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PHARMACOLOGY

INFLUENCE OF TIAGABINE ON THE ANTINOCICEPTIVE ACTION OF MORPHINE, METAMIZOLE AND INDOMETHACIN IN MICE

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Abstract: The influence of tiagabine at a dose of 3.2 mg/kg (single administration) and at a dose of 1.2 mg/kg (multiple administration – 10 days) on the antinociceptive effect of morphine (10 mg/kg), metamizole (500 mg/kg) and indomethacin (10 mg/kg – single dose and 1.4 mg/kg – multiple doses) was investigated in mice using the hot-plate and tail-flick tests. All drugs were injected intraperitoneally. Tiagabine was administered to mice 30 min before the analgesic drugs. Measurement of the reaction to a noxious stimulus was performed 60, 90 and 120 min after administration of tiagabine. The study was further conducted for 10 days with repeated drug doses. Tiagabine and morphine administered in single doses demonstrate an additive antinociceptive effect in the hot-plate test and a slightly synergistic effect in the tail-flick test. A single administration of tiagabine slightly increased the antinoceptive action of metamizole and indomethacin in both tests, but that effect is less pronounced than the antinociceptive action of tiagabine alone. Repeated administration of tiagabine with morphine abolishes the tolerance to morphine analgesia. Both single and repeated administration of tiagabine alone exerted the antinociceptive effect in the hot-plate test.

Keywords: antinociception; tiagabine; morphine; metamizole; indomethacin; interaction

Classic approach to pain relief comprises the use of non-steroidal anti-inflammatory drugs in mild pain treatment, and opioids in moderate and strong pain. Recently, pharmaceuticals which have no analgesic potential per se but can enhance the activity of classic analgesics are more often administered in pain treatment. Among them tricyclic antidepressants (which augment the opioid analgesia) play a vital role, along with anxiolytics and antiepileptic drugs. Administration of the above-mentioned pharmaceuticals to relieve the pain of different and often complex pathogenesis allows for successful treatment of this pathology, also with elimination of concomitant symptoms such as inflammation, edema, depression or anxiety. Use of different groups of drugs, beside unquestionable advantages, can trigger adverse interactions.

Tiagabine is a new generation antiepileptic drug, a specific inhibitor of reuptake of γ-aminobutyric acid (GABA) from the synaptic space to neurons and glia (1). Sparse literature on the subject seems to prove the use of tiagabine as an analgesic. The analgesic effect of tiagabine has been investigated in various mouse and rat analgesic tests. The antinociceptive action of tiagabine was described by Ipponi et al. in models of mechanical (paw pressure test), thermal (hot-plate test) and chemical (abdominal constriction test) stimuli-evoked pain (2). Tiagabine also showed analgesic activity in the mouse grid-shock analgesia test (3). Laughlin et al. demonstrated that tiagabine produced antinociception in a broad range of nociceptive tests (hot-plate, formalin- and dynorphin-induced chronic allodynia) (4). The promising results of studies in which tiagabine was administered as an analgesic, were also obtained in experimental models of neuropathic pain (5) as well as in clinical practice (6, 7).

The presented study concerns the influence of tiagabine on the antinociceptive effect of the selected analgesics (morphine, metamizole and indomethacin in mice), varying in the mechanisms of action, pharmacokinetic characteristics and the influence on different structures of the antinociceptive system.

EXPERIMENTAL

Materials

The experiments were carried out on male Swiss mice (18 – 26 g). The animals were grouped

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in cages under normal laboratory conditions at a temperature of 20-21°C, and natural day/night cycle with free access to commercial rat chow and water. All experiments were performed between 9.00 a.m. and 3.00 p.m. The drugs were injected intraperitoneally (i.p.) dissolved in 0.9% NaCl. Tiagabine (Gabitril, Sanofi, Winthrop, France) at a single dose of 3.2 mg/kg was given 30 min before the analgesic drugs: morphine – 10 mg/kg (Morphini sulfas, Polfa, Warszawa, Poland), metamizole – 500 mg/kg (Pyralginum, Polpharma, Starogard Gdański, Poland), indomethacin – 10 mg/kg (Metindol, Polfa, Kraków, Poland). In experiments with prolonged administration, all drugs were given for 10 days: tiagabine – 1.2 mg/kg/day, morphine – 10 mg/kg/day, metamizole – 500 mg/kg/day, indomethacin – 1.4 mg/kg/day.

Nociception tests

The hot-plate test was derived from that of Eddy and Leimbach (8). 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 ± 0.4°C. The maximum time of exposure was 60 s. Latencies to hind paw licking were determined 60, 90 and 120 min after the administration of tiagabine. The groups consisted of 8 – 12 mice each.

The tail-flick test of D'Amour and Smith modified for mice was used (9). 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 tip of the tail. The temperature of heat source was 70 ± 0.2°C and maximum time of exposure was 60 s. This noxious stimulation did not cause tissue damage. The latency was measured 60, 90 and 120 min after the administration of tiagabine. Each group consisted of 8 – 12 mice.

Statistical analysis

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

All the procedures used in these studies were approved by the Ethics Committee of the Medical University of Lódź, Poland (licence 115, permission Ł/BD/109).

RESULTS

Tiagabine used at a single dose of 3.2 mg/kg 30 min before the administration of morphine at a dose of 10 mg/kg increases the time to pain reaction in animals in a statistically significant way, in comparison with the animals receiving only morphine. The effect was evident in the hot-plate test (Fig. 1A) and weakly (90 and 120 min after the administration of tiagabine) in the tail-flick test (Fig. 1B). Tiagabine at a single dose administered 30 min before metamizole at a dose of 500 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 only metamizole (Figs 2A, 2B). In the hot-plate test, this effect was observed in the measurements after 60 and 90 min (Fig. 2A), whereas in the tail-flick test increased action of metamizole was seen in the measurements after 60 and 120 min (Fig. 2B). Tiagabine administered at a single dose together with indomethacin (10 mg/kg) increased the time to reaction to pain stimulus in comparison with mice which received indomethacin alone. This effect was observed in both tests (Figs 3A, 3B). Administration of tiagabine alone in a single dose of 3.2 mg/kg significantly prolonged latency to the pain reaction in comparison with mice receiving 0.9% NaCl but in the hot-plate test only (Figs 1, 2, 3A). This effect was also significantly more pronounced than that obtained after metamizole (Fig. 2A) and indomethacin (Fig. 3A). In the tail-flick test, the mice's reaction after tiagabine did not differ as compared with the control (0.9% NaCl) group (Figs. 1, 2, 3B). Tiagabine at the dose of 1.2 mg/kg administered for 10 days together with morphine (10 mg/kg) significantly prolonged the latency to pain reaction in mice in comparison with animals receiving morphine alone. The decreased sensitivity to the pain stimulus was observed both in the hot-plate and tail-flick tests (Figs 4A, 4B), but this effect was more pronounced in hot-plate test (Fig. 4A) Repeated doses of tiagabine together with metamizole (500 mg/kg for 10 days) had no effect on the analgesic activity in mice in the hot-plate test 60 and 120 min after the administration of the antiepileptic drug, whereas in the measurement after 90 min, a significant weakening of the effect of metamizole was observed (Fig. 5A). In the tail-flick test, there was no effect on the analgesic activity of metamizole 60 and 90 min after the administration of tiagabine. In the measurement after 120 min, the time to nociceptive reaction in the animals was shortened (Fig. 5B). Repeated doses of

tiagabine together with indomethacin (1.4 mg/kg for 10 days) did not significantly change the pain reaction in the hot-plate test in mice in comparison with animals receiving indomethacin alone (Fig. 6A), whereas in the tail-flick test decreased action of indomethacin was observed in the measurement after 90 and 120 min after the administration of the antiepileptic drug (Fig. 6B). The repeated administration of tiagabine alone prolonged the time to reaction to the nociceptive stimulus as compared with mice receiving 0.9% NaCl. This effect was significant in the hot-plate test (Figs. 4, 5, 6A) and weak in the tail-flick test (Figs. 4, 5, 6B). Tiagabine administered in repeated doses was found to have more potent antinociceptive effect than metamizole, both alone and combined with tiagabine – hot-plate test (Fig. 5A). Similarly, tiagabine in repeated doses has a more potent antinociceptive effect than indomethacin alone and indomethacin combined with tiagabine – hot-plate test (Fig. 6A).

DISCUSSION

Besides the opioidergic system, antinociception involves concerted action of adrenergic, serotoninergic and also GABA-ergic systems. They inhibit pain via neurons which are localized in the descending (efferent) nociceptive system. GABA is present in the central nervous system (CNS) in almost 60% of synapses. GABA, stimulating

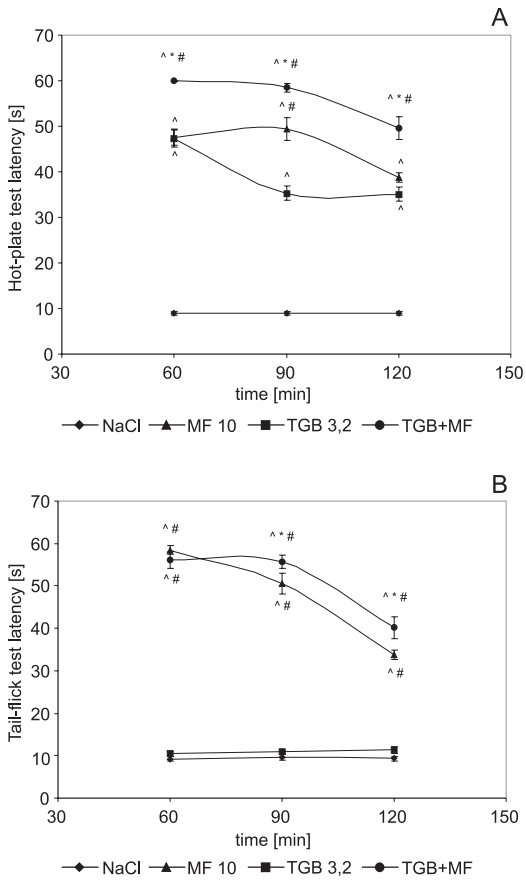

Figure 1. The antinociceptive effect in hot-plate (**A**) and tail-flick (**B**) tests after i.p. administration of 0.9 % NaCl (–◆– NaCl), morphine 10 mg/kg (–▲– MF 10), tiagabine 3.2 mg/kg (–■–TGB 3.2), tiagabine + morphine (–●– TGB + MF). ^ Significantly different from the control group receiving 0,9 % NaCl, $p < 0.05$, * significantly different from the morphine-treated group, $p < 0.05$, # significantly different from the tiagabine – treated group, $p < 0.05$, ANOVA-test.

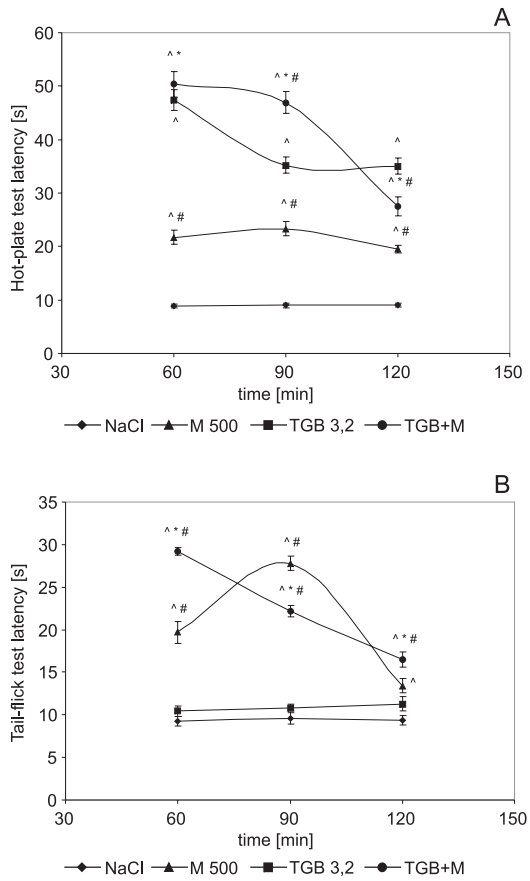

Figure 2. The antinociceptive effect in hot-plate (**A**) and tail-flick (**B**) tests after i.p. administration of 0,9 % NaCl (–◆– NaCl), metamizole 500 mg/kg (–▲– M 500), tiagabine 3.2 mg/kg (–■– TGB 3.2), tiagabine + metamizole (–●– TGB + M). ^ Significantly different from the control group receiving 0.9 % NaCl, $p < 0.05$, * significantly different from the metamizole-treated group, $p < 0.05$, # significantly different from the tiagabine – treated group, $p < 0.05$, ANOVA-test.

$GABA_A$ receptors (ionotropic in character), exerts a strong inhibitory effect on the CNS. The stimulation of $GABA_B$ receptors through Gi/Go membrane protein signaling leads to the inhibition of adenylate cyclase, increases K^+ channel conductance, and reduces Ca^{2+} channel conductance in the analgesic activity (10, 11, 12). This mechanism is to a large extent responsible for analgesic effect (13, 14).

The knowledge of mechanisms involved in the function of GABAergic system permits the use of drugs which inhibit the function of the CNS and – among others – suppress epileptic attacks. Among a number of antiepileptic pharmaceuticals, tiagabine, which is a third-generation antiepileptic drug, deserves attention. Tiagabine is a strong, selective and reversible inhibitor of GABA transporters to presynaptic endings and glia, which in consequence leads to the increase in GABA concentration in the synapse and augmentation of the receptor effect.

Laughlin et al. demonstrated a dose-dependent antinociceptive effect of tiagabine in acute and chronic pain tests in mice (4).

In presented studies, an attempt was made to estimate potential analgesia-enhancing properties of tiagabine administered with the selected pain-killing drugs. Tiagabine was given at doses described in the literature as having analgesic effect in animals with no toxic effect at the same time: a single dose was 3.2 mg/kg, and repeated doses were 1.2 mg/kg each

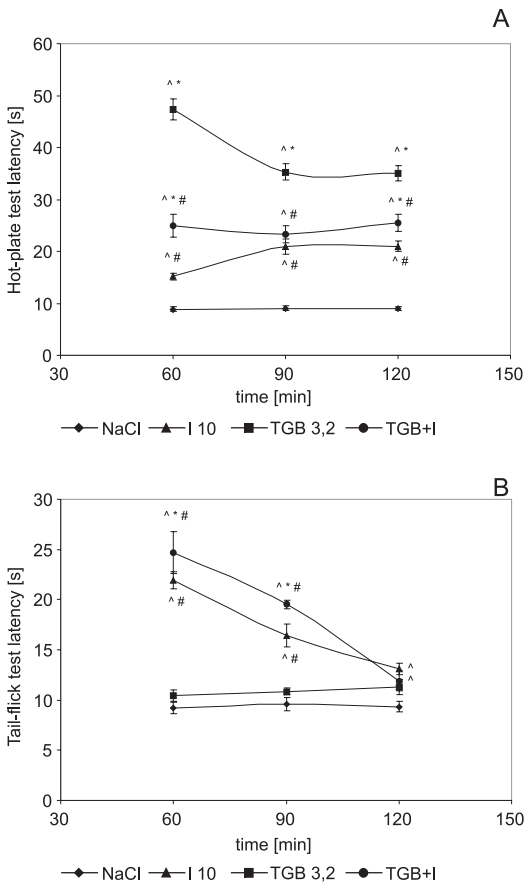

Figure 3. The antinociceptive effect in hot-plate (**A**) and tail-flick (**B**) tests after i.p. administration of 0.9 % NaCl (–◆– NaCl), indomethacin 10 mg/kg (–▲– I 10), tiagabine 3.2 mg/kg (–■– TGB 3.2), tiagabine + indomethacin (–●– TGB + I). ^ Significantly different from the control group receiving 0.9 % NaCl, $p < 0.05$, * significantly different from the indomethacin-treated group, $p < 0.05$, # signifficantly different from the tiagabine-treated group, $p < 0.05$, ANOVA-test.

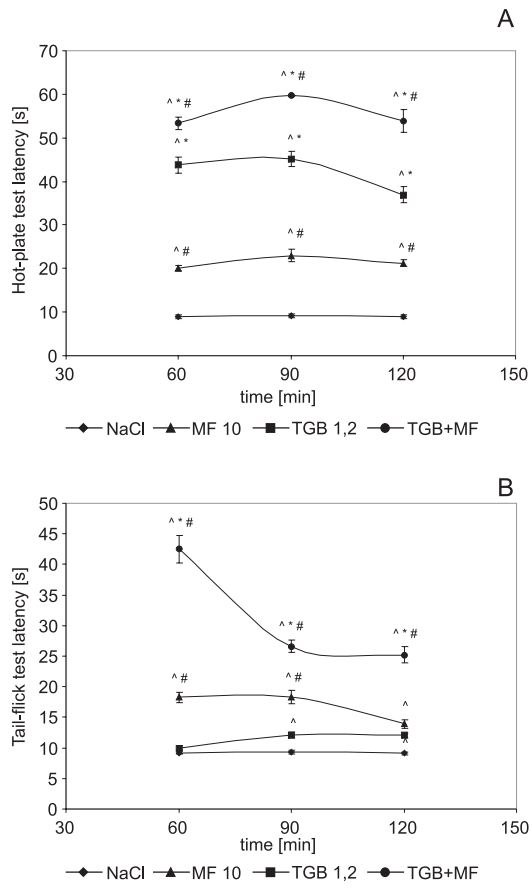

Figure 4. The antinociceptive effect in hot-plate (**A**) and tail-flick (**B**) tests after multiple (10 days) i.p. administration of 0.9% NaCl (–◆– NaCl), morphine 10 mg/kg (–▲– MF 10), tiagabine 1.2 mg/kg (–■– TGB 1,2), tiagabine + morphine (–●– TGB + MF). ^ Significantly different from the control group receiving 0.9 % NaCl, $p < 0.05$, * signifficantly different from the morphine-treated group, $p < 0.05$, # significantly different from the tiagabine-treated group, $p < 0.05$, ANOVA-test.

(2, 3). In order to establish tiagabine influence on the activity of analgesics, it was administered together with morphine (10 mg/kg), metamizole (500 mg/kg) and indomethacin (10 mg/kg and 1.4 mg/kg). These doses of analgesics administered in the previous studies exerted the analgesic activity in mice (15, 16).

Morphine, a narcotic drug, owes its strong analgesic activity to agonistic properties at opioid receptors, mainly μ opioid receptor. Their stimulation leads to the decrease in intracellular cAMP concentration. Stimulation of δ receptor causes the opening of potassium channels, hyperpolarization and inhibition of the conduction of pain stimuli. Agonism at all three subtypes of opioid receptors (μ, δ, κ) is connected with the closure of calcium channels which leads to the inhibition of the release of pain neuromediators: dopamine, acetylcholine, substance P (17-19).

Metamizole is a non-narcotic analgesic. Its mechanism of the analgesic activity results in the inhibition of cyclooxygenase in the CNS (13).

Indomethacin is a strong non-steroidal analgesic drug, effective in pain, especially in the course of inflammatory process. It inhibits prostanglandin synthesis a few hundred times stronger than acetylsalicylic acid (20).

In this studies, it was proved that tiagabine at a single dose showed antinociceptive activity observed in the hot-plate test while it did not have

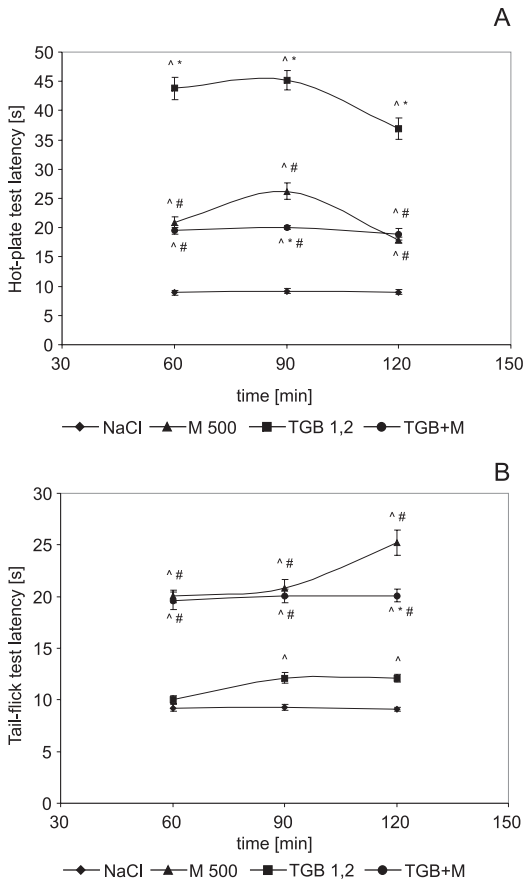

Figure 5. The antinociceptive effect in hot-plate (**A**) and tail-flick (**B**) tests after multiple (10 days) i.p. administration of 0.9 % NaCl (–◆– NaCl), metamizole 500 mg/kg (–▲– M 500), tiagabine 1.2 mg/kg (–■– TGB 1.2), tiagabine + metamizole (–●– TGB + M). ^ Significantly different from the control group receiving 0.9 % NaCl, $p < 0.05$, * significantly different from the metamizole-treated group, $p < 0.05$, # significantly different from the tiagabine-treated group, $p<0.05$, ANOVA-test.

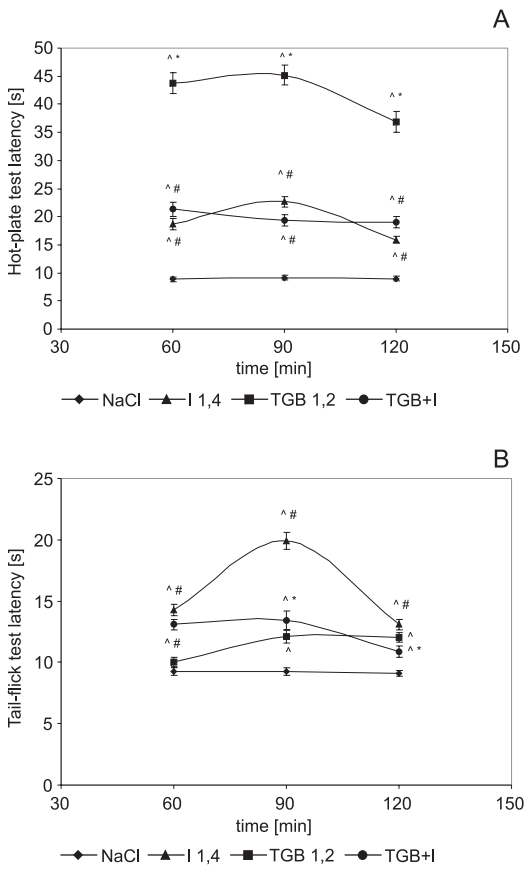

Figure 6. The antinociceptive effect in hot-plate (**A**) and tail-flick (**B**) tests after multiple (10 days) i.p. administration of 0.9 % NaCl (–◆– NaCl), indomethacin 1.4 mg/kg (–▲– I 1.4), tiagabine 3.2 mg/kg (–■– TGB 1.2), tiagabine + indomethacin (–●– TGB + I). ^ Significantly different from the control group receiving 0.9 % NaCl, $p < 0.05$, * significantly different from the indomethacin-treated group, $p < 0.05$, # signifficantly different from the tiagabine-treated group, $p < 0.05$, ANOVA-test.

such activity in the tail-flick test. Repeated administration of tiagabine significantly prolonged the latency of reaction to the pain stimulus in both tests applied, however, in the tail-flick test the effect was considerably weaker. Studies conducted by Tremont-Lukats et al. also revealed inefficacy of tiagabine in the tail-flick test in animals (21). Similarly, the studies by Laughlin et al. demonstrated that tiagabine administered i.t. does not exert antinociceptive effect in the hot-plate test as well as in dynorphin-induced allodynia, which suggests its mainly supraspinal action (4).

It is considered that the hot-plate test measures supraspinal analgesia whereas tail-flick test is an indicator of spinal analgesia (22).

GABA receptors are located in the majority of brain structures and there are significantly fewer of them in the spinal cord. It can be a cause of the obtained difference in the analgesic activity of tiagabine.

The observed antinociceptive properties of tiagabine seem to be closely connected with the mechanism of its activity, leading to the enhancement and prolongation of the synaptic effect of GABA. It seems that its influence on the $GABA_B$ receptor plays a role in the analgesic activity, resulting ultimately in the inhibition of cAMP synthesis and in consequence in the analgesic activity.

In presented studies, tiagabine and morphine administered together in a single dose demonstrated an additive analgesic effect in the hot-plate test, and a weak synergistic effect in the tail-flick test.

It was demonstrated that the analgesic effect of morphine, after repeated administration of this opioid alone to mice, was considerably weaker than after single administration. This confirms the development of tolerance to the analgesic effect of morphine. Repeated administration of morphine together with tiagabine resulted in the superposition of the antinociceptive effect of tiagabine on the opiod effect (in both tests). Tolerance to opioids is a consequence of weakening of their inhibitory effect on cAMP. The studies carried out by Collier et al. demonstrated that drugs which inhibit cAMP activity (e.g. antiserotoninergic) efficiently abolished experimentally induced morphine tolerance (23). Tiagabine, probably via Gi protein, inhibits cAMP activity which leads to the abolition of the tolerance caused by the long-term morphine administration observed in our experiment.

Administration of tiagabine at a single dose increased the antinociceptive activity of metamizole in the hot-plate test. In the tail-flick test this effect was weak. Repeated administration of tiagabine did not significantly change the analgesic effect of metamizole in either test, although the observed decrease of pain reaction time after metamizole administered simultaneously with tiagabine in both tests was insignificant.

It is, however, noteworthy that single administration of metamizole exerted a weaker antinociceptive effect than tiagabine in the hot-plate test. Also repeated, simultaneous administration of metamizole and tiagabine had a weaker antinociceptive effect than tiagabine alone (hot-plate test).

An interaction during the excretory process does not seem probable.

The mechanism of metamizole action is based on the inhibition of constitutive cyclooxygenase activity (COX 1) and cyclooxygenase induced by proinflammatory factors (COX 2) in the CNS (24). It was also revealed that the ability of metamizole to activate the opioidergic system in the periaqueductal grey matter may have essential therapeutic significance (25). The influence of tiagabine on the antinociceptive action of metamizole, observed in our studies, is connected mainly with supraspinal structures. This interaction seems to be pharmacodynamic. These drugs are metabolized by different enzymes, namely izoenzymes of cytochrome P-450: CYP2B1 and CYP2B2 are involved in the metabolism of metamizole (26), whereas tiagabine is metabolized by CYP3A4 (14, 27). Metamizole is bound with serum proteins to a small extent (approximately 20%), while almost all tiagabine is protein-bound (96%) (1, 11).

Recent studies demonstrated that metamizole delayed gastric emptying in rats (28, 29). This effect is suggested to be exerted through the central nervous system (CNS) and to be associated with the GABA-ergic system. Baclofen (a $GABA_B$ agonist) abolishes this effect. The reduction of the antinociceptive effect of tiagabine by metamizole, observed in our experiments, may be associated with a decrease in its absorption.

Single administration of tiagabine together with indomethacin slightly prolonged the latency of reaction to the pain stimulus in animals in both tests applied. The effect, however, is weaker than after tiagabine alone. Repeated administration of tiagabine did not change considerably the analgesic effect of indomethacin, although the insignificant decrease of pain reaction time in the tail-flick test was observed in mice.

Comparing the combined effect of indomethacin and tiagabine to tiagabine alone, it was observed that indomethacin reduces the analgesic effect of tiagabine similarly to metamizole.

The antagonistic effect on $GABA_B$ receptor could explain such influence. However, Olianas et al. demonstrated that basal adenyl cyclase activity increased by baclofen (a $GABA_B$ receptor agonist) was not affected by indomethacin (30).

Indomethacin is metabolized in the liver by cytochrome P450, mainly by its isoenzyme CYP2C9 and, to a lesser extent, CYP2C19. These isoenzymes, different from those involved in metabolism of tiagabine (CYP3A4), take part in synthesis of an active O-demethylated metabolite (31).

Liu et al. showed that inhibitors of cyclooxygenase: aspirin, ibuprofen, indomethacin and piroxicam attenuated the duration of loss of righting reflex induced by diazepam (32). These findings suggested that COX products may modulate GABAergic activity. This can explain the attenuation of the analgesic effect of tiagabine by indomethacin.

CONCLUSIONS

1. Tiagabine administered i.p. to mice exerts an antinociceptive effect in the hot-plate test, which suggests its supraspinal action.

2. A single dosing of tiagabine in combination with morphine demonstrates an additive antinociceptive effect (hot-plate test); multiple dosing leads to a reduction of morphine tolerance.

3. A single dosing of tiagabine in combination with metamizole or indomethacin weakly increases while repeated administration not increases the action of both analgesics. Metamizole and indomethacin may decrease the antinociceptive effect of tiagabine.

4. Antinociceptive effect of tiagabine alone is stronger than metamizole or indomethacin action.

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