Short communication

Behavioural Brain Research

The centrally acting non-narcotic antitussive tipepidine produces antidepressant-like effect in the forced swimming test in rats.

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Abstract

The antidepressant-like effect of tipepidine was studied in rats. Tipepidine at 20 and 40 mg/kg i.p. reduced immobility in the forced swimming test and tipepidine at 40mg/kg, i.p. increased climbing in the test. The drug at 40 mg/kg, i.p. had no effect on the loco-motor activity and motor coordination. These results suggest that tipepidine may be a novel drug with antidepressant-like activity.

Many currently available antidepressants have been developed based on the monoamine theory for the etiology of depression. First generation tricyclic antidepressants (TCA) such as imipramine elicit antidepressive effects by increasing the concentration of serotonin (5-HT) and norepinephrine (NE) at the synapse by inhibiting the 5-HT (SERT) and NE transporters (NET). The second generation of antidepressants was more selective in inhibiting NE and/or dopamine (DA) uptake (desipramine, nortriptyline, maprotiline) or 5-HT uptake (clomipramine), but demonstrated no significant improvements in side effect [1, 15, 16]. The third generation antidepressants are selective serotonin reuptake inhibitors (SSRI), and the side effects of the drugs were considerably reduced. The fourth generation antidepressants are 5-HT and NE reuptake inhibitors (SNRI). Several meta-analyses have suggested that treatment of major depressive disorder (MDD) with the SNRI results in a greater response or remission rather than the SSRIs [19, 24, 28, 29, 31, 32]. Currently, NE and DA reuptake inhibitor (NDRI), and 5-HT, NE, and DA reuptake inhibitors（SNDRI） are under development, which may lead to further clinical improvements. We hypothesized that new drugs such as tipepidine may lead to changes in the balance of monoamine levels in the brain resulting in improvement in symptoms of depression. These drugs may change the levels of monoamines in a different way than SSRIs, resulting in new therapeutic potentials for depression.

We have previously reported that dextromethorphan, a centrally acting antitussive, inhibits G protein-coupled inwardly rectifying potassium (GIRK) channel currents [30]. This was also the case for other centrally acting antitussives such as tipepidine. Potassium efflux through GIRK channels causes membrane hyperpolarization, and thus plays an important role in the inhibitory regulation of neuronal excitability [21, 26, 33]. Therefore, we hypothesized that tipepidine may have an antidepressant-like effect leading to increased 5-HT release via the

inhibition of GIRK channels in the raphe nucleus. Further, our recent preliminary data using in vivo microdialysis showed that this drug increases not only serotonin, but also DA levels in the prefrontal cortex of rats. Therefore, in this study, we investigated whether or not tipepidine has antidepressive-like activity in rats using the forced swimming test.

Experiments were carried out in male Wistar rats weighing 200g-240g . Rats were housed in Tokiwa TPX under a normal 12-h light:12-h dark period (light on at 08:00 h) at least for 3 days, with ad libitum access to food (standard pellets; CLEA JAPAN INC) and water. Ambient temperature was maintained at 22±2 ℃ and relative humidity was 60±20%. This study was approved by the committee of animal experimentation at Kumamoto and was conducted in strict accordance with the Guidelines of the Japanese Pharmacological Society for the Care and Use of Laboratory Animals.

This experiment was carried out using the method described by De vry et al. [6]. During the pretest session, the rats were individually placed in a transparent cylinder (height 40 cm, diameter 20 cm) containing 20 cm of water at 25 ±1 ℃. Twenty minutes later, they were removed from the water and dried off. Twenty-four hours later, animals were placed in the cylinder again and the total time of immobility (in seconds) during this session was recorded for 5 min. Two rats, separated by a nontransparent board placed between the two cylinders, were scored simultaneously. A rat was considered to be immobile when it remained floating in the water in an upright position, making only very small movements to keep its head above water. Climbing was defined as strong movements in and out of the water, executed with the forepaws, usually against the walls. Swimming was defined as movement (usually horizontal) throughout the cylinder. Six to twelve rats were used for each dose of drug, and each rat was

tested only once (pretest and one 5 min test). In the dose-response experiment, rats received triple injections with tipepidine (10, 20, and 40 mg/kg per one injection) or saline. Drug was given i.p., 23, 5 and 0.5 h before the test session, because it is reported that preferably three, pretest administrations provide more stable pharmacological results [22]. This protocol of drug administration was also used for the open field and rota-rod experiments. A 5-min swim test session was videotaped, and the time spent immobile, climb and swim during the 5-min was analyzed by observers blinded to treatment groups.

In order to examine general changes in motor activity, rats were also assessed for changes in locomotor activity. Motor activities were determined for 5 min at 30 min after the last of the triple injections. Rats were placed individually in a box made of vinyl chloride (1000 mm, 1000 mm square and 400 mm high), and locomotion was measured using video tracking software (Limelight, Neuroscience, Tokyo, JAPAN).

The motor coordination of the animals was evaluated by using a rota-rod apparatus (Muromachi, JAPAN). Rats were trained to walk themselves on the rotating rod for a total period of 2 min at 24 h before the experiment; rats were walked on the rotating rod with the tail catched by an observer for the first 1 min trial, and were then walked without being catched with the tail for the next 1 min trial. Rats that dropped out from the rotating rod within 10 sec after the last trial started were not used in the experiment. On the experimental day, the animals were placed on the rotating rod (10 r.p.m.) and the time they spent on it was measured. The cutoff time was 300 s.

Using the protocol describe by De vry et al [6], tipepidine (purchased from

Mitubishi-tanabe, Japan) was dissolved in saline (0.9% NaCl), and administrated i.p, prior to the forced swimming test.

All behavioral parameters were reported as the mean ± S.E.M.. The data from the forced swimming test was analyzed by ANOVA and Dunnett's test. The locomotor activity and motor coordination data were analyzed with the unpaired Student's t test. A p value of less than 0.05 was considered significant. All statistical analyses were carried out using SPSS for Windows.

The effect of tipepidine on the duration of immobility and climbing are shown in Fig. 1A and Fig. 1B, respectively. Tipepidine at 20 and 40 mg/kg i.p. induced significant reduction in immobility [$F(3, 27) = 5.66$ $P<0.01$], and at 40mg/kg, i.p. an increase in climbing in the test [$F(3, 27)=7.83$, $P<0.01$]. However, swimming time was not affected by this range of tipepidine [$F(3, 27)=0.91$, $P>0.1$] (Fig. 1C). Tipepidine at 40 mg/kg had no effect on the locomotor activity (Fig 2A) and motor coordination (Fig.2B) compared to control.

In this study, we found that tipepidine decreases immobility in the forced swimming test in rats. It is unlikely that this is simply due to activation of motor function, because the drug had little effect on the locomotor activity in the open field test. Many of the currently available antidepressants have also been shown to reduce immobility in the forced swimming test. However, the selective serotonin re-uptake inhibitor (SSRI) such as fluvoxamine does not. Furthermore, wake amines and anticholinergic drugs also reduce immobility time in the forced swimming test [18, 20, 25].

Tipepidine, an antitussive prescribed clinically in Japan, is not a wake amine, but rather has

an inhibitory effect on central nervous system function such as respiration. The present results, therefore, suggest that tipepidine may have an antidepressive-like effect.

Almost all antidepressants developed so far reduce the immobility time of rats in the forced swimming test, but differ in their effect on climbing and swimming behaviors. It has been reported that the drugs that increase the NE level in the brain increase the climbing behavior. Recent study revealed that increase in the DA level in the brain was also involved into increase in the climbing behavior in the forced swimming test [8]. Our preliminary study showed that tipepidine increased the DA level in the nucleus accumbens and prefrontal cortex of rats [10,11,12]. Therefore, it is suggested that tipepidine-induced increase in the climbing behavior might be due to the increase in the catecholamine levels in the brain. Apart from the above, drugs that increase the serotonin levels in the brain increase the swimming behavior in the forced swimming test [4]. Desipramine and milnacipran which are SNRIs and bupropion, a dopamine reuptake inhibitor, increase the climbing behavior and reduce immobility in the forced swimming test [5, 8, 23]. Fluoxetine an SSRI, increases the swimming behavior [5]. Venlafaxine an SNRI, increases both the climbing and swimming behaviors [23], but duloxetine, another SNRI, did not affect climbing and swimming behaviors [23]. In our study tipepidine increased the climbing behavior similar to desipramine, milnacipran, and bupropion. However, tipepidine, even at a dose of 40 mg/kg, did not significantly inhibit the locomotor activity examined with the open field test. Thus, the pharmacological properties of tipepidine seem to differ from those of desipramine and milnacipran and are rather similar to that of bupropion, which also had little effect on the locomotor activity [23].

The rota rod test has been used to evaluate pharmacological actions of psychotropic agents. Skeletal muscle relaxants and anticonvulsants often show impaired motor coordination in the rota rod method [7]. In this study, tipepidine had little effect on the motor

coordination in the rota rod test using rats.

In our preliminary study, we found that other antitussives which inhibit GIRK channel activated currents in brain neurons inhibited the forced swimming in rats. We did not investigate the mechanism of the antidepressive action of tipepidine in the present study. Noticeably, tipepidine had little effect on the SERT, NET and/or DA transporter (DAT) (personal communication; Mitsubishi Tanabe Pharma. Corp, Japan). Furthermore, it has been reported that dextromethorphan, a centrally-acting antitussive, had little or no affinity to the receptors of many neurotransmitters in the brain [3], although it is unknown as to whether tipepidine has any affinity to various receptors in the brain. Therefore, the followings merit to be discussed. GIRK channels are coupled to various G-protein coupled receptors (GPCRs) such as 5-HT_{1A}, α_2, D_2 receptors and others. GPCR-mediated activation of GIRK channels stabilizes the excitation of neurons through the hyperpolarization caused by outward currents carried by K^+. Thus, inhibition of GIRK channels should activate neurons, facilitating the release of the neurotransmitters. In a previous study, we reported that 5-HT inhibited the excitability of single neurons dissected from the raphe nucleus [30]. Further, in our own preliminary study, a drug that inhibits GIRK channels reversed the inhibition caused by 5-HT. Furthermore, it has been reported that dextromethorphan facilitated the release of 5-HT from slice preparations including the nucleus solitarii [13]. In addition, our preliminarily study using in vivo microdialysis directly shows that tipepidine and cloperasitine increase the levels of 5-HT and DA in the frontal cortex of rats. An increase in DA level was also found in the nucleus accumbens [10,11,12]. GIRK channels are also coupled to α_2 adrenergic receptors. We have reported that dextromethorphan also inhibited GIRK channel currents mediated by α_2 adrenergic receptors. Thus, tipepidine may increase NE levels, leading to an increase in the climbing behavior in the forced swimming test. More recently, our preliminary study also

revealed that drugs such as cloperastine inhibited DA receptor-mediated GIRK channel currents in single neurons. Taken together, it is likely that the antidepressive action of tipepidine shown here may be due to inhibition of GIRK channels that are coupled to 5-HT,α_2 adrenergic receptors and dopamine D_2 receptors. As described above, tipepidine has a very similar pharmacological profile to that of bupropion, having similar effects on immobility, climbing and swimming in the forced swimming test and on locomotor activity in the open field test. However, the chemical structure of tipepidine is quite different from that of bupropion. Bupropion also has no effect on GIRK channels [17]. Therefore, it is possible that the mechanisms of the behavioral effects of tipepidine may be different from that of bupropion. It is well known that reduction in the brain levels of NE and 5-HT may lead to some forms of depression [27]. DA is also postulated to play a critical role in depression [27]. Recent studies have shown that modulation of DA levels in the brain may be effective in treating depression that is resistant to standard treatment [2, 9].

In our microdialysis study, drugs such as tipepidine that inhibited GIRK channel activity remarkably increased the level of DA compared to the level of 5-HT [10,11,12]. This increase was found in the shell of the nucleus accumbens. It is, however, clear that tipepidine dose not have abuse potential, because tipepidine is commonly used as a cough-depressant drug in clinics and as the over-the-counter drug in Japan. In addition, doses of tipepidine used in the present study was antitussive effective dose. Furthermore, it has been reported that no abstinence syndrome occurred after successive administration of tipepidine for 100 days in dog [14].

Further studies are needed to elucidate the mechanism of the antidepressive activity of tipepidine seen in experimental animals. Nevertheless, drugs such as tipepidine that have an inhibitory actions on GIRK channels may provide new therapeutic potential for the treatment

of otherwise treatment-resistant depression via an increase in the levels of monoamines like DA.

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Fig. 1 Effect of tipepidine treatment in the forced swimming model of depression.

Rats (control; n=12, 20 mg/kg; n=7, 10 mg/kg and 40 mg/kg; n=6 each) were received a 20 min swimming pretest, and were retested 24 h later. Immobility (A), climbing (B), and swimming (C) in sec were measured during the second 5 min swimming test. Drug was administered i.p., 23, 5 and 0.5 hour before the second test. *$p<0.05$, **$p<0.01$, *** $p < 0.001$ as compared with saline control.

Fig. 2 Effect of tipepidine treatment in the locomotor activity and motor coordination.

Effect of tipepidine on the locomotor activity (A) and motor coordination (B) in rats. Forty mg of tipepidine was i.p. administered three times at 23, 5 and 0.5 hour before the open field test and the rota rod test. Locomotor activity and motor coordination was measured for 5 min. Six animals per group were used in the Open field test and 5-6 animals per group in the Rota rod test.

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