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Original Article

Phenothiazine-type antipsychotics may attenuate naloxone-precipitated withdrawal jumping in morphine-dependent mice

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ABSTRACT

Objectives: Withdrawal of opioids is usually associated with intolerable aversive symptoms. Our objective was to evaluate the efficacy of phenothiazine-type antipsychotics for reducing withdrawal symptoms during morphine abstinence.
Methods: Adult NRL mice were rendered physically dependent on morphine by escalating the doses of subcutaneous morphine for 3 days. Withdrawal jumping was precipitated by a subcutaneous injection of naloxone (50 mg/kg) on day 4. In study I, on an equimolar basis, we compared the efficacy of six phenothiazine antipsychotics in saline on reducing morphine withdrawal symptoms. One hour before naloxone injection, the mice were assigned to receive intramuscular (i.m.) saline or one of the six phenothiazine-type antipsychotics (0.3 μmol/kg). After naloxone injection, the tested mouse was immediately placed in a transparent acrylic cylinder, and the severity of withdrawal symptoms was assessed, via a computer connected to the floor of the cylinder, by counting the number of the withdrawal jumps over a 30-minute interval. In study II, we performed a dose–response test in these six phenothiazine antipsychotics (0.03, 0.3, and 3 μmol/kg, i.m., for each test drug) on the inhibition of naloxone-precipitated morphine withdrawal jumping.
Results: We found that all six phenothiazine-type antipsychotics attenuated the morphine withdrawal jumps, as compared with saline ($p < 0.05$). The effect is dose-dependent, with the potency ranking order as follows: fluphenazine = trifluprom azine > chlorpromazine = perphenazine > promazine = thioridazine ($p < 0.05$).
Conclusions: All six phenothiazine-type antipsychotics could attenuate morphine withdrawal symptoms; in particular, fluphenazine and trifluprom azine may potentially be the more appropriate candidates for the treatment of morphine withdrawal symptoms.

1. Introduction

Morphine is one of the most effective analgesics for moderate to severe pain. However, chronic morphine use usually has unwanted adverse effects, such as analgesic tolerance and physical dependence.[1] Tolerance causes morphine to gradually lose its effect, and usually it requires escalating doses to produce pain relief; morphine dependence is generally characterized by serious affective and physical disorders, such as irritability, anxiety, nausea, chills, sweating, diarrhea, sneezing, and muscular and abdominal pain, upon discontinuation. These symptoms are extremely aversive and become an obstacle to abstinence treatment.[2] Therefore, treatments that circumvent such dependence symptoms would be greatly beneficial to the abstainers.[2] Various pharmacological treatments have been studied and suggested to facilitate a safe transition to tide over the relapse prevention program. However, the available options are still limited.

Phenothiazine-type antipsychotics are a group of typical psychoactive drugs commonly used in management of acute

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psychosis and other serious psychiatric conditions. These drugs are also used for treating postoperative or chemotherapy-associated nausea and vomiting.[3] In addition, they are known to have antihistaminic effects and the ability to potentiate analgesics, sedatives, and general anesthetics.[3,4] These properties seem to be able to weaken a number of the opioid withdrawal symptoms. Moreover, phenothiazine-type antipsychotics possess potent anti-Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) activities.[5–7] CaMK II is suspected to play an important role in the development of opioid analgesic tolerance and physical dependence.[8] Phenothiazine-type antipsychotics could be bound to calmodulin to prevent the formation of Ca^{2+}/calmodulin complexes, thus blocking the activation of downstream Ca^{2+}/calmodulin dependent kinases.[6] One of the phenothiazine-type antipsychotics, trifluoperazine, has been demonstrated to attenuate morphine-induced conditioned place preference, a model for studying the reinforcing effects of drugs with dependence liability,[9,10] and also attenuate morphine analgesic tolerance in rats.[11] These pieces of evidence imply that phenothiazine-type antipsychotics drugs may be useful in treating somatic withdrawal symptoms. Therefore, the purpose of the present study was to investigate six phenothiazine-type antipsychotics for alleviation of morphine withdrawal symptoms in a rodent model.

2. Materials and methods

2.1. Animals

Adult male NRL mice, weighing between 25 g and 30 g, were purchased from the National Laboratory Animal Center, Taiwan, and used for the experiments. They were housed in groups of six in a climate-controlled room maintained at 21°C with approximately 50% relative humidity for at least 1 week before the experiment. Lighting was provided on a 12-hour light/dark cycle (lights on at 6:00 AM), with food and water available *ad libitum* except during the time of testing. All tests were performed in accordance with the recommendations and policies of the International Association for the Study of Pain. The protocol was approved by the Animal Investigation Committee of Chi Mei Medical Center.

2.2. Drugs

Morphine (hydrochloride) was purchased from the National Bureau of Controlled Drugs, Taiwan, whereas naloxone (hydrochloride dehydrate) and six different phenothiazine antipsychotics were purchased from Sigma-Aldrich (St. Louis, MO, USA). All drugs were prepared in 0.9% saline. Morphine and naloxone were injected subcutaneously, whereas antipsychotics were injected intramuscularly. The site for subcutaneous injection was 2 cm below the neck at the midline of the back of the tested mouse, whereas the site for intramuscular injection was the biceps femoris and semitendinosus of the right hind leg of the tested mouse. The volume of injection was 3 mL/kg for all medications at both injection sites.

2.3. Induction of morphine dependence

The mice were rendered dependent on morphine by administering morphine subcutaneously thrice daily (9:00 AM, 1:00 PM, and 5:00 PM) in consecutively increasing daily dose for 3 days, in accordance with previous reports.[12,13] Briefly, doses of morphine were given to mice as follows: 20 mg/kg × 3 on day 1, 40 mg/kg × 3 on day 2, and 80 mg/kg × 3 on day 3. On the contrary, six mice in the sham group received subcutaneous saline instead of morphine during the induction period.

2.4. Precipitation of morphine withdrawal jumping by naloxone

Morphine withdrawal jumping was precipitated by naloxone (50 mg/kg, s.c.) at 10:00 AM on day 4, as described in our previous reports.[12] Immediately after naloxone injection, animals were placed in individual Plexiglas observation cages (30 cm high × 11 cm long × 11 cm wide), and the number of jumping responses, defined as the simultaneous removal of all four paws from the horizontal surface, was counted by a computer connected to the sensor-equipped cage floor for 30 minutes.

2.5. Study design

In the first part of the study, we measured the effects of the six different phenothiazine type antipsychotics (fluphenazine, triflupromazine, chlorpromazine, perphenazine, promazine and thioridazine) on morphine withdrawal jumping. In the morning (9:00 AM) of day 4, 1 hour before naloxone injection, the mice received one of the six antipsychotics (0.3 μmol/kg i.m.), whereas the control group received saline injection.

In the second part of the study, we evaluated the dose–response effects of these six phenothiazine antipsychotics at doses of 0.03, 0.3, and 3 μmol/kg on attenuation of morphine withdrawal jumping. The test drugs were given 1 hour before naloxone injection, i.e., at 9:00 AM on day 4.

2.6. Statistical analysis

Data were expressed as mean ± SEM. In the first part of the study, we used the Student *t* test for individual comparison between the test drug- and saline-treated groups. In the second part of the study, we used analysis of variance (ANOVA) test to verify the linear trends between the dose–response curves. The ranking potency of the drugs was evaluated using two-way ANOVA with *post-hoc* Scheffe's comparisons. Statistical significance was set at $p < 0.05$.

3. Results

Following escalating doses of morphine for 3 days, all mice showed physical dependence on morphine, because naloxone precipitated significantly more withdrawal jumps during the 30-minute observation period in the saline group, whereas naloxone did not precipitate any jumping among mice in the sham group (Fig. 1). Pretreatment with every one of the six antipsychotics (0.3 μmol/kg, i.m.) 1 hour before naloxone injection significantly decreased the number of withdrawal jumping (Fig. 1; $^{*}p < 0.05$, $^{**}p < 0.01$, Student *t* test for individual comparison to saline group).

The dose–response relationship between each of the six phenothiazine-type antipsychotics was tested by the responses to three different doses (0.03, 0.3, and 3 μmol/kg, i.m.) of each of the tested drugs (Fig. 2). The ANOVA test for the trend revealed that the attenuation of the withdrawal jumping was dose-dependent for each drug ($p < 0.001$). The relative potencies between these six drugs were analyzed by two-way ANOVA, followed by *post-hoc* Scheffe's comparisons. It was revealed that the potency of fluphenazine was equal to that of triflupromazine ($p = 0.516$); triflupromazine was more potent than chlorpromazine ($p = 0.03$); chlorpromazine was equal to perphenazine ($p = 0.611$); perphenazine is more potent than promazine ($p = 0.044$); and promazine was as potent as thioridazine ($p = 0.657$) (Fig. 2). In summary, the ranking potency was as follows: fluphenazine = triflupromazine > chlorpromazine = perphenazine > promazine = thioridazine.