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INHIBITION OF 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE BY ANTIALLERGIC AGENTS

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SUMMARY — Some antiallergic agents having protective action against anaphylaxis were found to inhibit NAD^+-linked 15-hydroxyprostaglandin dehydrogenase partially purified from guinea pig lung. The concentrations required to inhibit the enzyme by 50% were 3.0 μM for baicalein, 9.8 μM for xanoxic acid and 350 μM for disodium cromoglycate. The inhibitory effects of these compounds on 15-hydroxyprostaglandin dehydrogenase were greater than on cAMP phosphodiesterase activity in the same tissue. Inhibitory effects of flavonoids other than baicalein were also investigated.

A close relationship between anaphylaxis and prostaglandins has been observed. E type prostaglandins have been reported to inhibit spontaneous histamine release from isolated rat peritoneal mast cells (1) and also inhibit the release induced by compound 48/80 (2). In several models of allergy, E type prostaglandins have been reported to inhibit anaphylactic release of histamine and SRS-A* with simultaneous stimulation of adenylate cyclase (3, 4). On the other hand, non-steroid anti-inflammatory agents, inhibitors of prostaglandin synthesis, increased the release of histamine and SRS-A from lung tissue during anaphylaxis (5).

15-Hydroxyprostaglandin dehydrogenase* is the first step enzyme in prostaglandin catabolism (6), and was assumed to play a major part in inactivation of prostaglandins in vivo (7). The dehydrogenase is abundant in lung tissue, and intraveneously infused prostaglandins were inactivated by 90% or more after a single passage through the pulmonary circulation (8).

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* Abbreviations: SRS-A, slow reacting substance of anaphylaxis; PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; 15-OH-PG dehydrogenase, 15-hydroxyprostaglandin dehydrogenase; DSCG, disodium cromoglycate; R-52,510, 2,3-dimethyl-5H-furo[3,2-g]chromen-5-one-7-carboxylic acid; R-52,511, 2,3-dimethyl-8H-furo[2,3-g]chromene-8-one-6-carboxylic acid; N-5', N-(3',4'-dimethoxycinnamoyl)anthranilic acid.

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In the present study, we found that some antiallergic agents having protective action against anaphylaxis inhibited the 15-OH-PG dehydrogenase partially purified from guinea pig lung. The inhibitory effects on the guinea pig lung dehydrogenase were compared with those on cyclic nucleotide phosphodiesterases in the same tissue. The inhibitory effects of flavonoids on the 15-OH-PG dehydrogenase were also investigated.

MATERIALS AND METHODS

Antiallergic agents DSCG* and N-5'* were obtained from Fujisawa Pharmaceutical Industries (Osaka, Japan) and Kissei Pharmaceutical Co. (Matsumoto, Japan), respectively. Baicalein was kindly provided by Prof. Michio Takido (Nihon University). 7-Isopropoxy-9-oxaxanthene-2-carboxylic acid (xanoxic acid) was synthesized in these laboratories according to the method described by Pfister et al. (9). R-52,510* and R-52,511* (10) were kindly provided by Prof. Yoshiyuki Kawase (Toyama University).

Chemicals The chemicals used were obtained from the following sources: PGE_1*, Fuji Chemical Industries (Tokyo, Japan); [adenine-U-^{14}C]cAMP, Radiochemical Centre; [^{14}C(U)]cGMP, New England Nuclear; flavone, quercetin, fisetin and naringenin, Tokyo Kasei (Tokyo, Japan); kaempferol, Fulka & Bushs (Switzerland); morin, E. Merck (Darmstadt); hesperetin, Sigma; wogonin and norwogonin were kindly provided by Prof. Michio Takido. Other reagents were of highest quality generally available.

15-OH-PG dehydrogenase The 15-OH-PG dehydrogenase was partially purified from guinea pig lungs as described previously (11). The activity was assayed by the formation of 15-keto-PGE_1 as described by Änggård and Samuelsson (12). The standard assay mixture, contained, in a total volume of 0.50 ml, 14 nmoles of PGE_1, 600 nmoles of NAD+, and 10 µmoles of potassium phosphate buffer (pH 7.4). The mixture was incubated at 37°C for 30 min and the reaction was terminated by dipping in an ice-water bath. The formation of 15-keto-PGE_1 was determined from the absorption at 500 nm after addition of 30 µl of 2N NaOH. The apparent molar extinction coefficient of 15-keto-PGE_1 was 31,000 under the conditions.

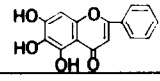
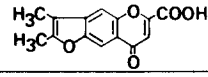
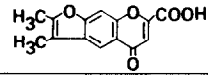
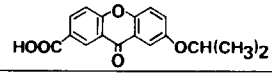
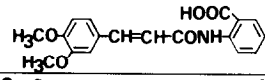
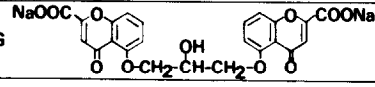
Cyclic nucleotide phosphodiesterases Guinea pig lungs were homogenized with 4 volumes of 0.17 M Tris-HCl (pH 8.0) containing 5 mM $MgSO_4$ at 0°C. The homogenate was centrifuged at 100,000 g for 1 hr. The clear supernatant fluid was used as a source of cyclic nucleotide phosphodiesterases. The activities were assayed by essentially the same method as described by Pichard and Cheung (13), using [^{14}C]cAMP and [^{14}C]cGMP as substrates at a concentration of 0.14 µM.

Assay of inhibitory activity DSCG was dissolved in distilled water. Xanoxic acid, R-52,510 and 52,511 were dissolved in 0.01N NaOH. N-5' and flavonoids were dissolved in dimethyl sulfoxide and brought to required concentrations using dimethyl sulfoxide or distilled water. The amount of dimethyl sulfoxide in the assay tubes was always less than 2%. The solvent blank was always assayed. In the case of 15-OH-PG dehydrogenase, the colour blank was assayed if necessary.

RESULTS

The concentrations of antiallergic agents required for half-maximal inhibition of 15-OH-PG dehydrogenase from guinea pig lung are shown in Table I.

Table I. Inhibition by antiallergic agents of NAD^+-linked 15-OH-PG dehydrogenase and cyclic nucleotide phsophodiesterases from guinea pig lungs. Values were expressed as the concentrations required for 50% inhibition of enzyme activities. Substrates at the following concentrations were used; a) 28 μM PGE_1 b) 0.14 μM cAMP, c) 0.14 μM cGMP. d) Protective action against passive cutaneous anaphylaxis in rats of intraveneously administered R-52,510 and R-52,511 was observed at these laboratories.

Compound	Structure	I_{50}(μM)			Reference to antianaphylaxis
		15-OH-PG dehydrogenase [a]	Phosphodiesterase		
			cAMP [b]	cGMP [c]	
Baicalein		3.0	9.7	40	(14)
R-52,511		8.0	74	54	d
R-52,510		8.7	34	66	d
Xanoxic acid		9.8	75	14	(9) (15)
N-5'		96	67	40	(16)
DSCG		350	650	90	(17)

Effects of these compounds on the low Km cAMP phosphodiesterase and the low Km cGMP phosphodiesterase from the guinea pig lung were also assayed. Baicalein, furochromone derivatives and xanoxic acid inhibited the dehydrogenase by 50% at concentrations on the order of 10^{-5} M. Inhibitory effects of these compounds on the 15-OH-PG dehydrogenase were greater than on the cyclic nucleotide phosphodiesterases, and concnetrations required for half-maximal inhibition of the dehydrogenase were one-tenth to one-third of those of cyclic nucleotide phosphodiesterases.

The concentrations of N-5' required for half-maximal inhibition of these three enzymes were on the order of 10^{-4} M. DSCG inhibited the 15-OH-PG dehydrogenase by 50% at a concentration of about 3×10^{-4} M, but its effect on the dehydrogenase was greater than on the cAMP phosphodiesterase.

These results indicate that most of the antiallergic agents used had greater inhibitory effects on 15-OH-PG dehydrogenase than on cyclic nucleotide phosphodiesterases.

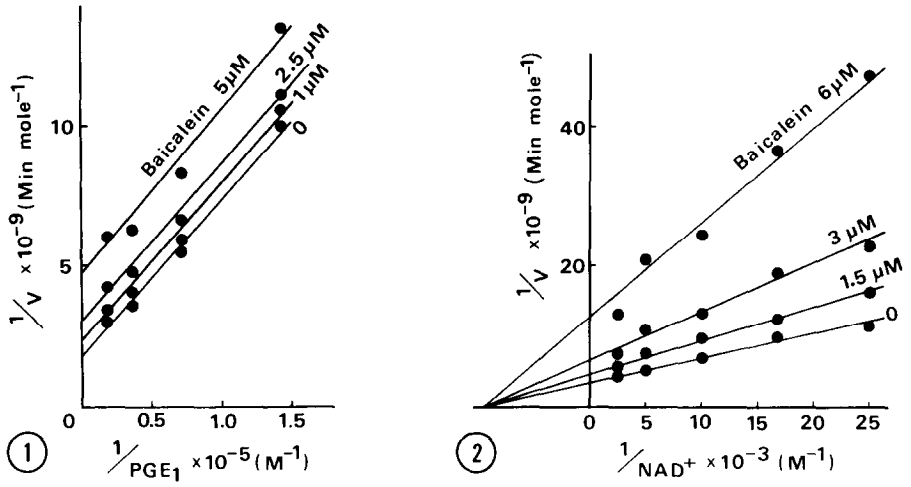
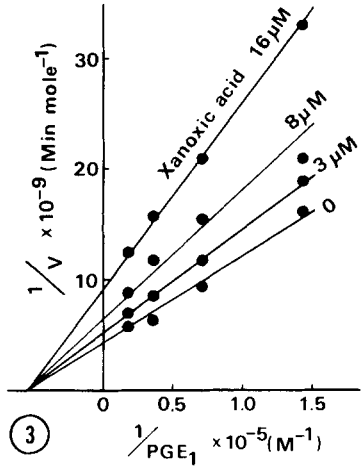


Fig. 1. Lineweaver-Burk plots for the inhibition of guinea pig lung 15-OH-PG dehydrogenase by baicalein. The cofactor (NAD^+) was fixed at a concentration of 1.2 mM.

Fig. 2. Lineweaver-Burk plots for the inhibition of guinea pig lung 15-OH-PG dehydrogenase by baicalein. The substrate (PGE_1) was fixed at a concentration of 168 μM.

Figure 1 shows the double reciprocal plots of the PGE_1 concentration *versus* reaction velocity of 15-OH-PG dehydrogenase from the guinea pig lung in the presence of baicalein at various concentrations. Baicalein exhibited an uncompetitive inhibition with regard to PGE_1. The inhibition of baicalein was noncompetitive with regard to NAD^+ (Fig. 2). On the contrary, xanoxic acid exhibited noncompetitive inhibition with regard to PGE_1 (Fig. 3), and uncompetitive inhibition with regard to NAD^+ (Fig. 4). DSCG inhibited the dehydrogenase in the same manner as xanoxic acid (data were not shown).

Since baicalein, a flavonoid, was found to be a potent inhibitor of 15-OH-PG dehydrogenase, other flavonoids were examined for inhibitory effects on the dehydrogenase and their inhibitory activities were compared with those on cyclic nucleotide phosphodiesterases. As shown in Table II, baicalein was the most potent inhibitor of the dehydrogenase from among the flavonoids tested. Other flavonoids inhibited the dehydrogenase by 50% at concentrations on the order of 10^{-5} M. The inhibitory effects of flavonoids on the 15-OH-PG dehydrogenase corresponded somewhat with their inhibitory



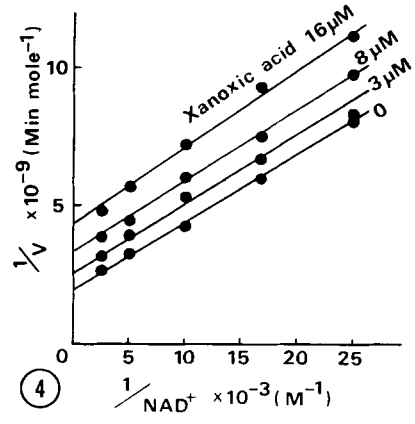


Fig. 3. Lineweaver-Burk plots for the inhibition of guinea pig lung 15-OH-PG dehydrogenase by xanoxic acid. The cofactor (NAD^+) was fixed at a concentration of 1.2 mM.

Fig. 4. Lineweaver-Burk plots for the inhibition of guinea pig lung 15-OH-PG dehydrogenase by xanoxic acid. The substrate (PGE_1) was fixed at a concentration of 168 μM.

effects on cAMP phosphodiesterase but not with their effects on cGMP phosphodiesterase.

DISCUSSION

Antiallergic agents which inhibit the release of mediators were found to inhibit 15-OH-PG dehydrogenase partially purified from guinea pig lung.

Table II. Inhibition by flavonoids of NAD^+-linked 15-OH-PG dehydrogenase and cyclic nucleotide phosphodiesterases from guinea pig lungs. Assay conditions were as described in Table I.

Type	Compound	Substituents							I_{50} (μM)		
									15-OH-PG dehydrogenae	Phosphodiesterase	
		5	6	7	8	2′	3′	4′		cAMP	cGMP
Flavone	Flavone	—	—	—	—	—	—	—	86	51	24
	Baicalein	OH	OH	OH	—	—	—	—	3.0	9.7	40
	Norwogonin	OH	OH	—	OH	—	—	—	13	24	>370
	Wogonin	OH	OH	—	OCH_3	—	—	—	22	40	29
Flavanol	Kaempferol	OH	—	OH	—	—	—	OH	17	13	6.4
	Quercetin	OH	—	OH	—	—	OH	OH	15	18	8.4
	Morin	OH	—	OH	—	OH	—	OH	76	66	37
	Fisetin	—	—	OH	—	—	OH	OH	35	20	13
Flavanone	Hesperetin	OH	—	OH	—	—	OH	OCH_3	17	81	30
	Naringenin	OH	—	OH	—	—	—	OH	42	16	5.4

This finding supported the close relationship between prostaglandins and anaphylaxis, and suggested that the inhibition of prostaglandin catabolism may be involved in the pharmacological action of these antiallergic agents.

The mechanism by which such agents inhibit the mediator release is still controversial and poorly understood. Practically the only and also the most popular theory on the enzyme level is that they act as phosphodiesterase inhibitors thereby increasing cyclic nucleotide levels (reviewed in 15). A possible role of the inhibition of prostaglandin catabolism by these antiallergic agents may be as follows: When the agents inhibit the NAD^+-linked 15-OH-PG dehydrogenase activity, PGE_1 or PGE_2* may accumulate in the tissue or body fluids, which would elevate the cAMP level in lung tissue or mast cells. On the other hand, the inhibitory effects of such agents on cAMP phosphodiesterases have been reported (15). So, these agents appear to raise the cAMP level both by stimulating cAMP synthesis and inhibiting its degradation, and finally to inhibit the release of anaphylactic mediators.

The fact that from among the flavonoids tested baicalein had the greatest inhibitory effect on 15-OH-PG dehydrogenase may correspond to the fact that baicalein is the only flavonoid which was reported to inhibit the release of mediators during anaphylaxis (14). Flavonoids have other versatile pharmacological activities such as diuretic activity or inhibition of capillary fragility (18). The inhibition of prostaglandin catabolism may also be involved in part in such pharmacological actions of flavonoids.

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