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Anxiolytic-like effects of baicalein and baicalin in the Vogel conflict test in mice

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Abstract

A previous receptor binding assay indicated that baicalein, one of the active principles of the Chinese herbal drug, Huangqin (Scutellariae Radix), interacts with the benzodiazepine binding site of $GABA_A$ receptors in mouse cortex membrane preparations with a K_i value of 13.1 μM. Therefore, the present study examined whether baicalein and its 7-glucuronide, baicalin, have anxiolytic-like effects in a Vogel conflict test adapted for ICR mice. The results showed that both baicalein (10 mg/kg, i.p.) and baicalin (20 mg/kg, i.p.) significantly increased the number of shocks accepted in the Vogel lick-shock conflict paradigm over 9 min, as did a benzodiazepine receptor agonist, chlordiazepoxide (5.0 mg/kg, i.p.) and a 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-*n*-propylamino)tetralin (0.5 mg/kg, i.p.). Since the total volume of water intake and the shock sensitivity of mice were not significantly changed after drug treatment, the effect of baicalein or baicalin was not due to an enhancement of thirst or shock tolerance. Furthermore, this anxiolytic-like effect of baicalein or baicalin was antagonized by co-administration with a benzodiazepine receptor antagonist, flumazenil (2 mg/kg, i.p.), but not with a 5-HT_{1A} receptor antagonist, pindolol (10 mg/kg, i.p.). It is concluded that the anxiolytic-like effect of baicalein or baicalin may be mediated through activation of the benzodiazepine binding site of $GABA_A$ receptors.

Keywords: Baicalein; Baicalin; Anxiolytic-like effect; Vogel conflict test; Chlordiazepoxide; Flumazenil

1. Introduction

The Chinese herbal drug, ‘‘Huangqin’’ (Scutellariae radix), is the dry root of *Scutellaria baicalensis* Georgi (Labiatae), which is used against bacterial infections of the respiratory and the gastrointestinal tracts (Chang and But, 1986; Tang and Eisenbrand, 1992). In addition, the decoction of Huangqin was shown to have a sedative effect (Chang and But, 1986). To explain its central nervous effect at the receptor level, the interactions of Huangqin water extract with receptors for some important central neurotransmitters were evaluated in radioligand receptor binding assays. It was found that Huangqin water extract interacted with the dopamine D1 ($K_i = 4 \pm 1$ mg/ml), D2 (19 ± 10 mg/ml), 5-HT_{1A} (4 ± 2 mg/ml) receptors, and the benzodiazepine binding site of $GABA_A$ receptors (0.018 ± 0.002 mg/ml), but not with the 5-HT_2 receptors and the GABA binding site of $GABA_A$ receptors (Liao et al., 1995). Due to the potent interaction of this extract with the benzodiazepine site, a subsequent study using a benzodiazepine binding assay-directed separation led to the identification of three benzodiazepine binding site-interactive flavones (baicalein, oroxylin A, and skullcapflavone II with a K_i value of 13.1, 14.6 and 0.36 μM, respectively) from Huangqin water extract (Liao et al., 1998). Although the affinity of skullapflavone II for the benzodiazepine sites is comparable to that of a benzodiazepine receptor agonist, chlordiazepoxide, the pharmacological effect of skullapflavone II has not been characterized due to its unavailability. According to the yield ratio (0.0058%), it was calculated that the content of skullapflavone II only has a minor role in the interaction of Huangqin water extract with the benzodiazepine sites. Thus, other different principles (for examples, baicalein and oroxylin A) with different content and affinities for

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the benzodiazepine sites should also contribute. Because the pharmacological characteristics (agonist, inverse agonist, or antagonist property) of these benzodiazepine site-interactive principles from Huangqin have not been examined, it was interesting to examine whether baicalein, like typical benzodiazepine receptor agonists, has an anxiolytic effect. Since its 9-glucuronide, baicalin also interacted with the benzodiazepine sites with a K_i value of 77 μM (Hui et al., 2000) and baicalin can be converted to baicalein by bacterial flora in the intestinal tract after oral administration (Akao et al., 2000), this study examined the anxiolytic-like effects of baicalein and baicalin using a Vogel conflict test adapted for ICR mice.

2. Materials and methods

2.1. Animals

Male ICR (Institute of Cancer Research) mice (25–30 g) were obtained from the Animal Center of National Taiwan University. They were maintained on a 12-h light and 12-h dark cycle (light on between 7:00 and 19:00) with food and tap water ad libitum.

2.2. Drugs

Chlordiazepoxide hydrochloride and DL-propranolol hydrochloride were purchased from Sigma. Pindolol, baicalein, and baicalin hydrate were purchased from Aldrich Chemical. Flumazenil was purchased from Tocris Cookson. *R*(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT HBr) was purchased from Research Biochemical International.

2.3. Vogel drinking conflict test

The apparatus for the Vogel conflict test is AnxioScan 99 (AccuScan Instruments, USA). The experimental protocol was according to Vogel et al. (1971) with some modifications. In brief, the mouse deprived of water for 24 h was put into the test cage (20 × 20 × 25 cm) for adaptation and allowed to lick water freely for 9 min. This 9-min test period was based on the finding in a pilot study, which showed no significant differences in the total volume of water intake by the mouse among 9-, 20-, and 40-min test periods (i.e., 2.7 ± 0.3 vs. 3.3 ± 0.3 vs. 3.0 ± 0.0 ml, respectively). After a further 24-h deprivation of water, the mouse was put into the test cage again for 9 min and received the punishment per 20 licks by electrical shock (0.45 mA for 2 s) delivered via the drinking spout and the grid floor. The number of shocks was recorded during this 9-min lick-shock paradigm. The test cage was cleaned between each test. The test drug was i.p. administered 30 min before the test. To establish the validity of this test, various doses of two control anxiolytic drugs, the benzodiazepine receptor agonist, chlordiazepoxide, and the 5-HT_{1A} receptor agonist, 8-OH-DPAT, were examined first.

To examine the effect of drug on the total volume of water intake by the animal during this 9-min test period, the same protocol was performed except that no punishment was received during the test. The total volume of the water intake was recorded.

To examine the effect of drug on the shock sensitivity of the animal, the same protocol was performed except that the electrical shock was increased 0.01 mA per 2-min interval from 0.35 mA until the animal flinched. This shock threshold was recorded.

2.4. Antagonism by flumazenil or pindolol

The antagonism by a benzodiazepine receptor antagonist, flumazenil, or a 5-HT_{1A} receptor antagonist, pindolol, was used to check the involvement of activation of the benzodiazepine site of $GABA_A$ receptors or 5-HT_{1A} receptors, respectively, in the anxiolytic-like effect of the test drug. The optimal antagonizing dose of flumazenil or pindolol was chosen based on the fact that alone, it had no significant effect on drinking behavior but that it abolished the anxiolytic effect of chlordiazepoxide or 8-OH-DPAT when co-administration with the anxiolytic dose of chlordiazepoxide or 8-OH-DPAT, respectively.

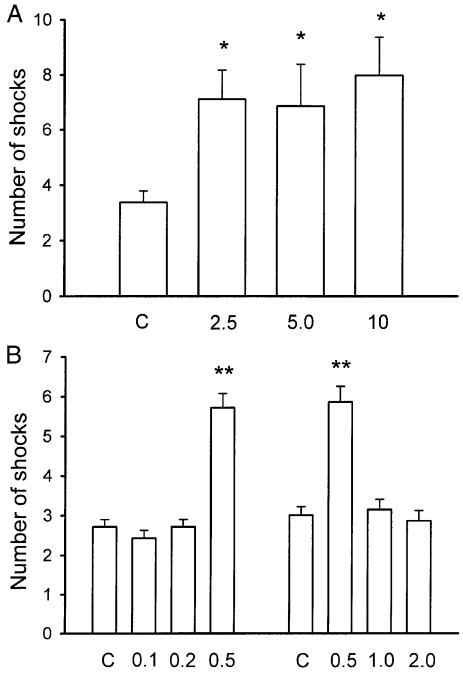

Fig. 1. Effects of chlordiazepoxide and 8-OH-DPAT on the number of shocks accepted in a Vogel drinking conflict test in mice. Various doses of (A) chlordiazepoxide (2.5–10 mg/kg) or (B) 8-OH-DPAT (0.1–0.5 or 0.5–2.0 mg/kg) were i.p. administered 30 min before the Vogel test and the number of shocks accepted by the animal in this lick-shock conflict paradigm over 9 min were recorded. Data are means ± S.E.M. ($n = 7–8$). *$P < 0.05$, **$P < 0.01$, as compared with the corresponding control (C).

Because pindolol also is a β-adrenoceptor antagonist, the antagonism by propranolol, a typical β-adrenoceptor antagonist, on the anxiolytic effect of 8-OH-DPAT was also examined.

2.5. Data analysis

The data were expressed as means ± S.E.M. and analyzed by one-way analysis of variance followed by Newman–Keuls test with a significance level of $P<0.05$.

3. Results

3.1. Effects of chlordiazepoxide and 8-OH-DPAT

As shown in Fig. 1A, chlordiazepoxide (2.5–10 mg/kg, i.p.) significantly increased the number of shocks accepted in the Vogel lick-shock conflict paradigm over 9 min. In an initial study, the effects of various doses of 8-OH-DPAT (0.5–2.0 mg/kg, i.p.) were examined and only 0.5 mg/kg was found to significantly increase the number of shocks. Using reduced doses (0.1–0.5 mg/kg, i.p.), a subsequent study also indicated that 8-OH-DPAT had a significant effect only at the dose of 0.5 mg/kg (Fig. 1B).

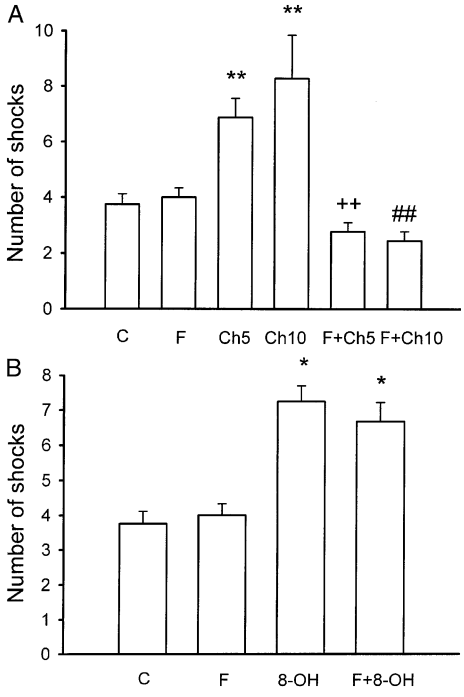

Fig. 2. Effects of flumazenil on the anxiolytic activity of chlordiazepoxide and 8-OH-DPAT in mice. Flumazenil (F, 2 mg/kg) was i.p. administered or co-administered with (A) chlordiazepoxide (Ch, 5.0 or 10 mg/kg) or (B) 8-OH-DPAT (8-OH, 0.5 mg/kg) 30 min before the Vogel test and the number of shocks accepted in this lick-shock conflict paradigm over 9 min were recorded. Data are means ± S.E.M. ($n=7–9$). *$P<0.05$, **$P<0.01$, as compared with the corresponding control (C); $^{++}P<0.01$, as compared with the Ch5 group; $^{\#\#}P<0.01$, as compared with the Ch10 group.

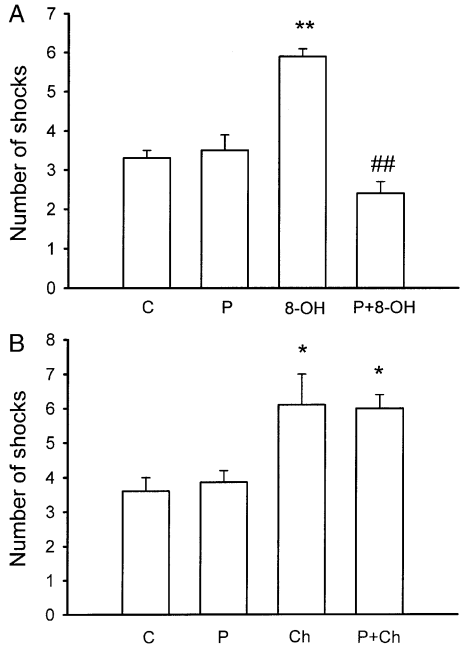

Fig. 3. Effects of pindolol on the anxiolytic activity of 8-OH-DPAT and chlordiazepoxide in mice. Pindolol (P, 10 mg/kg) was i.p. administered or co-administered with (A) 8-OH-DPAT (8-OH, 0.5 mg/kg) or (B) chlordiazepoxide (Ch, 5.0 mg/kg) 30 min before the Vogel test and the number of shocks accepted in this lick-shock conflict paradigm over 9 min were recorded. Data are means ± S.E.M. ($n=7–8$). *$P<0.05$, **$P<0.01$, as compared with the corresponding control (C); $^{\#\#}P<0.01$, as compared with the 8-OH group.

3.2. Effects of flumazenil and pindolol

As shown in Fig. 2A, flumazenil (2 mg/kg, i.p.) alone had no significant effect but reversed the chlordiazepoxide (5.0 and 10 mg/kg, i.p.)-induced increase in the number of shocks when co-administered with chlordiazepoxide. As shown in Fig. 3A, pindolol (10 mg/kg, i.p.) alone had no significant effect but reversed the 8-OH-DPAT (0.5 mg/kg, i.p.)-induced increase in the number of shocks. In contrast, the anxiolytic-like effect of chlordiazepoxide was not influenced by pindolol (Fig. 3B) and that of 8-OH-DPAT was not influenced by flumazenil (Fig. 2B). Since pindolol also is a β-adrenoceptor antagonist, the effect of a typical β-adrenoceptor antagonist, propranolol, was also examined on the 8-OH-DPAT-induced action. It was found that propranolol (10 mg/kg, i.p.) had no significant effect on the 8-OH-DPAT-induced increase in the number of shocks (data not shown).

3.3. Effects of baicalein and baicalin

As shown in Fig. 4A, baicalein at 10.0 mg/kg (i.p.) significantly increased the number of shocks whereas a

lower dose (5.0 mg/kg) and a higher dose (20.0 mg/kg) increased the number but the effect had no statistical significance. As shown in Fig. 4B, baicalin at 20.0 mg/kg (i.p.) significantly increased the number of shocks whereas lower doses (5.0 and 10.0 mg/kg) had no significant effects.

To examine whether the baicalein- or baicalin-induced increase in the number of shocks was due to an enhancement of thirst or shock tolerance, the effects of baicalein or baicalin on the total volume of water intake and the threshold of electric shock were evaluated. Baicalein (10.0 and 20.0 mg/kg, i.p.) had no significant effect on either the total volume of water intake (2.8 ± 0.1 and 2.8 ± 0.1 vs. control 2.8 ± 0.3 ml, $n = 7$) or the threshold for electric shock (0.39 ± 0.04 and 0.39 ± 0.04 vs. control 0.40 ± 0.07 mA, $n = 7$). Similarly, baicalin (10.0 and 20.0 mg/kg, i.p.) had no significant effect on either the total volume of water intake (2.8 ± 0.1 and 2.9 ± 0.2 vs. control 2.6 ± 0.3 ml, $n = 7$) or the threshold for electric shock (0.41 ± 0.01 and 0.40 ± 0.01 vs. control 0.40 ± 0.01 mA, $n = 7$).

To examine whether this anxiolytic-like effect of baicalein or baicalin was due to activation of the benzodiazepine binding sites or the 5-HT_{1A} receptors, the antagonizing effect of flumazenil or pindolol was evaluated. As shown in Fig. 5A and B, the anxiolytic-like effects of baicalein (10.0 mg/kg, i.p.) and baicalin (20.0 mg/kg, i.p.) were antagonized by flumazenil (2 mg/kg, i.p.) but not by pindolol (10 mg/kg, i.p.).

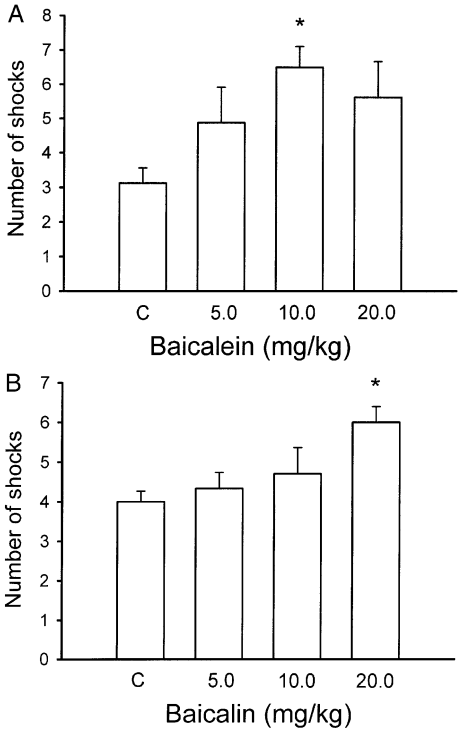

Fig. 4. Effects of baicalein and baicalin on the number of shocks accepted in a Vogel drinking conflict test in mice. Various doses of (A) baicalein (5.0–20.0 mg/kg) or (B) baicalin (5.0–20.0 mg/kg) were i.p. administered 30 min before the Vogel test and the number of shocks accepted in this lick-shock conflict paradigm over 9 min were recorded. Data are means ± S.E.M. ($n = 8–10$). *$P < 0.05$, as compared with the corresponding control (C).

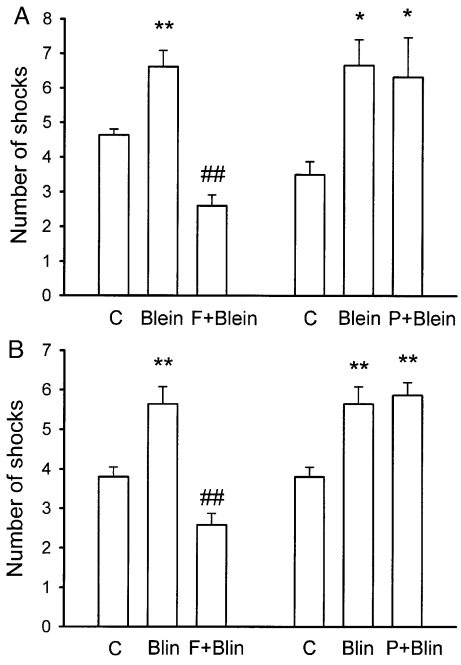

Fig. 5. Effects of flumazenil and pindolol on the anxiolytic activities of baicalein and baicalin in mice. Flumazenil (F, 2 mg/kg) or pindolol (P, 10 mg/kg) was i.p. administered or co-administered with (A) baicalein (Blein, 10.0 mg/kg) or (B) baicalin (Blin, 20.0 mg/kg) 30 min before the Vogel test and the number of shocks accepted in this lick-shock conflict paradigm over 9 min were recorded. Data are means ± S.E.M. ($n = 8–15$). *$P < 0.05$, **$P < 0.01$, as compared with the corresponding control (C); $^{\#\#}P < 0.01$, as compared with the corresponding Blein group or Blin group.

4. Discussion

In the Vogel drinking conflict test, the anxiolytic-like effect of a test drug is indicated by the increase in the number of shocks during a lick-shock paradigm. However, this increase in the number of shocks may be due to enhancement of drinking behavior by an increase in thirst or a decrease in the response to electric shock due to an increase in shock tolerance. The present study showed that both baicalein (10.0 mg/kg, i.p.) and baicalin (20.0 mg/kg, i.p.) increased the number of shocks but had no significant effects on either total volume of water intake or shock sensitivity, indicating that they have anxiolytic-like effects in the Vogel test. Because the effects of baicalein and baicalin were antagonized by the benzodiazepine receptor antagonist, flumazenil, but not by the 5-HT_{1A} receptor antagonist, pindolol, it is suggested that the anxiolytic-like effects of baicalein and baicalin are mediated via activation of the benzodiazepine site of $GABA_A$ receptors but not via the 5-HT_{1A} receptors. This suggestion is consistent with the reports that baicalein or baicalin has moderate affinity at benzodiazepine sites (Liao et al., 1998; Hui et al., 2000) but

has no significant interaction with the 5-HT_{1A} receptors even at 10^{-4} M in a receptor binding assay (Liao et al., unpublished data).

The benzodiazepine agonists are widely prescribed for their anxiolytic, muscle relaxant, sedative-hypnotic and anticonvulsant actions. In the search for benzodiazepine site ligands that conserve the therapeutic properties of benzodiazepines but are devoid of unwanted side-effects, Medina et al. (1997) demonstrated that some naturally occurring flavonoids (for example, chrysin and apigenin) are the ligands for the benzodiazepine binding sites and have anxiolytic effects. Three benzodiazepine binding site-interactive flavones (baicalein, oroxylin A, and skullcapflavone II) from a Huangqin water extract (Liao et al., 1998) were further added to the list of naturally occurring flavones as ligands for the benzodiazepine binding sites, though their related pharmacological activities were not characterized. The present results showed for the first time that baicalein and baicalin have anxiolytic-like effects and probably act via the activation of the benzodiazepine site of $GABA_A$ receptors in the central nervous system. However, further study is needed to find whether or not these two compounds, like the naturally occurring flavonoids, chrysin and apigenin, lack sedative, myorelaxant, anticonvulsant and amnesic effects (Wolfman et al., 1994; Viola et al., 1995; Salgueiro et al., 1997).

Akao et al. (2000) reported that baicalin is not well absorbed after oral administration but is converted to baicalein by the bacterial flora in the intestinal tract to be absorbed and baicalein is efficiently conjugated to baicalin in rat intestinal and hepatic microsomes. Therefore, this interchange between baicalein and baicalin in vivo will make the explanation and comparison for the in vivo pharmacological effects of these two compounds more complicated. In vitro, baicalein is more potent than baicalin to inhibit a histamine-induced increase in intracellular Ca^{2+} concentration in C6 rat glioma cells (Kyo et al., 1998). Baicalein also is more potent than baicalin for an antioxidant effect as evaluated by xanthine oxidase inhibition (IC_{50} = 3.12 vs. 215.19 μM) but baicalin is more potent for the activity on cytochrome c reduction (IC_{50} = 224.12 vs. 370.33 μM) and the scavenging activity of superoxide radical ($IC_{50} = 1.19 \times 10^5$ vs. 7.31×10^4 μ/g) (Shieh et al., 2000). Furthermore, baicalin is more potent than baicalein for the anti-proliferative activity on human bladder cancer cell lines KU-1 (IC_{50} = 3.4 vs. 30 μg/ml) and EJ-1 (IC_{50} = 4.4 vs. 22 μg/ml) but is equally potent on the murine bladder cancer cell line MBT-2 (IC_{50} = 0.93 vs. 0.98 μg/ml) (Ikemoto et al., 2000). However, baicalein is more potent than baicalin for the inhibition of cell growth in androgen-positive and -negative human prostate cancer lines LNCaP (IC_{50} = 29.8 vs. 60.8 μM) and JCA-1 (IC_{50} = 17.7 vs. 46.8 μM) (Chen et al., 2001). At a concentration of 10 mg/ml, the inhibitory activity of baicalein also is greater than that of baicalin on eotaxin production by interleukin-4 plus tumor necrosis factor-α-stimulated human fibroblasts (Nakajima et al., 2001). In vivo, s.c. administered baicalin is more potent than baicalein in the anti-inflammatory activity against carrageenan-induced paw edema in rats (Lin and Shieh, 1996). However, the present study showed that i.p. administered baicalein, on a weight basis or molar basis, is more potent than baicalin for anxiolytic activity in mice. This result is consistent with the report that baicalein has higher affinity for the benzodiazepine binding sites than baicalin (K_i = 5.7 vs. 77.1 μM) (Hui et al., 2000).

In summary, the present study extended the previous findings to demonstrate that baicalein and baicalin, two benzodiazepine binding site-interactive flavones, have anxiolytic-like effects in the Vogel drinking conflict test in mice.

Acknowledgements

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