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Inhibition of 15-Hydroxyprostaglandin Dehydrogenase by 9,11-Deoxyprostaglandins *in Vitro* and *in Vivo*

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SUMMARY

YAMAZAKI, M., K. OHUCHI, M. SASAKI, AND K. SAKAI. Inhibition of 15-hydroxyprostaglandin dehydrogenase by 9,11-deoxyprostaglandins *in vitro* and *in vivo*. *Mol. Pharmacol.* **19**:456–462 (1981).

Fourty-six prostaglandin analogues were studied to determine their ability to inhibit 15-hydroxyprostaglandin dehydrogenase partially purified from swine lung. The inhibition was high in prostaglandin analogues having the following structure: (a) cyclopentane ring with no substituent; (b) hydroxy or keto group at carbon atom 15: (c) lengths of side chains similar to those of prostaglandin: and (d) methylene group between carbon atoms 13 and 14. The most potent inhibitor was *rac*-13β,14β-methylene-15-ketoprostanoate, the K_i being 0.14 μM, and its inhibition was noncompetitive with regard to prostaglandin (PG) E_2, the substrate. This compound inhibited [^{3}H]PGE_1 degradation in the soluble fraction of guinea pig lung homogenates. One potent inhibitor, 15α-hydroxyprost-13-enoate (9,11-deoxy-PGE_1), was tested for its *in vivo* effect in anesthetized rats. When PGE_1 was intravenously infused together with 9,11-deoxy-PGE_1, its antilipolytic activity was significantly enhanced. Namely, the blood free fatty acid levels, which had been elevated by norepinephrine injection, were markedly decreased by the infusions of PGE_1 with the inhibitor.

INTRODUCTION

Prostaglandins have been shown to be inactivated rapidly by metabolic processes *in vivo* (1). The first step in the metabolism of prostaglandins, the oxidation of the secondary alcohol group at carbon atom 15 to a ketone (2, 3), is catalyzed by an NAD^+-linked PGDH[2] (EC 1.1.1.141) (4). This reaction is a key step in the control of the biological inactivation of prostaglandins, since oxidized prostaglandins possess little biological activity (5).

PGDH inhibition seems useful in potentiating or prolonging biological prostaglandin activity. Prostaglandin analogues such as prostanoic acid (6), 7-thia-13-prostynoic acid (7), 7-oxa-13-prostynoic acid (8), PGB_1 and 15-epi-PGE_1 (9), 7-oxa-$PGF_{1\alpha}$ (8), and alkyl side-chain-modified PGE analogues (10), when used with enzyme preparations partially purified from various tissues, reportedly inhibit PGDH, but these prostaglandin analogues were not potent enough to permit examination of their *in vivo* effectiveness.

The present study was undertaken in a search for potent PGDH inhibitors among synthetic prostaglandin analogues. Potent inhibitors were found in alkyl side-chain-modified analogues of prostanoic acid, and structure-activity relationships were studied between prostaglandin analogues with respect to their PGDH-inhibiting ability. A representative compound was examined for its effects *in vivo* when administered together with PGE_1 to rats. Enhanced antilipolytic activity in the presence of the inhibitor was taken as evidence of its *in vivo* effectiveness.

MATERIALS AND METHODS

Materials. The prostaglandin analogues used in the present study, all of which were racemic compounds, were synthesized at the Central Research Laboratories, Sankyo Company, Ltd. (11). These compounds were stored in an ethanol solution at −20°. [5,6(*n*)-^{3}H] PGE_1 was purchased from New England Nuclear Corporation, Boston, Mass. Nonradioactive PGE_1 and PGE_2 were obtained from Fuji Chemicals, Toyama, Japan, and *dl*-norepinephrine from Sankyo, Japan. Other reagents were of analytical grade.

Inhibition of purified enzyme. PGDH was partially purified from a high-speed supernatant fraction of swine lung homogenates by ammonium sulfate fractionation and chromatography *O*-(triethylaminoethyl)-cellulose

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[2] The abbreviations used are: PGDH, 15-hydroxyprostaglandin dehydrogenase; PGE_1, prostaglandin E_1; 15-keto-PGE_2, 15-keto-prostaglandin E_2; 9,11-deoxy-PGE_1, 9,11-deoxyprostaglandin E_1 (15α-hydroxyprost-13-enoate).

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and Sephadex G-100 by the method described by Änggård and Samuelsson (4). The standard assay system contained 14 nmoles of PGE_2, 0.6 μmole of NAD^+, 0.5 μmole of GSH, 40 μmoles of potassium phosphate buffer (pH 7.3), partially purified enzyme (protein, 20–40 μg), and various concentrations of the prostaglandin analogue to be tested, in a total volume of 0.5 ml. The reaction, started by adding PGE_2 dissolved in 5 μl of ethanol, was carried out at 45° for 20 min and terminated by dipping the tubes in an ice-water bath. The formation of 15-keto-PGE_2 was determined from the absorption at 500 nm after addition of 30 μl of 2 N NaOH. The apparent molar extinction coefficient of 15-keto-PGE_2 was 31,000 under these conditions (12). The concentrations producing a 50% inhibition of the reaction were estimated graphically from dose-response curves, composed of at least four points.

Substrate activity of the prostaglandin analogues was determined by an increase in the absorption at 340 nm due to NAD^+ reduction. The reaction components were the same as those for the standard assay system except that 56 nmoles of prostaglandin analogue were used in place of PGE_2.

Inhibition of PGE_1 metabolism in the soluble fraction of lung homogenates. Lungs of male guinea pigs were rapidly excised and homogenized in a Waring Blendor with 4 volumes of an ice-cold Bucher medium (13) (0.02 M KH_2PO_4, 0.072 M K_2HPO_4, 0.028 M nicotinamide, and 0.0036 M $MgCl_2$, pH 7.4) for 2 min. The homogenates were centrifuged for 15 min at 3,000 × *g*, and the supernatant fluid was centrifuged for 60 min at 105,000 × *g*. A portion (2 ml) of the final clear supernatant fraction was incubated at 37° for 30 min with 14 nmoles of [^{3}H]PGE_1 (specific activity, 0.14 μCi/nmole) in the presence and absence of 400 nmoles of 13,14-methylene-15-ketoprostanoate, the prostaglandin analogue used in this study. The reaction was terminated by the addition of 9 volumes of ethanol; after filtration and evaporation to a small volume, the residue was acidified to pH 3 and extracted three times with ethyl ether. The combined ether extracts were evaporated to dryness, and the residue was analyzed by reverse-phase partition chromatography. The column (1.2 cm × 18 cm) consisted of 4.5 g of hydrophobic Super-Cel with a C-47 solvent system supplemented with acetic acid (methanol:water:chloroform: 2-ethylhexanol:acetic acid, 140:160:15:15:2, v:v) (14, 15). Chromatography was carried out at 25° with the elution rate of 14 ml/hr. The fraction volume was 8.5 ml. An aliquot of each fraction was analyzed for radioactivity in toluene-dioxane (20:80) scintillation fluid using a Packard liquid scintillation spectrometer. To serve as a reference in the chromatography, PGE_1 (0.3 mg) was added and was detected by measuring absorption at 278 nm after conversion to PGB_1 by treatment with 0.5 M methanolic KOH (16).

Determination of free fatty acids in blood. Male rats (Wistar-Imamichi), weighing 380–450 g, were anesthetized with pentobarbital (50 mg/kg, s.c.) and fixed in the superior position. *dl*-Norepinephrine was injected into the rats s.c. at a dose of 0.75 mg/kg. After 30 min, an i.v. infusion of PGE_1 in the presence and absence of 9,11-deoxy-PGE_1, the prostaglandin analogue used in this study, was started and continued for 40 min. PGE_1 and 9,11-deoxy-PGE_1 were infused into the femoral vein of the rat at the rates of 0.5 and 250 μg/kg/min, respectively. The prostaglandin analogue was dissolved using an ultrasonic oscillator, in a mixture of 0.85% NaCl and 1.3% $NaHCO_3$ (3:2, v:v) at a concentration of 5 mg/ml. Blood samples (0.2 ml) were taken at intervals before, during, and after the infusion period from the vinyl catheter which was inserted into the cervical artery of the rat and were analyzed for free fatty acids by the method of Itaya and Ui (17). The free fatty acid concentration was expressed as microequivalents per milliliter with palmitic acid as a standard.

RESULTS

Structure-activity relationships. Inhibitory effects of prostaglandin analogues on swine lung PGDH were investigated with PGE_2 as the substrate. Table 1 shows the inhibition activity of analogues with a modified cyclopentane ring. 9,11-Deoxy-PGE_1 (No. 1-1) at low concentrations was found to inhibit PGDH, the inhibition being essentially unchanged with a 15-hydroxy epimer or by introducing a double bond at carbon atom 5, as in PGE_2. Introduction of a double bond between carbon atoms 11 and 12 (No. 2) or carbon atoms 8 and 12 (No. 3) slightly decreased the inhibition activity. Dehydration of the 13, 14-double bond to a triple bond did not affect the activity (Nos. 2-1 and 2-2). The inhibition was markedly reduced by substitution of the hydroxy, keto, hydroxymethyl, carboxy, carboxyethyl, or carboxymethyl group on the cyclopentane ring (Nos. 4–16). In these compounds, 9-deoxy-PGE_1 (No. 7-1) and 9-deoxy-PGE_2 (No. 7-3) had relatively higher inhibition activity and their activities were affected by configuration of the 15-hydroxy group.

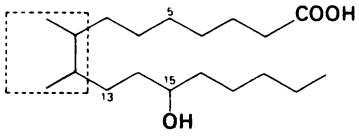
Substrate activity of analogues were also determined with the enzyme preparation (Table 1). 10-Keto-15α-hydroxyprost-13-enoate (No. 5-1), 9-deoxy-PGE_1 (No. 7-1), 9-deoxy-PGE_2 (No. 7-3), 11-hydroxymethyl-11-deoxy-PGE_1 (No. 8-1), and 9-hydroxymethyl-9-deoxy-PGE_1 (No. 10-1) reacted at 40–100% of the rate of PGE_2. On the other hand, 9,11-deoxy-PGE_1 (No. 1-1) and 9,11-deoxy-PGE_2 (No. 1-3) were less active substrates. The compound (No. 17) having no cyclopentane ring also served as substrate of PGDH.

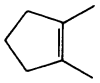
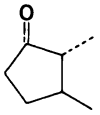
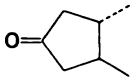
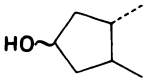
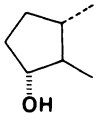
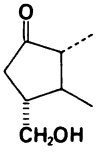
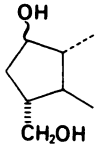
Table 2 shows the effects of modification of the side chains of 9,11-deoxy-PGE_1 on the inhibition activity. Oxidation of the 15-hydroxy group to a ketone (No. 18) or saturation of the double bond between carbon atoms 13 and 14 did not affect the inhibition activity (Nos. 19 and 20). Addition of a 13, 14-methylene group increased the activity, particularly in a 15-keto compound (Nos. 21 and 22). The configuration of the 13,14-methylene group was of importance in inhibition: the compound with β-configuration of the methylene group, 13β,14β-methylene-15-ketoprostanoate (No. 22-3), was about six times more potent than the α-methylene isomer (No. 22-2). Drastic reductions in the activity were produced by 15-methylation (No. 23), shortening the alkyl side chain by four carbon atoms (No. 24), or modification between carbon 16 and 20 (Nos. 25–27). Compounds without the hydroxy or keto group at carbon atom 15 were poor

TABLE 1

Inhibition and substrate activities of cyclopentane ring-modified analogues of prostaglandins for PGDH reaction

The inhibition activity was assayed under the standard conditions with 28 μM PGE_2 as the substrate; I_{50} refers to the concentration of compound required for 50% inhibition. Substrate activity was assayed with a concentration of compound at 112 μM and is expressed as relative rate with PGE_2 at 28 μM as 100%.

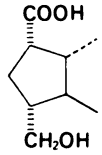
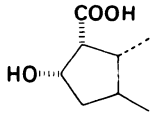
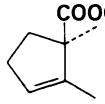
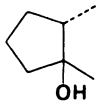
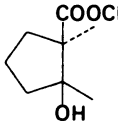
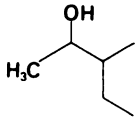


No.	Cyclopentane ring	Bond[a]		Configuration[b] of 15-OH	Inhibition activity (I_{50})	Substrate activity (PGE_2 = 100%)
		C_{5-6}	C_{13-14}			
					μM	%
1-1		s	d	α	7.5	25.2
1-2		s	d	β	8.0	6.3
1-3		d	d	α	5.8	27.2
1-4		d	d	β	7.0	4.7
2-1		s	d	Mixed	10	
2-2		s	t	Mixed	11	
3		s	d	Mixed	13	
4		s	d	Mixed	48	
5-1		s	d	α	125	45.9
5-2		s	d	β	130	11.7
6		s	d	Mixed	68	
7-1		s	d	α	41	104
7-2		s	d	β	200	12.7
7-3		d	d	α	27	64.3
7-4		d	d	β	127	11.0
8-1		s	d	α	240	53.2
8-2		s	d	β	640	4.7
9 ($PGF_{1\alpha}$)		s	d	α	1500	22.0
10-1		s	d	α	>1000	69.5
10-2		s	d	β	>1000	1.4
11		s	d	Mixed	670	

[a] s, Single bond; d, double bond; t, triple bond.

[b] Configuration of the hydroxy group at carbon atom 15. Mixed, α, β mixture.

TABLE 1 *cont'd.*

No.	Cyclopentane ring	Bond[a] C_{5-6}	Bond[a] C_{13-14}	Configuration[b] of 15-OH	Inhibition activity (I_{50})	Substrate activity (PGE_2 = 100%)
					μM	%
12-1		s	d	α	>1000	8.5
12-2		s	d	β	>1000	0.8
13		s	d	Mixed	1670	
14		s	t	Mixed	30	
15		s	t	Mixed	950	
16		s	t	Mixed	>1000	
17		s	d	Mixed	49	31

inhibitors (No. 28–30). Shortening the carboxy side chain by five carbon atoms also reduced the activity (No. 31).

Dixon plots of experiments in which 13,14-methylene-15-keto-prostanoate (No. 22-1) was used as an inhibitor are shown in Fig. 1A. This compound inhibited the reaction in a noncompetitive manner with regard to PGE_2, the K_i being 0.6 μM. 13,14-Methylene-15-hydroxyprostanoate (No. 21-1) and 9,11-deoxy-PGE_1 (No. 1-1) showed also noncompetitive-type inhibition. In contrast, 9-deoxy-PGE_2 (No. 7-3) was a competitive inhibitor, the K_i being 7.6 μM (Fig. 1B). Likewise, 9-deoxy-PGE_1 (No. 7-1) showed competitive inhibition, the K_i being 14 μM.

Inhibition of PGE_1 metabolism in lung homogenates. The effect of 13,14-methylene-15-ketoprostanoate (No.

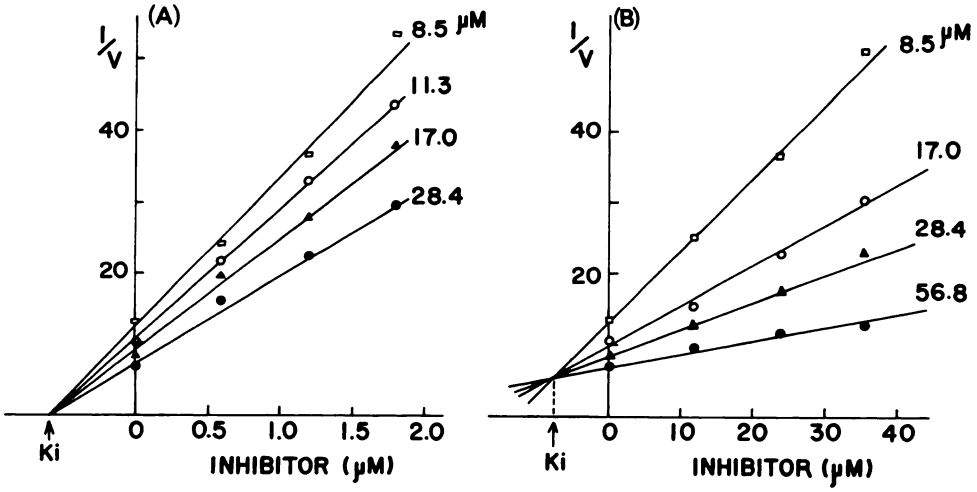


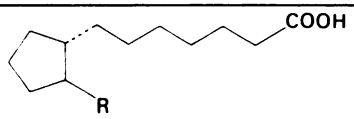
FIG. 1. *Dixon plots of PGDH inhibition by 13,14-methylene-15-ketoprostanoate (A) and 9-deoxy-PGE_2 (B)*

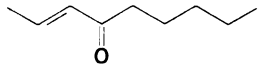
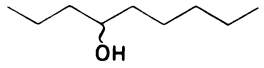
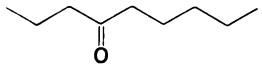
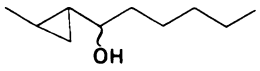
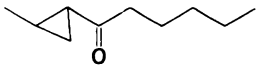
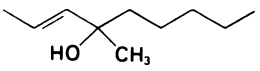
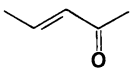
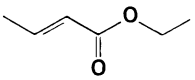
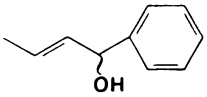
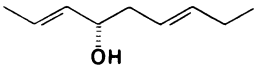
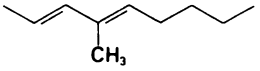
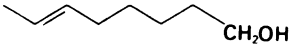
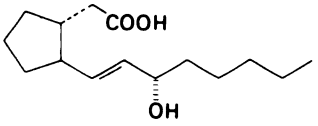
Initial velocity, *V*, was expressed as nanomoles of 15-keto-PGE_2 formed per minute. Concentrations marked are of PGE_2. Reactions occurred under standard assay conditions.

TABLE 2

Inhibition and substrate activities of side chain-modified analogues of 9,11-deoxyprostaglandin for PGDH reaction

Assay conditions were the same as those described in Table 1.



No.	R	Configuration of 13,14-methylene[a]	Inhibition activity (I_{50})	substrate activity (PGE_2 = 100%)
			μM	%
18			9.2	
19			6.2	11.3
20			7.0	
21-1		Mixed	4.5	4.0
-2		α, α	7.0	
22-1		Mixed	0.6	
-2		α, α	0.8	
-3		β, β	0.14	
23-1			89	0
-2		(1-Methyl ester)	>1000	
24			160	
25			164	
26			60	8.3
27			20	14.5
28			57	
29			80	
30			110	
31			360	9.5

[a] Mixed, α, β mixture.

22-1) on the metabolism of [^{3}H]PGE_1 in the soluble fraction of guinea pig lung homogenates was investigated. After incubation at 37° for 30 min, radioactive materials were extracted with ethyl ether and were analyzed by reverse-phase partition chromatography. In the absence of the prostaglandin analogue, PGE_1 was completely transformed into less polar metabolites, 13, 14-dihydro-PGE_1 and 13,14-dihydro-15-keto-PGE_1 (Fig. 2A), but in its presence, the main radioactive peak corresponded to reference PGE_1, indicating that this compound strongly inhibited PGE_1 metabolism in the crude tissue extract (Fig. 2B).

Decrease in norepinephrine-induced blood free fatty acid levels. The effect of 9,11-deoxy-PGE_1 (No. 1-1) was investigated in rats *in vivo* when this compound was infused intravenously together with PGE_1. The antilipolytic effect of PGE_1 on the mobilization of free fatty acids was studied in the anesthetized rats. Subcutaneous

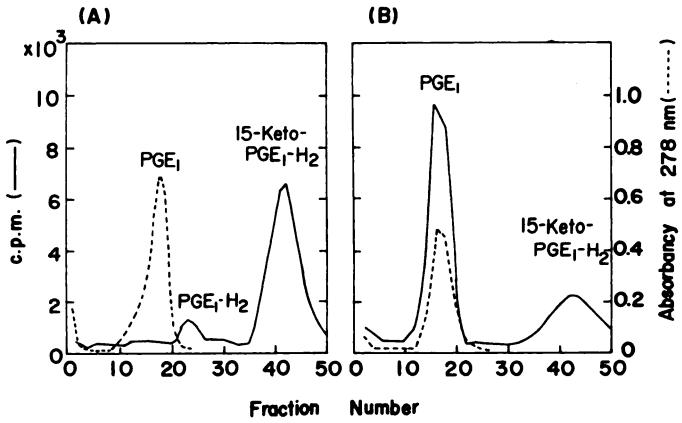


FIG. 2. *Reverse-phase partition chromatography of the products of [³H]PGE₁ after incubation with the soluble fraction of guinea pig lung homogenates in the presence and absence of 13,14-methylene-15-ketoprostanoate*

Tritium-labeled PGE_1 (14 nmoles) (specific activity, 0.14 μCi/nmole) was incubated with 2.0 ml of the 105,000 × *g* supernatant fraction of a 20% lung homogenate in the presence (B) and absence (A) of 400 nmoles of 13,14-methylene-15-ketoprostanoate. After incubation at 37° for 30 min, the reaction products were extracted with ethanol and ether and were analyzed by reverse-phase partition chromatography. The fraction volume was 8.5 ml. Aliquots (0.5 ml) of the fraction were assayed for radioactivity. Nonradioactive PGE_1 was added as a reference (λ_{max} = 278 nm) after the incubation (- - -). *PGE_1-H_2*, 13,14-dihydro-PGE_1; *15-keto-PGE_1-H_2*, 13,14-dihydro-15-keto-PGE_1.

injection of norepinephrine increased the blood free fatty acid level by about 2.5-fold 30 min after the injection, and this elevated level continued for 60 min (Fig. 3). When PGE_1 was infused in the rats for 40 min, the free

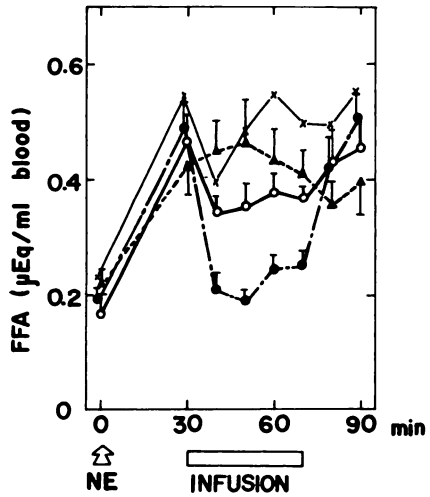


FIG. 3. *Reduction of blood free fatty acid levels in rats by infusions of PGE_1 and 9,11-deoxy-PGE_1*

Norepinephrine was injected s.c. at a dose of 0.75 mg/kg at time zero, and 30 min afterward PGE_1 and 9,11-deoxy-PGE_1 were infused into the femoral vein for 40 min at rates of 0.5 and 250 μg/kg/min. Blood samples were obtained from the cervical artery and analyzed for free fatty acids. ▲- - -▲, vehicle (physiological saline) at a rate of 0.02 ml/min (N = 5); ○——○, PGE_1 (N = 4); ●—·—●, PGE_1 plus 9,11-deoxy-PGE_1 (N = 6); and x——x 9,11-deoxy-PGE_1 (N = 3). The reductions in the free fatty acid level caused by PGE_1 plus 9,11-deoxy-PGE_1 were significantly greater than the decrease caused by PGE_1 alone, $p < 0.01$. N, number of animals. *NE*, norepinephrine. *FFA*, free fatty acids. Vertical bars indicate standard error of the mean.

fatty acid concentration decreased during the infusion period but never returned to the basal level observed before the norepinephrine injection. When PGE_1 was infused together with the prostaglandin analogue, the free fatty acid concentration decreased to the basal level and remained there throughout the infusion period. Infusions of the prostaglandin analogue alone did not show such a reduction of the free fatty acid level.

DISCUSSION

The present study has demonstrated potent PGDH inhibitors in 9,11-deoxyprostaglandins. Studies on structure-activity relationships revealed that the following structures were important in the inhibition activity: (a) prostanoic acid structure without a substituent on the cyclopentane ring; (b) presence of the hydroxy or keto group at carbon atom 15; and (c) presence of a 13,14-methylene group. Although prostanoic acid (No. 28) has been reported as an inhibitor of the enzyme (6, 7), its I_{50} was about 8–10 times higher than 15-hydroxy or 15-ketoprostanoate (Nos. 19 and 20). This result indicates that the 15-hydroxy or 15-keto group is important in the inhibition.

Addition of the 13,14-methylene group increased the inhibition activity. The configuration of the 13,14-methylene group was of importance in inhibition, since the two isomers of the methylene group differed in activity by 5 times. The compound with β-configuration of the methylene group, 13β,14β-methylene-15-ketoprostanoate (No. 22-3), was the most potent inhbitor tested. Its inhibition potency seems to be higher than those of reported inhibitors of the enzyme (18, 19). Raduchel *et al.* (20) and Schaaf (21) reported that 13α,14α-methylene-$PGF_{2\alpha}$ was not a substrate for PGDH, whereas 13β,14β-methylene-$PGF_{2\alpha}$ was substrate for the same enzyme. It is of interest that the configuration of the methylene group affected the substrate and inhibition activity toward PGDH.

Änggård and Sammuelsson (14) reported that PGE_1 was rapidly metabolized to 13,14-dihydro and 13,14-dihydro-15-keto metabolites by incubation with the soluble fraction of guinea pig lung homogenates. In the present study, when PGE_1 was incubated with the soluble fraction of homogenates in the presence of 13,14-methylene-15-ketoprostanoate, most PGE_1 was recovered unchanged from the incubation medium. This result indicates that the prostaglandin analogue inhibited PGDH in the crude lung preparation and that PGE_1 was metabolized exclusively through this enzyme reaction.

The effects of the prostaglandin analogues in rats *in vivo* were investigated with 9,11-deoxy-PGE_1 as a representative compound. Norepinephrine-induced free fatty acid levels in rat blood were markedly decreased by the intravenous infusions of PGE_1 with 9,11-deoxy-PGE_1. This result is comparable to the study by Bergström *et al.* (22), who showed that intra-arterial infusions of PGE_1 had a more pronounced effect than did intravenous infusions on the plasma free fatty acid levels in the norepinephrine-treated dogs. They explained their results by binding and/or inactivation of PGE_1 in the lungs.

It is reasonable to assume that the enhanced antilipolytic activity in the presence of the prostaglandin analogue

is ascribed to inhibition of PGDH *in vivo*, since the prostaglandin analogue is a potent inhbitor of PGDH *in vitro*. Although high concentrations of the analogue were required (ratio of PGE_1 to the analogue, 1:500), this may be attributed to the abundant activity of PGDH in lung tissue (1). However, it is possible that rate of metabolism has been modified by other factors, for example, inhibition of transport of PGE_1 into the lung (23) or changes in excretion or metabolism other than PGDH. Further experiments to elucidate these matters are needed.

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