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Selective effect of oleamide, an endogenous sleep-inducing lipid amide, on pentylenetetrazole-induced seizures in mice

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Abstract

The anti-seizure effect of oleamide, an endogenous sleep-inducing fatty acid amide, was studied in mice. Oleamide, in the dose range 43.7–700.0 mg kg^{-1}, significantly and dose-dependently inhibited the seizures induced by pentylenetetrazole. However, oleamide showed no inhibitory action on the seizures induced by picrotoxin, strychnine, caffeine or semicarbazide. These results provide the first evidence for the anti-seizure effect of oleamide, and suggest that this effect may be selective to the seizure model induced by pentylenetetrazole.

Introduction

Oleamide (*cis*-9,10-octadecenoamide) is a brain lipid amide isolated from the cerebrospinal fluid of sleep-deprived cats (Cravatt et al 1995). Evidence indicates that oleamide induces sleep (Cravatt et al 1995; Basile et al 1999; Yang et al 1999), facilitates memory extinction (Murillo-Rodriguez et al 2001), and decreases locomotor activity (Basile et al 1999; Yang et al 1999) and body temperature (Cravatt et al 1995) when intraperitoneally administered to rats and mice. In-vitro it has been shown that oleamide potentiates 5-$HT_{2A/2C}$ receptor-mediated chloride currents in transfected *Xenopus* oocytes (Huidobro-Toro & Harris 1996) and modulates 5-HT-mediated signal transduction at different subtypes of mammalian 5-HT receptors (Thomas et al 1997), and it seems that oleamide acts at an apparent allosteric site on the 5-HT_7 receptor and elicits functional responses via activation of this site (Thomas et al 1997, 1999). Guan et al (1997) reported that oleamide inhibited cell gap junction communication without affecting calcium wave propagation in cultured glial cells, which may constitute one of the important mechanisms of the sleep-inducing action of this lipid amide. In addition to its actions on 5-HT receptors and cell gap junction communication, oleamide also potentiates γ-aminobutyric acid (GABA) receptor activity and especially potentiates the peak Cl^- current when applied with GABA to benzodiazepine-sensitive $GABA_A$ receptors (Yost et al 1998).

Since the disclosure of oleamide as an endogenous sleep-inducing agent, extensive studies have focused on the investigation of the biochemical mechanisms of action (Huidobro-Toro & Harris 1996; Thomas et al 1997, 1999), or the structure–activity relationship (Boger et al 1998a, 1998b) of this lipid amide. The studies on the pharmacological properties to date, especially in intact animals, of oleamide have mainly focused on its sleep-inducing activities (Basile et al 1999; Yang et al 1999). Being an inhibitor of cell gap junction communication or a modulator of GABA receptors, it is rational to assume that this substance might possess other central inhibitory actions. In order to expand the pharmacological knowledge of this endogenous biologically active lipid, the present study investigates the possible anticonvulsant effect of oleamide.

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Materials and Methods

Male Swiss mice weighing 18–22 g were used for all experiments. Animals were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. They were housed in groups of 10 animals per cage, with food and water *ad libitum*, except during actual testing. All animal treatments were strictly in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University.

Oleamide, synthesized by the Department of Organic Chemistry of Shenyang Pharmaceutical University (purity > 98%), was dissolved in corn oil and administered intraperitoneally (i.p.) to mice at the dose range 43.75–700 mg kg^{-1}. Diazepam (5 mg kg^{-1}, i.p.) was used as a positive control. The blank control animals received only corn oil. All injections were given at a volume of 0.2 mL per 10 g body weight. Thirty minutes after the injection of oleamide, the animals were administered intraperitoneally with 8.5 mg kg^{-1} of picrotoxin (Sigma Chemicals, USA), 85.0 mg kg^{-1} of pentylenetetrazole (Sigma Chemicals, USA), 1.5 mg kg^{-1} of strychnine nitrate (Shanghai Tianfeng Pharmaceutics, China), 600.0 mg kg^{-1} of caffeine sodium benzoate (Shenyang Medicine Co., China), or 200.0 mg kg^{-1} of semicarbazide hydrochloride (Sigma Chemicals, USA). Mice were observed at once after the administration of the seizure-inducing agents for 30 min, and the latency of seizure onset was recorded.

The statistical significance of the drug effects was determined by means of analysis of variance (ANOVA) or general linear models (GLM) using SAS statistical software (SAS Institute Co., Cary, NC, USA). When significant effects were found, differences between groups were subsequently tested by Dunnett's test.

Results

Intraperitoneal injection of oleamide, at the doses of 43.75, 87.5, 175.0, 350.0 and 700.0 mg kg^{-1}, showed a dose-dependent inhibition of pentylenetetrazole-induced seizure in mice. The latency of seizure onset was significantly delayed 2–3-fold compared with the vehicle controlled group. Oleamide at a dose of 350.0 mg kg^{-1} or more displayed an effect nearly equivalent to 5.0 mg kg^{-1} of diazepam (Table 1). However, in the same dose range, oleamide did not affect the latencies of seizure onset induced by picrotoxin, semicarbazide hydrochloride, strychnine nitrate or caffeine sodium benzoate. Diazepam showed a significant anticonvulsant effect in all these models (Table 2).

Discussion

In the present study several seizure-inducing agents with different mechanisms of action were used to test the possible anticonvulsant property of oleamide. Interestingly, oleamide only significantly antagonized the seizures induced by pentylenetetrazole, not by other agents. In contrast, diazepam showed a significant anticonvulsant effect in all seizure models. Every seizure-inducing agent used in the present study possesses its own unique mechanism of action to induce seizures. Picrotoxin is a GABA-gated chloride channel blocker (Bitran et al 1991). Semicarbazide is a glutamate decarboxylase inhibitor that induces seizures by decreasing the content of GABA in the brain (Hara et al 1991). Strychnine blocks glycine effects by binding to glycine receptors (Young & Snyder, 1973). The major mechanism of action of caffeine is that it acts as an antagonist at adenosine receptors (Daly et al 1981). Pentylenetetrazole is an agent often used to induce seizures in the screening of anticonvulsant drugs. However, its mechanism of action is not very clear. Although it has been reported that pentylenetetrazole acts at the picrotoxin site of the $GABA_A$ receptor complex (Huang et al 2001), there is evidence showing a differentiation of pentylenetetrazole and picrotoxin in their convulsion-inducing activities. For example, picrotoxin- and bicuculline- but not pentylenetetrazole-induced seizure thresholds are altered by prenatal diazepam exposure, suggesting that the induction of seizures by pentylenetetrazole is not solely dependent on action of that compound at any of the sites at which the other drugs act (Bitran et al 1991). It is also reported $GABA_B$ receptors are involved in a pentylenetetrazole seizure model (Snead 1992). Therefore, according to the action of oleamide observed in the present study, it is further demonstrated that pentylenetetrazole has a different mechanism of action with picrotoxin and semicarbazide to induce seizures. Thus, the present results suggest that a more specific mechanism of action might exist for the anticonvulsant property of oleamide.

It is reported that oleamide alone has no activity on $GABA_A$ receptors, but potentiates GABA activation of benzodiazepine-sensitive $GABA_A$ receptors (Yost et al 1998). However, this result cannot be used to explain why oleamide did not antagonize picrotoxin- or semicarbazide-induced seizure, and the different anticonvulsant activities between oleamide and diazepam. It should be noted that the potentiation of oleamide on GABA activation of $GABA_A$ receptors cannot be affected by the benzodiazepine antagonist flumazenil or the inverse agonist β-carboline (Yost et al 1998), suggesting that the sites of action of oleamide and the other two compounds do not overlap. Recently it has been reported that oleamide does not directly mimic GABA or operate as a neurosteroid-, benzodiazepine- or barbiturate-like modulator of $GABA_A$ receptors, which suggests that it exerts its effects indirectly or at a novel recognition site on the $GABA_A$ complex (Coyne et al 2002). Although depletion of the $GABA_A$ receptor β-3 subunit eliminates the hypnotic actions of oleamide (Laposky et al 2001), there is no evidence showing that pentylenetetrazole acts at this site to induce seizure. Considering the specific antagonism of oleamide on pentylenetetrazole, it is worthwhile testing, at molecular level, whether or not these two drugs act at the same site.

Table 1 Effect of oleamide on the seizures induced by pentylenetetrazole in mice.

Group	Dose (mg kg^{-1})	Number of mice	Seizure latency (min)
Control	—	10	8.5 ± 0.9
Oleamide	43.75	10	16.2 ± 1.7*
	87.5	10	16.8 ± 2.5*
	175.0	10	20.2 ± 3.8*
	350.0	10	24.1 ± 3.2*
	700.0	10	28.4 ± 3.7*
Diazepam	5.0	10	26.8 ± 2.5*

Oleamide was administered intraperitoneally in the dose range 43.75–700 mg kg^{-1} 30 min before pentylenetetrazole (85.0 mg kg^{-1}, i.p.). Diazepam (5 mg kg^{-1}, i.p.) was also administered 30 min before pentylenetetrazole. The data are expressed as mean ± s.e.m. of 10 mice in each group. *$P < 0.05$ compared with blank control group.

Table 2 Effect of oleamide on seizures induced by different chemoconvulsants in mice.

Group	Number of mice	Dose (mg kg^{-1})	Seizure latency (min)			
			Picrotoxin	Strychnine	Caffeine	Semicarbazide
Control	10	—	8.3 ± 0.7	3.3 ± 0.5	6.7 ± 1.3	94.6 ± 37.1
Oleamide	10	43.75	10.4 ± 1.9	3.6 ± 0.6	7.5 ± 1.4	97.2 ± 43.6
	10	87.5	11.4 ± 1.9	3.3 ± 0.5	9.4 ± 1.7	90.4 ± 35.3
	10	175.0	11.6 ± 1.5	3.3 ± 0.6	8.7 ± 1.8	87.3 ± 34.4
	10	350.0	11.0 ± 1.7	4.0 ± 0.5	7.4 ± 1.7	79.6 ± 20.9
	10	700.0	8.8 ± 1.2	3.8 ± 0.5	7.9 ± 1.2	94.5 ± 26.9
Diazepam	10	5.0	29.7 ± 0.7*	8.5 ± 1.2*	22.1 ± 2.9*	166.2 ± 13.3*

Oleamide was administered intraperitoneally in the dose range 43.75–700 mg kg^{-1} 30 min before the chemoconvulsants. Diazepam (5 mg kg^{-1}, i.p.) was also administered 30 min before the chemoconvulsants. The data were expressed as mean ± s.e.m. of 10 mice in each group. *$P < 0.05$ compared with blank control group.

The inactivation of glial cell gap junction channels by oleamide may influence a higher order neuronal function. The inhibition of the gap junction by oleamide may involve many mechanisms (Lerner 1997), such as perturbations in the bulk membrane fluidity or the membrane–protein interface that would affect the conformation of the membrane-bound proteins as well as direct interaction with the gap junction proteins (Guan et al 1997; Lerner 1997). It is possible that the inhibition of gap junction communication partially contributes to the anticonvulsant effect of oleamide. However, this could not fully explain the selectivity of oleamide on pentylenetetrazole-induced seizures because of the lack of the effect of oleamide on the seizures induced by other reagents. The same deduction could be employed to exclude the possibilities that the effect of oleamide on pentylenetetrazole-induced seizures might be via mechanisms including CB1 mimicry (Mendelson & Basile, 1999; Lambert et al 2001), state-dependent blockage of Na^+ channels (Verdon et al 2000) and hypnotic action, which are strikingly similar to those displayed by sedatives or anticonvulsants.

In summary, the present study provides the first evidence that oleamide exhibits an anticonvulsant effect, and this anticonvulsant effect is selective to the seizures induced by pentylenetetrazole. The data suggest that oleamide acts via a more specific mechanism of action relative to the site of action of pentylenetetrazole. Further studies on this action will have implications for the potential usefulness of this new biologically active signalling substance in the treatment of certain kinds of seizure-related diseases.

Conclusion

The present study demonstrates that oleamide, an endogenous sleep-inducing substance, possesses a selective anticonvulsant action induced by pentylenetetrazole, implying the potential for the clinical utility of this agent in the treatment of seizure-related diseases.

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