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Original Research Article

Co-administration of Nimodipine with Morphine in Rat, delayed the development of tolerance to analgesic effect of Morphine: The Tail-Flick test study: New insight for treatment of chronic pain

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Abstract
Background: Opioids like morphine produce side effects ranging from nausea and vomiting, pruritus, oversedation, dizziness and urinary retention to respiratory depression. Particularly, on chronic administration, it leads to development of tolerance. Combining opioids with certain other drugs (adjuvant analgesics) like ketamine, which is an N-methyl-D-aspartate (NMDA) receptor antagonist, not only increases the analgesia, but also reduces the dose of opioids. Previous research done in our laboratory and outside suggests that nimodipine, an L-type calcium channel blocker (L-CCBs), could be one such adjuvant drug.
Aims & Objectives: Study of morphine-induced analgesia and the development of morphine tolerance & effect of nimodipine on morphine-induced analgesia and tolerance
Study Design: Experimental Design**:** The experimental work was divided into 4 parts-
Part I - Study of morphine induced analgesia and the development of morphine tolerance.
Part II- Study the effect of nimodipine on morphine-induced analgesia and tolerance.
Material & Methods: Adult Wistar rats (n=24) received either normal saline, L-CCB (Nimodipine), Morphine or both drugs (Morphine + Nimodipine). Tail-Flick test was done after 40 minutes of injection
Statistics: To compare the control with treated groups, statistical analysis of the values of Tail-flick latency was done by Kruskal Wallis one way ANOVA, followed by "Tukey's Multiple Comparison Test" (multiple range 't' test) ($p<0.05$ was taken to be significant*)*.
Results & Conclusion: In the present study it has been seen that, co-administration of nimodipine with morphine, has significantly increases the analgesic effect of morphine and also delayed the development of tolerance.
Keywords: Morphine, Pain, Nimodipine, Tail-Flick test, Tolerance, L-VGCCBs

1. Introduction

Opioids like morphine produce side effects ranging from nausea and vomiting, pruritus, oversedation, dizziness and urinary retention to respiratory depression.[1] Particularly, on chronic administration, it leads to development of tolerance.[2] Combining opioids with certain other drugs (adjuvant analgesics) like ketamine, which is an N-methyl-D-aspartate (NMDA) receptor antagonist, not only increases the analgesia, but also reduces the dose of opioids.[3] Previous research done in our laboratory and outside suggests that nimodipine, an L-type calcium channel blocker (L-CCBs), could be one such adjuvant drug.[4]

Majority of cancer patients suffers from moderate to severe pain, particularly at advanced stages of the disease. WHO has developed a three-step "LADDER" for management of cancer related pain. Non-opioids, mild-opioids and strong-opioids are prescribed in sequential order till pain is relieved. However, high opioids doses lead to serious side effects like respiratory depression, hallucinations, myoclonus, constipation, somnolence and agitation. High degree of tolerance develops to morphine and related opioids, if drug is using for long-term. Development of tolerance is partly pharmacokinetic (enhanced rate of metabolism) but mainly pharmacodynamic (cellular tolerance).[5] Recently

NMDA antagonists and inhibitors of nitric oxide synthetase have been found to reduce morphine tolerance and dependence in animals. Adjuvant analgesics are drugs which are primarily used for treatment of other disease but which are known to relieve pain under certain conditions.[6] Many of these drugs have an opioid sparing effect as drugs with different mechanism of actions can potentiate the analgesic effect. Tricyclic antidepressants are one such group of drugs, being used for treatment of neuropathic component of cancer pain.[7] Their beneficial effects are possibly due to increased availability of serotonin and norepinephrine in descending pain modulatory circuits.

Other adjuvant drugs are corticosteroids, anticonvulsants, like carbamazepine, N-methyl-D-aspartate (NMDA) receptor antagonist, psycho stimulants, radionuclides etc.

However the role of L-type voltage-gated calcium channel antagonist (L-VGCC antagonist) as adjutants to opioids needs to be investigated. A number of Pre-clinical and clinical studies have observed that various L-VGCC antagonists increase the analgesic effect of opioids.

L-VGCC antagonists comprise of three chemically different groups-

- Phenylalkylamines- e.g. Verapemil
- Benzothiazepines- e.g. Diltiazem
- Dihydropyrimidines- e.g. Nimodipine, Nifedipine

These L-VGCC antagonists' binds to alpha-1 subunit of calcium channel and prevent Ca^{2+} influx. Nimodipine, a VGCC antagonist was observed to reduce opioid requirement in cancer patients for management of pain, in a double-blind placebo controlled study, though contradictory finding has also been reported.[8] In a preclinical study, Nifedipine (5mg/kg once daily) and Verapemil(10mg/kg) was able to potentiate the analgesic effect of morphine (20mg/kg, once daily for 5 days followed by 30 mg/kg once daily for 3 days). Other studies have also reported similar findings.[9] Also, nimodipine was observed to inhibit the expression of tolerance to chronic systemic administration of sufentanil, a highly selective mu-opioid receptor agonist.[10] Both autoradiography and radio-receptor assay have shown that nimodipine and sufentanil co-administration lead to higher expression of L-VGCC in area of nervous system concerned with pain transmission like the dorsal horn of spinal cord and periaqueductal gray. The increase in L-VGCCs could be due to compensatory increase as a result of closure of existing L-VGCCs by nimodipine. Interestingly, morphine has been reported to block N and P/Q- types of VGCCs. [11] Moreover, it has been reported that administration of nimodipine produced super sensitivity to analgesic effect of sufentanil. Super sensitivity is defined as a shift of the dose-response curve to the left, relative to control position. In a recent study the effect of two different L-VGCC antagonists (nifedipine and nimodipine) on morphine-induced antinociception was studied by the tail-flick test (40 min after morphine administration) in adult Wistar rats. A fixed-dose of nimodipine or nifedipine (2 mg/kg, once daily) was combined with a fixed dose of morphine (10 mg/kg, twice daily) for 10 days. Co-administration of L-VGCC antagonists significantly increased the antinociceptive effect of morphine, even 12 hr after administration. Also, nimodipine was more effective than nifedipine. Nimodipine was further studied using higher and escalating doses of morphine (20-30 mg/kg twice daily for 14 days). Nimodipine increased the antinociceptive effect of morphine in the latter part of the study (days nine to fourteen) though significant difference was observed on 11^{th} evening and 12th morning. No obvious adverse effects were observed in the present study. The results show for the first time that nimodipine is more effective than nifedipine and that these L-VGCC antagonists continue to be effective, even 12 hr after administration in the tail-flick test.[12] The possible reasons for the superiority of nimodipine as compared to other L- VGCC antagonist could be that nimodipine-

Is more lipophilic than other L-VGCC antagonist and able to penetrate better into the CNS.

Is more effective than other VGCC antagonist in inhibiting Ca^{2+} uptake as shown by uptake of [45] Ca^{2} by Neuroblastoma (NCB-20 cell line) cells.

Decrease the release of substance P from neurons of dorsal root ganglia.

Inhibit the release of glutamate from synaptosomes prepared from cerebral cortex.

Dilate the cerebral blood vessels at much lower dose than that required for peripheral vasodilatation (Cerebro-selective).[13]

L-VGCCs have been shown to be predominantly present on the cell surface of basal dendrite and cell bodies of neurons, where they are well positioned to transduce calcium regulated signaling events to the nucleus.[14] It forms an important route of calcium ions entry to the cell and regulates gene transcription and synaptic plasticity. Calcium ions are important intracellular second messengers.[15] In contrast to all other second messengers, concentration of calcium ions is regulated by entry into and removal from cytoplasm. Calcium ions entering the cytoplasm through plasma

membrane channels like VGCCs or release from internal stores like endoplasmic reticulum, binds to small molecular weight proteins like calmodulin.

2. Materials and Methods

Male Albino Wistar rats (n=24) were used in the present study. These rats were obtained from Experimental Animal Facility of AIIMS after prior approval of the project by Institutional Animal Ethics Committee. The animals were kept in cages, with no more than 3 animals in one cage. They were maintained at a 12 hours: 12 hours light/dark cycle with water and food available *ad libitum*. Rats were randomly divided in 4 groups of 6rats/group for the present study.

Group I- Control group- treated with physiological saline

Group II- Morphine group-treated with morphine (10mg/kg) subcutaneously twice daily for 14 days.

Group III- Nimodipine group-treated with nimodipine (2 mg/kg) intraperitonially (ip) once daily for 14 days.

Group IV- Morphine + Nimodipine group- treated with morphine (10mg/kg/sc) twice daily and nimodipine (2mg/kg/ip) once daily for 14 days.

2.1 Tail-Flick Apparatus: (Figure-1)

Tail Flick apparatus (Ugo Basile, Italy) was used for rapid and precise screening of morphine analgesia in the present study. The instrument emits infrared radiation through a small opening in it. This infrared radiation produces a sense of heat, which induces withdrawal reflex in the animal. The time period between the start of intervening radiation and withdrawal is called "Latency period." The time at which the rat withdraws its tail is easily recorded as the instrument timer has an automatic shut off. Thus the withdrawal latency to the nearest 0.1 second could be recorded.

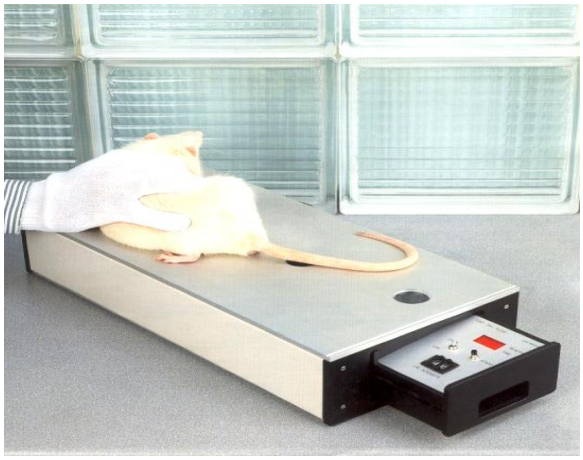

Figure 1: Tail Flick Apparatus (UGO BASILE)

2.1.1 Tail-Flick test-

The basal pain sensitivity in the rats was evaluated by the tail-flick test. The D'Armour and Smith (1941) first described this test. The tail-flick test was also used as a parameter for measuring the analgesic response to morphine. It is one of the most commonly used tests done in rats and guinea pigs. The rat was kept for 15 mins inside a loose fitting perpex restrainer with tail outside, for acclimatization before starting the experiment. In this test, a small part of the animal's tail is subjected to a radiant heat stimulus to evoke a tail-flick reaction. When the heat is focused on the tail, the animal quickly removes its tail from the heat source. Radiant heat is superior to any other form of thermal stimulation. It acts as a selective stimulus for nociceptors and does not stimulate the tactile receptors, and is thus an appropriate stimulant to perform the tail-flick test. The test is highly effective for study of opioid analgesia. The time interval between application of heat and withdrawal of the tail is the ***Latency*** to the tail-flick reaction. The intensity of heat delivered can be controlled to keep the baseline latency (the reading before any intervention or administration of drugs) between 2-4 seconds. The tail-flick response is produced independently from change in tail-skin temperature. The response depends on the intensity of the radiant heat and surface area stimulated. However it also depends on the site of stimulation. For morphine like drugs it is more sensitive when the distal part of the tail (5cm from the tip) is stimulated. [16] Any increase in reaction time after administration of drug (or any intervention) is interpreted as an *analgesic response*. However the maximum time limit of stimulus is 10 sec which is known as cut off time. If the rat does not flick the tail by 10 sec, tail is removed from the stage. In such instances the cut off time was taken as the response time. The practical advantage of this is to avoid tissue damage, so that repeated testing is possible with accurate results.

2.1.2 Selection of Experimental Animals

Male Albino Wistar rats weighing (200-250 g) were selected for study and all animals were subjected to a screening test with the help of tail-flick test. Animals with tail-flick latency between 2-4 seconds were selected for study. Any rat not giving latency within this range was not included in this study.

2.1.3 Baseline Recording

A baseline of tail-flick response was taken one day before the starting of each experiment. To record the baseline value, animals were put inside restrainers for 30 minutes, and this was repeated for 4-5 times, to acclimatize the animals. Later, a total of three readings were taken and their average calculated. The weight of each rat was determined, which was used to calculate the dose of each drug.

2.1.4 Drugs:

Normal saline- Normal saline is 0.9% NaCl (sodium chloride or salt), and it was purchased from market.

Morphine- Ampoules of morphine sulfate (15 mg/ml/ampoule) were obtained from a Government Licensed dealer after getting requisite permission from Office of The Commissioner Of Excise, L&N Block; Vikas Bhawan; New Delhi. It was procured in small batches because of restriction in its availability due to its abuse potential.

Nimodipine: It is a white powder like substance**.** Nimodipine is a L-subtype voltage gated calcium channel antagonist, purchased from Sigma (USA). It is insoluble in water. However its solubility was previously standardized in our laboratory in a vehicle (mixture of chemicals), consisting of: normal saline, polyethylene glycol (PEG) and absolute alcohol in **2:2:1** ratio.

Preparation for 1ml of drug-

Nimodipine	:	1mg
Saline	:	400µl
PEG	:	400µl
Absolute alcohol	:	200µl
Total	:	1000µl or 1ml

Since nimodipine is very light sensitive drug, so that entire procedure was performed in a dark room with indirect lighting. Also during preparation of nimodipine solution the entire procedure was performed in a laminar flow under aseptic condition. Dose of nimodipine (2mg/kg) and route of its administration was previously standardized in our laboratory. [17]

2.1.5 Experimental Design: The experimental work was divided into 2 parts-

Part I - Study of morphine induced analgesia and the development of morphine tolerance.

Part II- Study the effect of nimodipine on morphine-induced analgesia and tolerance.

Each part of the study is further described below:

Part I - Study of morphine induced analgesia and the development of morphine tolerance-

Group I (n= 6): Saline Group

This was the control group, which was injected normal saline, subcutaneously two times a day at 12 hours interval for 14 days. The injection was given at the lateral aspect of thigh. The volume of normal saline was equivalent to the dose of morphine, in volume. The injections were given with help of sterile tuberculin syringe. Tail-flick response was taken after 40 minutes of injection.

Group II (n= 6): Morphine Group

The animals in this group were treated with morphine sulphate (10mg/kg of body weight) twice a day at 12 hours interval for 14 days**.** Injections of morphine were given with tuberculin syringe, subcutaneously over the lateral aspect of thigh. Successive injections were given in alternate limbs. Tail-flick response was taken after 40 minutes of injection. Decrease in values of tail-flick near the latter-half of the experiment indicated the development of tolerance to morphine.

Part II- Study the effect of nimodipine co-administration on morphine analgesia and tolerance-

Group III (n=6): Nimodipine Group

The animals in this group were treated with nimodipine 2mg/kg body weight, once a day for 14 days. Injections of nimodipine were given into the peritoneal cavity. Tail-flick response was recorded after 60 minutes of injection. The intraperitoneal route has been selected because of the quick passage of the drug into the blood stream.

Group IV (n=6): Morphine + Nimodipine Group –

The animals in this group were treated with nimodipine 2mg/kg intraperitonially in the morning. After 20 minutes of the injection of nimodipine, morphine was given at the dose of 10mg/kg subcutaneously. However, morphine injection was given alone in the evening. The combined treatment of both drugs was given for 14 days. Tail-flick response was recorded after 40 minutes of morphine injection. The time interval between injections and recording of tail-flick latency has been standardized previously in our laboratory for both morphine and nimodipine, and it depends on the peak antinociceptive effect.

2.2 Statistical analysis

To compare the control with treated groups, statistical analysis of the values of Tail-Flick Latency in at morning & evening was done by Kruskal Wallis one way ANOVA, followed by "Tukey's Multiple Comparison Test" (multiple range 't' test) ($p<0.05$ was taken to be significant)

3. Results

3.1 Part I & Part II - Effect of nimodipine on morphine induced analgesia and tolerance

Morphine analgesia was studied on Groups I to IV, and assessment of analgesia was done by tail-flick test. The values of tail-flick latency were almost equal to baseline values for group I, throughout the experiment, while for group II, values of tail-flick latency were almost equal to the cut off time (9.15±1.762), at day 1, but gradually the values decreases over the time period of experiment and at the end of experiment, tail-flick values reaches to base line value. This pattern of gradual decreases of latency is interpreted as the development of tolerance to analgesic effect of morphine. The effect of nimodipine co-administration with morphine on

morphine analgesia and tolerance was studied in groups III and group IV. Group III was taken to observe whether nimodipine alone had an analgesic effect? As shown in Table- 1 & 2, tail-flick latency for nimodipine was the same as for saline. Values of tail-flick latency for group IV were higher in comparison with group II, which means co-administration of nimodipine with morphine had higher analgesic effect than morphine alone which also indicated delay in the development of tolerance to morphine.

Table -: 1- Values of tail-flick Latency in the morning (Mean ± SEM)

Days	Group I (Saline)	Group II (Morphine)	Group III (Nimodipine)	Group IV (Morphine + Nimodipine)
Baseline	3.36±0.328	3.3±0.326	3.01±0.53	2.95±0.24
Day 1	3.45±0.712	9.15±1.762	2.8±0.36	10±0
Day 2	2.75±0.0544	7.633±2.545	3.1±0.45	10±0
Day 3	3.85±0.493	7.817±1.843	3±0.40	10±0
Day 4	2.7±0	6.4±2.236	2.6±0.33	9.2±0.90
Day 5	2.78±0.087	5.1±2.072	2.4±0.27	9.7±0.65
Day 6	2.5±0.766	4.517±1.254	2.3±0.10	8.15±1.6
Day 7	2.7±0.19	4.65±1.847	2.3±0.11	8.35±1.9
Day 8	2.65±0.383	4.183±1.635	2.5±0.27	8±1.77
Day 9	2.4±0.109	4.033±1.958	2.3±0.17	6.98±1.3
Day 10	2.95±0.54	3.31±0.856	2.9±0.57	5.65±0.66
Day 11	2.9±0.32	3.51±1.55	2.6±0.35	5.9±1.4
Day 12	2.8±0.32	3.28±0.82	2.75±0.35	5.5±1.2
Day 13	2.85±0.273	4.25±1.84	2.6±0.36	5.3±1.6
Day 14	2.75±0.164	3.58±0.716	2.5±0.35	5.5±0.95

Table -: 2- Value of tail-flick Latency in the evening (Mean ± SEM)

Days	Group I (Saline)	Group II (Morphine)	Group III (Nimodipine)	Group IV (Morphine + Nimodipine)
Baseline	3.36±0.326	3.3±0.328	2.8±0.36	2.95±0.24
Day 1	3.45±0.712	10± 0	2.8±0.22	10±0
Day 2	2.75±0.054	8.783±1.87	2.9±0.19	10±0
Day 3	3.85±0.493	7.71±2.16	3.2±0.28	8.9±1.1
Day 4	2.7±0.236	5.9±1.14	3.08±0.3	8.45±1.3
Day 5	2.78±0.87	4.63±1.6	3.3±0.29	8.25±1.34
Day 6	2.5±0.152	5.16±1.51	3.1±0.44	7.7±1.8
Day 7	2.7±0.132	5.31±2.1	3.6±0.16	7.7±1.8
Day 8	2.65±0.38	4.6±1.0	3.15±0.53	5.7±2.2
Day 9	2.4±0.109	5±1.97	3.45±0.52	5.75±2.0
Day 10	2.95±0.054	4.71±2.69	3.1±0.45	5.35±1.01
Day 11	2.9±0.32	3.41±1.78	3.3±0.46	5.08±1.2
Day 12	2.8±0.32	3.51±1.89	3.3±0.25	5.11±1.4
Day 13	2.85±0.27	4.3±1.51	2.95±0.31	4.7±1.2
Day 14	2.75±0.16	3.11±0.69	2.7±0.17	4.88±1.2

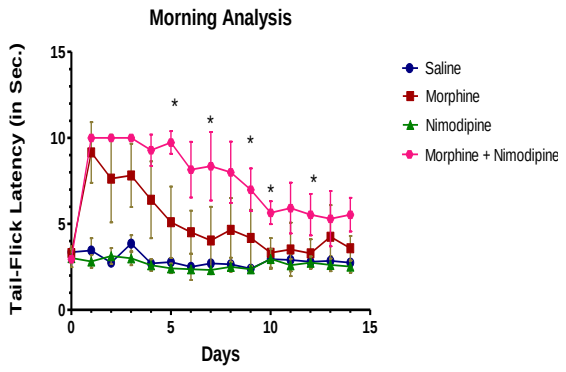

Figure: 2- Line graph

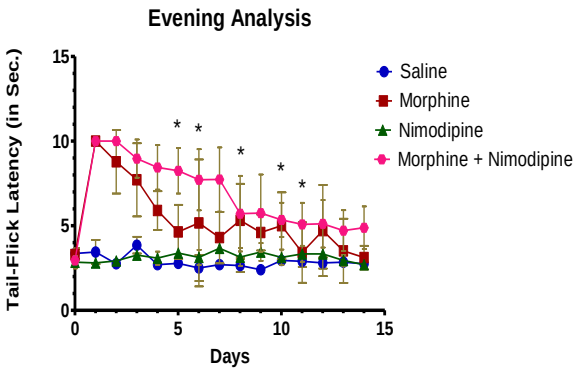

Figure: 3- Line graph

The tail-flick response time significantly ($p<0.05$) higher in morphine+nimodipine treated group (*) in comparison with morphine treated group, from day 4 to day 12, while for saline and nimodipine treated group it was almost same and just equal to the baseline value.

4. Discussion

In the present work the effect of nimodipine, a L type -VGCC antagonist on morphine induce analgesia was studied. Previous studies have shown that -

- Mu-opioid receptors are expressed by specific regions of the central nervous system concerned with transmission of pain like the dorsal horn of the spinal cord.
- Up-regulation of mu-opioid receptors in the spinal cord of morphine-tolerant rats. [18]
- Increased expression of N-type VGCCs in amagdyla of morphine tolerant rat.
- Nimodipine increased the antinociceptive effects of morphine after intraspinal administration. [19]
- Intrathecal co-administration of morphine and nimodipine produces higher antinociceptive effect by synergistic interaction as evident by injecting different doses of each drug in rats. [20]
- Nimodipine is more effective than nifedipine in attenuating morphine tolerance on chronic co-administration in the rat tail-flick test. [21]

So on the basis of these previous observations, it was hypothesized that nimodipine induced potentiation of morphine analgesia might be due to decreased intracellular Ca^{2+} concentration in post-synaptic neurons, which leads to decreased formation of Ca^{2+} /calmodulin complex, resulting in decreased activity of calcium/calmodulin Kinase IV (CaMKinase IV), which is an important factor for activation of cAMP response element binding protein (CREB) and its phosphorylation to p-CREB.

Development of Morphine Tolerance-

Morphine has been used for centuries to alleviate severe pain; however, the clinical usefulness of morphine is often limited by the development of analgesic tolerance, physical dependence, and addiction. Although the mechanisms underlying opioid tolerance are unclear, early adaptive responses, including β-arrestin-mediated uncoupling from G-protein signaling and receptor internalization, have been suggested to be crucial.[22]

Long-term morphine administration clearly induces behavioral tolerance in animals and humans but fails to strongly desensitize mu-receptors. Instead, long-term morphine use causes adenylyl cyclase (AC) supersensitization, which may underlie opioid tolerance and dependence at the cellular level.[23] Adaptations in adenylyl cyclase activities have been noted in several brain regions, including the ventral tegmental area (VTA) and nucleus accumbens (NAc) areas (which are critical for opioid drug reinforcement), and in the locus coeruleus and dorsal raphe nucleus which are critical for opioid withdrawal [24]. Although adenylyl cyclases have been suggested to mediate some of the actions of opioids, a lack of specific inhibitors has slowed progress in defining the roles of different AC isozymes. To date, genes for 10 ACs have been cloned, each with a distinct expression pattern in the central nervous system and the peripheral sensory nervous system. Among them, AC1 and AC8 are uniquely stimulated by Ca^{2+}/calmodulin in the brain.[25] AC1 and AC8 are widely distributed in the different brain regions including VTA, NAc, locus coeruleus, and dorsal raphe nucleus. Many studies have shown the involvement Ca^{2+}/calmodulin in morphine action.[26] Therefore, by using AC1 and AC8 single knockout (KO) mice as well as double KO mice. Shuang *et al*., have assessed short-term tolerance, long-term tolerance, and long-term withdrawal after morphine in both wild-type and KO mice to define the roles of these cyclases in the cellular and behavioral adaptations to opiate. Results of this study has measured morphine-induced locomotion and place preference to assess the roles of AC1 and AC8 in morphine reinforcement and then tested the contributions of AC1 and AC8 to morphine cAMP-response element binding protein (CREB) activation to further characterize the underlying mechanisms.[27]

Several studies have also examined the involvement of CREB activation in morphine response.[28] For example, a single morphine injection was reported to increase the number of p-CREB-positive cells in VTA. Moreover, long-term morphine exposure increases cAMP response element-mediated transcription in both the VTA and the locus coeruleus. The mechanisms linking opioid receptor activation to p-CREB are not clear. CREB phosphorylation may involve cAMP-activated protein kinase A, calcium^{2+}/ calmodulin-dependent protein kinase, or mitogen-activated protein kinase (MAP) pathways. In a recent study, Shuang *et al*., 2006 found that an increase of p-CREB induction in VTA by long-term morphine treatment was significantly reduced in the DKO mice, suggesting that AC1 and AC8 mediate the CREB activation in these neurons. However, the presence of a significant residual p-CREB in morphine-injected DKO mice indicates that Ca^{2+}stimulated AC1 and AC8 are not solely responsible.[29] This conclusion is consistent with a recent report showing that a Ca^{2+} independent form of adenylyl cyclase, AC5, has also been implicated in morphine actions within the striatum. The cellular mechanisms underlying morphine effects are not completely clear, but it is known that morphine disinherits VTA dopaminergic cell firing

by inhibiting neighboring GABAergic neurons. A reduction of cAMP/CREB signaling in these GABAergic neurons might prevent morphine effects in the nucleus accumbens and reduce the reinforcing properties of morphine. In conclusion, the study supports a fundamental reconsideration of the roles of calmodulin-stimulated adenylyl cyclases in the morphine response and tolerance to its antinociceptive effect and demonstrates that AC1 and AC8 contribute to the initial stages of morphine tolerance. Shuang *et al*., 2006 also found that AC1 and AC8 contribute to the expression of the somatic signs of opiate withdrawal and severity of morphine physical dependence. Finally, they found that AC1, AC8, and CREB contribute to the reinforcing properties of morphine.[30]

5. Conclusion

Morphine is the most effective analgesic drug used for management of chronic pain, but development of tolerance to its analgesic effect is a major limiting factor.

Co-administration of nimodipine, a L-type VGCC antagonist with morphine, significantly increases the analgesic effect of morphine and also delays the development of tolerance. It was hypothesized that tolerance could be due to increased Ca^{2+} entry into neurons, which then leads to Phosphorylation of CREB and transcription of pronociceptive gene like c-fos.

Molecular basis of this potentiation of morphine analgesia with nimodipine co-administration might be due to blockade of L-type VGCCs, which are predominatly present on post-synaptic neurons.

The present study indicates that antagonist of L-VGCCs, particularly nimodipine, may enhance the analgesic potency of opioids like morphine and also delayed the development of opioid tolerance.

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