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Synergistic interaction between hesperidin, a natural flavonoid, and diazepam

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Abstract

It has been recently reported the presence in Valeriana of the flavone 6-methylapigenin and the flavanone glycoside hesperidin. The apigenin derivative is a ligand for the benzodiazepine binding site in the γ-aminobutyric acid receptor type A ($GABA_A$) and has anxiolytic properties. Hesperidin has sedative and sleep-enhancing properties but is not a ligand for the benzodiazepine binding site. 6-Methylapigenin is able to potentiate the sleep-enhancing effect of hesperidin. In this work we demonstrate that this property is shared with various $GABA_A$ receptor ligands, among them the agonist diazepam, which was used to study the potentiation as measured in the hole board test. Isobolar analysis of the results showed the interaction being synergistic. We discarded pharmacokinetic effects or a direct action of hesperidin on the benzodiazepine binding site.

A possible use of hesperidin properties to decrease the effective therapeutic doses of benzodiazepines is suggested.

Keywords: Flavonoid; Hesperidin; Diazepam; Synergy; Sedation; Sleep-enhancing action

1. Introduction

Enhancement of the inhibitory effect of γ-aminobutyric acid (GABA) acting on its type A receptor in the mammalian brain explains the pharmacological and therapeutic actions of benzodiazepines which bind to a specific site in the $GABA_A$ receptor. However, the usefulness of benzodiazepines as anxiolytics, sedatives, anticonvulsants and muscle relaxants is compromised by the occurrence of several adverse effects such as ataxia, amnesia, alcohol intolerance and residual sedation as well as the related problems of tolerance and dependence after chronic use. Molecular biology studies have established the heterogeneity of $GABA_A$ receptors and the pharmacological and electrophysiological study of the different subtypes has allowed the search of new ligands with improved selectivity which eventually may lead to the development of safer drugs (Möhler et al., 2002, 2004; Vicini and Ortinski, 2004).

Flavonoids have recently attracted interest as new chemical entities with activity on the central nervous system (CNS) (Wang et al., 1999; Marder and Paladini, 2002). We have demonstrated that some naturally occurring flavonoids possess a selective and relatively mild affinity for the central benzodiazepine binding site in the $GABA_A$ receptor, and exert anxiolytic but not depressant effects in rodents (Medina et al., 1990; Wolfman et al., 1994; Viola et al., 1995). Several flavone derivatives with added electronegative groups have been synthesized by our group and found to have enhanced affinities for the benzodiazepine binding site. These compounds are potent anxiolytics in rodents without exerting sedative or myorelaxant effects (Medina et al., 1997; Marder and Paladini, 2002).

The presence of two neuroactive flavonoids in the sedative plants *Valeriana wallichii* and *Valeriana officinalis* has been recently described by us (Wasowski et al., 2002; Marder et al., 2003). They are the flavone 6-methylapigenin and the flavanone 2*S*(−)-hesperidin (Fig. 1). The apigenin

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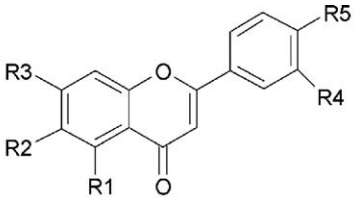

Compound	R1	R2	R3	R4	R5
6-methylapigenin	OH	CH_3	OH	H	OH
Chrysin	OH	H	OH	H	H
Apigenin	OH	H	OH	H	OH
6-methylflavone	H	CH_3	H	H	H
6,3'-dinitroflavone	H	NO_2	H	NO_2	H

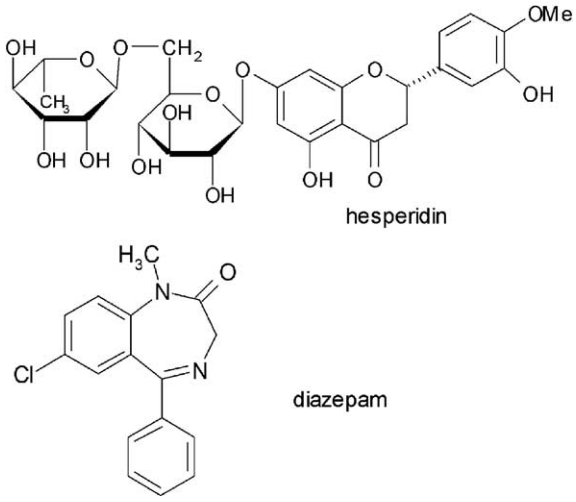

Fig. 1. Molecular structure of the drugs used in the behavioral assays.

derivative exhibits medium–high affinity for the benzodiazepine binding site (K_i=495 nM), and has anxiolytic effects in mice. Hesperidin, in turn, has sedative and sleep-enhancing properties but it is not a ligand for the benzodiazepine binding site. When hesperidin and 6-methylapigenin are jointly injected i.p. in mice during a thiopental-induced sleeping time test, the resultant effect suggests a greater than additive interaction between both drugs (Marder et al., 2003), 6-methylapigenin, however, does not potentiate the sedative action of hesperidin in the hole board test at the doses tested. In this paper we show that a potentiation occurs when hesperidin interacts with other benzodiazepine binding site ligands in the thiopental-induced sleeping time and in the hole board tests. The nature of this interaction was evaluated by an isobolar analysis of the data obtained with hesperidin and the classical agonist diazepam in the hole board assay. The analysis demonstrates that synergism is present when these drugs are co-injected. Although the mechanism of the CNS activity of hesperidin is still unknown, we have discarded a direct action of hesperidin on the plasma levels of diazepam after injection and/or direct effect on the benzodiazepine binding site.

The main goal of pharmacotherapy using a combination of different drugs with similar effects is to increase the efficacy of the treatment while decreasing its toxicity. Multiple-drug therapy has been applied in medical areas such as hypertension, chemotherapy, antibiotic therapy, asthma, etc. (Berenbaum, 1989; Rosow, 1997). The recent findings of Campbell et al. (2004) showing that flavonoids enhance diazepam modulatory action at recombinant $GABA_A$ receptors, plus the synergistic interaction in vivo between hesperidin and diazepam described here, suggest that flavonoids, besides being potentially valuable single drugs, may also be used with advantage in combination with benzodiazepines.

2. Materials and methods

2.1. Drugs and injection procedures

The drugs used to perform the behavioral experiments are shown in Fig. 1 and were obtained as follows: hesperidin isolated by us from *V. wallichii* as described by Marder et al. (2003) was used in the sodium thiopental-induced sleeping time test; genuine hesperidin from SIGMA, USA, was used in the hole board test; diazepam was from Hoffmann-La Roche; 6-methylapigenin was isolated by us from *V. wallichii* as described by Wasowski et al. (2002); apigenin, chrysin and 6-methylflavone were from SIGMA, USA; 6,3′-dinitroflavone was synthesized by us (Marder et al., 1995).

The drugs were dissolved by the sequential addition of: dimethylsulfoxide up to a final concentration of 5%, a solution of 0.25% Tween 80 up to a final concentration of 20%, and saline to complete 100% volume. Sodium thiopental (Fada, Biochemie Gesellschaft m.b.H., Kundl/Tirol, Austria) was dissolved in saline. The rodents were i.p. injected 20 min before performing the pharmacological tests. The volume of i.p. injections was 0.15 ml/30 g of body weight. In each session, a control group receiving only vehicle was tested in parallel with those animals receiving drug treatment.

2.2. Animals

Adult male Swiss mice weighing 25–30 g were used in the pharmacological assays. Animals were housed in a controlled environment (20–23 °C), with free access to food and water and maintained on a 12 h/12 h day/night cycle. Housing, handling, and experimental procedures complied with the recommendations of the European