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Antinociceptive activity of coniine in mice

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ABSTRACT

Ethnopharmacological relevance: Hemlock was used as an analgesic in certain ethnopharmacological traditions and there has been no record about the antinociceptive effect of coniine which is the major alkaloid compound of Hemlock.
Aim of this study: The present study was undertaken to evaluate the possible antinociceptive activity of coniine.
Material and methods: Antinociceptive activity of coniine was tested dose in Hotplate test (thermal pain model) and in Writhing test (chemical pain model) in different nociception models.
Results: Coniine caused a prolongation in reaction time in Hotplate test at 20 mg/kg dose. In addition, it was observed that coniine decreased the number of writhes in Writhing test. Both data indicated an antinociceptive effect of coniine. A rotarod test was also conducted in order to clarify, whether this activity was related with a loss of locomotion or with an analgesic activity. None of the chemical agents at those doses used in experiments caused a loss of locomotor activity. It was also shown that antinociceptive effect of morphine was potentialized by coniine which was inhibited by nicotinic receptor blocker mecamylamine (1 mg/kg).
Conclusion: Coniine has antinociceptive effect via the nicotinic receptors. A pharmacological assessment about the painless death of Socrates due to Hemlock (coniine) toxicity has also been presented by using this data.

1. Introduction

Coniine is the main toxic alkaloid ingredient of poison Hemlock, *Conium maculatum* L., a toxic plant well known since ancient times (Baumann, 1993). Poisoning of Socrates with a poison mixture was well documented and poison Hemlock had been identified as the main ingredient (Bloch, 2001). Also coniine is among the first isolated alkaloids (Cordell et al., 2001). With the progress in chemistry in the second half of 19th century, isolation and identification of chemical structure of coniine was established. Because of its popularity as a toxic substance since historical times, pharmacological studies concerning coniine had started quite early, in the 1890s. First publication concerning physiological actions of coniine was published in 1898 (Moore and Row, 1898). Pharmacological action mechanisms of coniine is well studied, revealing that coniine exerts its toxic effects via nicotinic receptors (Forysth et al., 1996; Cooper et al., 1996). A ganglionic stimulation is followed by a ganglionic blockade and finally suffocation occurs because of the paralysis of phrenic nerves (Bowman and Sanghvi, 1963). Although it was once a popular research molecule, after realizing that it is too toxic in humans, coniine became a subject in areas such as toxicology in husbandry (Candrian et al., 1984) or biosynthesis of alkaloids nowadays (Reynolds, 2005). There are various publications concerning involvement of nicotinic acetylcholine receptors on analgesia (Bannon et al., 1998; Lawand et al., 1999). However, there has been no record about coniine in antinociception.

The aim of this study was to investigate possible antinociceptive effects of coniine and whether this effect was mediated via nicotinic receptors. In order to evaluate antinociceptive effects of coniine, thermal and chemical pain models, namely Hotplate test and Writhing test, were performed.

This study also aims to make a contribution to a historical event. Death of Socrates with poison Hemlock had been well documented in history. It has been narrated by Plato and other authors as Socrates experienced no pain during his exile with a mixture of poison (Jowett, 1998). Ingredient of this poison potion was suggested as poison Hemlock mainly, but for some, it may also include opium (Bloch, 2001). In our study possible potentiation between these two ingredients in analgesia is also assessed.

2. Materials and methods

2.1. Animals

Swiss-Albino male mice weighing 25–35 g were used for antinociception experiments. All mice were kept in a room where

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temperature (24 ± 2 °C) and relative humidity (55 ± 15%) were kept within stable limits. A light–dark cycle of 12 h dark/12 h light was applied. Animals had free access to food and water, except during the time of experiments. The Guiding Principles in the Care and Use of Laboratory Animals together with The Recommendations from the Declaration of Helsinki were strictly adhered to the execution of all the procedures described within this study. This project was approved by the Institutional Experimental Animal Care and Use Ethics Committee of Hacettepe University (Approval Number: 2008/12-5; date: 14.02.2008) before the commencement of any intervention.

2.2. Hotplate test

Hotplate test was conducted as the thermal pain model (MacDonald, 1946). A hotplate system with a surface temperature of 52 °C was set for the assessment of analgesia. Prior to treatment, the reaction time of each mouse (licking or retraction of the hindpaw or jumping response) was recorded three times as an indicator of hypo- or hyper-sensitive mice. Those animals were excluded from experiments. Records were taken in 10 min intervals and up to a period of 1 h. Increase in reaction time is accepted as an indicator of analgesia. In order to avoid possible tissue injury, cut-off time was chosen as 30 s. Each experimental group was formed by 6 mice.

2.3. Writhing test

Writhing test was performed as the chemical pain model (Siegmund, 1957). Abdominal writhing is a model of visceral pain and nociceptive stimulus was induced with an 0.6% acetic acid solution intraperitoneally injected to mice (0.1 ml/10 g of body weight). Number of writhes were recorded for 15 min starting 5 min after i.p. injection. Each experimental group was formed by 6 mice.

2.4. Experimental design

Chemical and thermal nociceptive stimulus were given 10 min after the morphine and/or coniine application. Initially drug applications were performed solitarily and effects of morphine and coniine were assessed apart from each other. Then, morphine and coniine were applied in coincidence in order to assess their possible interactions in antinociception. In order to evaluate the possible role of nicotinic receptors on antinociception, saline and standard drug group received mecamylamine alone.

2.5. Toxicity

Animals were monitored for toxicity of drugs during experiments and up to 1 week. Any occasion of death or existence of complications were recorded. In addition, animals treated with drugs were tested with a rotarod device with a rotation speed of 12 r.p.m. in order to determine the possible effects of drugs at the applied doses on motor performance. Outcomes of this test were used for the assessment of locomotor activity of mice. Any negative alteration from the control was accepted as a loss of locomotor capacity.

2.6. Drugs

During the experiments following drugs were used; morphine HCl (Sigma, USA) at 0.1, 1, 5 and 10 mg/kg, mecamylamine HCl (Merck Sharp & Dohme) at 1 and 3 mg/kg, coniine (Sigma, USA) at 10 and 20 mg/kg doses and acetic acid (Merck, USA). The drugs were dissolved in saline and were administered intraperitoneally in a volume of 0.1 ml/10 g of body weight. Dilutions from stock solution were performed on a daily basis.

2.7. Statistical analysis

All data are expressed as the mean ± SEM. Data were analysed with Student's *t*-test, Mann–Whitney *U*-test and one way repeated analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test. Results were assigned as significant when $P < 0.05$. Graphics and statistics were prepared at Graph Pad Software Prism 3.0 and Microsoft Excel.

3. Results

3.1. Hotplate test

Coniine at 10 mg/kg dose increased reaction time in Hotplate test, however, this prolongation was not significant (control: 9 ± 0.45 s, coniine: 12.83 ± 1.38 s, $P > 0.05$). The prolongation became evident at dose of 20 mg/kg (coniine 16.33 ± 2.4 s, $P < 0.05$; Fig. 1). Starting from 25 mg/kg toxicity symptoms such as fasciculations, dyskinesia, convulsions and death were observed in a dose dependent manner. Accordig to this observation we chosed 20 mg/kg dose for coniine that is not toxic and yet maximum in potency for the rest of experiments.

As presented in Fig. 2 morphine also increased reaction time in 0.3 and 1 mg/kg doses (control: 9 ± 0.45 s, morphine 0.3: 10.33 ± 1.28 s, morphine 1: 11.83 ± 1.01 s, $P > 0.05$) and became statistically significant at 5 mg/kg dose (morphine 5: 16.67 ± 2.25 s, $P < 0.05$). Morphine at 10 mg/kg avoided mice to show any response against thermal stimulus until cut-off time (30 s; $P < 0.01$).

Increase in reaction time caused by application of coniine in 10 mg/kg dose was not changed by the concomitant administra-

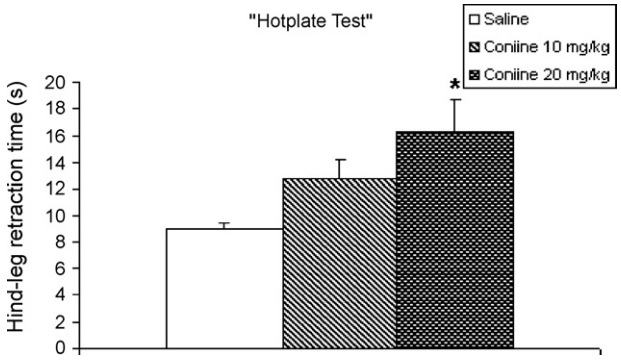

Fig. 1. Antinociceptive activity of coniine in Hotplate test (*$P < 0.05$).

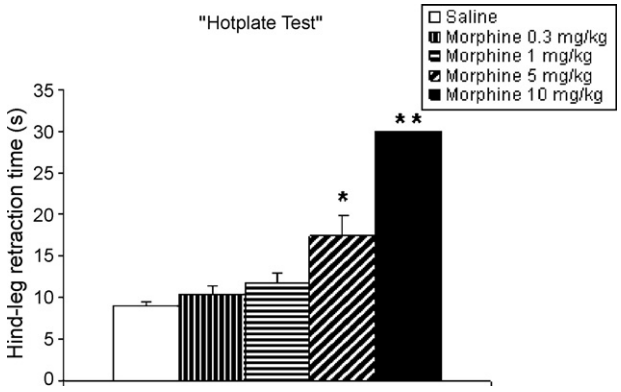

Fig. 2. Antinociceptive activity of morphine in Hotplate test (*$P < 0.05$; **$P < 0.01$).