Original article

Experimental study on the antinociceptive effect of retigabine in rats

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Abstract

Introduction: Retigabine is a new anticonvulsant which activates a low-threshold voltage-gated potassium channels. Anticonvulsant drugs reduce the abnormal hyperexcitability and may have analgesic effects in animals and humans. The aim of this study is to determine the antinociceptive effect of retigabine in rats.

Materials and methods: Thirty-two male Wistar rats were devided into four groups (n = 8). They were treated intraperitoneally with 0.9% NaCl, metamizole natrii and retigabine in a doses of 5 and 15 mg/kg bw. The antinociceptive effect was evaluated with hot plate test, test with mechanical pressure and formalin test.

Results: A single dose retigabine 15 mg/kg bw increased the withdrawal latency when compared with controls on the second and third hour in the hot plate test ($p<0.05$). During the first phase of the formalin test retigabine attenuates the flinching behavior while administrated in doses 5 and 15 mg/kg bw compared with controls. Administration of retigabine in dose 15 mg/kg bw significantly reduced the time of licking during the second phase of the test compared to the controls ($p<0.005$).

Conclusions: Retigabine is active against chemical, mechanical and thermal induced acute pain in rats. The drug is found effective in doses 5 and 15 mg/kg bw. A significant increase in the nociceptive threshold was observed when the higher dose (15 mg/kg bw) was administrated. The presence of KCNQ channels in the neuronal pathways of pain suggests that the antinociceptive effect of the compound may be a result of the activation of low-threshold potassium channels.

Keywords: retigabine, nociception, rats.

Introduction

Retigabine is an anticonvulsant, approved by European Medicines Agency in January 2011 under the trade name Trobalt® (GlaxoSmithKline)[1]. It is approved for adjunctive treatment of partial-onset seizures in adults[2]. The mechanism of action of retigabine is complex. The drug activates low-threshold voltage-gated potassium channels (KCNQ, Kv7 channels), causing hyperpolarization of the membrane potential. It may influence the neuronal mediation of γ-aminobutyric acid (GABA), glutamate, glutamine and dopamine[3, 4]. Flupirtine, an centrally acting non-opioid analgesic, possess the same mechanism of action – hyperpolarisation of the postsynaptic membrane as a result of increased potassium current[5].

Neuronal hyperexcitability events can occur not only in brain structures but also within pain transmission pathways. They may be a result of nerve injury or peripheral inflammation and can lead to persistent pain and sensory abnormalities (allodynia, hyperalgesia, spontaneous pain). Anticonvulsant drugs reduce the abnormal hyperexcitability and can have analgesic effects in animals and humans[6].

In the following report we describe the data obtained with retigabine in different models of acute pain in comparison with metamizole as reference compound.

The aim of this study is to determine the antinociceptive effect of retigabine in rats.

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Material and methods

All experiments were approved by the Animal Health and Welfare Directorate at Bulgarian Food Safety Agency with permit No 88/09.01.2014.

Animals

Thirty-two male Wistar rats (weight of 180 – 200g) were devided into four groups (n = 8). They were treated intraperitoneally as follows: 1-st group (control) – treated with 0.9% NaCl in the presence of 0.5 % methylcellulose, 2-nd group (positive control) – treated with metamizole natrii in a dose of 150 mg/kg bw, 3-rd group – treated with retigabine in a dose of 5 mg/kg bw and 4-th group – treated with retigabine in a dose of 15 mg/kg bw.

Rats were kept under standard laboratory conditions (temperature 22 ±1°C, humidity 45% and 12-h light cycle). The rodents received food and water ad libitum.

DRUGS

Retigabine (Trobalt® 100 mg, distributed by GlaxoSmithKline) was dissolved in 0.9% NaCl containing 0.5 % methylcellulose.

Experimental procedures

1. Antinociceptive test with thermal stimulus (hot plate test).

The test was conducted with Hot Plate Analgesy Meter, Ugo Basile, Italy immediately after the administration of the drugs (h0) and on the 60-th (h1), 120-th (h2) and 180-th (h3) minute. The animals were placed on metal surface heated to a temperature of 55 ± 0.5°C. The antinociceptive response was measured by the latency observed from the time the rat was placed on the heated surface until the first behavioral sign of nociception. As an endpoint of the test we used the following behavior changes: licking a hind paw, vocalization, or an escape response. The timer was stopped by a foot-operated pedal and the rat was immediately removed from the hotplate. A maximum hotplate latency of 30 sec was used to prevent tissue damage to the rats' paws. The results are shown as mean value±SEM.

2. Antinociceptive test with mechanical pressure (analgesimeter).

Rats were tested immediately after the treatment with the substances with Analgesimeter (Ugo Basile, Italy) and on the 60-th (h1), 120-th (h2) and 180-th (h3) minute after drug administration. The test is using mechanical pressure on the rat hind paw. Linearly increasing down force (16 g/s) is applied between the third and fourth metatarsal. Nociceptive threshold is measured as the strength of the pressure at which the rat withdraws testing paw (PPT-units). The maximal possible pressure (cut-off limit) is 250 grams. The results are shown as mean value±SEM.

3. Formalin test.

The animals were treated intraperitoneally with the substances. After 30 minutes 50 μL of a 0.5% solution of formalin was injected subcutaneously into the dorsal surface of the right hind paw of the rat[7]. Rats were observed for 30 minutes after the injection. Also, licking/biting of the right hind paw was recorded using a digital time-out stopwatch as total licking time (s) during the first 10 minutes and between the 20-th and 30-th minute after formalin injection. The results are shown as mean value ± SEM.

Statistical analysis

Data were analyzed using SPSS 19.0. One sample Kolmogorov-Smirnov test was performed to study the normal distribution. One way ANOVA with Tuckey post hoc test was used in case of normal distribution; non-parametric Wilcoxon signed rank test and Mann Whitney test were conducted in the other case.

Results were considered significant at $p<0.05$.

Observations and results

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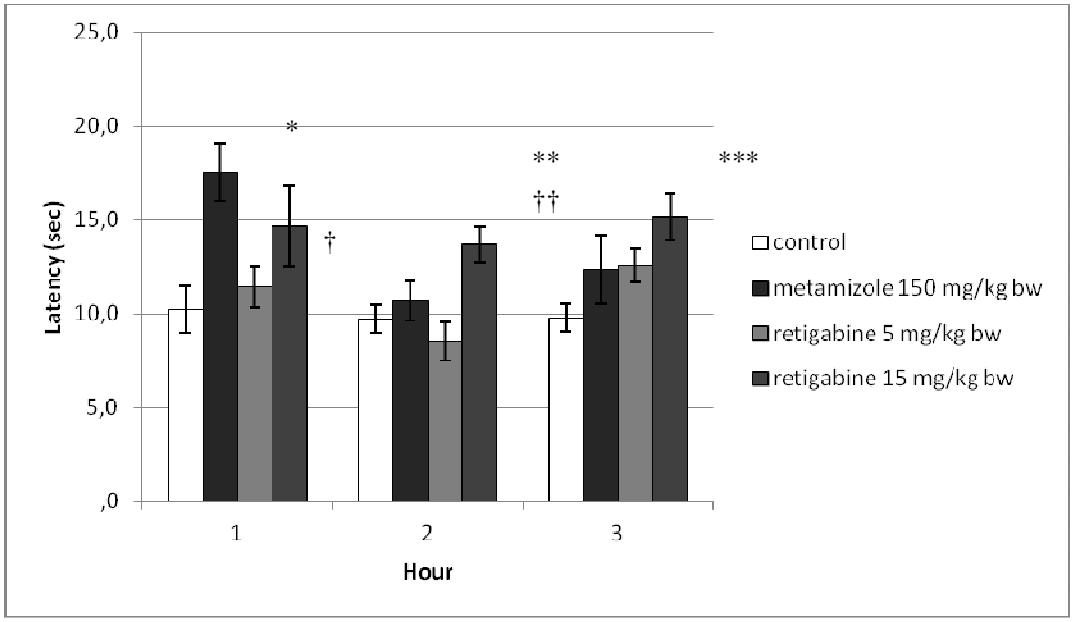
1. Antinociceptive test with thermal stimulus (hot plate test).



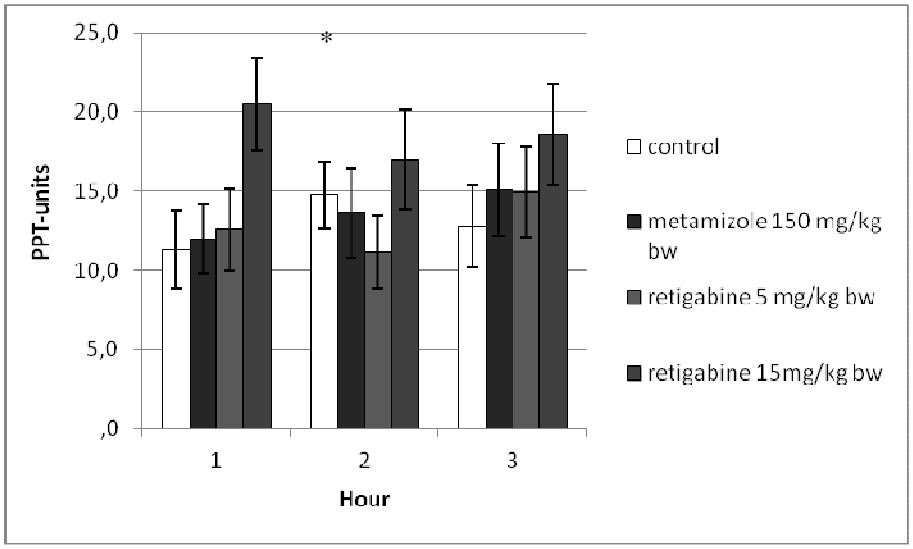
* - p<0.05 compared with controls at 1-st hour; ** - p<0.05 compared with controls at 2-nd hour; *** - p<0.05 compared with controls at 3-rd hour; † p<0.05 - compared with metamizole at 1-st hour; †† p<0.005 - compared with retigabine 5 mg/kg bw at 2-nd hour.

Figure 1. Antinociceptive effect of retigabine in dose 5 and 15 mg/kg bw evaluated with "hot plate" test.

On the first hour after the drug administration the group treated with metamizole natrii (17.53sec±1.53) showed increased withdrawal latency compared with the controls (10.23sec±1.26) on the same hour (p<0.05). Treatment with retigabine in dose 5 mg/kg bw reduced the reaction time of the animals (11.43sec±1.11) in comparison with metamizole natrii (17.53sec±1.53) 1 hour after the treatment (p<0.05). A single dose retigabine 15 mg/kg bw increased the withdrawal latency (13.68 sec± 0.96) when compared with controls (9.69sec±0.74) on the second hour (p<0.05).

This effect was observed also on the 3-rd hour after the treatment and the latency of rats, received 15 mg/kg bw retigabine (15.14sec±1.25) remained significantly higher than controls (9.79sec±0.73; p<0.05). Retigabine in dose 15 mg/kg bw prolonged the withdrawal latency compared with the group, treated with a lower dose of retigabine (8.51sec±1.01; p<0.005) on the 2-th hour after treatment. We observed a well defined antinociceptive effect in rats, treated with metamizole natrii and 15 mg/kg bw retigabine. The latency of the animals in those groups remained higher than controls during all three tests (Figure 1).

2. Antinociceptive test with mechanical pressure (analgesimeter).

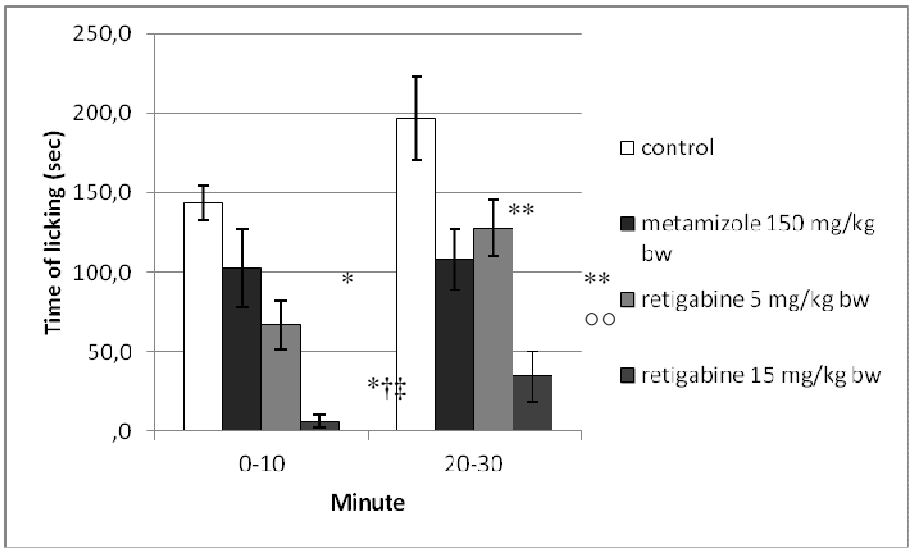


* - p<0.05 compared with controls at 1-st hour.

Figure 2. Antinociceptive effect of metamizole 150 mg/kg bw and retigabine in dose 5 mg/kg bw and 15 mg/kg bw, evaluated with analgesimeter (n=8).

Retigabine in dose 15 mg/kg bw prolonged the withdrawal latency on the first, second and third hour after intraperitoneal administration, but a significant difference with the controls was observed only on the first hour (20.50 ±2.95) vs (11.31 ±2.46) (Figure 2).

3. Formalin test.



* - p<0.005 compared with controls (0-10 minute); † - p<0.005 compared with metamizole (0-10 minute); ‡- p<0.05 compared with retigabine 5 mg/kg bw (0-10 minute); ** - p<0.005 - compared with controls (20-30 minute); ○○ - p<0.05 - compared with retigabine 5 mg/kg bw (20-30 minute).

Figure 3. Effects of metamizole 150 mg/kg bw and retigabine in dose 5 and 15 mg/kg bw in the formalin test.

During the first phase of the formalin test retigabine attenuates the flinching behavior while administrated in dose 5 mg/kg bw (67.13sec ±15.14; $p<0.05$) and 15 mg/kg bw (6.50sec ±3.50; $p<0.005$) compared with controls (143.88sec ±10.78). In the group treated with retigabine in dose 15 mg/kg bw we observed significant decrease in the time of licking (6.50sec ±3.50) compared with the group, received metamizole natrii (102.75sec±24.62; $p<0.005$) and lower dose retigabine (67.13sec ±15.14; $p<0.05$) during the first 10 minutes of the test. Administration of retigabine in dose 15 mg/kg bw significantly reduced the time of licking (34.75sec ±15.90) during the second phase of the test compared to the controls (196.75sec ±15.14; $p<0.005$). When comparing the effects of the two doses of retigabine, the animals treated with higher dose (15 mg/kg bw) showed significant decrease in time of licking (34.75sec ±15.90) in comparison to the group, treated with a lower dose retigabine (128.13sec ±17.68; $p<0.05$) during the second phase of the test (Figure 3).

Discussion

The methods "hot plate", analgesimeter and formalin test are widely used for studying antinociceptive effect of different compounds in rats[8]. In our experiments retigabine was found to be effective in all three models of acute pain in both doses (5 and 15 mg/kg bw) and the antinociceptive effect is dose-dependent. Retigabine 15 mg/kg bw injected intraperitoneally in rats effectively elevated the pain threshold on the 2-nd and 3-rd hour in hot plate test. The same dose retigabine increased the paw withdrawal latency compared with the control group in the test using mechanical stimulus with significant difference on the 1-st hour after the treatment.

In the hot plate test and the analgesimeter the application of high-intensity thermal or mechanical stimulus to the skin leads to activation of high-threshold sensory fibers and the discharge is transferred to dorsal-horn neurons. The neuronal firing reaches the medulla, mesencephalon and thalamus using ventrolateral tracts. In summary the applicfation of thermal stimulus results in behavior change (escape response) or paw withdrawal[8]. The formalin test is a commonly used model of persistent pain and consists of two phases: first phase (0-10 minute after the formalin injection) and second phase (20-30 minute). The licking and/or biting behavior during the first phase is a result on direct chemical stimulation of the nociceptors. Injection of formalin solution leads to peripheral inflammatory processes and subsequent sensitization of nociceptive spinal neurons, which results in nociceptive behavior 20-30 minutes after the subcutaneous administration[9].

Our data obtained in the first phase of formalin test in rats showed significant decrease of the time of licking in the groups, treated with retigabine in doses 5 and 15 mg/kg bw. The same result was observed during the second phase of the test but only in rats treated with 15 mg/kg bw i.p.

Munro G. et al.[6] studied the antinociceptive effect of retigabine after intraperitoneal injection. Treatment with the anticonvulsant in doses 3, 6 and 10 mg/kg bw significantly reduced flinching behavior of the rats during the first phase of the test. Formalin-induced flinching and licking during the second phase was influenced significantly when doses of 6 and 10 mg/kg were administrated.

Retigabine was found to be effective against visceral pain, induced by capsaicin in mice. The drug not only suppressed the number of licking

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behavior in mice, but also increased the latency to first licking[10].

Passmore G et al.[11] used a model of inflammatory pain to evaluate the analgesic effect of retigabine. The drug showed antinociceptive effect when used in dose 5 mg/kg bw orally. This effect was prevented by co-administration of M-channel blocker, which suggests the role of Kv7 channels in the signaling pathways of pain. According to these authors the sensory neurons of rat dorsal root ganglia express KCNQ channels. The excitability of small-diameter, predominantly nociceptive neurons can be influenced by KCNQ openers and blockers. The analgesic action of retigabine in an animal model of inflammatory pain can be explained by reduced responses of nociceptive neurons in the dorsal horn - a result of enhanced M-current. Moreover, those authors showed that individual neurons from dorsal root ganglion express Kv7.2, Kv7.3 and Kv7.5 subunits from KCNQ family. Enhancement of the M-current leads to hyperpolarization and prevents spike generation. Also retigabine has greater potential to influence C-fiber responses than Aδ-fiber responses. The responses to mechanical and thermal stimuli correspond to C and Aδ-fibers evoked activity. Rivera-Arconada et al.[12] also studied the mechanism of antinociceptive effect of retigabine. According to them KCNQ channels, expressed in spinal neurons could regulate the neuronal excitability. M-channels were found in dorsal horn neurons and motor neurons. They observed significant depression of synaptic responses in most neurons after application of retigabine.

The tests for nociception, used in our studies required unaffected locomotor activity. Hirano et al. [10] studied the locomotor activity of mice treated with 10 mg/kg bw retigabine intraperitoneally and found no significant difference with controls. Rostock et al.[13] used open field apparatus to evaluate the effects of retigabine on muscle tone. They observed hyperexcitability, increased muscle tone in the limbs and a flat body posture after intraperitoneal injection of 10 mg/kg bw retigabine. More importantly they found no side effects on the muscle tone 45 minutes after the injection. We conducted hot plate test and test with mechanical pressure on the paw 60, 120 and 180 minutes after drug administration. Based on data, obtained by Rostock et al.[13] we can suggest that the antinociceptive effect of retigabine evaluated in our studies is not due to a general disturbance of motor function.

Hayashi et al.[14] reported impaired exploratory activity in rats 30 minutes after oral administration of 1, 3, 10 and 30 mg/kg bw retigabine. These authors measured the activity for 10 minutes (30 to 40 minute after treatment) and found statistical significance with controls in doses 10 and 30 mg/kg bw. Our findings are consistent with Hayashi et al's [14], who reported analgesic effect of retigabine in models of imflammatory pain.

Conclusions

Retigabine is active against mechanical, thermal and formalin-induced acute pain in rats. The drug is found effective in doses 5 and 15 mg/kg bw. A significant increase in the nociceptive threshold was observed when the higher dose (15 mg/kg bw) was administrated. The presence of KCNQ channels in the neuronal pathways of pain suggest that the antinociceptive effect of the compound may be a result of the activation of low-threshold potassium channels.

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