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Diterpene Sclareol Glycol Inhibits Clonidine-Induced Aggressive Responses in Mice

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GEORGIEVA, J. V. *Diterpene sclareol glycol inhibits clonidine-induced aggressive responses in mice.* PHARMACOL BIOCHEM BEHAV **34**(3) 503–505, 1989.—The effects of a reversible activator of adenylate cyclase sclareol glycol (SG), a semisynthetic diterpene of the labdane family, on the aggressive behavior induced by a high dose of clonidine in mice were studied. SG was applied at doses well below the lethal dose. Aggressive behavior induced by clonidine at a dose of 30 mg/kg IP was decreased in a dose-dependent manner by SG (1, 5, 25 mg/kg IP). The aggressive responses were abolished by doses of 50 and 100 mg/kg. It is suggested that the inhibitory effects of SG on clonidine-induced aggressive behavior are realized mainly via its effect on adenylate cyclase and perhaps involving synaptic transmitter action.

Diterpene Sclareol glycol Clonidine aggressive behavior

SCLAREOL glycol (SG) (13,14,15,16-tetranorlabdane-8α,12-diol) is a semisynthetic analogue of diterpenes isolated from *Salvia sclarea* L. growing in Bulgaria. SG belongs to the labdane family of diterpenes. Our previous studies (6) have shown that SG applied intraperitoneally (IP) to rats and mice, at doses well below the lethal dose, provokes mainly general restlessness of the animals. SG at a low dose stimulates locomotor activity while at a high dose it decreases this activity (7); SG reverses the reserpine-induced hypokinesia and potentiates the hypokinesia caused by a low dose of apomorphine (7). SG induces dose-dependent changes in rectal temperature in rats and reverses the reserpine-induced hypothermia (9). In order to get more detailed information on the central action of SG, we examined the effects of this diterpene on aggressive behavior in mice (biting and attacking when they are housed in pairs) induced by a high dose of clonidine (13,17).

METHOD

Animals

The experiments were carried out on male albino mice (22–25 g). Food and tap water were provided ad lib except during trials. All experiments were performed at an environmental temperature of 22 ± 1°C, at a natural day-night lighting cycle.

Measurement of Aggressive Responses

Each pair of mice (4 pairs per group) was placed in the same transparent plastic box (20 × 15 × 17 cm) and aggressiveness (the aggressive responses in scores according to the point scale, the number of biting attacks and the latency to the first biting attack in min) was assessed every 10 min for 60 min after injection of clonidine to both animals. Rating was made on a 0–4 point scale (1): 0—no aggressive manifestation; 1—intermittent attack posturing and contact with other mouse, no vocalization; 2—intermittent attacking and nibbling, with mild vocalization; 3—continuous attacking, biting and vocalization; 4—continuous attacking, biting, inflicting wounds and emitting vocalizations. Means ± S.E.M. were calculated and the statistical significance of the differences between the means of the groups with various drug doses and the control were determined by Student's *t*-test.

Drugs

The experimental animals were injected with SG (1, 5, 25, 50 or 100 mg/kg IP) 10 min before clonidine hydrochloride (Boehringer Ingelheim) (30 mg/kg IP). The controls were injected IP with the solvent 10 min before clonidine. Sclareol glycol (Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia) was dissolved in 0.9% saline with 1% Tween and used as a fine suspension. Drugs were prepared ex tempore and administered in a volume of 0.1 ml/kg.

RESULTS

A single injection of 30 mg/kg of clonidine elicited aggressive responses within 5–10 min, causing bleeding wounds mainly on the tail and the back. Aggressiveness was most pronounced within 10–30 min and was not observed at all at 60th min. Mice seldom assumed the upright fighting posture and defence or escape reaction. Marked tremor, piloerection and attacking also appeared 5–10 min after clonidine, diminishing within 30–40 min.

TABLE 1

EFFECTS OF SCLAREOL GLYCOL ON CLONIDINE-INDUCED AGGRESSIVE RESPONSES

Pretreatment mg/kg	Latency to the First Attack (min)	Total Number of Attacks	Time-Course of Clonidine-Induced Aggressive Behavior[1] 10 min	20 min	30 min	40 min	50 min	60 min
Control (SG solvent)	6.5 ± 1.02	34.5 ± 2.7	1.0 ± 0	2.25 ± 0.2	1.25 ± 0.2	1.0 ± 0	0.25 ± 0.2	0
SG 1	6.5 ± 0.42	26.0 ± 4.8	0.75 ± 0.2	1.0 ± 0§	1.0 ± 0§	0.25 ± 0.2§	0	0
SG 5	7.2 ± 1.11	22.0 ± 5.6§	0.5 ± 0.2†	0.75 ± 0.2§	0.75 ± 0.2†	0.25 ± 0.2§	0	0
SG 25	9.2 ± 1.18*	16.0 ± 3.9§	0.25 ± 0.2†	0.5 ± 0.2§	0.25 ± 0.2§	0	0	0
SG 50	0	0	0	0	0	0	0	0
SG 100	0	0	0	0	0	0	0	0

Sclareol glycol (SG) was administered IP 10 min before clonidine (30 mg/kg IP). Data represent means ± S.E.M. *$p<0.05$; †$p<0.02$; ‡$p<0.01$; §$p<0.001$; significant difference vs. control group, determined by Student's t-test. Each group consists of 8 mice (4 pairs). Total number of attacks means the attacks counted for a 60-min period of observation.

[1]Aggressive behavior at 10-min intervals represents the mean aggressiveness score according to 4-point scale.

The aggressive behavior induced by clonidine was depressed by SG depending on the dose. Thus, SG at a dose of 1 mg/kg significantly reduced the aggressive responses at the 20th to 40th min; at a dose of 5 mg/kg it significantly inhibited the aggressive responses at 10th to 40th min and abolished them at the 50th min. At a dose of 25 mg/kg, SG significantly inhibited the aggressive responses at the 10th to 30th min and abolished them at the 40th and 50th min. At doses of 50 and 100 mg/kg of SG clonidine-induced aggressive responses were not observed at all (Table 1). SG applied to mice (10 mice per group) at a dose of 50 or 100 mg/kg IP decreased the spontaneous activity of animals.

DISCUSSION

The present results suggest that the diterpene sclareol glycol has a dose-dependent inhibitory action on clonidine-induced aggressive behavior in mice. When explaining this effect one should have in mind that SG in a concentration of 10^{-6} M reversibly activates the catalytic subunit of adenylate cyclase (estimated in vitro in rat brain tissue slices) which leads to an increased availability of 3′,5′-AMP in the brain (19). SG (10^{-6} M) also increases the 3′,5′-AMP availability in monolayer tissue cultures from rat anterior pituitary (6). This was assumed to mediate the locomotor depressant action of both SG (7) and forskolin (another thoroughly studied diterpene of the labdane family) (2, 15, 16, 20, 21). The inhibition of epileptic seizures produced by forskolin (8,14), the decrease of rectal temperature, the reversal of reserpine-induced hypothermia, etc., are also thought to be due to the increased brain 3′,5′-AMP availability (20,21).

The depressant effect of SG on clonidine-evoked aggression might also have been a result of an interference in some brain neurotransmitter systems. Clonidine has been proposed to produce aggressive behavior by blocking adenosine receptors (18). The clonidine-produced aggression is reduced to a certain degree after administration of adenosine and its N^6-substituted analogues (18). Clonidine-induced self-injurious behavior is also antagonized by adenosine (10). However, the recent studies performed by Daly *et al.* (4) have shown that clonidine (considered an arylamino heterocycle in analogy to 8-aryl xanthines—potent antagonists of A_1- and A_2-adenosine receptors) has no effect on [^{3}H] phenylisopropyladenosine-binding to rat brain A_1-adenosine receptors (in rat cerebral cortical membranes) and on the activation of adenylate cyclase via interaction of N-ethyl-carboxamidoadenosine with A_2-adenosine receptors in human platelet and rat pheochromocytoma cell membranes. The clonidine analogue DPI [2-(3,4-dihydroxyphenylamino)imidazoline] does inhibit binding of [^{3}H]phenylisopropyladenosine to A_1 receptors in a noncompetitive manner. Thus, the binding studies do not support the view that clonidine is an antagonist of adenosine receptors. We have no evidence for effects of SG on adenosine receptors and, thus, we could not further discuss the SG-clonidine-adenosine interactions. What we could suggest, however, is that the hypotensive and hypothermic effect of adenosine and its analogues [for review see (5)] might be responsible for their inhibitory effects on clonidine aggression.

It has also been postulated that clonidine at high doses (10–50 mg/kg) produces aggression via α_1-adrenoceptor stimulation (3, 11, 13). This is supported by the evidence for reduced clonidine aggression by prazocin, the α_1-selective antagonist (12). It could also be assumed that SG is able to inhibit the clonidine-induced aggressive behavior via decreased susceptibility of α_1-adrenoceptors. Participation of brain GABA, dopamine, etc., neurotransmitter systems in the realization of the SG inhibitory effect on clonidine-induced aggressive behavior should also be considered because these systems are involved in realization of the effects of SG on convulsive seizures (8) and locomotor activity (7).

In conclusion, the diterpene sclareol glycol of the labdane family exerts pronounced inhibitory effects on aggressive responses induced by a high dose of clonidine. These effects of SG might be explained mainly by its interactions with adenylate cyclase (stimulation of catalytic subunit) and perhaps with other neurotransmitter systems in the CNS.

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