Tipepidine enhances the antinociceptive-like action of carbamazepine in the acetic acid writhing test

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Abstract

Several antidepressants have been used to treat severe pain in clinics. Recently, we reported that the centrally acting non-narcotic antitussive (cough suppressant drug), tipepidine produces an antidepressant-like effect in the forced swimming test, although the mechanism of action appears to be quite different from that of known antidepressants. In the present study, we investigated whether a combination of tipepidine and carbamazepine acts synergistically to induce an antinociceptive effect in the acetic acid-induced writhing test in mice. Prior to studying the combination of tipepidine and carbamazepine, the analgesic action of tipepidine alone was also examined in mice. Tipepidine at 5–40 mg/kg i.p. significantly reduced the number of writhes induced by acetic acid in mice. Carbamazepine at 20 mg/kg i.p. also significantly reduced the writhing reaction. Furthermore, co-administration of carbamazepine (5 and 10 mg/kg, i.p.) and tipepidine (2.5 mg/kg i.p.) significantly decreased the number of writhes induced by acetic acid. This finding suggests that a combination of carbamazepine and tipepidine may be a new strategy for the treatment of neuropathic pain such as what occurs in trigeminal neuralgia, because the use of carbamazepine is often limited by its adverse effects and by reduction of its analgesic efficacy by microsomal enzyme induction.

Key words: Tipepidine, Antitussives, G-protein coupled inwardly rectifying potassium ion channels, Antinociceptive-like action, Carbamazepine

1. Introduction

Recently, we reported that the centrally acting non-narcotic antitussive (cough suppressant drug) tipepidine produces an antidepressant-like effect in the forced swimming test, which is the most widely used model for assessing pharmacological antidepressant activity (Kawaura et al., 2009). Antidepressants that increase neuronal transmission in the serotonin and/or noradrenaline systems are likely to produce relief from pain, because an increase in monoamine levels in the descending serotonin and noradrenaline pathways likely plays a role in the antinociceptive effect. Indeed, several antidepressants including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), and serotonin noradrenaline reuptake inhibitors (SNRIs) have been used to treat several types of pain in clinics. (O'Malley et al., 1999; Sindrup and Jensen, 1999, Briley, 2004). In addition to antidepressants, the antiepileptic drug carbamazepine is a well-known first choice drug that has been widely used as an analgesic to treat neuropathic pain, especially pain caused by trigeminal neuralgia (Backonja, 2002; Jensen, 2002). However, the use of carbamazepine is often limited by its adverse effects such as somnolence, dizziness, gait abnormalities, and hematological changes (Backonja, 2002; Jensen, 2002). Furthermore, the chronic use of carbamazepine reduces its analgesic efficacy due to microsomal enzyme induction (Benedetti et al., 2005). Recently, Aoki et al. (2006) reported that the antinociceptive effect of carbamazepine is enhanced when combined with antidepressants such as imipramine, fluvoxamine, or milnacipran. In this context, it is important to determine whether a combination of carbamazepine with tipepidine enhances the antinociceptive effect because 1) tipepidine has a different pharmacological mechanism for producing the antidepressant-like effect than known antidepressants including SSRIs and SNRIs (Kawaura et al., 2009), and 2)

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tipepidine has a very high safety profile, having been used as a cough suppressant drug in infants for about 50 years in Japan. Therefore, in this study, we investigated the effect of tipepidine on the analgesic activity of carbamazepine using the acetic acid-induced writhing test in mice. Prior to studying the combination of carbamazepine and tipepidine, the analgesic action of tipepidine alone was examined in mice.

2. Materials and Methods

2.1. Animals

Male ddY mice (SLC Inc. Japan) weighing 26–45 g were used in this study. The animals were maintained in a constant room temperature (22 ± 2 °C) under a 12-h light–dark cycle (light on 08:00–20:00). Food and water were available *ad libitum*. this study was approved by the Committee of Animal Experimentation of Kumamoto University and was conducted in strict accordance with the Guidelines of the Japanese Pharmacological Society for the Care and Use of Laboratory Animals.

2.2. Acetic acid-induced writhing test

Analgesia was evaluated using the acetic acid-induced writhing test. Acetic acid (1%) was injected at a volume of 10 ml/kg i.p., and the mice were individually placed in a plastic cage ($30 \times 8.5 \times 8.0$ cm) for observation. The writhing reaction was counted 5 min after acetic acid injection for 10 min. A writhe was defined as stretching of the hind limbs accompanied by a contraction of the abdominal muscles. Tipepidine (2.5–40

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mg/kg), carbamazepine (2.5, 5, 10 and 20 mg/kg), or vehicle (10 ml/kg) were injected

intraperitoneally 20 min before the injection of acetic acid. In the combination studies,

carbamazepine (2.5, 5 and 10 mg/kg) and tipepidine (2.5 mg/kg) were co-administered

20 min before the injection of acetic acid. The number of writhes during the 10-min

period was recorded by observers blinded to the treatment groups.

2.3. Drugs

Carbamazepine was purchased from Sigma, Japan. Tipepidine was obtained from

Mitsubishi-Tanabe Pharm. Corp., Japan. Tipepidine was dissolved in saline.

Carbamazepine was dissolved in 40% dimethyl sulfoxide (Wako, Japan) and then

diluted with saline. All drugs were administered in a volume of 10 ml/kg.

2.4. Statistical analysis

The data are shown as the mean ± S.E.M. All results were analyzed by analysis of

variance (ANOVA) followed by Dunnett's test. A p value of less than 0.05 was

considered significant. All statistical analyses were carried out using SPSS Ver.13

JAPAN for Windows.

3. Results

3.1. Effects of tipepidine and carbamazepine on the writhing reaction in mice

The results for tipepidine and carbamazepine are shown in Figs. 1 and 2. Tipepidine at 5–40 mg/kg i.p. significantly decreased the number of writhes induced by acetic acid (1.0% (v/v)) in mice [$F(5, 37) = 6.14$, $P < 0.001$] (Fig. 1). Carbamazepine at 20 mg/kg i.p. also significantly decreased the writhing reaction [$F (4, 47)=4.47$, $P<0.01$] (Fig. 2), but not at 2.5, 5 and 10 mg/kg, i.p.

3.2. Effects of co-administration of carbamazepine with tipepidine on the writhing reaction in mice

The results of co-administration of carbamazepine (2.5, 5 or 10 mg/kg i.p.) with tipepidine (2.5 mg/kg. i.p.) are shown in Fig. 3. Out of these three doses of carbamazepine, coadministration of 2.5 mg/kg with tipepidine (2.5 mg/kg) had no effect on writhing behaviors induced by acetic acid. However, dosing of carbamazepine at 5 mg/kg and significantly inhibited the number of acetic acid induced writhes, when co-administered with tipepidine (2.5 mg/kg). [$F(3, 43)=9.12$, $P<0.001$].

4. Discussion

This study demonstrated that tipepidine inhibits acetic acid-induced writhing behaviors in mice. The effect was dose-dependent at doses of 2.5–40 mg/kg i.p. In the acetic acid-induced writhing test, drugs that affect motor activity may give false-positive

and/or false-negative results. Tipepidine has little or no effect on motor function even at a dose as high as 40 mg/kg, when tested with the open-field test and the rota-rod test (Kawaura et al., 2009). Therefore, the present result indicates that tipepidine may have antinociceptive-like activity in mice.

Aoki et al. (2006) reported that imipramine, a classical tricyclic antidepressant, inhibited acetic acid-induced writhing behavior more potently than fluvoxamine or milnacipran. The difference in the potency was attributed to the antagonistic activity of imipramine on various neurotransmitter receptors, especially histamine H_1 receptors (Mochizuki et al., 2002). This finding is interesting because tipepidine has little effect on various neurotransmitter receptors including histamine H_1 receptors. Further studies are necessary to clarify the mechanism of the antinociceptive action of tipepidine.

Interestingly, tipepidine showed antidepressant-like activity, similar to imipramine and other antidepressants, and enhanced the antinociceptive activity of carbamazepine. Co-administration of sub-optimal doses of tipepidine and carbamazepine significantly reduced acetic acid-induced writhing behavior. Although it is hard to refer to mechanisms of the combination effect of both drugs, the following merits to be discussed. Recent evidence suggests that neuronal tetrodotoxin-resistant sodium channels play a key role in the generation of nociceptive impulses in peripheral nerve fibers in both physiological as well as pathophysiological conditions (Brock et al., 1998; Novakovic et al., 1998). More recently, carbamazepine was reported to inhibit tetrodotoxin-resistant sodium currents in dorsal root ganglion neurons (Bräu et al., 2001; Stummann et al., 2005), suggesting that these effects may be involved in the mechanism of the antinociceptive effects of carbamazepine.

On the other hand, the neurochemical mechanism involved in the antinociceptive activity of tipepidine is unknown. The descending inhibitory pathways in the spinal cord are comprised of serotonin and noradrenaline systems (Wall and Melzack, 1999). The stimulation of these pathways may induce an antinociceptive action through increases in monoamine levels in synaptic clefts. This idea is supported by the finding that pharmacological blockade of monoamine receptors inhibited the antinociceptive effect of antidepressants (Otsuka et al., 2001; Yokogawa et al., 2002; Duman et al., 2004). We found that tipepidine increases monoamine levels in the brain by inhibiting G-protein coupled inwardly rectifying potassium ion (GIRK) channels, which are coupled to G-protein-coupled receptors such as adrenaline α_2 receptors and serotonin 5-HT$_{1A}$ receptors (Inoue et al., 2009a, 2009b). Because tipepidine has little affinity for transporters such as noradrenaline, serotonin, and dopamine transporters, tipepidine may cause an antinociceptive effect by increasing monoamine levels in the spinal cord via inhibition of GIRK channels.

Aoki et al. (2006) suggested that antidepressants may enhance the inhibitory effect of carbamazepine on nociceptive impulses in dorsal root ganglion neurons via the activation of descending inhibitory pathways, without ruling out the possibility that the pharmacokinetic interactions between carbamazepine and imipramine or fluvoxamine may also occur at a metabolic level (Daniel and Netter, 1988; Monaco and Cicolin, 1999; Szymura-Oleksiak et al., 2001; Spina and Perucca, 2002). Thus, tipepidine likely enhances the antinociceptive effect of carbamazepine by inhibiting nociceptive impulses in dorsal root ganglion neurons via the activation of descending inhibitory pathways. However, the mechanism of activation of tipepidine on the descending inhibitory pathways appears to be different from that of antidepressants such as imipramine and

fluvoxamine. Further studies are needed to elucidate the detailed mechanisms of the potentiating effect of the combination of tipepidine and carbamazepine on the antinociceptive effect in mice, including studies on the pharmacokinetic interactions between carbamazepine and tipepidine.

5. Conclusions

An opimal dose of carbamazepine in combination effect of an antinociceptive-like action with tipepidine was considered to be 5 mg/kg, when 2.5 mg/kg of tipepidine was used. The use of carbamazepine is often limited by frequent adverse effects such as somnolence, dizziness, gait abnormalities, and hematological changes (Backonja, 2002; Jensen, 2002), and by reduction of its analgesic efficacy by microsomal enzyme induction (Benedetti et al., 2005). Therefore, the present finding suggests that a combination of carbamazepine and tipepidine may be a new strategy for treating neuropathic pain such as pain caused by trigeminal neuralgia, although further studies are necessary to confirm this combination effect using other methods evaluating antinociceptive actions.

Acknowledgements

Legends

Fig. 1. The effects of tipepidine (TP; 2.5–40 mg/kg i.p.) on acetic acid-induced writhing in mice (n = 5–10). Data are the mean ± S.E.M. * $P< 0.05$, ** $P < 0.01$ vs Saline control.

Fig. 2. The effects of carbamazepine (2.5, 5, 10 and 20 mg/kg i.p.) on acetic acid-induced writhing in mice (n = 7–14). Data are the mean ± S.E.M. ** $P< 0.01$ vs DMSO control.

Fig. 3. The effects of co-administration of carbamazepine (2.5, 5 and 10 mg/kg i.p.) and tipepidine (2.5 mg/kg i.p.) on acetic acid-induced writhing in mice (n = 10–14). Data are the mean ± S.E.M. ** $P<0.01$, *** $P<0.001$ vs DMSO+Saline control.

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