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Behavioural pharmacology of polygalasaponins indicates potential antipsychotic efficacy

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Abstract

Polygalasaponins were extracted from a plant (*Polygala tenuifolia* Willdenow) that has been prescribed for hundreds of years to treat psychotic illnesses in Korean traditional medicine. Previous in vitro binding studies suggested a potential mechanism for its antipsychotic action, as polygalasaponin was shown to have an affinity for both dopamine and serotonin receptors [Psychopharmacol. Bull. 31 (1995) 139.]. In the present study we have investigated the functional in vivo actions of this material in tests that are predictive of dopamine and serotonin antagonist activities. Polygalasaponin (25–500 mg/kg) was shown to produce a dose-related reduction in the apomorphine-induced climbing behaviour (minimum effective dose [ED_{min}] 25 mg/kg ip, 250 mg/kg sc and po), the 5-hydroxytryptamine (5-HTP)-induced serotonin syndrome (ED_{min} 50 mg/kg ip) and the MK-801-induced hyperactivity (ED_{min} 25 mg/kg ip) in mice. This compound also reduced the cocaine-induced hyperactivity (ED_{min} 25 mg/kg ip) in rats. These results demonstrated that polygalasaponin has dopamine and serotonin receptor antagonist properties in vivo. This might suggest its possible utility as an antipsychotic agent.

Keywords: Polygalasaponin; Antipsychotics; Behavioural pharmacology; Climbing behaviour; Dopamine; Serotonin

1. Introduction

A number of studies have demonstrated that the newer antipsychotic agents, such as olanzapine, clozapine, and risperidone, exert their actions through an interaction with multiple receptors within the CNS. Dopamine and serotonin receptors have been shown to be particularly important in mediating the "atypical" properties of these newer agents (Meltzer, 1989; Meltzer and Nash, 1991; Tran et al., 2000).

Various traditional herbal remedies have also been suggested to possess activity at multiple sites within the CNS (Chung et al., 1995). For example, *Polygala tenuifolia* Willdenow, a plant that has been used as an expectorant in Korean traditional medicine, has also been used as a sedative and as an antipsychotic agent (Chung et al., 1992). The dried root of this plant contains polygalitol, tenuigenin, polygalasaponin, and xanthone derivatives. Sapogenins were firstly isolated from this plant in 1947 (Sakuma and Shoji, 1981) and some of the polygalasaponins showed considerable inhibitory activity against the action of cAMP (Nikaido et al., 1982).

In competitive binding studies using tritiated radioligands to dopamine-2 and serotonin-2 receptors in rat brain membrane preparations (Chung et al., 1992), a crude extract (100 ml of water soluble extracts from 20 g of the plant by boiling at least for 8 h) of *P. tenuifolia* Willdenow inhibited specific binding in a concentration-dependent manner. In particular, the maximal inhibition level of specific binding to dopamine-2 and serotonin-2 receptors were up to 70% and 91.7% of that exhibited by reference compounds such as spiperone and ketanserin, respectively. These results suggested that *P. tenuifolia* Willdenow contains active ingredients that have potential antipsychotic action by inhibiting the activities of both dopamine-2 and serotonin-2 receptors in vitro.

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In the present study we have therefore investigated the functional in vivo actions of this compound in animal behavioural tests which are considered to be predictive of dopamine and serotonin antagonist activities.

2. Method

2.1. Animals

Female TO mice (Tuck Farms, Battlebridge, Essex) (20–25 g) were used in studies of the apomorphine-induced climbing, the 5-hydroxytryptamine (5-HTP)-induced serotonin syndrome and MK-801-induced hyperactivity. Male Lister Hooded rats (Harlan Olac, Bicester) (180–250 g) were used in the cocaine-induced hyperactivity study. All animals were housed in conventional plastic or metal cages in groups of up to 16 mice and 5 rats, in a room maintained at 21 ± 1 °C on a 12-h light/dark cycle. All the animals had free access to food and water.

2.2. Drugs

Apomorphine (Sigma), MK-801 (RBI), pargyline (Sigma), cocaine (Sigma), 5-HTP (Sigma), and polygalasaponin (Chung et al., 1995) were dissolved in water. Polygalasaponin was isolated from the roots of *P. tenuifolia* Willdenow by means of a series of sequential separation procedures using Diaion HP-20, silica gel, and RP-18 column chromatographies. The chemical structures of the active compounds were identified by NMR spectroscopy. A patent for the isolation procedure from the plant and the resultant potential antipsychotic activity of polygalasaponins was obtained (Korean Patent No. 0262072). Apomorphine (2.5 mg/kg) and 5-HTP (50 mg/kg) were injected subcutaneously using a volume of 0.2 ml. Pargyline (78 mg/kg), MK-801 (0.3 mg/kg), and cocaine (40 mg/kg) were administered via an intraperitoneal route in a volume of 0.5 ml. Polygalasaponin (25–500 mg/kg) was administered by three different routes, intraperitoneally (in a volume of 0.5 ml), subcutaneously (0.2 ml), and orally (0.5 ml). If necessary, the pH of the solution was adjusted using 1 N NaOH.

2.3. Apomorphine-induced climbing experiments

Each animal was placed in a cylindrical wire mesh cage (height 13 cm, diameter 14 cm, and mesh size 3 mm) for 1 h prior to the experiments. Various doses of polygalasaponin (25–500 mg/kg) were administered by three different routes 30 min before subcutaneous administration of apomorphine (2.5 mg/kg). Climbing behaviour was assessed at 5-min intervals for up to 40 min, starting 10 min after the apomorphine administration using the following scoring system: 0 — no paws on the cage, 1 — one paw on the cage, 2 — two paws on the cage, 3 — three paws on the cage, 4 — four paws on the cage. The score recorded for each animal was based on the position of the animal at the moment it was first observed. The animals were also assessed for other changes in behaviour. These changes were recorded but not quantified. An observer made all observations unaware of the specific drug treatments.

2.4. 5-HTP-induced serotonin syndrome experiments

The syndrome was measured after placing a mouse in a Perspex cage for a 30-min habituation period. The mice were then injected with pargyline (75 mg/kg) in order to prevent the rapid degradation of 5-HTP. Thirty minutes later, polygalasaponin (12.5 to 100 mg/kg) was intraperitoneally administered. After a further 30 min, the mice received 5-HTP (50 mg/kg sc). The mice were returned to the test cages and the serotonin syndrome was assessed every 10 min after 20 min through 50 min. Five behaviours such as tremor, hindlimb extension, forepaw treading, head weaving, and head twitch were monitored using the following scoring system, 0 — absent, 1 — moderate, 2 — marked. An observer made all observations unaware of the specific drug treatments.

2.5. MK-801-induced hyperactivity experiments

Mouse locomotor activities were measured in clear Perspex boxes (30 × 30 × 30 cm) with a metal base covered with 2 cm of fine sawdust. Each box had five equally spaced pairs of horizontal photocell beams, 2 cm above the sawdust. Each beam interruption was recorded as a photocell count. All the boxes were connected to a personal computer, which recorded the number of photocell interrupts made in each cage for every minute of the test period. Mice were placed in pairs in the photocell boxes for a 30-min period. After this time had elapsed the animals were injected intraperitoneally with various doses of polygalasaponin or vehicle and then returned to the test boxes and the recording was begun immediately. After a 30-min period had elapsed, the animals were injected with MK-801 (0.3 mg/kg) and the recording was continued immediately. Activity was measured for 90 min. The Perspex boxes allowed for continuous visual monitoring of the animals in addition to the automated measure of locomotor activity. The photocell counts that were measured every minute in each locomotor activity box were grouped into 10-min time intervals.

2.6. Cocaine-induced hyperactivity experiments in rats

Male Lister Hooded rats were used in this study. The animals were removed from the holding room and randomly assigned to treatment groups. Animals received either the vehicle or polygalasaponin (25 to 50 mg/kg) by the intraperitoneal route and were placed in the activity cages. Following the 30 min of habituation period, the animals received cocaine (40 mg/kg ip) and were returned to the activity cages for a further 90 min. Activity was measured as light beam interruptions per 10-min period.

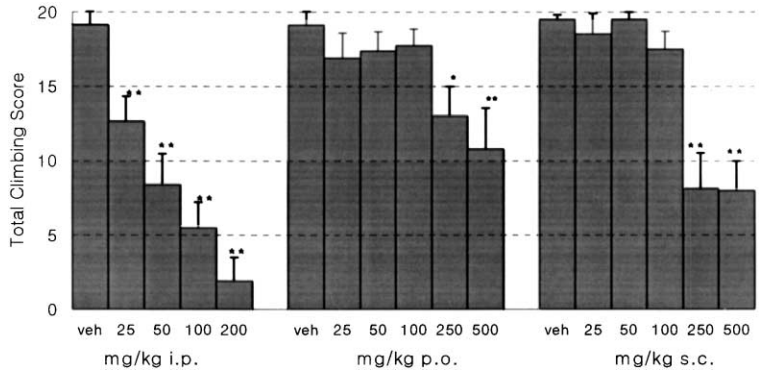

Fig. 1. The effect of polygalasaponin on climbing behaviour induced by apomorphine (2.5 mg/kg sc). Each column represents the mean ± S.E. total climbing score for groups of six mice assessed at 5-min intervals for 20 min, starting 10 min after the apomorphine treatment. A score of 20 is the maximum possible. * $P<.05$, ** $P<.01$.

2.7. Statistical analysis

In all our studies, comparisons between groups were carried out using a one-way ANOVA procedure for independent groups, followed by a post hoc least square means multiple comparison test within SAS Proc GLM (SAS, 1985). A probability of $P<.05$ was considered to be significant. The minimum effective dose (ED_{min}) was defined as being the lowest dose tested that produced a statistically significant effect.

3. Results

3.1. Apomorphine-induced climbing

Polygalasaponin (25–500 mg/kg) produced a dose-related inhibition of apomorphine-induced climbing behaviour (Fig. 1). The ED_{min} preventing the climbing response were 25 mg/kg by the intraperitoneal route and 250 mg/kg by the subcutaneous and oral routes. However, the higher doses of polygalasaponin administered by the intraperitoneal route appeared to produce an initial abdominal discomfort immediately after administration, because the animals displayed some writhing and then became hypoactive. Apart from some diarrhoea at the highest dose administered by the oral route, no other adverse events were observed when the compound was administered by either the subcutaneous or the oral routes.

3.2. 5-HTP-induced behavioural syndrome

Polygalasaponin reduced all the five behaviours produced by 5-HTP (50 mg/kg) in a dose-related fashion. The ED_{min} for the overall suppression of these behaviours was 50 mg/kg by the intraperitoneal route; however, lower doses of polygalasaponin significantly reduced the tremor response (Fig. 2).

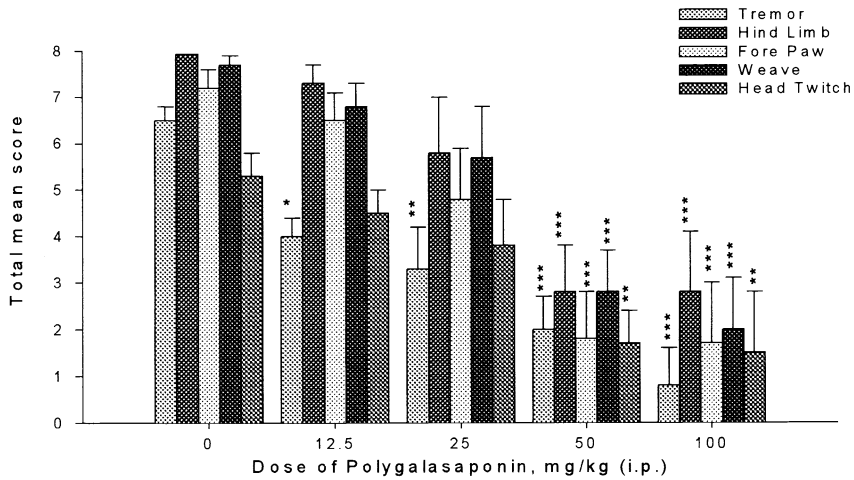

Fig. 2. The effect of polygalasaponin (intraperitoneal) on the serotonin syndrome induced by 5-HTP (50 mg/kg sc). Each column represents the mean ± S.E. total score for groups of six mice assessed at 10-min intervals for 30 min, starting 20 min after the 5-HTP treatment. A score of 8 is the maximum possible. * $P<.05$, ** $P<.01$, *** $P<.001$.

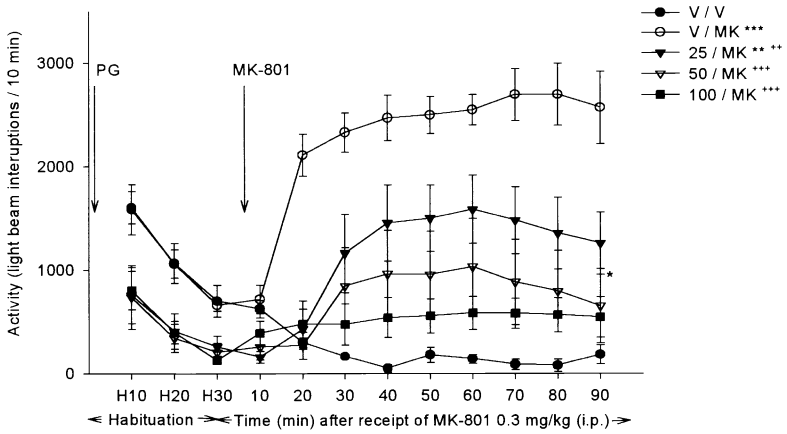

Fig. 3. The effect of polygalasaponin (intraperitoneal) on the hyperactivity induced by MK-801 (0.3 mg/kg ip). Each point represents the mean ± S.E. number of light beam interruptions for groups of six pairs of mice. ** $P<.01$, *** $P<.001$ compared to the vehicle + vehicle, $^{++}$ $P<.01$, $^{+++}$ $P<.001$ compared to the vehicle + MK-801 group. PG — polygalasaponin; V/V — vehicle/vehicle; V/MK — vehicle/MK-801; 25/MK — polygalasaponin 25 mg/kg/MK-801; 50/MK — polygalasaponin 50 mg/kg/MK-801; 100/MK — polygalasaponin 100 mg/kg/MK-801.

3.3. MK-801-induced hyperactivity

Polygalasaponin inhibited hyperactivity induced by MK-801 (0.3 mg/kg) in a dose-related fashion ($ED_{min} < 25$ mg/kg ip). The suppression by polygalasaponin persisted from 20 through 90 min after MK-801 injection. Immediately after intraperitoneal injection of polygalasaponin, a reduction in spontaneous activity was also observed (Fig. 3).

3.4. Cocaine-induced hyperactivity

Polygalasaponin produced a partial inhibition of the hyperactivity induced by cocaine (40 mg/kg) in rats. This suppression by polygalasaponin was evident from 40 through 90 min after the cocaine injection. However, the inhibition did not appear to be dose-related. Intraperitoneal injection of polygalasaponin also induced a reduction in spontaneous movements in rats (Fig. 4).

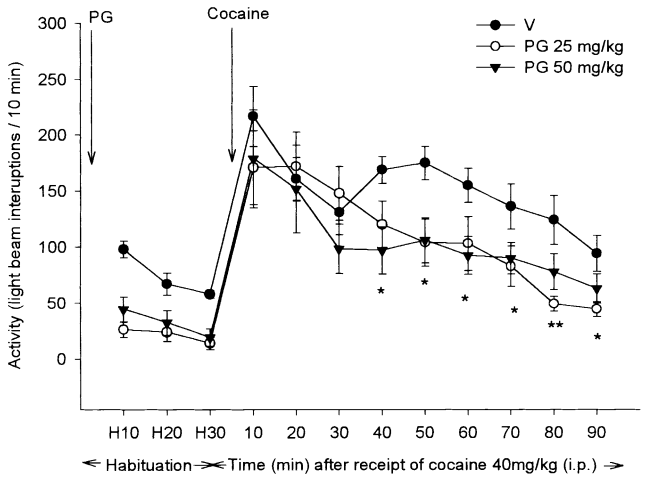

Fig. 4. The effect of polygalasaponin (intraperitoneal) on the hyperactivity induced by cocaine (40 mg/kg ip). Each point represents the mean ± SE number of light beam interruptions per 10 min for groups of eight rats. * $P<.05$, ** $P<.01$ compared to the cocaine only (V) group. PG — polygalasaponin; V — vehicle.

4. Discussion

These behavioural data demonstrate that polygalasaponin shows clear pharmacological actions in vivo. The compound prevented the behaviours that are mediated by dopamine and serotonin receptors in the central nervous system. This data also showed the same results as those of earlier in vitro studies which demonstrated that the compound has an affinity for both dopamine and serotonin receptors (Chung et al., 1995) and suggests a possible mechanism of the antipsychotic activity for the herbal extract used in traditional medicine.

Antagonism of apomorphine-induced climbing behaviour is one of the most widely used tests that are predictive of dopamine antagonist properties in vivo (Moore and Axton, 1988; Arnt, 1982). Both the selective D_1 antagonist SCH23390 and the selective D_2 antagonist clebopride antagonised the climbing behaviour produced by apomorphine (Moore and Axton, 1990), which demonstrates that the climbing response requires both D_1 and D_2 receptor activation (Moore and Axton, 1988; Vasse et al., 1988). Various antipsychotics such as olanzapine, clozapine, and haloperidol showed the preventive effects on this behaviour (Moore et al., 1992). The polygalasaponin extracted from the herbal plant *P. tenuifolia* Willdenow also reduced this behaviour in a dose-related manner. This response occurred in all the routes of administration albeit the intraperitoneal route was significantly the most effective. Possibly, some initial abdominal writhing could partly have contributed to the enhanced potency when administered by the intraperitoneal route.

A number of studies have suggested that the inhibition of the serotonin system, more specifically, serotonin-2

receptors, may contribute to the "atypical" property of antipsychotics such as clozapine, olanzapine, and risperidone (Meltzer, 1989; Meltzer and Nash, 1991). The serotonin-2 antagonists prevented the components of the 5-HTP-induced syndrome, especially the head twitch response (Colpaert and Janssen, 1993). Olanzapine and clozapine had also been shown to inhibit the head twitches induced by 5-HTP, whereas haloperidol produced only a slight effect at the highest dose tested (Moore et al., 1992). Polygalasaponin demonstrated a dose-related inhibition of the entire 5-HTP-induced syndrome, suggesting that the compound may interact at more than one subtype of the serotonin system.

The locomotor stimulant properties of MK-801, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, have been proposed for use as a possible animal model for antipsychotic drug action (Carlsson, 1995). It has been demonstrated that NMDA antagonist-induced hyperactivity is mediated via serotonin-2A receptor activation and serotonin-2 receptor antagonists have been shown to attenuate the locomotor activity induced by MK-801 (Schmidt and Fadayel, 1996; O'Neill et al., 1997). Our data demonstrate that polygalasaponin significantly reduced MK-801-induced hyperactivity. Although spontaneous activity was also reduced by this compound at similar doses, other agents that reduce spontaneous activity, such as haloperidol, have little effect on MK-801-induced hyperactivity (O'Neill et al., 1997). This suggests that the antagonism of the response is not due solely to a reduction in spontaneous activity.

Numerous studies have demonstrated that dopamine antagonists prevent the hyperactivity following cocaine administration in mice and rats. For example, haloperidol and clozapine in a dose-dependent manner attenuated the locomotor effects of cocaine (O'Neill and Shaw, 1999). SCH23390, the selective D_1 antagonist, also significantly suppressed the locomotor response produced by cocaine. These data suggest that both D_1 and D_2 receptors are involved in cocaine-induced hyperactivity (O'Neill and Shaw, 1999; Moore, 1999). In the present study, polygalasaponin produced a partial reduction in the hyperactivity produced by cocaine.

The data in the present study demonstrated that polygalasaponin can prevent both dopamine-2 and serotonin-2 receptor mediated behaviours and that this in vivo activity may suggest the therapeutic potential of this pharmacological agent in the treatment of psychotic disturbances.

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