Effect of Potassium Channel Modulators on Morphine Withdrawal in Mice

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Abstract: The present study was conducted to investigate the effect of potassium channel openers and blockers on morphine withdrawal syndrome. Mice were rendered dependent on morphine by subcutaneous injection of morphine; four hours later, withdrawal was induced by using an opioid antagonist, naloxone. Mice were observed for 30 minutes for the withdrawal signs i.e., the characteristic jumping, hyperactivity, urination and diarrhea. ATP-dependent potassium (K⁺ ATP) channel modulators were injected intraperitoneally (i.p.) 30 minutes before the naloxone. It was found that a K⁺ ATP channel opener, minoxidil (12.5–50 mg/kg i.p.), suppressed the morphine withdrawal significantly. On the other hand, the K⁺ ATP channel blocker glibenclamide (12.5–50 mg/kg i.p.) caused a significant facilitation of the withdrawal. Glibenclamide was also found to abolish the minoxidil’s inhibitory effect on morphine withdrawal. The study concludes that K⁺ ATP channels play an important role in the genesis of morphine withdrawal and K⁺ ATP channel openers could be useful in the management of opioid withdrawal. As morphine opens K⁺ ATP channels in neurons, the channel openers possibly act by mimicking the effects of morphine on neuronal K⁺ currents.

Keywords: K⁺ ATP channels, minoxidil, glibenclamide, morphine dependence, opioid withdrawal
Introduction
The problem of dependence and tolerance is the major limiting factor in medical opioid usage.\(^1\) If this problem can be dissociated or tackled somehow, opioid use can again become popular as they are the most effective drugs for the management of pain.\(^2\) Extensive research is going on for the development of non-addicting opioids and/or agents that can prevent or reverse the addiction processes. New therapeutic agents are also explored for their ability to inhibit opioid-induced abstinence syndrome and self-administrative behavior.\(^3\) Some of the drugs used are based on specified mechanisms, and others have no rationale, indicating that the addiction processes may possibly involve multiple molecular mechanisms operating at different sites in the body.\(^4\)

While several mechanisms have been implicated for the development of opioid dependence and manifestation of the withdrawal syndrome, the management of the established withdrawal syndrome has remained largely restricted to the substitution of the causative agent by a less addictive or weaker opioid agonist, which has obvious limitations.\(^5,6\) In this regard, the use of clonidine, an alpha-2 agonist for the suppression of withdrawal effects, has met with limited success but has provided an important lead for future research to explore possible mechanisms that are distal to the opioid receptor and are affected during opioid dependence. Pharmacological manipulations of these mechanisms can yield therapeutic strategies that employ non-opioid agents for the control of opioid dependence and withdrawal.\(^7\) To evolve such alternative therapeutic modalities, it is imperative that a clear understanding of the role of neurotransmitters/second messengers and ionic fluxes across the membrane during the development of dependence and in the control of withdrawal symptoms is essential.\(^8,9\)

Extensive research work has been done to elucidate the role of various neurotransmitter systems (viz. opioidergic, catecholaminergic, cholinergic, serotonergic, GABAergic and glutamatergic systems) in the development of withdrawal syndrome.\(^10,11\) In addition, the importance of second messengers like cAMP and calcium has also been elucidated to some extent. As far as the ionic fluxes are concerned, the role of calcium and potassium has been considered important and explored by some researchers.\(^12-16\) Particularly, the T-type and L-type calcium channels have been implicated in the process of opioid dependence and withdrawal.\(^17,18\)

ATP-dependent potassium (K\(_{ATP}\)) channels have been reported to be involved in several actions of morphine following mu-receptor stimulation.\(^19\) K\(_{ATP}\) channel blockers have also been found to antagonize morphine analgesia.\(^20\) But the precise role of K\(_{ATP}\) channels in the genesis of opioid dependence and withdrawal syndrome remains to be clarified. Therefore, it was thought worthwhile to investigate the role of K\(_{ATP}\) channels using the channel modulators, namely an opener (minoxidil) and a blocker (glibenclamide), as pharmacological tools in morphine withdrawal syndrome.

Material and Methods

Animals
Male albino Swiss mice weighing 20–25 g were housed five per cage at room temperature under a standard light/dark cycle with free access to food and water. All the animals were acclimatized to the laboratory conditions for at least two days prior to the initiation of any experiment. Each animal was used for only one experiment. The experiments were performed between 9:00 a.m. and 5:00 p.m.

Drugs
Morphine sulphate was procured through the official agencies of Government of India. Naloxone hydrochloride, glibenclamide and minoxidil were supplied by Sigma-Aldrich Corporation. All drugs were dissolved in normal saline (0.9%).

Experimental procedure
Mice were rendered dependent on morphine by subcutaneous (s.c.) injection of morphine sulphate; withdrawal was induced four hours later by naloxone (s.c.), as per the method described elsewhere.\(^21,22\) The magnitude of withdrawal was evaluated by scoring different withdrawal signs within the next 30 minutes after naloxone challenge. A positive jumping response (where a mouse jumps more than four times during the observation period)\(^21\) was assigned a score of 4, hyperactivity response was a score of 3, diarrhea a score of 2 and urination a score of 1.

In the first series of experiments, morphine in a dose of 125 mg/kg followed 4 hours later by 10 mg/kg naloxone, produced a full-fledged withdrawal
(positive jumping) in 80%–100% of the animals. Thirty minutes before naloxone, normal saline (control group) or minoxidil in different doses was administered intraperitoneally (i.p.) to observe their influence on withdrawal.

In the second series of experiments, morphine at a dose of 100 mg/kg followed by 2 mg/kg naloxone induced full-fledged withdrawal in 20% of the animals. Thirty minutes before naloxone, saline (control group) or glibenclamide in different doses was administered (i.p.) to observe their effect on withdrawal.

In addition, we also tested the interaction between glibenclamide and minoxidil, and their effects on withdrawal. For this purpose, we administered minoxidil 50 mg/kg i.p. 30 minutes prior to naloxone (10 mg/kg) challenge to the morphine-treated (125 mg/kg) mice. Glibenclamide (25 mg/kg i.p.) was given 15 minutes prior to minoxidil.

**Statistical analysis**
Median scores of withdrawal were calculated for each group of five mice in the study. The significance of the difference between the withdrawal scores of two groups was calculated by nonparametric statistical analysis by employing the Mann-Whitney U-test. The difference between values were considered significant at \( P < 0.05 \).

**Results**
In this study, we investigated the role of drugs modifying the \( K_{ATP} \) channels in naloxone-precipitated morphine withdrawal syndrome. We used minoxidil, a \( K^+ \) ATP channel opener, and glibenclamide, a \( K^+ \) ATP channel blocker.

**Effect of minoxidil pre-treatment on naloxone-induced withdrawal**
Minoxidil at a dose of 12.5 mg/kg inhibited the naloxone-induced withdrawal signs so that stereotyped jumping was observed in 50%, hyperactivity in 80%, and diarrhea and urination in 90% of the animals; 10% of the animals did not show any withdrawal sign. The median score was found to be 8 \( (P > 0.05) \) compared to the control group, where the median score was 10.

Minoxidil pre-treatment at a dose of 25 mg/kg further inhibited withdrawal. The signs comprised of stereotyped jumping in 10%, hyperactivity in 60%, diarrhea in 40% and urination in 50% of the animals; 40% of the animals did not show any sign of withdrawal. The median score in this group was 6 \( (P < 0.01) \). A 50 mg/kg dose of minoxidil inhibited withdrawal so much that 50% of the animals showed no sign of withdrawal. Hyperactivity was observed in 12.5% and diarrhea and urination in 50% of the animals. The median score in this group was 1.5 \( (P < 0.01) \).

Therefore, minoxidil produced a dose-dependent inhibition of the withdrawal syndrome (Fig. 1).

**Effect of glibenclamide pre-treatment on naloxone-induced withdrawal**
Glibenclamide at a dose of 12.5 mg/kg facilitated the naloxone-induced withdrawal signs so that stereotyped jumping was observed in 40%, hyperactivity in 70%, and diarrhea and urination in all of the animals. The median score was found to be 6 \( (P > 0.05) \) compared to control group where median score was 3.

Glibenclamide pre-treatment at a dose of 25 mg/kg further facilitated withdrawal. The signs comprised of jumping in 60%, hyperactivity in 80%, diarrhea in 90% and urination in 100% of the animals. The median score in this group was 9 \( (P < 0.05) \). At a dose of 50 mg/kg, glibenclamide facilitated withdrawal so much that 60% of the animals showed the stereotyped jumping. Hyperactivity was observed in 90%, and diarrhea and urination in 100% of the animals. Median score in this group was 10 \( (P < 0.05) \).

Therefore, glibenclamide produced a dose-related facilitation of morphine withdrawal syndrome (Fig. 2).
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Glibenclamide abolished the inhibitory effects of minoxidil, as stereotyped jumping was observed in 60% of the animals. Hyperactivity was seen in 80%, and diarrhea and urination in 90% of the animals. No withdrawal sign was seen in 10% of the animals. Therefore it was interesting to note that glibenclamide could also block the inhibitory effect of minoxidil on the development of morphine withdrawal syndrome (Fig. 3).

Discussion

The opening of potassium channels shifts the membrane potential to the hyperpolarized state and the excitability of the cell is reduced. This mechanism forms the basis of the inhibitory effects of several transmitter substances, such as that of acetylcholine on the heart and enkephalins on central neurons, and is shared by the group of synthetic agents known as the potassium channel openers. It is now known that cromakalin, aprikalim, pinacidil, minoxidil, diazoxide and nicorandil exhibit potassium channel opening properties. These agents act specifically on K\textsubscript{ATP} channels. While other types of potassium channels, like those blocked by tetraethyammonium and quinine, are not affected.

One would expect that K\textsubscript{ATP} channel openers would hyperpolarize the membranes in the central nervous system and make the neurons less excitable. One of their pharmacological effects is their anticonvulsant activity.

Our results show that minoxidil causes a significant dose-dependent inhibition of all the observed withdrawal signs like jumping, hyperactivity, diarrhea and urination. In an earlier study, cromakalin and diazoxide were also found to inhibit several signs of morphine withdrawal (number of jumps, episodes of fore-paw tremors and body weight loss). The exact mechanism by which these K\textsubscript{ATP} channel openers inhibit morphine withdrawal is difficult to specify. However, as morphine opens K\textsubscript{ATP} channels in the neuronal cells, possibly K\textsubscript{ATP} channel openers mimic the effects of morphine on neuronal K\textsuperscript{+} currents and therefore may act as substitutes for this drug during morphine withdrawal.

As stated earlier, morphine withdrawal is inhibited by several non-opioid drugs such as clonidine, which acts on alpha-2 adrenoceptors; (-)-N6-(R-phenylisopropyl)-adenosine (R-PIA), which acts on adenosine A\textsubscript{1} receptors; and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), which acts on 5-HT\textsubscript{IA} receptors. Stimulation of all these receptors also promotes the opening of the K\textsubscript{ATP} channels in neurons and possibly inhibits morphine withdrawal as a consequence.

In our study, glibenclamide showed a dose-dependent facilitation of withdrawal. This was also

![Figure 2. Effect of glibenclamide pre-treatment on naloxone (2 mg/kg) precipitated withdrawal. Note: *P < 0.05 as compared to control.](image)

![Figure 3. Effect of glibenclamide on minoxidil-induced inhibition of naloxone (10 mg/kg) precipitated withdrawal.](image)
statistically significant. Similarly, other studies showed that glibenclamide antagonizes nicotinic-induced facilitation of morphine withdrawal.35 Glibenclamide has also been shown to antagonize morphine analgesia,20 as it specifically blocks the $K_{ATP}$ channels in the central nervous system through which morphine acts.19

Next, the interaction of glibenclamide with minoxidil was tested. It was found that glibenclamide could also abolish minoxidil’s effect on morphine withdrawal. A previous study conducted on memory recall processes also observed a similar interaction, where diazoxide blocked the effect of glibenclamide.36

The study clearly indicated that $K_{ATP}$ channels play an important role in the genesis of morphine withdrawal, and their pharmacological modulation could be valuable in the management of opioid withdrawal.

Conclusion

The $K_{ATP}$ channel opener minoxidil was found to suppress morphine withdrawal dose-dependently, while the $K_{ATP}$ channel blocker glibenclamide caused a dose-dependent facilitation of morphine withdrawal. The results are statistically significant and give convincing evidence that the $K_{ATP}$ channels play an important role in the genesis of opioid withdrawal. As the study suggests that potassium channel openers could be useful in the management of opioid withdrawal, clinical trials are needed to confirm their efficacy for this purpose.

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Disclosures

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