Evaluation of acute effects of melatonin on ethanol drinking in ethanol naïve rats

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Introduction
Ethanol dependence impacts millions of individuals worldwide. It is a complex disorder that will require the use of different therapeutic approaches to treat the disease effectively. There has been some progress with pharmacotherapy for ethanol-dependent individuals. However, there remains a critical need for the development of novel and additional therapeutic approaches. The effect of ethanol could be mediated by many different neurotransmitters within the central nervous system. Reinforcing properties of drugs are associated with their capacity to increase neuronal activity in critical brain areas. Cocaine, amphetamine, ethanol, opiates, cannabinoids, and nicotine all reliably increase extracellular fluid dopamine levels in the nucleus accumbens.11

Melatonin (N-acetyl-5-methoxytryptamine), is the hormone produced by the pineal gland. It has been shown to have several potential therapeutic benefits. Its acute toxicity is extremely low as seen in both animal and human studies. It may cause minor adverse effects, such as headache, insomnia.

ABSTRACT
Objective: The objective was to evaluate the acute effect of melatonin on ethanol drinking in ethanol naïve rats and to determine the specificity of the effect of melatonin on ethanol intake as compared to an intake of plain tap water or sugar water.

Materials and Methods: A total of three experiments (2 weeks duration each) using different drinking solutions (ethanol, plain tap water, sugar water) was conducted in individually housed male wistar rats of 5 weeks age. Each animal had access to bottles containing drinking solutions for 2 h a day. In each experiment, on day 1, day 2, day 4, day 5, day 8, day 9, day 11, day 12 rats received drinking solutions. Each individual rat received single doses of saline, melatonin (50 mg and 100 mg/kg), and naltrexone on day 2, 5, 9, and 12, 1-h before receiving drinking solution. The order of drug administration is permuted such a way that each animal received the drugs in a different order in different experiments.

Results: Melatonin has significantly decreased ethanol consumption by the rats and effect is dose-dependent. Naltrexone also has caused a significant reduction in the ethanol consumption. The maximum reduction in ethanol consumption was seen with melatonin 100 mg/kg dose compared to melatonin 50 mg/kg and naltrexone. There was no statistically significant effect of melatonin on plain water and sugar solution intake.

Conclusions: Melatonin decreases ethanol consumption in ethanol naïve rats. The effect of melatonin is similar to naltrexone affecting selectively ethanol consumption, but not plain water and sugar water consumption.

KEY WORDS: Dependence, ethanol, melatonin, naltrexone

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In animal studies, lethal dose 50 could not be established. Even 800 mg/kg bodyweight (high dose) did not have lethal effects.[10] Melatonin is categorized by the USA Food and Drug Administration as a dietary supplement. Oral bioavailability of melatonin is around 30–50%. It has a half-life of 30–50 min. Melatonin administration can entrain the circadian clock by a direct action on the suprachiasmatic nucleus (SCN). Studies suggest an important role of circadian genes in limbic regions of the brain, outside of the central circadian pacemaker in the SCN, and rhythms in the development of ethanol dependence.[31]

Ethanol modulates the activity of the mesolimbic dopamine system by acting through opioid receptors. Recently, a study has reported the reversal effect of melatonin on the expression of morphine-induced rewarding effect.[18] Based on these facts, we predicted that melatonin would reduce ethanol drinking by its effect on the opioid system or circadian rhythm.

A major goal of ethanol research is to understand the neural underpinnings associated with the transition from ethanol use to ethanol dependence. The preclinical research uses a wide array of techniques to assess the molecular, cellular, and behavioral events associated with the transition to ethanol dependence. These techniques are used in conjunction with animal models that mimic various components of ethanol dependence in humans.[35] This study was planned to examine the acute effect of melatonin on ethanol drinking in ethanol naïve rats and to determine the specificity of the effect of melatonin on ethanol intake as compared to an intake of plain tap water or sugar water.

Materials and Methods

Male wistar rats (5 weeks of age) were procured from the Central Animal House, Kasturba Medical College, Mangalore. This study was conducted after getting approval from the Institutional Animal Ethics Committee. All animals were allowed to habituate to the animal facilities for 5 days before initiation of the experiment. During the acclimation period, rats were housed 5 per cage in standard polycarbonate cages. Rooms were controlled for temperature (21 ± 1°C) and photoperiod (12:12 L: D). Food and water were provided ad libitum, except when ethanol was substituted for water for 2 h as described below. All tests were performed between 09:00–14:00 to minimize the confounding effects of circadian rhythms. All procedures were performed in compliance with CPCSEA guidelines.

Drugs and Drinking Solutions

Ethanol

The ethanol-drinking solution was prepared from absolute anhydrous ethanol 99.9% ethanol (Manufactured by: Changshu Yangyuan Chemicals China) diluted to 10% (v/v) using tap water. The 10% sugar-water drinking solution was prepared from food-grade plain white sugar (Manufactured by: Soubhagyalaxmi Sugars Ltd, India) dissolved in tap water at 10% (W/V) concentration.

Study Drug

Melatonin tablet (Aristo Pharmaceuticals) was dissolved in water at concentrations 6 mg/ml and administered orally (50 mg/kg and 100 mg/kg). Doses of melatonin were selected based on previous literature showing the effects of melatonin in opioid dependence.[16]

Standard Drug

Naltrexone: 1 mg/kg.

Ethanol Consumption in Ethanol Naïve Rats

A within-subjects design was used. A total of three experiments using different drinking solutions (ethanol, plain tap water, sugar water) was conducted. Duration of each experiment was 2 weeks. Each animal was individually housed and had access to bottles containing drinking solutions. The drinking solution bottles remained in place for 2 h. Intakes were recorded at the end of a 2 h session. Precautions were taken to prevent any leakage. In addition, a specially designed metal cup was placed inside the rat cages in such a way that, it can collect fluid leaked from the drinking bottle if any. The volume of fluid in the metal cup (if present) was taken into consideration while measuring the amount of ethanol/fluid consumed by the rats. After the 2 h period, the drinking solution bottles were replaced with water bottles.

In each experiment, on day 1, day 2, day 4, day 5, day 8, day 9, day 11, day 12 (i.e., Monday–Tuesday, and Thursday–Friday, with Wednesday off and weekend off for 2 weeks) rats received drinking solutions [Table 1]. Each individual rat received saline, melatonin (two doses), and naltrexone, 1-h before receiving drinking solution. The schedule of the drug administration is shown in Table 2. All drugs are administered orally. Thus, before an animal receives the drug, they always had 1-day of access to drinking solution without drugs, and 1 or 2 days where they were left undisturbed [Table 1]. The rationale for allowing the rats to experience the drinking solutions without drugs on alternate days is to reduce the chance that a taste aversion might develop from always pairing a drinking solution with drugs. The rationale for leaving the animals undisturbed is to separate episodes of the 2 days cycle and to reduce the chance for carry over effects of drugs. Each experiment began with 16 animals, and the order in which the four drugs are administered is permuted such that each of the 16 animals received the drugs in a different order. This design will ensure that each rat will serve as its own control.[9]

Statistical Analysis

The effect of different drugs on the mean intake of drinking solutions by the rats in all the three experiments were compared using paired student “t”-test. A (P < 0.05) was considered as significant.

Results

Table 3 shows the effect of melatonin, naltrexone, and normal saline on ethanol drinking in male wistar rats. Melatonin has significantly decreased ethanol consumption by the rats and effect is dose-dependent. Naltrexone also has caused a significant reduction in the ethanol consumption. The maximum reduction in ethanol consumption was seen with melatonin 100 mg/kg dose compared to melatonin 50 mg/kg and naltrexone. There was an increase in ethanol consumption after the administration of normal saline. Normal saline was the first drug administered (i.e., on the 2nd day of ethanol consumption) in this experiment [Table 2]. Therefore, the ethanol consumption before normal saline administration, which is on the 1st day was
lower as ethanol consumption in the beginning is usually lower due to the bitter taste.

Table 4 shows the effect of melatonin, naltrexone, and normal saline on plain water consumption in male Wistar rats. There was no statistically significant effect of melatonin on plain water intake. However, naltrexone has caused a significant increase in the plain water consumption, which could be due to lower predrug water consumption that is, before the administration of naltrexone.

Table 5 shows the effect of melatonin, naltrexone, and normal saline on a sugar solution drinking in male Wistar rats. There was an increase in the consumption of sugar solution due to the palatability of sugar solution, which is acting as a natural reward. Hence, there was a progressive increase in the consumption of sugar solution during the 2 weeks course of the experiment. Melatonin 50 mg/kg being the last drug given in this experiment, the maximum increase in the consumption of sugar solution was seen after its administration. This result shows that melatonin or naltrexone did not have any effect on normal reinforcement of consumption of sweet fluids.

### Discussion

The drinking in the dark (DID) model to evaluate the effect of drugs on ethanol drinking in rats has shown to be displaying predictive validity with opioid and dopaminergic mechanism, as described by Kamdar et al. The same model is followed in the present study. The DID model is useful to evaluate the process of rapid initiation of the high level of drinking, which ultimately progresses to ethanol dependence.

Our study has shown that melatonin has significantly reduced ethanol consumption in ethanol naïve rats, the effect being in a dose-dependent manner. This effect of melatonin seems to be specific as it did not have any effect on intake of plain water and sugar solution. Naltrexone, an opioid antagonist, has proven to reduce the ethanol consumption clinically, as well as in several preclinical studies. In this study, also naltrexone has significantly reduced ethanol consumption, and the effect was specific to ethanol as it did not affect plain water or sugar solution consumption.

Both melatonin and naltrexone did not have any effect on normal reinforcement for drinking sweet fluids or consumption of plain water. Ethanol, plain water, and sugar water consumption are influenced by different motivational pathways; that is motivation for ethanol is different from motivation for natural rewards. The fact that naltrexone did not affect consumption of plain water or sugar water confirms that opioid signaling is involved in ethanol consumption, but not in natural motivation to drink water or palatable fluids like sugar water. Our observation has shown that similar to naltrexone, melatonin also selectively affects ethanol drinking without having a significant effect on plain water or sugar water consumption. Hence, these results suggest that melatonin also affects ethanol consumption, possibly through opioid mechanisms (may be indirectly), similar to naltrexone.
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Table 4:
Effect of drugs on plain water drinking in rats (n=16)

| Drugs            | Plain water consumption (ml) | t    | P    | CI     |
|------------------|-----------------------------|------|------|--------|
|                  | Before the drug administration |       |      |        |
| Normal saline    | 1.34±0.31                   | 0.80 | 0.44 | -0.80-0.37 |
| Melatonin 50 mg/kg | 1.56±0.23                   |      |      |        |
| Melatonin 100 mg/kg | 1.03±0.14                   | 0.73 | 0.45 | -0.42-0.86 |
| Naltrexone       | 0.63±0.11                   | 2.58 | 0.02 | -0.91-0.09 |
|                  | After the drug administration |       |      |        |
| Normal saline    | 2.06±0.17                   | 2.00 | 0.09 | -0.90-0.03 |
| Melatonin 50 mg/kg | 1.56±0.23                   |      |      |        |
| Melatonin 100 mg/kg | 1.03±0.14                   | 0.73 | 0.45 | -0.42-0.86 |

Values were expressed as mean±SEM. Student t-test. SEM=Standard error of mean, CI=Confidence interval

Table 5:
Effect of drugs on sucrose solution drinking in rats (n=16)

| Drugs            | Sugar water consumption (ml) | t    | P    | CI     |
|------------------|-----------------------------|------|------|--------|
|                  | Before the drug administration |       |      |        |
| Naltrexone       | 5.69±0.44                   | 4.04 | 0.004 | -5.35-1.65 |
| Normal saline    | 9.19±0.97                   |      |      |        |
| Melatonin 100 mg/kg | 11.31±0.89                  | 1.85 | 0.09 | -2.59-0.19 |
|                  | After the drug administration |       |      |        |
| Naltrexone       | 11.31±0.89                  | 1.85 | 0.09 | -2.59-0.19 |
| Normal saline    | 10.33±2.06                  | 0.56 | 0.58 | -1.28-0.75 |
| Melatonin 50 mg/kg | 13.27±0.98                  | 4.73 | <0.001 | -2.62-0.98 |

Values were expressed as mean±SEM. Student t-test. SEM=Standard error of mean, CI=Confidence interval

Alternatively, it can be argued that melatonin reduced ethanol consumption by causing sedation or affecting locomotor activity. However, the fact that melatonin selectively affects ethanol drinking, but not plain water or sugar solution rules out this possibility. To the best of our knowledge, there were no other studies, which evaluated the effect of melatonin on ethanol drinking.

Dopamine signaling is known to be involved in reinforcing properties of dependence-producing agents including ethanol dependence. The dopaminergic mesolimbic system plays a significant part in the motivational and reinforcement mechanisms related to behavior. Ethanol increases dopaminergic transmission in the mesolimbic pathway and increases the firing rate of dopaminergic neurons enhancing dopamine release. The transient surge in dopamine level in these areas occurring with the administration of dependence-producing agents contributes to reinforcement properties. A transient surge in dopamine also occurs when rodents taste sweet fluids, which may underlie reinforcement of sugar water drinking in rodents.

Direct evidence of a role for dopamine in ethanol reward comes from the finding that rats that operantly self-administer ethanol will stimulate its release in the nucleus accumbens. During chronic ethanol abuse, larger amounts of ethanol may need to be consumed to evoke dopamine release, in order to obtain the pleasurable effects of ethanol intake. During ethanol withdrawal, dopamine release will be reduced, thereby reducing the firing of related neurons leading to dysphoria, malaise, and depression. Ethanol, acting through opioid receptors in the ventral tegmental area and nucleus accumbens, modulates the activity of the mesolimbic dopamine system. Melatonin has shown to be reversing the morphine-induced rewarding effects. Hence, we can postulate that melatonin may have an effect on opioid receptors. Thus, melatonin may modulate ethanol consumption by acting through opioid receptors.

The limitations of our study should be considered. The model used in our study, may be helpful to evaluate the effect of drugs on the process of rapid initiation in ethanol drinking, which may be one of the preliminary steps in the development of ethanol dependence. However, we could not procure rodent strains known to have high ethanol drinking for our study. The present study used a single bottle instead of two bottle choices, which are traditionally used in DID models. However, this issue of using the single bottle as the limitation was examined in detail by Rhodes et al. They have studied the ethanol consumption in two groups of rats: One group using a single bottle of ethanol and the other group going for two bottle choices (one bottle of ethanol and another bottle of plain water). The results of their study showed consumption of ethanol by the rats did not differ much in two groups. The only difference was that single bottle animals reached a blood ethanol level much faster than two bottle choice animals. Our study did not aim at evaluating the effect of melatonin on other important features of alcoholism such as dependence and tolerance. These aspects will be evaluated in our future studies.

Conclusions

Melatonin has shown to decrease ethanol consumption in ethanol naïve rats, the effect being dose-dependent. The effect of melatonin is similar to naltrexone, affecting selectively ethanol consumption, but not plain water and sugar water consumption. Both melatonin and naltrexone did not have any effect on normal reinforcement for drinking sweet fluids or consumption of plain water. Hence, the results of this study suggest that melatonin may affect the process of rapid initiation of ethanol drinking,
which may be one of the preliminary steps in the development of ethanol dependence.

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**Conflicts of Interest**

There are no conflicts of interest.

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