of Experimental Animals (CPCSEA) on 15/11/2011, the present study was conducted in the laboratory of the department of pharmacology of our institute. The study was carried out from 01/01/2012 to 30/06/2013.

Experimental Animals Male and female Albino rats weighing 150-250 gm were used for the study. All the animals were kept under uniform conditions. Male and female rats were housed in a separate colony cage with free access to food and water *ad libitum*. They were acclimatized for 7 days before they were used and maintained on natural light and dark cycle. The rats fasted from 8 am on the day of experimentation. The tests were carried out between 1100 to 1400 hours. Animals were repeated for analgesic test after a gap of 7 days. Total of 60 rats were required. Experiments were performed at the same time of the day to exclude diurnal variation in pharmacological effects.

Drugs: Inj Nimodipine (Nimocard, New Medicon Pharma Lab Ltd) 10 mg/50 ml vial

Inj Fentanyl citrate (Verfen, Verve Health care ltd) 100 mcg / 2 ml amp

Inj Pethidine HCl (Verpat-50, Verve Health care ltd) 50 mg/ml amp

Injectable preparations of the above drugs were further diluted in distilled water to make solutions of desired strengths, prepared freshly at the time of experiments. These drugs were injected intraperitoneally (i.p.) in a volume of 0.5 ml/100 gm of rats. Control group of animals received the same volume of vehicle (distilled water).

Method: To study the analgesic effect of drugs tail-flick method was used to induce nociception in rats. Analgesic effect of nimodipine was evaluated by using three graded doses (200 mcg/kg, 400 mcg/kg, and 800 mcg/kg). Sub effective doses for fentanyl and pethidine were determined by trial and error method. A sub-effective dose was selected to leave scope to assess potentiation of therapeutic response if and when it is seen in combination with nimodipine and opioid.

To study the effect of nimodipine on opioid-induced antinociception, nimodipine (400mcg/kg) was administered in combination with sub-effective doses of fentanyl and pethidine.

The animals were grouped in the number of 8 (n=6)

Group 1: Control (Distilled water 0.5 ml/100gm)

Group 2: Nimodipine (200 mcg/kg)

Group 3: Nimodipine (400 mcg/kg)

Group 4: Nimodipine (800 mcg/kg)

Group 5: Fentanyl (15 mcg/kg)

Group 6: Nimodipine (400 mcg/kg) + Fentanyl (15 mcg/kg)

Group 7: Pethidine (10 mg/kg)

Group 8: Nimodipine (400 mcg/kg) + Pethidine (10 mg/kg)

Tail-Flick Method: [12] In tail flick method rat was kept in a rat holder, and the distal one-third of the tail was placed on the bridge. Below the bridge is a nichrome wire, which radiates heat and induces pain. As soon as the wire radiates heat, after a few seconds the rat flicks the tail. The time taken by the

rat for flicking the tail was taken as the endpoint. Reaction time was recorded by stopwatch.

Cut off time was kept 15 seconds to avoid injury too. Rats having basal reaction time not exceeding 15 seconds were included in the study. Drugs or vehicle were administered intraperitoneally and reaction time was noted before (baseline) and 30 and 60 minutes after the drugs or vehicle administration in each rat. Percentage analgesia (%) was calculated using the before and after drug reaction times by the following formula:

% Analgesia = After drug – Before drug × 100 / 15 (Cut off time in sec.) – Before drug

Statistical Analysis: Reaction time was presented as mean ± SD, and % analgesia was expressed in percentage. The difference in reaction time at different time periods after drug administration compared to that at baseline within the group was estimated using Repeated Measures ANOVA followed by Dunnett's Test. The difference in reaction time at different time periods between different groups was estimated by using One Way ANOVA followed by Tukey's Multiple Comparison Test. $p<0.05$ was considered as statistically significant. Data were analyzed statistically using Stata version 10.0 software.

RESULTS:

Evaluation of the Analgesic Effect of Nimodipine Table 1: Nimodipine at 200 mcg/kg, 400 mcg/kg and 800 mcg/kg did not show any significant difference in reaction time after 30 and 60 min as compared to baseline and as compared to control.

TABLE 1: REACTION TIME AND % ANALGESIA TO NIMODIPINE USING TAIL-FLICK METHOD IN RATS

Drugs	Reaction time (seconds) (Mean ± SD)			% Analgesia	
	Base line	After 30 min	After 60 min	After 30 min	After 60 min
Control	5 ± 1.26	5.33 ± 1.36	5.16 ± 0.75	3.33	1.66
Nimodipine (200mcg/kg)	5.16 ± 1.16	5.66 ± 0.51	5.5 ± 1.04	5.08	3.45
Nimodipine (400mcg/kg)	5.33 ± 1.03	6 ± 1.09	5.83 ± 0.75	6.93	5.17
Nimodipine (800mcg/kg)	5 ± 0.63	5.66 ± 0.81	5.33 ± 1.03	6.66	3.33
Comparison among groups by One way ANOVA followed by Tukey's multiple comparison test				After 30 min: F = 0.44, p = 0.726 After 60 min: F = 0.59, p = 0.628	

TABLE 2: REACTION TIME AND % ANALGESIA TO NIMODIPINE AND FENTANYL ALONE AND IN COMBINATION USING TAIL-FLICK METHOD IN RATS

Drugs	Reaction time (seconds) (Mean ± SD)			% Analgesia	
	Base line	After 30 min	After 60 min	After 30 min	After 60 min
Control	5 ± 1.26	5.33 ± 1.36	5.16 ± 0.75	3.33	1.66
Nimodipine (400mcg/kg)	5.33 ± 1.03	6 ± 1.09	5.83 ± 0.75	6.93	5.17
Nimodipine (15mcg/kg)	5.66 ± 0.81	8 ± 2.52	6.83 ± 1.47	25.05	12.56
Nimodipine (400mcg/kg) + Fentanyl (15 mcg/kg)	5 ± 0.89	12.83 ± 2.4	9 ± 2.09	78.33	40
Comparison among groups by One way ANOVA followed by Tukey's multiple comparison test				After 30 min: F = 18.1, p = <0.0001 After 60 min: F = 8.74, p = 0.0007	

TABLE 3: REACTION TIME AND % ANALGESIA TO NIMODIPINE AND PETHIDINE ALONE AND IN COMBINATION USING TAIL-FLICK METHOD IN RATS

Drugs	Reaction time (seconds) (Mean ± SD)			% Analgesia	
	Baseline	After 30 min	After 60 min	After 30 min	After 60 min
Control	5 ± 1.2	5.33 ± 1.36	5.16 ± 0.75	3.33	1.66
Nimodipine (400mcg/kg)	5.33 ± 1.03	6 ± 1.09	5.83 ± 0.75	6.93	5.17
Pethidine (10 mg/kg)	5 ± 0.63	7.16 ± 3.54	6 ± 1.78	21.66	10
Nimodipine 400 (mcg/kg) + Pethidine 10 (mg/kg)	5.83 ± 0.75	12.83 ± 2.04	8.83 ± 1.16	76.33	32.71
Comparison among groups by One way ANOVA followed by Tukey's multiple comparison test				After 30 min: F = 14.16, p = <0.0001 After 60 min: F = 11.1, p = 0.0002	

Effect of Nimodipine on Opioid-Induced Antinociception Table 2 and 3: Combination of nimodipine (400 mcg/kg) with opioids (fentanyl 15 mcg/kg and pethidine 10 mg/kg) produced a highly significant increase in reaction time after 30 and 60 min as compared to individual effect of the drugs.

Analgesic effect was maximally seen with nimodipine and fentanyl combination (78.33% after 30 min and 40% after 60 min).

DISCUSSION: A potential advantage of using combination therapy is that analgesic effects can be maximized while the incidence of adverse effects is minimized. Therefore using combinations of medications that offer analgesic synergism should allow a reduction in required dosage and decrease the incidence of adverse effects.

In the present study, no analgesic effect was observed with nimodipine and with sub-effective doses of opioids (fentanyl or pethidine) alone, but the combination of nimodipine with opioids (fentanyl or pethidine) produced significantly greater analgesia. The analgesic effect of this combination at 30 min was more than the effect at 60 min. Analgesic effect was maximally seen with nimodipine and fentanyl combination (78.33% after 30 min and 40% after 60 min). Fentanyl is a more potent opioid than pethidine, and this might be the reason for its highly significant analgesic effect in combination with nimodipine.

Opioid agonists produce analgesia by binding to opioid receptors. At molecular level, all opioid receptors are linked to G-protein located in the brain and spinal cord regions involved in the transmission and modulation of pain. At molecular level opioids inhibit adenylyl cyclase leading to a decrease in intracellular cAMP, inhibit voltage-gated Ca^{2+} channels on presynaptic nerve terminals and activate K+ channels and subsequent increase in K+ conductance.

Because Ca^{2+} influx is needed for the stimulus-induced neurotransmitter release, opioids decrease the release of neurotransmitters from nociceptive nerve terminals. Thus, the neuronal hyperpolarisation coupled with decreased neurotransmitter release in CNS and myenteric neurons, hamper the transmission of the nociceptive information at the first central synapse [13]. Calcium ions are unique and vitally important second messenger system within neurons. There are evidence suggesting the involvement of calcium ions in nociception 7, 8, 9 and L- and N- types VGCCs responsible for neurotransmitter release from sensory neurons of the dorsal column of spinal cord [10]. Previous research on nimodipine has indicated that it is more lipophilic than other L-type calcium channel blockers thus capable of crossing the blood-brain barrier, decreased release of substance P from the neurons of dorsal root ganglia and inhibited the release of glutamate from synaptosomes prepared from cerebral cortex [11].

So the potentiation of analgesic action of opioids with the co-administration of L- type calcium channel blocker, nimodipine could be due to additional closure of L-type VGCCs which are located on postsynaptic terminals in the neurons concerned with the transmission of pain.

CONCLUSION: In conclusion, nimodipine alone exhibited no analgesic activity. However, a combination of nimodipine with opioids (fentanyl or pethidine) had an enhanced analgesic effect. Thus in the present study nimodipine potentiated the analgesic effect of opioids (fentanyl or pethidine) suggesting a synergistic interaction between these drugs. Such combination would help to overcome the problems of tolerance, physical dependence, and abuse potential associated with opioid therapy, particularly on chronic administration.

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CONFLICT OF INTEREST: Nil

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