

Potassium Channel Openers Exhibit Cross-tolerance with Morphine in Two Experimental Models of Pain

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ABSTRACT

Objective: The study was performed to assess the effect of potassium channel openers on morphine tolerance and vice-versa.

Methods: Swiss albino mice of either gender weighing between 25–30 g were used for the study. The study assesses the effect of potassium channel openers (cromakalim, diazoxide and minoxidil) on morphine tolerance and vice-versa, using formalin and tail-flick tests.

Results: The antinociceptive effect of cromakalim and minoxidil was significantly reduced when administered to morphine-tolerant mice, in both the behavioural tests. However, reduced analgesic effect of diazoxide was observed on morphine-tolerance in the formalin test but not in the tail-flick test. Tolerance was observed when morphine was administered to animals chronically treated with any of the potassium channel openers. The same effect was observed when morphine was injected into a group treated with a combination of morphine and any of the potassium channel openers.

Conclusions: This study, therefore, suggests that both morphine and potassium channel openers are cross-tolerant. However, such interaction occurs at the level of potassium channels rather than at the level of receptors.

Keywords: Antinociception, cromakalim, morphine, tolerance

Los Abridores de Canales de Potasio Exhiben Tolerancia Cruzada con la Morfina en dos Modelos Experimentales de Dolor

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RESUMEN

Objetivo: El estudio fue realizado para evaluar el efecto de los abridores de canales de potasio en la tolerancia a la morfina, y viceversa.

Métodos: Para el estudio, se usaron ratones albinos suizos de ambos sexos que pesaban entre 25–30 g. El estudio evalúa el efecto de los abridores de canales de potasio (cromacalina, diazóxido y minoxidil) en la tolerancia a la morfina, y viceversa, usando la prueba de la sacudida de la cola y la prueba de la formalina.

Resultados: El efecto antinociceptivo de la cromacalina y el minoxidil fue significativamente reducido cuando se le administró a los ratones tolerantes a la morfina, en ambas pruebas conductuales. Sin embargo, se observó un efecto analgésico reducido de diazóxido sobre la tolerancia a la morfina en la prueba de la formalina, pero no en la prueba de la sacudida de la cola. Se observó tolerancia al administrar morfina a animales crónicamente tratados con cualquiera de los abridores de canales de potasio. El mismo efecto fue observado cuando se inyectó la morfina al grupo tratado con una combinación de morfina y cualquiera de los abridores de canales de potasio.

Conclusiones: Por consiguiente, este estudio sugiere que tanto la morfina como los abridores de canales de potasio son tolerantes cruzados. Sin embargo, tal interacción ocurre a nivel de los canales de potasio más bien que a nivel de los receptores.

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Palabras claves: Antinocicepción, tolerancia, cromacalina, morfina

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INTRODUCTION

Morphine and other opiates are the most commonly used drugs for the treatment of severe pain. The usefulness of long-term opiate administration for pain is limited by a factor known as tolerance, which is the decreased effect of a drug after repeated exposure (1). The openers of the ATP-gated potassium channels are a class of drugs that share with opioids the ability to open potassium channels and enhance potassium efflux from the cell (2). Previous studies suggest that the ATP-gated potassium channel openers either interact directly with the opioid receptors or release an endogenous opioid, which can bind to its specific opioid receptor (3, 4). It has also been observed that the function of potassium channels is altered in morphine tolerance and the amplitude of the neuronal inwardly rectifying potassium conductance induced by mu-opioid receptor agonists is lower in morphine-tolerant than in control animals (5–8). However, the interaction between morphine and potassium channel openers on tolerance induced by both morphine and potassium channel openers, when administered peripherally, has not been examined. Whether such functional changes reflect modifications in the properties of potassium channels is not well known, but it has been reported that chronic administration of morphine downregulates the levels of mRNA for certain non-ATP-dependent potassium channels (9).

Hence, the present study was performed using formalin- and tail-flick tests, to assess –

- (a) the effect of potassium channel openers (cromakalim, minoxidil and diazoxide) in morphine-tolerant mice
- (b) the effect of potassium channel openers and morphine on tolerance induced by potassium channel openers
- (c) the effect of co-administration of potassium channel openers with morphine in the development of morphine tolerance in mice

MATERIALS AND METHODS

Swiss albino mice of either gender weighing between 25-30 g were used for the study. The animals were divided into different groups having 10 mice/group. These mice were procured from the Central Animal House, University College of Medical Sciences, Delhi. The animals were kept in temperature-controlled rooms at 22 ± 2 °C, with 12 hours dark/light cycles and free access to food and water. All the experiments were performed at daytime between 0930 and 1530 hours. Care of the animals was in accordance with the guidelines for the care of laboratory animals and ethical guidelines for research in experimental pain with conscious

animals (10). The study was duly approved by the Institutional Animal Ethics Committee.

Morphine sulfate was obtained from Civil Drug Laboratories, Delhi. Cromakalim, diazoxide and minoxidil were purchased from Sigma, USA. Morphine sulfate was dissolved in 0.9% saline. Cromakalim (in doses of 1 and 5 mg/kg) and minoxidil (in doses of 30 and 50 mg/kg) were dissolved in absolute alcohol at a concentration of 10 and 40 mg/ml respectively and further diluted with 0.9% saline. Diazoxide (in doses of 30 and 50 mg/kg) was dissolved in 0.1N sodium hydroxide (NaOH) at a concentration of 10 mg/ml, and further diluted with 0.9% saline. All the drugs were injected intraperitoneally 30 minutes prior to the behavioural testing. Tolerance to morphine was produced in animals by administering escalating doses of morphine, starting from 5 mg/kg to 35 mg/kg with the first dose given at 1530 hours on the first day and thereafter twice daily, at 0930 and 1530 hours, with an increment of 5mg/kg/day, for 7 days. Tolerance to potassium channel openers was produced by injecting fixed doses of cromakalim (1 mg/kg), diazoxide (30 mg/kg) or minoxidil (30 mg/kg), twice daily at 0930 and 1530 hours for 7 days.

For the interaction study, animals of the groups cromakalim with morphine, diazoxide with morphine and minoxidil with morphine, received potassium channel openers 30 minutes before morphine. The cromakalim with morphine group received a fixed dose of cromakalim (1 mg/kg) twice daily along with escalating doses of morphine, following the same schedule used for producing morphine tolerance. Similarly, the diazoxide with morphine and minoxidil with morphine group received diazoxide and minoxidil respectively in a dose of 30 mg/kg twice daily along with escalating doses of morphine. The drug treatment of various groups continued for 7 days, followed by a withdrawal period of 72 hours, after which the analgesic response of morphine and potassium channel openers was assessed using formalin and tail-flick tests.

Formalin Test (FT): This procedure was similar to one previously described by Shibata *et al* (11). The animals were exposed to the observation chamber 15 minutes prior to the formalin injection for acclimatization. The formalin test was carried out in a 30x30x30 cm clear plexiglass box. A mirror was mounted at 45° angle below the transparent floor to allow an unobstructed view of the rat's paw. Then, 0.02 ml of 1% formalin was injected subcutaneously into the dorsal surface of the right hindpaw. Nociception was evaluated by quantifying paw licking time during the first five minutes (early phase) and at 25–30 minutes (late phase). The five-

minute period in the late phase was selected on the basis of response seen in the preliminary studies.

Tail Flick Test (TFT): Analgesia was assessed by the method as described by D'Amour and Smith (12), using a standard tail-flick apparatus. The intensity of the thermal stimulus was adjusted to provide average baseline tail-flick latency of 2–3 seconds. A cut-off time of 10 seconds was used to avoid any injury to the tail. Animals not responding within the cut-off time were removed and assigned a latency of 10 seconds.

The data showing duration of licking response and tail-flick latency were expressed as Mean \pm SEM (Standard Error of Mean) and the data were analysed by one-way ANOVA followed by post-hoc Tukey's test, using SPSS v12 software. *P*-values less than 0.05 were considered significant.

RESULTS

Morphine in a dose of 5 mg/kg caused significant ($p < 0.001$) inhibition of formalin-induced biphasic licking response (Table 1). Morphine also caused a significant ($p < 0.001$) increase in the tail-flick latency (TFL). Cromakalim, diazoxide and minoxidil in a dose of 5, 50 and 50 mg/kg respectively caused a significant ($p < 0.001$) decrease in licking response in both the phases of formalin-induced pain (Table 1). The potassium channel openers also caused a significant increase in the TFL at the same dose (Table 1).

Administration of morphine (5 mg/kg) to morphine-tolerant mice, after a withdrawal period of 72 hours, caused a significant ($p < 0.001$) increase in the licking response in both the phases of the FT (Table 1). While in the TFT, significant ($p < 0.001$) decrease in the TFL was observed (Table 1).

When a test dose of cromakalim (5 mg/kg) or minoxidil (50 mg/kg) or diazoxide (50 mg/kg) was administered to morphine-tolerant mice, then tolerance to their analgesic action was observed in both the phases of the FT and TFT (Table 1).

When morphine (5 mg/kg) was administered to the group chronically treated with either of the potassium channel openers, then tolerance was seen in each of the group in both FT as well as TFT (Table 2). When mice chronically treated with potassium channel openers were given a test dose of the respective potassium channel opener, then significant reduction in the analgesic response of potassium channel openers was observed in both FT and TFT (Table 2).

When mice chronically treated with both morphine and either of the potassium channel openers were given a test dose of morphine (5 mg/kg), then significant reduction in the analgesic action of morphine was observed in both the behavioural tests (Table 3).

Table 1: Effect of morphine and potassium channel openers on FT and TFT in morphine-tolerant mice.

Chronic Treatment	Test Dose (mg/kg)	Formalin Test Mean licking (in sec) \pm S.E.M.		Tail Flick Latency Mean (in sec) \pm S.E.M.
		Early Phase	Late Phase	
Saline	Saline	116.67 \pm 3.17	83.50 \pm 1.72	2.66 \pm 0.16
Saline	Morphine (5)	24.63 \pm 1.83 ^a	9.25 \pm 1.35 ^f	10.0 \pm 0.0 ^k
Morphine*	Morphine (5)	54.0 \pm 3.15 ^b	26.50 \pm 1.61 ^g	5.52 \pm 0.22 ^l
Saline	Cromakalim (5)	8.38 \pm 0.68	1.18 \pm 0.08	5.25 \pm 0.21
Morphine*	Cromakalim (5)	46.17 \pm 1.87 ^c	42.16 \pm 1.17 ^h	3.07 \pm 0.06 ^m
Saline	Diazoxide (50)	9.38 \pm 1.34	1.13 \pm 0.48	5.48 \pm 0.19
Morphine*	Diazoxide (50)	40.17 \pm 0.91 ^d	42.17 \pm 1.08 ⁱ	3.57 \pm 0.11 ⁿ
Saline	Minoxidil (50)	17.25 \pm 2.50	3.50 \pm 1.34	8.15 \pm 0.40
Morphine*	Minoxidil (50)	67.0 \pm 3.21 ^e	35.83 \pm 2.66 ^j	2.80 \pm 0.18 ^o

* administered escalating doses of morphine

^a $p < 0.001$ as compared to early phase of saline group

^b $p < 0.001$ as compared to early phase of morphine (5 mg/kg) group

^c $p < 0.001$ as compared to early phase of cromakalim (5 mg/kg) group

^d $p < 0.001$ as compared to early phase of diazoxide (50 mg/kg) group

^e $p < 0.001$ as compared to early phase of minoxidil (50 mg/kg) group

^f $p < 0.001$ as compared to late phase of saline group

^g $p < 0.001$ as compared to late phase of morphine (5 mg/kg) group

^h $p < 0.001$ as compared to late phase of cromakalim (5 mg/kg) group

ⁱ $p < 0.001$ as compared to late phase of diazoxide (50 mg/kg) group

^j $p < 0.001$ as compared to late phase of minoxidil (50 mg/kg) group

^k $p < 0.001$ as compared to early phase of saline group of tail-flick test

^l $p < 0.001$ as compared to morphine (5 mg/kg) group of tail-flick test

^m $p < 0.001$ as compared to cromakalim (5 mg/kg) group of tail-flick test

ⁿ $p < 0.001$ as compared to diazoxide (50 mg/kg) group of tail-flick test

^o $p < 0.001$ as compared to minoxidil (50 mg/kg) group of tail-flick test

Table 2: Effect of morphine and potassium channel openers on FT and TFT in mice tolerant to potassium channel openers

Chronic Treatment (mg/kg)	Test Dose (mg/kg)	Formalin Test Mean licking (in sec) ± S.E.M.		Tail Flick Latency Mean (in sec) ± S.E.M.
		Early Phase	Late Phase	
Saline	Morphine (5)	24.63 ± 1.83	9.25 ± 1.35	10.0 ± 0.0
Cromakalim (1)	Morphine (5)	46.83 ± 1.05 ^a	22.50 ± 0.89 ^e	5.17 ± 0.18 ⁱ
Diazoxide (30)	Morphine (5)	58.33 ± 2.60 ^a	23.83 ± 1.17 ^e	4.73 ± 0.31 ⁱ
Minoxidil (30)	Morphine (5)	61.17 ± 3.45 ^a	31.50 ± 1.54 ^e	3.55 ± 0.20 ⁱ
Saline	Cromakalim (5)	8.38 ± 0.68	1.18 ± 0.08	5.25 ± 0.21
Cromakalim (1)	Cromakalim (5)	35.5 ± 2.75 ^b	20.17 ± 1.11 ^f	3.43 ± 0.18 ^j
Saline	Diazoxide (50)	9.38 ± 1.34	1.13 ± 0.48	5.48 ± 0.19
Diazoxide (30)	Diazoxide (50)	29.67 ± 0.96 ^c	21.83 ± 0.54 ^g	3.13 ± 0.14 ^k
Saline	Minoxidil (50)	17.25 ± 2.50	3.50 ± 1.34	8.15 ± 0.40
Minoxidil (30)	Minoxidil (50)	62.67 ± 4.11 ^d	23.17 ± 0.79 ^h	2.77 ± 0.28 ^l

^a p < 0.001 as compared to early phase of morphine (5 mg/kg) group

^b p < 0.001 as compared to early phase of cromakalim (5 mg/kg) group

^c p < 0.001 as compared to early phase of diazoxide (50 mg/kg) group

^d p < 0.001 as compared to early phase of minoxidil (50 mg/kg) group

^e p < 0.001 as compared to late phase of morphine (5 mg/kg) group

^f p < 0.001 as compared to late phase of cromakalim (5 mg/kg) group

^g p < 0.001 as compared to late phase of diazoxide (50 mg/kg) group

^h p < 0.001 as compared to late phase of minoxidil (50 mg/kg) group

ⁱ p < 0.001 as compared to morphine (5 mg/kg) group of tail-flick test

^j p < 0.001 as compared to cromakalim (5 mg/kg) group of tail-flick test

^k p < 0.001 as compared to diazoxide (50 mg/kg) group of tail-flick test

^l p < 0.001 as compared to minoxidil (50 mg/kg) group of tail-flick test

Table 3: Effect of morphine (5 mg/kg) on tolerance induced by a combination of morphine and potassium channel openers

Chronic Treatment	Test Dose (mg/kg)	Formalin Test Mean licking (in sec) ± S.E.M.		Tail Flick Latency Mean (in sec) ± S.E.M.
		Early Phase	Late Phase	
Saline	Morphine (5)	24.63 ± 1.83	9.25 ± 1.35	10.0 ± 0.0
Morphine	Morphine (5)	54.0 ± 3.15 ^a	26.50 ± 1.61 ^b	5.52 ± 0.22 ^c
Cromakalim (1)* + Morphine	Morphine (5)	53.67 ± 3.13 ^a	38.17 ± 1.54 ^b	4.32 ± 0.09 ^c
Diazoxide (30) + Morphine*	Morphine (5)	64.0 ± 1.57 ^a	33.67 ± 0.96 ^b	4.62 ± 0.16 ^c
Minoxidil (30) + Morphine*	Morphine (5)	64.5 ± 3.68 ^a	64.5 ± 1.50 ^b	4.67 ± 0.37 ^c

* administered escalating doses of morphine

^a p < 0.001 as compared to early phase of morphine (5 mg/kg) group

^b p < 0.001 as compared to late phase of morphine (5 mg/kg) group

^c p < 0.001 as compared to morphine (5 mg/kg) group of tail-flick test

DISCUSSION

Pain is a complex experience which in addition to conveying sensory information about its modalities such as location, type and intensity of the painful stimulus, has profound autonomic emotional effects. The experience of pain is the final product of a complex information processing network. Different models of pain are being used to assess nociception in preclinical studies using laboratory animals. In the present study, acute thermal pain was modelled by the tail-flick test

whereas persistent pain was assessed by the formalin test (11,12). The tail-flick test uses heat as a noxious stimulus, in response to which the animal's tail is flicked away from the heat source, which is a spinally mediated flexion reflex.

The formalin test involves subcutaneous injection of formalin into the dorsal surface of the rat paw, to produce moderate, persistent pain that bears a resemblance to most clinical pain (11). Formalin produces a biphasic pain response *ie* the early phase and a late phase. The early phase

starts immediately after the injection of formalin and lasts for 8–10 minutes. During this phase, excessive licking/biting behaviour is observed, which is due to the peripheral stimulation of C-fibers (13). Subsequent to the early phase, there is a period of 5–10 minutes when the animal displays very little behaviour suggestive of nociception. The second phase or late phase starts approximately 15–20 minutes after formalin injection and lasts for 20–40 minutes. The licking/biting behaviour during this phase is due to the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord. These functional changes initiated by the C-fiber barrage during the early phase seem to persist during this phase. Repeated treatment with opioids produce different types of changes in cellular responsiveness such as alternation in calcium influx (14–19), increase in dihydropyridine sites (20–22), receptor uncoupling and internalization (23, 24), sustained changes in G-proteins (25), the adenylate cyclase system (26, 27) and changes in resting membrane potential due to an alternation in sodium pumping (28). Development of opioid tolerance has been found to be associated with adaptive changes in the function of voltage-dependent calcium channels resulting in up-regulation of L-type calcium channels (20, 29). This is due to the increase in potassium efflux from the cell by potassium channel openers causing hyperpolarisation of the cell's membrane potential, which in turn decreases the calcium entering, thereby lessening the amount of neurotransmitter released by the cell. Thus, the amplitude of the neuronal G-protein activated inwardly rectifying potassium conductance (GIRK) induced by agonists of mu-opioid receptors is decreased (5, 6, 30–33) and there is increased opioid inhibition of presynaptic GABA release (30–33). Studies with GIRK knockout mice have demonstrated that GIRK channels become uncoupled from postsynaptic mu-opioid receptors in peri-aqueductal gray neurons after chronic morphine administration (34–38).

In the present study, administration of escalating doses of morphine for 7 days, induced tolerance to antinociceptive effect of morphine when tested after 72 hours, in the formalin and tail-flick tests. The potassium channel openers (cromakalim, diazoxide and minoxidil) induced antinociception in both the behavioural tests when administered alone.

When potassium channel openers were administered to morphine-tolerant animals, the antinociceptive effect was reduced as compared to respective drugs when administered *per se*, in both the behavioural tests. These results suggest that there may be a possible cross-tolerance between opioids and potassium channel openers (Table 1). Repeated treatment with either of the potassium channel openers reduced the antinociceptive effect of the respective potassium channel openers as well as morphine, in both the behavioural tests. These results further suggest that opioids and potassium channel openers may be cross-tolerant (Table 2). Such interaction between morphine and potassium channel openers on tolerance induced by both morphine and

potassium channel openers, when administered peripherally, has not been previously reported.

In the animals that received chronic administration of morphine in combination with either of the potassium channel openers, tolerance was observed to the antinociceptive effect of the test dose of morphine in both behavioural tests. This observation is in line with a recent study which has also reported the development of tolerance to morphine following chronic concomitant administration of both morphine and potassium channel openers (39). However, we have achieved the same effect using a lower dose of morphine (5 mg/kg). These results further indicate that the development of tolerance to the analgesic action of morphine may be occurring at the level of potassium channels rather than at the level of receptors, leading to cross-tolerance between morphine and potassium channel openers (5, 6, 39). However, an earlier study has reported lack of cross-tolerance between ATP-gated potassium channel openers and morphine (3). Nevertheless, in this study, we have provided evidence of cross-tolerance between these drugs. In addition, we also suggest that potassium channel openers have the potential to be used as analgesic agents, either alone or in combination with opioids, which may reduce the dose and adverse effects of opioids.

REFERENCES

1. McQuay H. Opioids in pain management. *Lancet* 1999; **353**: 2229–32.
2. Duty S, Weston AH. Potassium channel openers: pharmacological effects and future uses. *Drugs* 1990; **40**: 785–91.
3. Welch SP, Dunlow LD. Antinociceptive activity of intrathecally administered potassium channel openers and opioid agonists: a common mechanism of action. *J Pharmacol Exp Ther* 1993; **267**: 390–9.
4. Lohmann AB, Welch SP. ATP-gated potassium channel openers enhance opioid antinociception: indirect evidence for the release of endogenous opioid peptides. *Eur J Pharmacol* 1999; **385**: 119–27.
5. Christie MJ, Williams JT, North RA. Cellular mechanisms of opioid tolerance: Studies in single brain neurons. *Mol Pharmacol* 1987; **32**: 633–8.
6. Zhang G, Lagrange AH, Ronnekleivi OK, Kelly MJ. Tolerance of hypothalamic b-endorphin neurons to mu-opioid receptor activation after chronic morphine. *J Pharmacol Exp Ther* 1996; **277**: 551–8.
7. Gonzalez LG, Portillo E, Del Pozo E, Baeyens JM. Changes in [3H] glibenclamide binding to mouse forebrain membranes during morphine tolerance. *Eur J Pharmacol* 2001; **418**: 29–37.
8. Gross ER, Hsu AK, Gross GI. GSK-3 beta inhibition and potassium-ATP channel opening mediate acute opioid-induced cardioprotection at reperfusion. *Basic Res Cardiol* 2007; **102**: 341–9.
9. Mackler SA, Eberwine JH. Cellular adaptation to opiates alters ion channel mRNA levels. *Proc Natl Acad Sci* 1994; **91**: 385–9.
10. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animal. *Pain* 1983; **16**: 109–10.
11. Shibata M, Ohkubo T, Takahashi H, Inoki, R. Modified formalin test: characteristic biphasic pain response. *Pain* 1989; **38**: 347–52.
12. D'Amour FE, Smith DL. A method for determining loss of pain sensation. *J Pharmacol Exp Ther* 1941; **72**: 74–9.
13. Heapy CG, Jamieson A, Russell NJW. Afferent C-fibre and A-delta activity in models of inflammation. *Br J Pharmacol* 1987; **90**: 164.
14. Ben-Sreti MM, Gonzalez JP, Sewell RD. Effect of elevated calcium and calcium antagonists on 6,7-benzomorphan-induced analgesia. *Eur J Pharmacol* 1983; **90**: 385–91.

15. Bongiani F, Carla V, Moroni F, Pellegrini-Giampietro DE. Calcium channel inhibitors suppress the morphine-withdrawal syndrome in rats. *Br J Pharmacol* 1986; **88**: 561–7.
16. Del Pozo E, Caro G, Baeyens JM. Analgesic effects of several calcium channel blockers in mice. *Eur J Pharmacol* 1987; **137**: 155–60.
17. Welch SP, Olson KG. Opiate tolerance-induced modulation of free intracellular calcium in synaptosomes. *Life Sci* 1991; **48**: 1853–61.
18. Dogrul A, Yesilyurt O, Deniz G, Isimer A. Analgesic effects of amlodipine and its interaction with morphine and ketorolac-induced analgesia. *Gen Pharmacol* 1997; **29**: 839–45.
19. Dogrul A, Yesilyurt O. Effects of intrathecally administered aminoglycoside antibiotics, calcium-channel blockers, nickel and calcium on acetic acid-induced writhing test in mice. *Gen Pharmacol* 1998; **30**: 613–6.
20. Ramkumar V, El-Fakahany EE. Prolonged morphine treatment increases rat brain dihydropyridine binding sites: Possible involvement in the development of morphine dependence. *Eur J Pharmacol* 1988; **146**: 73–83.
21. Ramkumar V, El-Fakahany EE. Increase in [3H]nitrendipine binding sites in the brain in morphine-tolerant mice. *Eur J Pharmacol* 1984; **102**: 371–2.
22. Zharkovsky A, Totterman AM, Moisis J, Ahtee L. Concurrent nifedipine administration attenuates the withdrawal signs and the increase of cerebral dihydropyridine binding sites after chronic morphine treatment in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 1993; **347**: 483–6.
23. Johnson SM, Fleming WW. Mechanisms of cellular adaptive sensitivity changes: Applications to opioid tolerance and dependence. *Pharmacol Rev* 1989; **41**: 435–88.
24. Kennedy C, Henderson G. Chronic exposure to morphine dose not induce dependence at the level of the calcium channel in human SH-SY5Y cells. *Neuroscience* 1992; **49**: 937–44.
25. Ammer H, Nice L, Lang J, Schulz R. Regulation of G proteins by chronic opiate and clonidine treatment in the guinea pig myenteric plexus. *J Pharmacol Exp Ther* 1991; **258**: 790–6.
26. Wang L, Gintzler AR. Altered mu-opiate receptor-G protein signal transduction following chronic morphine exposure. *J Neurochem* 1997; **68**: 248–54.
27. Wang L, Medina VM, Rivera M, Gintzler AR. Relevance of phosphorylation state to opioid responsiveness in opiate naive and tolerant/dependent tissue. *Brain Res* 1996; **723**: 61–9.
28. Fleming WW. Cellular adaptation: Journey from smooth muscle cells to neurons. *J Pharmacol Exp Ther* 1999; **291**: 925–31.
29. Diaz A, Ruiz F, Florez J, Pazos A, Hurlle MA. Regulation of dihydropyridine-sensitive calcium channels during opioid tolerance and supersensitivity in rats. *J Pharmacol Exp Ther* 1995; **274**: 1538–44.
30. Chieng B, Christie MD. Local opioid withdrawal in rat single periaqueductal gray neurons in vitro. *J Neurosci* 1996; **16**: 7128–36.
31. Ingram SL, Vaughan CW, Bagley EE, Connor M, Christie MJ. Enhanced opioid efficacy in opioid dependence is caused by an altered signal transduction pathway. *J Neurosci* 1998; **18**: 10269–76.
32. Hack SP, Vaughan CW, Christie MJ. Modulation of GABA release during morphine withdrawal in midbrain neurons in vitro. *Neuropharmacology* 2003; **45**: 575–84.
33. Bagley EE, Gerke MB, Vaughan CW, Hack SP, Christie MJ. GABA transporter currents activated by protein kinase A excite midbrain neurons during opioid withdrawal. *Neuron* 2005; **45**: 433–45.
34. Ikeda K, Kobayashi T, Kumashiro T, Yano R, Sora I, Niki H. Molecular mechanisms of analgesia induced by opioids and ethanol: is the GIRK channel one of the keys. *Neurosci Res* 2002; **44**: 121–31.
35. Blednov YA, Stoffel M, Alva H, Harris RA. A pervasive mechanism for analgesia: activation of GIRK2 channels. *Proc Natl Acad Sci* 2003; **100**: 277–82.
36. Mitrovic I, Margeta-Mitrovic M, Bader S, Stoffel M, Jan LY, Basbaum AI. Contribution of GIRK2-mediated postsynaptic signaling to opiate and alpha 2-adrenergic analgesia and analgesic sex differences. *Proc Natl Acad Sci* 2003; **100**: 271–6.
37. Marker CL, Lujan R, Loh HH, Wickman K. Spinal G-protein-gated potassium channels contribute in a dose-dependent manner to the analgesic effect of mu- and delta- but not kappa-opioids. *J Neurosci* 2005; **25**: 3551–9.
38. Ingram SL, Fossum EN, Morgan MM. Behavioral and electrophysiological evidence for opioid tolerance in adolescent rats. *Neuropsychopharmacology* 2007; **32**: 600–6.
39. Khalilzadeh O, Anvari M, Khalilzadeh A, Sahebgharani M. Involvement of amlodipine, Diazoxide and Glibenclamide in development of morphine tolerance in mice. *Int J Neurosci* 2008; **118**: 503–18.