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Cholinergic activation affects the acute and chronic antinociceptive effects of morphine

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HIGHLIGHTS

- Donepezil and rivastigmine enhance and prolong acute antinociceptive effects of morphine.
- Muscarinic receptors are involved in these effects of cholinesterase inhibitors.
- Cholinergic drugs inhibit the development but not expression of tolerance to morphine analgesia.

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ABSTRACT

Current studies indicate that the cholinergic and opioid systems interact to modulate pain. In the present work, we investigated the influence of the cholinesterase inhibitors, donepezil (0.5; 1 or 3 mg/kg, i.p.) and rivastigmine (0.03; 0.5 or 1 mg/kg, i.p.), on the acute antinociceptive effects of morphine (5 mg/kg, i.p.) in the hot plate test in mice. Herein, both inhibitors were found to enhance and prolong the analgesic effects of morphine without affecting latencies themselves. In an extension of this work, we determined which cholinergic receptors subtype mediates the enhancement of analgesic effects of morphine, following inhibition of cholinesterases. In this part of the study, scopolamine (0.5 mg/kg, i.p.), a muscarinic cholinergic receptors antagonist, but not mecamylamine (3 mg/kg, i.p.), a nicotinic cholinergic receptors antagonist, reversed the enhancing effects of donepezil (3 mg/kg, i.p.) and rivastigmine (1 mg/kg, i.p.) on the morphine antinociception. Moreover, both cholinesterase inhibitors attenuated the development of tolerance to the antinociceptive effects of morphine. In contrast, acute administration of donepezil (3 mg/kg, i.p.) or rivastigmine (1 mg/kg, i.p.) on the day of expression of tolerance, had no effect on the already developed morphine tolerance. What is more, in both set of experiments, rivastigmine was slightly more potent than donepezil due to the broader inhibitory spectrum of this drug on acetylcholine degradation. Thus, our results suggest that the cholinesterase inhibitors, donepezil and rivastigmine, may be administered with morphine in order to enhance the latter's analgesic effects for the treatment of acute and chronic pain.

1. Introduction

The synaptic level of endogenous neurotransmitter acetylcholine (ACh) is regulated by cholinesterases - enzymes which inactivate the endogenous neurotransmitter. There are two known forms of cholinesterases: acetylcholinesterase and butyrylcholinesterase [30]. Inhibition of cholinesterases increase the level of ACh in the synaptic cleft, and thus stimulate the cholinergic system influencing two major types of cholinergic receptors, nicotinic (N) and muscarinic (M). Cholinesterase inhibitors such as donepezil and rivastigmine, are approved by the U.S. Food and Drug Administration (FDA) for the treatment of mild to moderate Alzheimer's disease [71,76]. Donepezil is a highly selective and reversible acetylcholinesterase inhibitor with relatively low affinity to butyrylcholinesterase [33,74]. Rivastigmine is pseudo-reversible inhibitor of both acetyl- and butyrylcholinesterase [52].

Morphine is widely used in the management of acute and chronic pain [7,59,64]. Analgesic effects of morphine are mainly due to the agonist activity at the μ opioid receptors [56], however, current literature indicates that the cholinergic and opioid systems interact to modulate pain [1,42,67]. Acquired data has revealed that the cholinesterase inhibitor, neostigmine, when added to fentanyl (μ opioid receptor agonist), prolonged surgical analgesia [5], while another cholinesterase inhibitor, physostigmine, when combined with morphine, enhanced analgesia in

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the postoperative period in humans [9,72]. Moreover, neostigmine, in combination with morphine, produced more profound analgesia in the hot plate test in rats, and the observed effect were synergistic in action [3]. Other data [51] indicated that this neostigmine-morphine interaction measured in the hind-paw withdrawal test in rats, was simply additive. Furthermore, cholinesterase inhibitors might possess analgesic activity themselves [15,44,58].

At the level of the central nervous system, N and M cholinergic receptor agonists modulate the perception of pain in experimental animals by affecting descending inhibitory pain pathways [22,38,39]. In several studies, direct injection of ligands of cholinergic receptors have revealed their role in analgesia at the level of the centrally located brain structures such as the periaqueductal gray (PAG) [31], rostral ventromedial medulla (RVM) [54], hypothalamus [23], nucleus raphe magnus (NRM) [11] and amygdala [55]. Furthermore, small cholinergic neurons and choline acetyltransferase mRNA have been identified in the nuclei of rostral ventrolateral medulla (RVLM) [45]. Moreover, immunohistochemical analysis have shown that the major source of cholinergic innervation in RVLM or NRM is pedunculopontine tegmentum nucleus (PPT) [27,75]. In addition, pharmacological activation of cholinergic neurons in PPT using nicotine, the N cholinergic receptors agonist, induced antinociception. This effect was reversed by direct administration of both mecamylamine (N cholinergic receptors antagonist) and pirenzepine (M cholinergic receptors antagonist) to the NRM [37].

As mentioned earlier, morphine is widely used in the management of chronic pain. Still, chronic use of this opioid has some limitations which affect clinical usefulness of the drug [34,70]. The most important of these with regard to the therapy of pain seems to be the development of tolerance to the antinociceptive effects of morphine. This brings about the effect that administration of increasing doses of the drug is needed over time to maintain the same level of pain relief [19]. Although the mechanisms underlying morphine-induced tolerance are not completely understood, literature data reveal a number of adaptive mechanisms responsible for these processes [18,29,48,53,57,63]. However, to our knowledge, the majority of previous and on-going studies have not dealt with the role of the cholinergic system in the mechanism of this phenomenon. In spite of the available data, it is difficult to clearly determine whether substances that increase ACh level in the synaptic cleft can prevent the development of tolerance to the analgesic effects of morphine. As it has been shown in laboratory animals, physostigmine did not alter the development of tolerance to the analgesic effects of morphine in the tail-flick test [10], while nicotine attenuated the development of morphine tolerance in the same test [32]. Moreover, another study has indicated that scopolamine, a non-selective M cholinergic receptors antagonist, also attenuated the development of morphine tolerance in the tail-flick test in rats [78]. On the other hand, Monteiro et al. [49] reported that a novel M_2 receptor agonist, LASSBio-981, prevented and reversed morphine-induced tolerance in the rat model of neuropathic pain.

Herein, we assessed the influence of cholinesterase inhibitors, donepezil and rivastigmine on the acute antinociceptive effects of morphine, as well as their potential antinociceptive activity in the hot plate test in mice. Involvement of N or M cholinergic receptors in the influence of cholinesterase inhibitors on the acute antinociceptive effects of morphine was also investigated. Additionally, we also examined the influence of both drugs on the development and expression of morphine-induced tolerance. Furthermore, the locomotor activity test was performed in order to exclude the possibility that the antinociceptive action of all compounds could be related to non-specific disturbances in the locomotor activity of the animals.

2. Materials and methods

2.1. Animals

Male Swiss mice (HZL, Warsaw, Poland), weighing 25–30 g at the initiation of the experimental procedure, were used in our experiments. These mice were housed five per cage with standard laboratory feed (Bacutil, Motycz, Poland) and water ad libitum. Furthermore, the animals were kept under a 12/12 h light/dark cycle and in a controlled temperature (22 ± 2 °C). The mice were adapted to the laboratory conditions for at least one week prior experimentation. All behavioral studies were performed between 9:00 a.m. and 2:00 p.m. In our work, different cohorts of mice were used for the hot-plate and locomotor activity tests. The experimental protocols and housing conditions were performed according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, the European Community Council Directive of November 2010 for Care and Use of Laboratory Animals (Directive 2010/63/EU), and were approved by the Local Ethics Committee.

2.2. Drugs

Morphine (5 mg/kg; Polfa, Kutno, Poland), donepezil hydrochloride (0.5, 1, 3 mg/kg; Sigma-Aldrich, St. Louis, MO, USA), rivastigmine hydrochloride (0.03, 0.5, 1 mg/kg; Sigma-Aldrich, St. Louis, MO, USA), scopolamine hydrochloride (0.5 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) and mecamylamine hydrochloride (3 mg/kg; Abcam Biochemicals, Bristol, UK) were dissolved in sterile saline (0.9% NaCl) and given intraperitoneally (i.p.). Morphine and cholinergic agents were freshly prepared on each day of experimentation and were given in a volume of 2 ml/kg. Saline was administered in an equivalent volume and by the same route. Morphine dose and its administration regimen was based on the literature data [47]. Doses of cholinergic compounds were selected from the previous work [24,25] where mecamylamine (3 mg/kg, i.p.) and scopolamine (0.5 mg/kg, i.p.) effectively blocked the effects of cholinesterase inhibitors without affecting locomotor activity of the animals.

2.3. Behavioral tests

2.3.1. Hot-plate test

Twenty-four hours before all experiments, mice were habituated to the experimental procedure (two measurements) in order to minimize novelty-induced antinociception [62]. To determine nociceptive reaction, mice were placed on a hot-plate (Ugo Basile, Italy) maintained at a constant temperature of 55 ± 1 °C with a cut-off time of 20 s to avoid tissue damage [46]. Before drug administration, the baseline latency response (average for two measurements in seconds) for the mouse to lift either of the hind paws or a jump with all four feet off of the hot-plate induced by the thermal stimulus was first measured. The animals were then injected with cholinergic agents and saline/morphine according to the experimental paradigm, and the post-treatment latency responses were determined. The antinociceptive effects of morphine or saline with/without cholinergic agents were expressed as a percent maximum possible effect (% MPE), which was calculated according to the following equation: $[(T^1 - T^0) / (20 - T^0)] \times 100$, where T^0 and T^1 are the pre-drug and post-drug latencies for hot-plate response, respectively.

2.4. Experimental procedures

2.4.1. The effect of cholinesterase inhibitors, donepezil or rivastigmine on the acute antinociception induced by morphine in the hot-plate test in mice. Influence of scopolamine or mecamylamine on donepezil or rivastigmine effects

On the day of experiment, the baseline latency response (average of two measurements in seconds) was first assessed. The animals were then injected with donepezil (0.5; 1 or 3 mg/kg, i.p.) or rivastigmine (0.03; 0.5 or 1 mg/kg, i.p.) in combination with saline or morphine (5 mg/kg, i.p.). Of note: donepezil or rivastigmine were given 20 min prior saline/morphine administration. The control group received saline in the same volume and by the same route. Post-treatment latency