Biological properties of griseolic acid, a cyclic AMP phosphodiesterase inhibitor with an adenine group

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Griseolic acid inhibited cAMP phosphodiesterase (PDE) at low concentrations, the I_{50} being of the order of 0.01–0.1 μM. Administration of griseolic acid to rats increased the cAMP level in liver and plasma several-fold. It increased glycogen degradation in mouse liver and stimulated lipolysis in isolated rat fat cells. Griseolic acid did not block the adenosine-elicited accumulation of cAMP in guinea pig brain slices. It had no effect on cAMP-dependent protein kinase from rat liver nor on the adenyl cyclase from rat brain.

Griseolic acid *Phosphodiesterase inhibitor* *cAMP* *cGMP* *Glucagon* *Theophylline*

1. INTRODUCTION

Cyclic nucleotide phosphodiesterase (PDE) catalyzes the hydrolysis of cAMP or cGMP to 5′-AMP or 5′-GMP. Since PDE is the only enzyme known to degrade cAMP or cGMP, its cellular activity is critical in governing the extent and duration of the action of these nucleotides.

During an extensive search for PDE inhibitors in microbial culture broth, we discovered griseolic acid from the culture filtrate of *Streptomyces griseoaurantiacus* which had been isolated from a soil sample collected in Kyoto-fu, Japan [1]. Griseolic acid has a unique structure, resembling cAMP with an adenine group in the molecule [2] (fig.1). It inhibited PDE in homogenates of various tissues at low concentrations and the inhibition was competitive with regard to cAMP, the substrate [1].

This paper describes the effect of griseolic acid on cyclic nucleotide levels in cells and tissues together with its physiological effects.

Abbreviation: I_{50}, concentration required for 50% inhibition

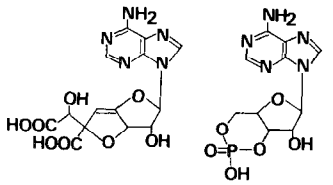

Fig.1. Structure of griseolic acid ($C_{14}H_{13}O_8N_5$; M_r 379) and cAMP.

2. EXPERIMENTAL

2.1. Assays for PDE activity

The preparation and assay of cyclic nucleotide phosphodiesterases from rat brain were as described [1].

Canine cardiac PDE was separated into 3 major forms by DEAE-cellulose chromatography using the stepwise elution procedure described by Thompson et al. [3,4]. Assay of separated PDEs was carried out essentially according for the method described by Pichard and Cheung [5] with 0.25 μM cAMP or cGMP as substrate.

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2.2. *Determination of cAMP and cGMP in the liver and plasma of rats*

Male Wistar rats (200–220 g body wt) were fasted for 18 h, and griseolic acid dissolved in saline was injected subcutaneously at a dose of 10 mg/kg. After 50 min the rats were anesthetized with pentobarbital and blood taken from the carotid artery. Simultaneously, a leaf of the liver was excised, quickly perfused with cold saline and frozen with clamps cooled in liquid nitrogen. The cAMP and cGMP levels were determined using radioimmunoassay kits (Yamasa Shoyu, Chiba, Japan) according to Honma et al. [6].

2.3. *Lipolysis of fat cells*

Isolation of fat cells from epididymal adipose tissue of rats was performed according to Rodbell [7]. Fat cells (3×10^5 cells) were incubated with 100 μg griseolic acid or theophylline in 1.0 ml Krebs Ringer bicarbonate (pH 7.4) supplemented with 4% bovine serum albumin at 37°C with a gas phase of 95% O_2: 5% CO_2 for 2 h with gentle shaking. Reaction was terminated by addition of an equal volume of cold 7.5% trichloroacetic acid and after centrifugation at 3000 rpm for 10 min, the supernate was subjected to determination of glycerol [8] and cAMP [6].

2.4. *Determination of blood glucose and liver glycogen in mice*

Male ddy mice (20–22 g body wt) were deprived of diet at 9 a.m. and the experiments started usually at 10 a.m. Griseolic acid, glucagon or the vehicle (saline) was injected into the tail vein. At time intervals, mice were decapitated, blood glucose determined with a Glucometer (Miles), and a portion of the liver excised into ice-cold saline. The liver glycogen level was determined by the method of Van der Vies [9] with mouse liver glycogen [10] as a standard.

2.5. *Adenosine-elicited accumulation of cAMP in guinea pig brain slices*

Cerebral cortex slices (0.26 × 0.26 mm) were prepared from male guinea pigs (about 300 g body wt), and incubated at 37°C in a nylon cloth dipped in Krebs-bicarbonate glucose medium gassed with 95% O_2: 5% CO_2 to lower the basal cAMP level [11,12]. The medium was changed twice. After about 60 min, the slices were suspended in fresh medium gassed as above, and an aliquot transferred to 2 ml (final) Krebs-bicarbonate glucose medium containing the agents tested. Incubation was performed at 37°C for 5 min with the same gas phase and the reaction terminated by addition of 0.1 ml of 100% trichloroacetic acid. After homogenization and centrifugation, the cAMP content of the supernate was determined by radioimmunoassay [6], and protein of the pellet determined according to Bradford [13].

2.6. *Other determinations*

cAMP-dependent protein kinase was purified from rat liver by the method of Kumon et al. [14], and the effect of griseolic acid on the enzyme measured in the presence and absence of 1 μM cAMP. Rat brain adenyl cyclase was prepared and assayed essentially according to Piascik et al. [15]. In the assay of calf intestinal adenosine deaminase (Sigma), formation of inosine was determined by HPLC [16,17].

3. RESULTS AND DISCUSSION

3.1. *Inhibitory activity on PDE*

Griseolic acid is a more potent inhibitor of cyclic nucleotide PDE than theophylline and papaverine. The I_{50} values (in μM) of griseolic acid, theophylline and papaverine with 0.14 μM cAMP (0.14 μM cGMP in parentheses) as substrate were 0.16 (0.63), 360 (196) and 3.6 (13.6), respectively, with the supernate of rat brain homogenate as the enzyme. The inhibitory effect of griseolic acid toward canine cardiac PDEs separated into 3 major forms (PDE I, II, III) was also investigated. The I_{50} values (in nM) for PDE I, II and III with 0.25 μM cAMP (0.25 μM cGMP) as substrate were 74 (35), 50 (22) and 18 (65), respectively.

3.2. *Elevation of cyclic nucleotide levels in rats*

cAMP and cGMP levels were determined in the liver and plasma 50 min after subcutaneous administration of griseolic acid (10 mg/kg) to rats. In the liver, the cAMP level was increased 5-fold and that of cGMP 3-fold (fig.2). In plasma, the cAMP level was increased 9-fold but the cGMP level only 1.3-fold. When the experiments were performed by oral administration of griseolic acid (30 mg/kg), the cAMP level was increased about 2-fold in plasma.

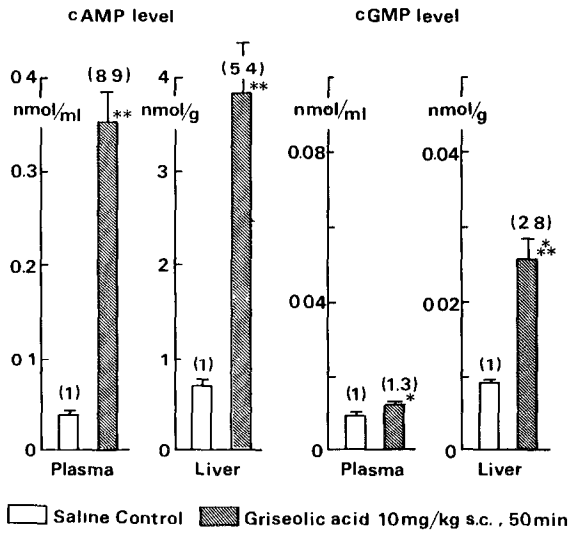

Fig.2. Cyclic nucleotide levels in liver and plasma of rats after griseolic acid administration. The columns show mean values ± SE ($n=5$) as nmol/ml plasma and nmol/g wet liver. Relative values for control are shown in parentheses. $*p<0.05$, $**p<0.01$, $***p<0.001$ by Students t-test for comparison with control value.

3.3. *Effect on glycogenolysis in mice*

When griseolic acid was administered intravenously to mice (1 mg/kg), the blood glucose level was increased by about 2-fold and the liver glycogen level was decreased to 30% of the original level (fig.3). Similar changes in blood glucose and liver glycogen were observed by administration of glucagon (0.03 mg/kg), although the duration was shorter. Glucagon is known to exert its effect through the cAMP system by stimulation of adenyl cyclase [18]. Therefore, these results may be explained by increase in cAMP level in the liver.

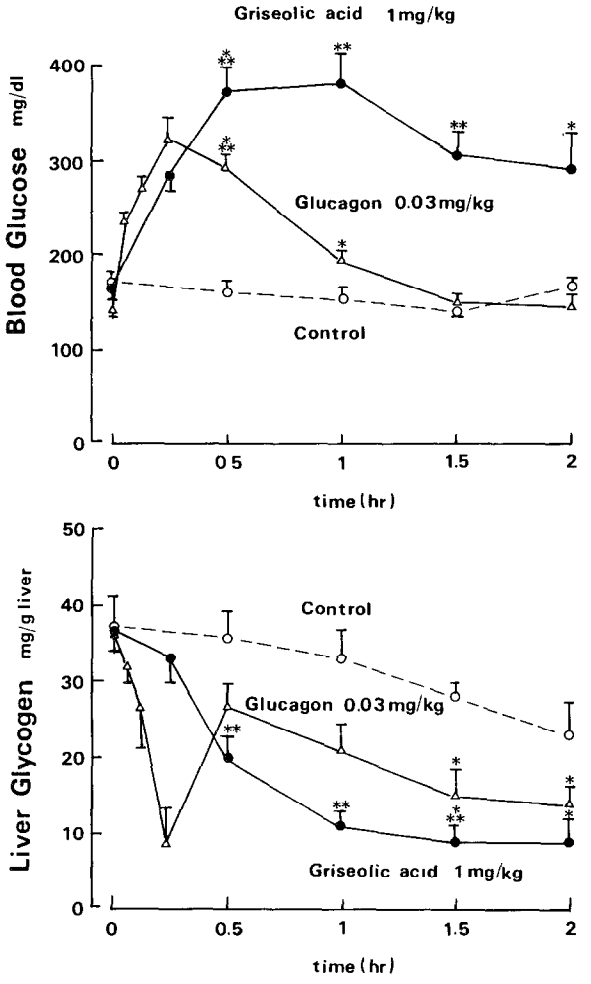

Fig.3. Blood glucose and liver glycogen levels after griseolic acid administration to mice. Values are expressed as average of 5 mice ± SE. $*p<0.05$, $**p<0.01$, $***p<0.001$ by Students t-test for comparison with control values.

3.4. *Effect on lipolysis in fat cells*

The effects of griseolic acid on lipolysis and formation of cAMP were investigated with fat cells isolated from epididymal adipose tissue. Griseolic acid showed a stimulatory effect on lipolysis similar to theophylline (fig.4A). Under our conditions, griseolic acid caused accumulation of cAMP in the incubation medium containing fat cells (fig.4B). On the other hand, theophylline caused only a slight accumulation of cAMP. The lipolytic effect of theophylline may be due to properties other than its ability to inhibit PDE, in part, to its binding activity to adenosine receptor in fat cells [19].

3.5. *Cyclic AMP formation in cortex slices*

The effect of griseolic acid on the adenosine receptor was investigated on adenosine-elicited accumulation of cAMP in guinea pig brain cortex slices. In this system, theophylline blocked the adenosine-elicited accumulation of cAMP [11,12, 20]. Griseolic acid, in contrast, had a stimulatory effect on the effect of adenosine, AMP, ADP and ATP (fig.5). The effect on cAMP accumulation of

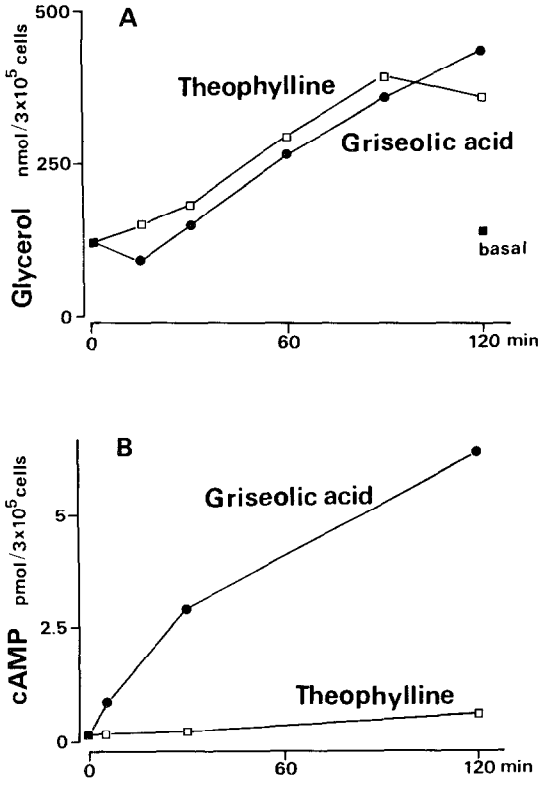

Fig.4. Effect of griseolic acid on lipolysis and cAMP levels in rat fat cells. Experiments were performed in duplicate.

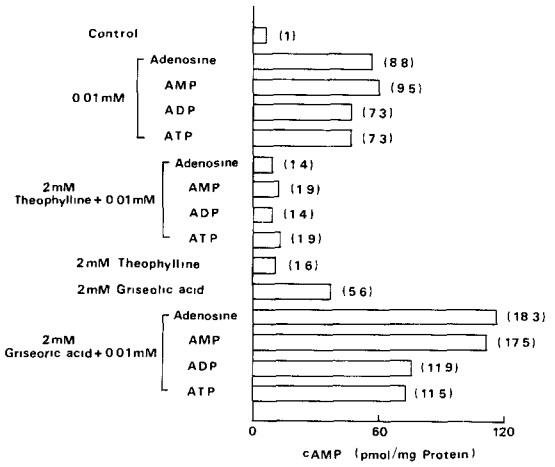

Fig.5. Effect of griseolic acid on adenosine-elicited accumulation of cAMP in cerebral cortex slices of guinea pigs. The columns show the mean values of duplicate experiments, and relative values for control are shown in parentheses.

2 mM griseolic acid alone was less than that of 10 μM adenosine. Griseolic acid seemed to have no binding activity to the adenosine receptor.

3.6. *Properties other than PDE inhibition*

Although griseolic acid has a similar structure to cAMP, it showed neither agonist nor antagonist activity on cAMP-dependent protein kinase partially purified from rat liver at 10^{-4} M, and showed no effect on adenyl cyclase activity of rat brain and on the adenosine deaminase from calf intestine.

Griseolic acid showed no inhibitory effect on platelet aggregation nor relaxation of guinea pig ileum even at μg/ml, whereas papaverine, another well-known PDE inhibitor, relaxes the smooth muscle.

Griseolic acid may become a unique and useful tool in biology to study the role of cyclic nucleotides. Potential new uses of griseolic acid or its analogues as a therapeutic agent will also continue to be investigated.

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