PHARMACOLOGY

INFLUENCE OF OXCARBAZEPINE ON THE ANTINOCICEPTIVE ACTION OF MORPHINE AND METAMIZOLE IN MICE

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Abstract: Numerous methods of management applied in order to obtain higher therapeutic efficacy of drugs with minimum adverse effects include taking advantage of interactions taking place between individual agents. Analgesics are combined with drugs belonging to other therapeutic groups, including, more and more frequently, antiepileptic agents. The influence of oxcarbazepine (10 mg/kg) on the antinociceptive effect of morphine (10 mg/kg) and metamizole (500 mg/kg) was investigated in mice using the hot-plate and tail-flick tests. All drugs were injected intraperitoneally (*i.p.*). Oxcarbazepine was administered 30 min prior to the injection of analgesic drugs. The reactions to noxious stimuli were measured 30, 60 and 90 min after the administration of an analgesic. The study was further conducted for 10 days with repeated drug doses. Single administration of oxcarbazepine enhanced the antinociceptive effect of a single dose of morphine, and 10-day administration led to a decrease of morphine tolerance in the hot-plate test. Oxcarbazepine administered in a single dose did not affect significantly the antinociceptive effect of metamizole in either of the tests. Multiple administration of oxcarbazepine enhanced the antinociceptive effect of metamizole in the hot-plate test. Oxcarbazepine alone, administered in a single and repeated doses, demonstrated an antinociceptive effect, but only for the hot-plate test, which indicates involvement of supraspinal structures in antinociception.

Keywords: oxcarbazepine, morphine, metamizole, antinociception, mice

Pain is a serious burden for the organism. The knowledge of pain, its mechanisms and treatment is very extensive, but despite that it is still insufficient. New analgesics characterized with higher therapeutic efficacy and causing fewer adverse effects are being sought. One of the directions of this search involves attempts at combination of typical analgesics with adjuvant drugs, whose main indications are different, but which, in addition to their primary effect, demonstrate antinociceptive activity or enhance the effects of analgesic agents.

In adjuvant analgesia, antiepileptic drugs of second generation (e.g. gabapentin, pregabalin, lamotrigine, oxcarbazepine) and first generation (phenytoin, valproate, carbamazepine) play an important role.

Oxcarbazepine, a ketone derivative of carbamazepine, is a relatively novel antiepileptic drug. The mechanism of action of oxcarbazepine involves primarily modulation of the sodium channels (1), inhibition of the high-treshold calcium (2) channels, enhancement of the potassium rectifier current (3) and reducing glutaminergic transmission (4, 5).

Antineuralgic proporties of oxcarbazepine have been demonstrated in animal models of neuropathic (6) and inflammatory pain (7) and in painful diabetic neuropathy (8-10), trigeminal neuralgia (11) and paroxysmal painful symptoms in multiple sclerosis (12).

The mechanisms of analgesic action of oxcarbazepine are not fully understood. Beside a blockade of ion currents, there is an evidence indicating that some receptors, e.g. adenosine A1 receptor and α_2-adrenoreceptor are also involved in analgesic action of oxcarbazepine (13, 14).

The presented study is concerned with the impact of oxcarbazepine on the antinociceptive effect of morphine and metamizole in mice. The analgesics are often used in therapy, however, the mechanism of interaction with oxcarbazepine is still unknown.

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EXPERIMENTAL

Materials

The experiments were carried out on Swiss male mice (18-27 g). The animals were grouped in cages under normal laboratory conditions at a temperature of 20-21°C, and natural day/night cycle with free access to commercial chow food and water. All experiments were performed between 8.30 a.m. and 3.30 p.m. The drugs were injected intraperitoneally (*i.p.*) dissolved in 0.9% NaCl. Oxcarbazepine (Trileptal, Novartis Pharma AG, Switzerland) at the dose of 10 mg/kg was administered 30 min before the analgesic drugs: morphine (Morphinum sulfas, Polfa Warszawa, Poland) at the dose of 10 mg/kg and metamizole (Pyralginum, Polpharma S.A. Starogard Gdański, Poland) at the

dose of 500 mg/kg. Oxcarbazepine doses were selected on the basis of literature data (15, 16), and those of the analgesics – on the basis of previous own experience (17, 18). In the prolonged administration experiments, all drugs were administered for 10 days.

Methods

The hot-plate test was derived from that of Eddy and Leimbach (19). A plastic cylinder (20 cm high, 14 cm in diameter) was used to confine a mouse to a heated plate surface. The temperature of the plate was maintained at $50 \pm 0.4°C$. The maximum time of exposure was 60 s. Latencies to hind paw licking were determined 30, 60 and 90 min after the administration of an analgesic. The groups consisted of 8 – 12 mice each.
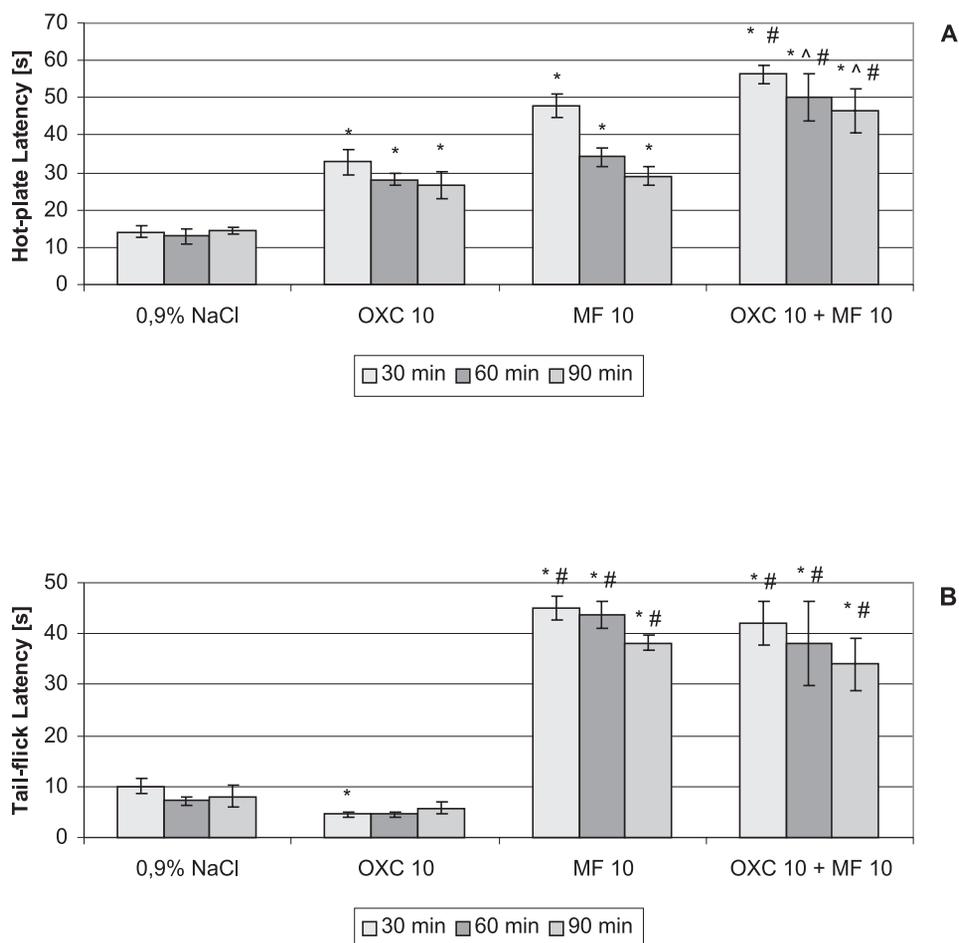

Figure 1. The antinociceptive effect in hot-plate (A) and tail-flick (B) tests after *i.p.* administration (single dose) of saline (0.9 % NaCl), morphine 10 mg/kg (MF 10), oxcarbazepine + morphine (OXC 10 + MF 10); * significantly different from the control group receiving 0,9 % NaCl, p < 0.05, ^ significantly different from the morphine – treated group, p < 0.05, # significantly different from the oxcarbazepine – treated group, p < 0.05, ANOVA-test
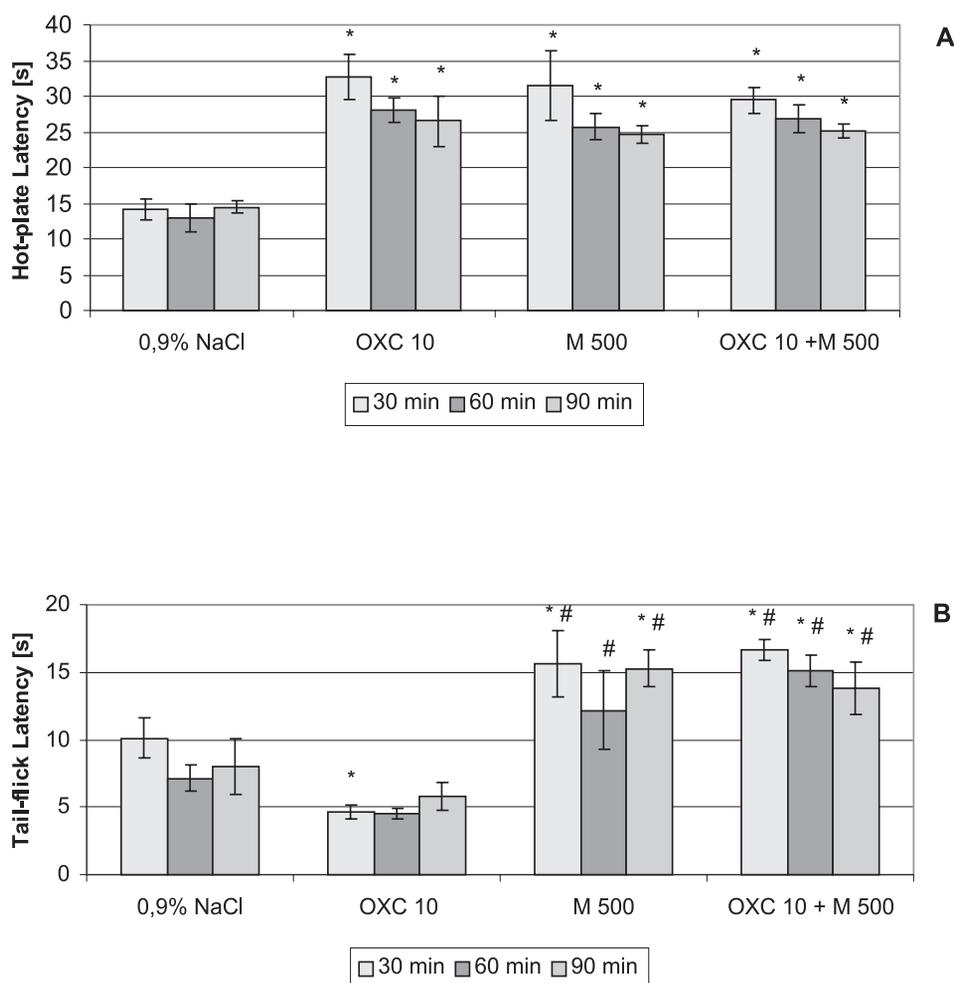
Figure 2. The antinociceptive effect in hot-plate (A) and tail-flick (B) tests after *i.p.* administration (single dose) of saline (0.9 % NaCl), metamizole 500 mg/ kg (M 500), oxcarbazepine + metamizole (OXC 10 + M 500); * significantly different from the control group receiving 0,9 % NaCl, p < 0.05, # significantly different from the oxcarbazepine – treated group, p < 0.05, ANOVA-test

The tail-flick test of D'Amour and Smith (20) modified for mice was used. The mice were placed in retention boxes. The latency of tail withdrawal was determined by focusing a radiant heat source on the tail at about 3 cm from the tail tip. The heat source temperature was $70 \pm 0.2^{\circ}C$ and the maximum time of exposure was 60 s. This noxious stimulation did not cause tissue damage. The latency was measured 30, 60 and 90 min after the administration of an analgesic. Each group consisted of 8 – 12 mice.

All procedures used in these studies were approved by the Ethical Committee of Medical University of Łódź, Poland (licence 115, permission Ł/BD/161).

Statistical analysis

Group data are expressed as the means ± SEM. The normality of the distribution was checked using Kolmogorow-Smirnow test with Lillieforse correction. Inter-group comparison was carried out using two-way analysis of variance (ANOVA) test. Statistical evaluation was performed by means of LSD test. Statistical differences were considered significant if p value was lower than 0.05.

RESULTS

Oxcarbazepine administered alone in a single dose (10 mg/kg), as well as in multiple ones (10 mg/kg/day administered for 10 days) increased the latency of reaction to nociceptive stimuli in comparison with mice receiving 0.9% NaCl, but only with respect to the hot-plate test (Fig. 1A, 2A, 3A and 4A). No significant effect of oxcarbazepine on reaction latency in comparison with control animals

receiving 0.9% NaCl was observed in the tail-flick test (Fig. 1B, 2B, 3B and 4B).

Morphine at a single dose (10 mg/kg) prolonged in a statistically significant manner the reaction time in both the hot-plate and tail-flick tests in comparison with mice which received 0.9% NaCl. The effect was higher even several times (Fig. 1A and B). After 10 doses of morphine, the latency of reaction to nociceptive stimuli was prolonged significantly in comparison with the group of untreated animals in both tests. However, the response after multiple doses of morphine was considerably less pronounced than after a single dose (Fig. 3A and B). Oxcarbazepine administered to mice in a single dose of 10 mg/kg dose 30 min before the administration of morphine prolonged significantly the latency of nociceptive reaction to the hot-plate test in mice

(Fig. 1A). The single dose of oxcarbazepine administered with morphine did not affect the action of morphine in the tail-flick test (Fig. 1B).

Oxcarbazepine administered for 10 days (10 mg/kg/day) in a combination with morphine (10 mg/kg/day) prolonged significantly nociceptive reaction latency in comparison with animals treated with morphine alone, but only in the hot-plate test. This effect was observed in the measurements after 30 and 60 min (Fig. 3A). The tail-flick test did not demonstrate a significant effect of oxcarbazepine on the reaction latency in animals who also received morphine (Fig. 3B).

Metamizole used in a single dose (500 mg/kg) prolonged significantly the latency of nociceptive reaction in both tests. The effect was evident in the hot-plate test (Fig. 2A) and weaker (30 and 90 min
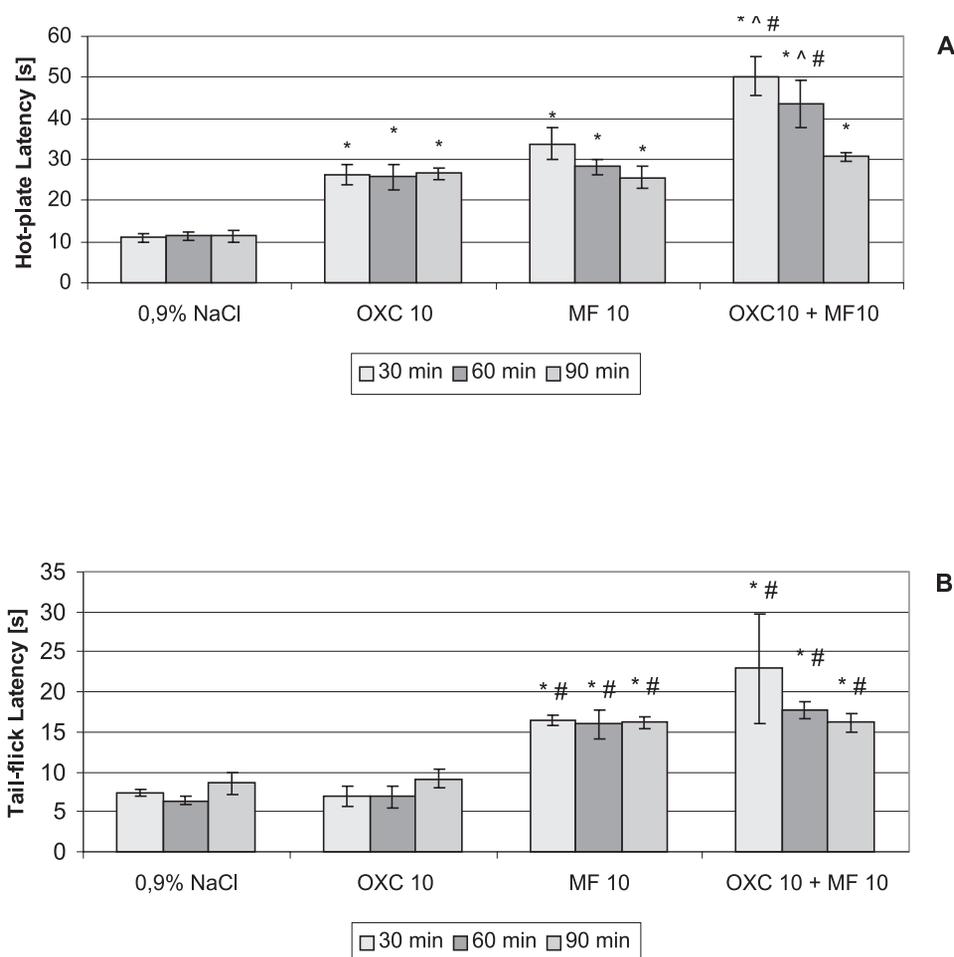
Figure 3. The antinociceptive effect in hot-plate (A) and tail-flick (B) tests after *i.p.* administration (multiple dose) of saline (0.9 % NaCl), morphine 10 mg/kg (MF 10), oxcarbazepine + morphine (OXC 10 + MF 10); * significantly different from the control group receiving 0,9 % NaCl, $p < 0.05$, ^ significantly different from the morphine – treated group, $p < 0.05$, # significantly different from the oxcarbazepine – treated group, $p < 0.05$, ANOVA-test
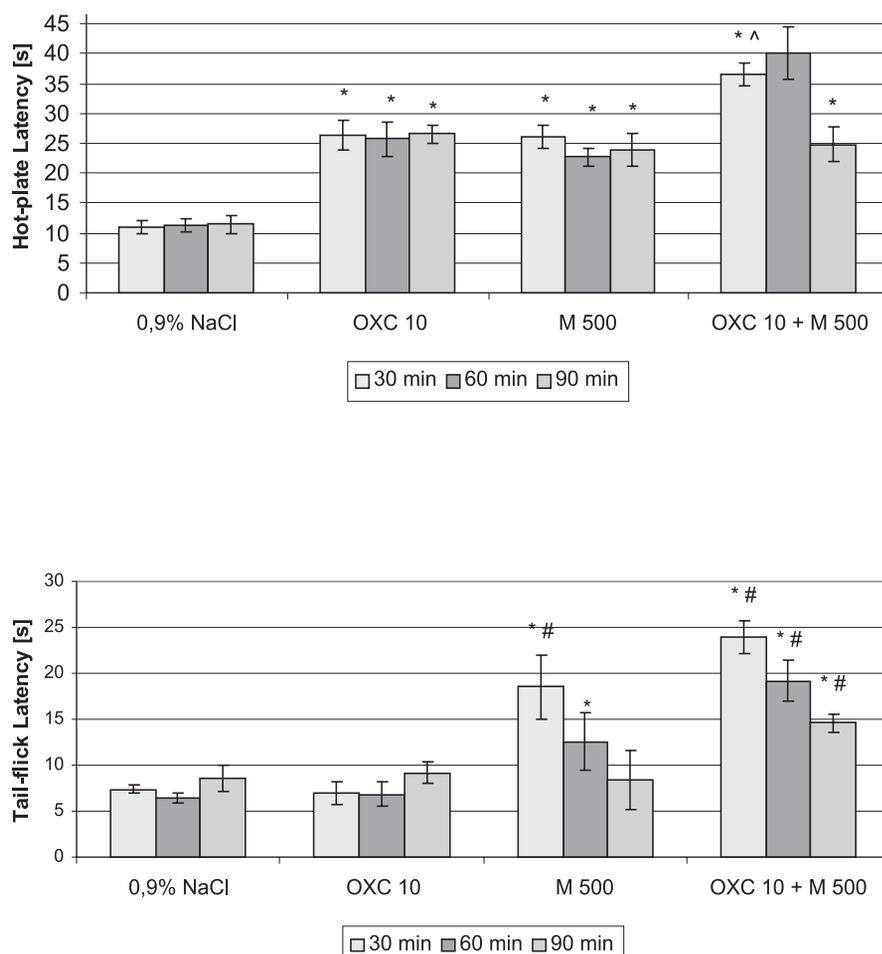
Figure 4. The antinociceptive effect in hot-plate (A) and tail-flick (B) tests after *i.p.* administration (multiple dose) of saline (0.9 % NaCl), metamizole 500 mg/kg (M 500), oxcarbazepine + metamizole (OXC 10 + M 500); * significantly different from the control group receiving 0,9 % NaCl, p<0.05, ^ significantly different from the metamizole- treated group, p < 0.05, # significantly different from the oxcarbazepine – treated group, p < 0.05, ANOVA-test

after the administration analgesic) in the tail-flick test (Fig. 2B). Repeated use of metamizole (500 mg/kg/day) demonstrated a weaker antinociceptive effect than after a single dose in both tests (Fig. 4A and B). In tail-flick test, this effect was observed in measurements after 30 and 60 min (Fig. 4B).

The single dose of oxcarbazepine administered with metamizole did not affect the action of metamizole in any test (Fig. 2A and B). Repeated (10 days) use of oxcarbazepine together with metamizole significantly prolonged the latency of nociceptive reaction in mice in comparison with the group of animals which received metamizole alone, but only in hot-plate test. Reduced sensitivity to noxious stimuli was observed in the measurements after 30 and 60 min (Fig. 4A).

DISCUSSION

Among the systems involved in antinociception, the opioid system plays the most important role. Morphine is the classic agent activating this system. The mechanism of analgesic effect of morphine involves stereospecific binding to opioid receptors, predominantly μ, and, to a lesser extent, δ and κ. Stimulation of the μ receptors leads to a decrease of intracellular cAMP levels. As a result of stimulation of the δ receptor, the potassium channels open, which leads to hyperpolarization and inhibition of nociceptive transmission. Agonism to all three receptor subtypes (μ, δ and κ) is associated with closing the calcium channels, which leads to inhibition of nociceptive neurotransmitters

(dopamine, acetylcholine, substance P) release (21). Morphine exerts its antinociceptive effect at various levels of the nervous system: it raises the nociceptive threshold in the cerebral cortex, inhibits nociceptive transmission in the thalamus, inhibits presynaptic substance P release from neuron I in the spinal dorsal horns, activates serotoninergic neurons in the descending pathways.

Morphine inhibits also the activity of adenylate cyclase activated by prostaglandins. This mechanism is responsible for enhanced antinociceptive effect after the administration of morphine in combination with cyclooxygenase inhibitors and is used successfully e.g. in the so-called analgesic ladder. Morphine-induced antinociception involves spinal and supraspinal structures.

In our studies, we have demonstrated the antinociceptive effect of morphine in both tests (hot-plate and tail-flick). The tail-flick test is a method which allows to assess the involvement of spinal cord structures in a nociceptive reaction, whereas in the hot-plate test, the nociceptive reaction is coordinated predominantly by supraspinal structures (22). Thus, the involvement of both the spinal cord and the supraspinal structures in the effect of morphine has been confirmed.

Both after a single dose and after 10 doses of morphine, both in the hot-plate and in the tail-flick test, significantly prolonged latency of nociceptive reaction was observed in mice in comparison with untreated animals. However, the response was much less pronounced after multiple doses than after a single dose of morphine, which indicates the development of tolerance to the analgesic effect of the drug.

Our studies have demonstrated a significant increase of the antinociceptive effect of morphine administered in combination with oxcarbazepine, both after single and multiple doses, but only in the hot-plate test. No such effect has been observed in the tail-flick tests, Thus, the observed effects indicate that, the mechanism of analgesic interaction of oxcarbazepine with morphine is bound up with supraspinal structures.

In case of multiple morphine and oxcarbazepine dosing, a reduction of opioid tolerance by oxcarbazepine was observed.

The synergism between morphine and oxcarbazepine may result from the mechanism of action of both drugs with respect to their effect on calcium and potassium channels. The role of calcium channels in antinociception has been extensively documented and described. Reduction of intracellular concentration of calcium ions is responsible for the antinociceptive effect of calcium channel blockers (23-25).

Modification of the function of potassium channels by administration of ATP-dependent potassium channel blockers may lead to attenuation or abolition of antinociception (26), and their opening – to induction or enhancement of antinociceptive effect (27-29). The enhancement of morphine-induced effect by oxcarbazepine may have a character of a pharmacodynamic interaction taking place at the level of calcium and potassium channels.

In our experiments, oxcarbazepine reduced the development of tolerance to the antinociceptive effect of morphine.

The mechanism of morphine tolerance reduction by oxcarbazepine seems to be due to similar effect of morphine and oxcarbazepine on cAMP levels. Tolerance to opioids develops, among others, as a result of decrease of their inhibitory effect on cAMP (30-32). Blocking of protein G controlled calcium channels by oxcarbamazepine is associated with a decrease of cAMP level and may lead to restoration of low neuronal cAMP concentration, and, consequently, to reduction of tolerance.

Oxcarbazepine administered in a single dose did not affect significantly the antinociceptive effect of metamizole in either of the tests. Repeated co-administration of oxcarbazepine with metamizole caused a more potent effect than metamizole alone. Metamizole administered to mice repeatedly demonstrated a weaker antinociceptive effect than after a single dose. The observed effect may suggest the development of tolerance to the drug. Metamizole – a non-narcotic analgesic agent, has a primary antinociceptive effect involving inhibition of cyclooxygenases activity, particularly in the CNS. However, there is evidence that also involvement of the opioid system can have considerable therapeutic significance (33, 34). The development of morphine-like tolerance to the antinociceptive effect of metamizole has also been demonstrated for repeated use of the drug (33, 35, 36).

Increasing the effect of metamizole by oxcarbazepine may be similar in character and probably also in the underlying mechanism, to the effect of the drug on morphine tolerance.

Oxcarbazepine alone, administered in single as well as repeated doses, demonstrated an antinociceptive effect, but only for the hot-plate test. No such effect was observed in the tail-flick test. The studies of Kiguchi et al. also revealed inefficacy of oxcarbazepine in the tail-flick test in normal mice. However, that effect was revealed in diabetic animals (37). Oxcarbazepine inhibits the activity of voltage-dependent sodium channels, which plays a particular role in its anticonvulsant effect.

The mechanism of analgesic action of oxcarbazepine has not been elucidated yet. An important role may be played by adenosine receptors A1. Recent studies proved that anti-hyperalgesic effects of oxcarbazepine in inflammatory pain are attenuated by treatment with adenosine receptor antagonists (13, 38). That mechanism is probably connected with the central effect due to oxcarbazepine's interaction with central A1 and A2 receptors.

It seems that α_2 receptors are engaged in the analgesic activity of oxcarbazepine. In the rat model of inflammatory pain, it was indicated that yohimbine (selective α_2-adrenoreceptor antagonist) significantly depresses the effect of oxcarbazepine whereas clonidine (α_2-adrenoreceptor agonist) increases analgesic action (14, 39).

A study by Stepanowic et al. (40) indicated that opioidergic mechanisms are not involved in the antihyperalgesic effects of oxcarbazepine because naloxone did not alter the antihyperalgesic effects of oxcarbazepine in inflammatory pain. However, the antinociceptive effect of oxcarbazepine observed in our experiments is associated rather with modification by the drug of calcium and potassium channels involved in this process by influencing the levels of cAMP. The antinociceptive effect of oxcarbazepine is probably exerted at the level of supraspinal structures, which is indicated by its effectiveness in the hot-plate test. In that mechanism an important role can be played by α_2 adrenoreceptor because it is also an anatomic place for adenosine A1 receptors and agonists of opioidergic receptors. Stimulation of the opioidergic system leads to a decrease of cAMP and inhibition of calcium ions inflow.

CONCLUSIONS

1. Oxcarbazepine administered in a single as well as repeated doses, demonstrated an antinociceptive effect connected with supraspinal structures.

2. Single administration of oxcarbazepine enhanced the antinociceptive effect of a single dose of morphine.

3. Multiple administration of oxcarbazepine led to a decrease of morphine tolerance.

4. Repeated administration of oxcarbazepine increased the antinociceptive effect of metamizole in the hot-plate test.

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