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Research report

Assessment of luteolin (3′,4′,5,7-tetrahydroxyflavone) neuropharmacological activity

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Abstract

Since the discovery that certain flavonoids (namely flavones) specifically recognise the central BDZ receptors, several efforts have been made to identify naturally occurring $GABA_A$ receptor benzodiazepine binding site ligands. Flavonoid derivatives with a flavone-like structure such as apigenin, chrysin and wogonin have been reported for their anxiolytic-like activity in different animal models of anxiety. Luteolin (3′,4′,5,7-tetrahydroxyflavone) is a widespread flavonoid aglycon that was reported as devoid of specific affinity for benzodiazepine receptor (BDZ-R) binding site, but its psychopharmacological activity is presently unknown. Considering (1) the close structural similarity with other active flavones, (2) the activity of some of its glycosilated derivatives and (3) the complexity of flavonoid effects in the central nervous system, luteolin was submitted to a battery of tests designed to evaluate its possible activity upon the CNS and its ability to interact with the BDZ-receptor binding sites was also analysed.

Luteolin apparently has CNS activity with anxiolytic-like effects despite the low affinity for the BDZ-R shown *in vitro*. Our findings suggest a possible interaction with other neurotransmitter systems but we cannot rule out the possibility that luteolin's metabolites might show a higher affinity for the BDZ-R *in vivo*, thus eliciting the evident anxiolytic-like effects through a GABAergic mechanism.

Keywords: Luteolin; Flavonoids; Flavones; Central nervous system; Anxiolytics; GABA

1. Introduction

Flavonoids are a large group of plant secondary metabolites that share a basic phenylbenzopyrone feature and are found in all vascular plants where they occur in several structurally and biosynthetically related classes [1]. They are important constituents of the human diet [2] and can also be found in expressive amounts in many medicinal plants [3]. Amongst the wide range of biological and pharmacological properties of these compounds we find a series of reports on their activity in the central nervous system (CNS) (for reviews see [4–6]). Since the discovery that certain flavonoids (namely flavones) specifically recognise the central BDZ receptors [7,8], efforts have been made to identify naturally occurring $GABA_A$ receptor benzodiazepine binding site ligands [5] to understand their interaction with these receptors [9–12] and to establish the CNS activity of different natural [13] and synthetic flavonoids [14,15]. Amongst these reports flavonoid derivatives with a flavone-like structure such as apigenin [16,17], chrysin [18] and wogonin [19] have been reported for their anxiolytic-like activity in different animal models of anxiety. These flavonoids with BDZ-receptor specificity and/or anxiolytic activity have been isolated from medicinal plants traditionally used in folk medicine for their anxiolytic/sedative properties such as *Passiflora coerulea* [20], *Matricaria recutita* [16], *Tilia tomentosa* [21], *Jatropha cilliata* [22], *Salvia guaranitica* [23], *Matricaria chamomilla* [17], *Zizyphus jujuba* [24]. Recently, we have reported on the isolation of luteolin-7-*O*-(2-rhamnosylglucoside) from *Passiflora edulis* Sims and demonstrated its anxiolytic-like activity [25].

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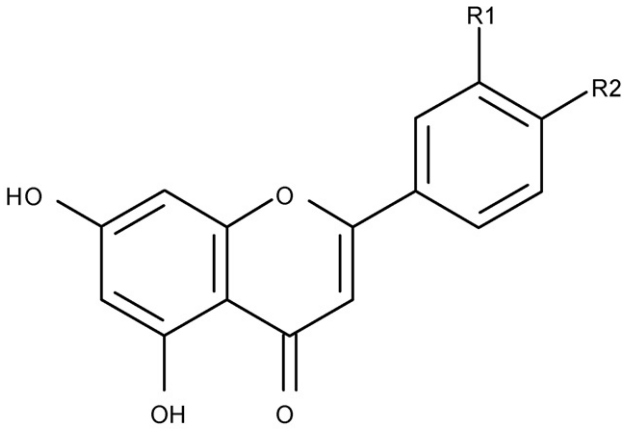

	R1	R2
Chrysin	-	-
Apigenin	-	OH
Luteolin	OH	OH

Fig. 1. Luteolin's structure is close to that of other flavones that have been reported for its anxyolitic activity like apigenin or chrysin.

Luteolin is a widespread flavonoid aglycon that was reported as devoid of specific affinity for BDZ-receptor binding site [21], but its psychopharmacological activity is presently unknown. Considering (1) the close structural similarity with other active flavones (Fig. 1), (2) the activity of some of its glycosilated derivatives [25,26,22] and (3) that flavonoid effects in the central nervous system are complex and can involve different mechanisms [27] besides the interaction with the benzodiazepine binding sites (BDZ-bs) at the $GABA_A$ receptors, we became interested in the possible psychopharmacological profile of action of luteolin. This substance, purchased from a commercial source, was submitted to a battery of tests designed to evaluate its possible activity upon the CNS and to an eventual understanding of mechanisms underlying its activity(ies). As we were also interested in analysing the ability of luteolin to interact with the BDZ-receptor binding sites, we have also evaluated this substance in a radioreceptor binding assay with [^{3}H]flunitrazepam.

2. Material and Methods

2.1. Animals

Male adult Swiss mice from our breeding stock, weighing 20–25 g, were used. Animals were placed in groups of 10 with free access to water and food, except during the experiments. They were kept on a 12/12 h day/night cycle (lights on at 07:00 a.m.) at controlled room temperature (23 ± 2 °C) and were allowed to adapt to the laboratory conditions for, at least, 1 week before the beginning of the behavioral experiments. Each animal was used just once. All experiments were conducted in accordance with international standards of animal welfare recommended by the Brazilian Society of Neuroscience and Behavior. The experimental protocols were approved by the local Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used and all behavioral testing was performed during the animal's day light period between 09:00 a.m. and 01:00 p.m.

2.2. Drugs

Diazepam i.v. solution (Dienpax ®, Sanofi-Winthrop Lab., Brazil) was diluted with distilled water and used in the dose of 1 mg/kg as reference drug (positive control) for anxiolytic, sedative, muscle relaxant and anticonvulsant activities. Luteolin, the flavonoid compound, was purchased from Extrasynthése (Genay, France). [^{3}H]flunitrazepam was obtained from Amersham Biosciences.

2.3. Treatments

Luteolin was freshly suspended (in an ultrasound bath) in a suitable amount of distilled water to be acutely (1 h) or repeatedly (14 days) administered *per os* (*p.o.*) by an intragastric cannula. Doses of luteolin (0.1–50 mg/kg) as well as the time intervals were determined in preliminary tests. Control groups received only distilled water in equivalent volumes by the same route. The behavioral tests were performed in a soundproof room between 09:00 a.m. and 01:00 p.m. to reduce the confounding influence of diurnal variation in spontaneous behavior.

2.4. Procedures

2.4.1. Motor performance evaluation

Muscle relaxant effects were evaluated using the horizontal-wire test that consists of a stretched copper wire placed 20 cm above the ground [28]. Motor coordination was assessed using a rota-rod apparatus. This equipment has a 2.5 cm bar, rotating at 12 rpm, divided in six parts and placed at a height of 25 cm. Latency to fall from the rotating bar and number of falls in a period of 1 min test were registered [29].

2.4.2. Elevated plus-maze test (EPM)

The elevated plus-maze was slightly modified from that used by Lister [30]. Briefly, it consisted of two open arms (30 cm × 5 cm × 0.25 cm) and two enclosed arms (30 cm × 5 cm × 15 cm), extending from a central platform (5 cm × 5 cm) and raised 50 cm above floor level. The maze floor was constructed from black Plexiglas and the walls from clear Plexiglas. The conventional spatial–temporal measures recorded were the number of entries (all four paws on open or enclosed arms and expressed as percentage of total entries), the time spent on open arms (expressed as percentage of time spent on closed plus open arms), number of entries on enclosed arms and the time on the central platform. Ethologically derived measures were grooming, rearing, stretched attend postures (SAP), head-dipping (HD) and defecation as an emotionally related parameter [31]. A selective increase in the parameters of exploration of the open arms of the maze reveals an anxiolytic effect [32].

2.4.3. Hole-board test

The hole-board consisted of a square box made of transparent Plexiglas (50 cm × 50 cm × 30 cm), 10 cm above table surface, with equally distributed nine holes, 2 cm in diameter. The area of the hole-board is divided with white ink into 24 smaller areas. During 5 min we registered the number of head-dips, grooming behavior, rears and also of displacements between the different areas (locomotor activity) [33].

2.4.4. Potentiation of barbiturate-induced loss of righting reflex

One hour after treatment with luteolin, animals were administered (i.p.) with sodium pentobarbital (50 mg/kg). Latency for the loss of the righting reflex and its total duration was registered for three consecutive hours [34].

2.4.5. Catalepsy test

Animals' forepaws were placed over a horizontal glass tube standing 5 cm above floor surface, each 10 min interval for up 1 h. Catalepsy was evaluated as the time until removal of the forefeet from the tube. Two different sets of