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Antidepressant-like effect of centrally acting non-narcotic antitussive caramiphen in a forced swimming test

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Abstract

Recently, we reported that a centrally acting non-narcotic antitussive (cough suppressant drug), tipepidine produces an antidepressant-like effect in the forced swimming test in rats. Because pharmacological properties of tipepidine apparently differ from those of typical antidepressants developed to date, we speculated that caramiphen, another centrally acting antitussive, has an antidepressant-like effect. That effect of caramiphen was studied in rats using the forced swimming test. Caramiphen at 20 and 40 mg/kg i.p. significantly reduced immobility. At 40 mg /kg i.p., it increased climbing behavior. Even at 40 mg /kg, this drug had no effect on locomotor activity. Results suggest that a centrally acting antitussive possessing inhibition of GIRK channels has an antidepressant-like effect.

Key words: Antitussives, Antidepressant-like effect, Caramiphen, Forced swimming test, G-protein coupled inwardly rectifying K⁺ (GIRK) channel

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 in rats [9]. It is particularly interesting that the pharmacological properties of tipepidine apparently differ from those of typical antidepressants developed to date. Tipepidine increased climbing behavior as did desipramine (noradrenaline reuptake inhibitor: NRI) and milnacipran (serotonin and noradrenaline reuptake inhibitor: SNRI). Unlike these two drugs, tipepidine had no effect on locomotor activity examined in the open field. Furthermore, tipepidine exhibited no effect on swimming behavior, differing from fluoxetine (selective serotonin reuptake inhibitor; SSRI) [1,14] and venlafaxine (SNRI) [14]. Another SNRI, duloxetine, failed to affect climbing and swimming behaviors [14]. Consequently, behavioral–pharmacological properties of tipepidine were apparently similar to those of bupropion, a dopamine reuptake inhibitor (DRI), because bupropion increased climbing behavior and because it had no effect on locomotor activity, reducing immobility in the forced swimming test [14]. Tipepidine had little or no effect on monoamine transporters such as serotonin transporter, norepinephrine transporter, and dopamine (DA) transporter (personal communication: Mitsubishi–Tanabe Pharma Corp., Japan). Furthermore, the drug had little effect on various neurotransmitter receptors such as dopamine D₁, D₂, 5-HT_{1A}, 5-HT₂, and adrenergic α_1 and α_2 receptors (personal communication: Mitsubishi–Tanabe Pharma Corp., Japan). These findings suggest that tipepidine may produce an antidepressant-like effect through actions that differ from those of antidepressants developed to date. In a previous report, we noticed that antitussives including tipepidine have an inhibitory action on G-protein-coupled inwardly rectifying K⁺ (GIRK) channel activated currents [10,16]. Potassium efflux through GIRK channels causes membrane hyperpolarization and thereby plays an important

role in the inhibitory regulation of neuronal excitability [11, 15]. It is particularly interesting that GIRK channels are coupled to various G-protein coupled receptors (GPCRs) such as 5-HT_{1A}, adrenergic α_2 , dopamine D₂ receptors and others [3,4,5,11]. Consequently, inhibition of GIRK channels activates neurons, thereby facilitating the release of the neurotransmitters. Our preliminary study also revealed that tipepidine increases the level of monoamines in the brain [6,7,8]. In this context, it is of interest to know whether or not a drug possessing inhibitory action on GIRK-channel activated currents has an antidepressant-like effect in the forced swimming test of experimental animals. Therefore, effect of caramiphen, other antitussives possessing inhibitory action on GIRK channel activated currents, on the forced swimming of rats was studied.

Materials and Methods

Animals

Forced swimming test and open field test were conducted using male Wistar rats weighing 200–240 g. All 41 rats used for this study were purchased (Kyudo Inc., Japan) and housed in plastic cages (Tokiwa Inc., Japan) 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 and water. Ambient temperature was maintained at 22±2°C; the relative humidity was 60±20%. This study was approved by The Committee of Animal Experimentation at Kumamoto University, and was conducted in strict accordance with the Guidelines of the Japanese Pharmacological Society for the Care and Use of Laboratory Animals.

Forced swimming test

The forced swimming test was carried out using the method described by De Vry et al. [2]. During the

pretest session, the rats were individually placed in a transparent cylinder (height 40 cm, diameter 20 cm) with 20-cm-deep water at $25\pm 1^{\circ}\text{C}$. 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 slight 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. Each rat (Control, $n=7$; 10 mg/kg, $n=6$; 20 mg/kg, $n=5$; and 40 mg/kg, $n=5$) was tested only once (pretest and one 5 min test). In the dose–response experiment, rats received triple injections with caramiphen (10, 20 and 40 mg/kg per injection) or saline. Drug was given i.p. at 23, 5, and 0.5 h before the test session because preferably three, pretest administrations provide more stable pharmacological results [12]. This protocol of drug administration was also used for the open field test. A 5-min swim test session was videotaped. The time spent immobile, climbing, and swimming during the 5-min was analyzed by observers blinded to treatment groups.

Open field test

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 ($n=9$ per group) were placed individually in a box made of vinyl chloride (1000 mm square, 400 mm height). Then locomotion was measured using video tracking software (Limelight; Neuroscience Inc.,

Tokyo, Japan). In this experiment, rats were at first studied with the triple injections of caramiphen at only 40 mg/kg, i.p. per injection or saline. Because this dose had little effect on locomotor activity in rats, no study was done with low doses of 10 and 20 mg/kg.

Statistical analysis

All behavioral parameters were expressed as the mean \pm S.E.M. The data from the forced swimming test were analyzed using one way-ANOVA followed by Dunnett's test. The locomotor activity was analyzed using Student's *t*-test. A *p* value of less than 0.05 was considered significant. All statistical analyses were conducted using software (SPSS ver.13 for Windows; SPSS Inc.).

Results

The effects of caramiphen on the duration of immobility, climbing and swimming are shown in Figs. 1A, 1B, and 1C, respectively. Caramiphen at 20 and 40 mg/kg i.p. induced significant reduction in immobility [$F(3, 19)=7.31, P<0.01$]. At 40 mg/kg i.p., it induced an increase in climbing in the test [$F(3, 19)=4.18, P<0.05$]. However, swimming time was unaffected by this dosage of caramiphen [$F(3, 19)=2.26, P>0.1$]. Caramiphen at 40 mg/kg i.p. showed no effect on locomotor activity (Fig. 2) compared to saline control.

Discussion

Results of the forced swimming test in rats show that caramiphen dose-dependently decreases immobility. In this study, caramiphen at 40mg/kg, i.p. did not affect locomotor activity. Therefore, it

is unlikely that this decrease is caused simply by activation of motor function. Many currently available antidepressants have also been shown to reduce immobility in the forced swimming test [12,13,14], although SSRIs such as fluvoxamine do not. Furthermore, wake amines also reduce the immobility period in the forced swimming test and increase locomotor activity [13]. Caramiphen, an antitussive drug, is not a wake amine, but rather has an inhibitory effect on central nervous system functions such as respiration. The results, therefore, suggest that caramiphen may have an antidepressant-like effect.

Almost all antidepressants developed to date reduce the immobility time of rats in the forced swimming test, but they differ in their effects on climbing and swimming behaviors in the test. Desipramine (NRI), milnacipran (an SNRI), and bupropion (DRI) increase the climbing behavior and reduce immobility in the forced swimming test [1,14]. Fluoxetine an SSRI, increases swimming behavior [1,14]. Venlafaxine (an SNRI) increases both climbing and swimming behaviors [14], but duloxetine, another SNRI, does not affect climbing and swimming behaviors [14]. In our previous study, tipegidine was shown to increase climbing behavior, but it had no effect on swimming behavior, suggesting that this drug might have pharmacological properties differing from those of antidepressant drugs developed to date. In addition, tipegidine showed no effect on motor activities when determined by open field testing and the rota-rod test, differing from desipramine (NRI) and milnacipran (SNRI). Interestingly, caramiphen showed a similar pharmacological property to that of tipegidine in the forced swimming test. It had no effect on motor activities. Tipegidine at 20 mg/kg reduced the immobility time to 54.9% of the control time. Increased dosage of 40 mg/kg reduced the time to 26.6% of control [9]. Caramiphen at the same dosage inhibited the immobility time in the forced swimming test to a similar degree (20 mg/kg, 50.94%; 40 mg/kg, 25.2%). In addition, tipegidine and caramiphen at 40 mg/kg both increased climbing behavior to 225.7 [9] and 237.0% of control and had no effect on

the swimming behavior. Considering all these observations together, it is reasonable to speculate that the mechanism of the antidepressant-like effect of caramiphen might resemble that of tipepidine.

A previous report described a novel pharmacological profile of the anti-depressant-like effect of tipepidine, addressing its inhibitory action on GIRK channels. Caramiphen-like tipepidine has an inhibitory action on GIRK-channel activated currents. As discussed in that earlier report, GIRK channels are coupled to various G-protein coupled receptors (GPCRs) such as 5-HT_{1A}, adrenergic α_2 , dopamine D₂ receptors and others [3,4,5,11]. The GPCR-mediated activation of GIRK channels stabilizes the excitation of neurons through the hyperpolarization caused by outward currents carried by K⁺ [11,15]. Consequently, inhibition of GIRK channels can be expected to activate neurons, thereby facilitating the release of neurotransmitters. Our preliminary study using *in vivo* microdialysis showed directly that caramiphen and tipepidine increase the levels of 5-HT and DA in the frontal cortex of rats. An increase in the DA level was also found in the nucleus accumbens [6,7,8]. Taken together, it is likely that the antidepressive action of caramiphen shown here is attributable to inhibition of GIRK channels that are coupled to 5-HT, adrenergic α_2 receptors, and dopamine D₂ receptors. In addition to tipepidine, caramiphen was found to have antidepressant-like action with a similar pharmacological profile to that of tipepidine. This finding further supports the hypothesis that the novel antidepressant-like action of caramiphen and tipepidine is caused at least partly through inhibitory action on the GIRK channels in the brain, although further studies are necessary to elucidate the mechanism of the novel antidepressant-like activity of caramiphen.

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Legends

Fig. 1 Effect of caramiphen treatment on the forced swimming model of depression.

Rats (Control, $n=7$; 10 mg/kg, $n=6$; 20 mg/kg, $n=5$; and 40 mg/kg, $n=5$) were tested only once (pretest and one 5-min test). Those of the dose–response experiment received a 20-min swimming pretest. They were retested 24 h later. Immobility (A), climbing (B), and swimming (C) were measured during the second 5 min swimming test. The drug was administered i.p. 23, 5, and 0.5 h before the second test.

* $p<0.05$, ** $p<0.01$ compared with saline control.

Fig. 2 Effect of caramiphen treatment on locomotor activity.

Effect of caramiphen on locomotor activity in rats ($n=9$ per groups). The drug was administered i.p. 23, 5, and 0.5 h before the test.

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Figure.1

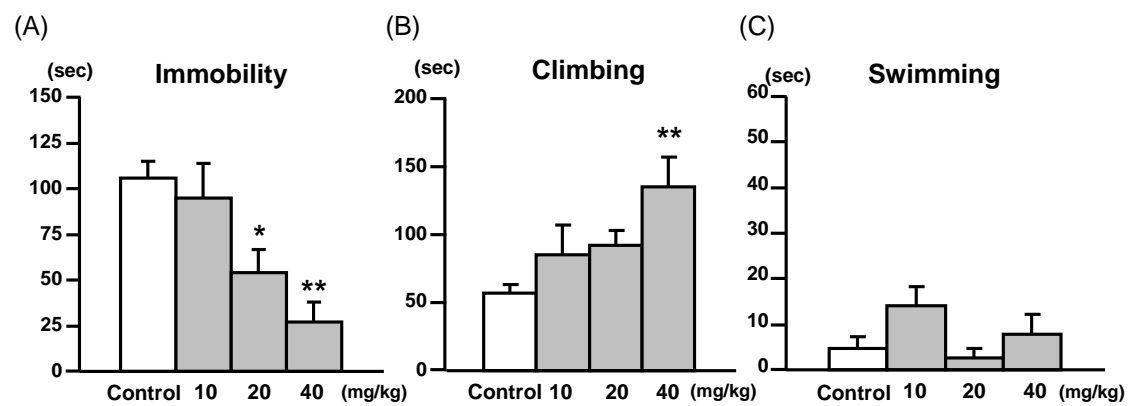


Figure.2

