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MODULATION OF PROSTACYCLIN SYNTHETASE AND UNMASKING OF PGE_2 ISOMERASE IN BOVINE CORONARY ARTERIAL MICROSOMES

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Prostacyclin (PGI_2), a major product of prostaglandin endoperoxide (PGH_2) metabolism in blood vessels, has potent vasodilator and platelet activity. Therefore, modulation of PGI_2 synthetase activity is of prime physiological importance in the regulation of blood vessel function. In this study, PGI_2 synthetase activity of bovine coronary arterial microsomes could be altered over a 2-3 fold range by GSH or dithiothreitol in a concentration-dependent manner and over a microsomal protein range of 10-200 µg. Modulation of coronary artery PGI_2 synthetase activity was also seen in vessels from sheep, dog and man. These data suggest that coronary artery PGI_2 synthetase activity is unusually sensitive to the redox state or sulfhydryl oxidation of the enzyme. The present data also unmask an active PGE_2 isomerase, previously reported to be absent in bovine coronary arterial microsomes.

Prostaglandin E_2 (PGE_2) isomerase and prostacyclin (PGI_2) synthetase are the major endoperoxide (PGH_2)-metabolizing enzymes present in microsomes isolated from vascular tissue. PGE_2 isomerase activity specifically requires the addition of reduced glutathione (GSH) (1). The effect of GSH on PGI_2 synthetase is minimal with any alterations being a decrease in PGI_2 formation most likely due to a shunting of available substrate to the GSH activated PGE_2 isomerase. However, thiol antioxidants other than GSH and nonthiol antioxidants have been reported to increase the formation of 6-keto-$PGF_1\alpha$, the stable breakdown product of PGI_2, in microsomes isolated from bovine coronary microvessels (2) and rat aorta (3). We report here the concentration-dependent modulation of the formation of 6-keto-$PGF_1\alpha$ by GSH and dithiothreitol (DTT) and unmasking of PGE_2 isomerase in microsomes isolated from bovine coronary artery.

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Abbreviations: PG, prostaglandin; PGI_2, prostacyclin; TLC, thin-layer chromatography, DTT, dithiothreitol.

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MATERIALS AND METHODS

Preparation of Coronary Arterial Microsomes. Sheep and dog hearts were obtained from anesthetized animals, bovine hearts were obtained from a local slaughterhouse, the human heart was obtained from a seventeen-year-old male at the time of harvesting of the kidneys for renal transplantation. All hearts were immediately placed in ice-cold 0.1 M phosphate buffer, ph 7.4 for transport to the laboratory. The left anterior descending and left circumflex coronary arteries were dissected free, cleaned, pooled, minced and homogenized in three volumes of the ice-cold phosphate buffer using a Polytron homogenizer. The homogenate was centrifuged at 10,000 x g for 15 min and the supernatant was strained through cheesecloth and centrifuged at 105,000 x g for 60 min. All procedures were carried out at 0`-4`C. The microsomal pellet obtained was suspended in phosphate buffer and stored at -55`C. Protein assayed by the method of Lowry, et al. (4).

Assay of Microsomal Metabolites of PGH_2. The incubation mixture contained coronary arterial microsomes in 100 1 of 0.1 M potassium phosphate buffer, pH 7.4, any additions, e.g. GSH and [1-^{14}C]PGH_2 (15,000 cpm). The reaction was initiated by the addition of the microsomal fraction to a 0`C tube containing $PGH_2$ (previously blown dry under a $N_2$ stream), vortexed and incubated at 37`C for 2 min. The reaction was stopped, the products extracted by adding 400 1 of ethyl acetate:methanol:0.2 M citric acid, pH 2.0 (15:2:1), vortexed and centrifuged The upper organic layer was spotted for TLC on Analtech silica gel GHL plates along with authentic prostaglandin standards (Upjohn Co.) and developed using the solvent system ethyl acetate:acetic acid:hexane:water (54:12:25:60, organic phase). The migration of authentic prostaglandin standards was located by exposing the plates to iodine vapor and that of radiolabelled products by radiochromatogram scan. [1-^{14}C] PGH_2 preparation from (1-^{14}C) arachidonic acid (50-60mCi/mmol, Amersharm) and all other procedures were as previously described in detail (5-8).

RESULTS

The data in Table 1 show an increase in the formation of 6-keto-$PGF_1\alpha$ by bovine coronary arterial microsomes in response to increasing concentrations

Table 1
Effect of GSH Concentration on Activities of Bovine Coronary Arterial Prostacyclin Synthetase and PGE_2 Isomerase

	Prostacyclin Synthetase		PGE_2 Isomerase	
GSH	PGH_2 concentration		PGH_2 concentration	
(mM)	2.5 µM*	10 µM**	2.5 µM*	10 µM**
--	47 ± 8	129 ± 12	56 ± 7	246 ± 9
0.1	58 ± 3	127 ± 16	55 ± 2	254 ± 24
0.5	56 ± 4	191 ± 30	73 ± 5	329 ± 34
1	65 ± 8	167 ± 19	74 ± 6	315 ± 11
1.25	--	268 ± 65	--	308 ± 45
1.5	80 ± 9	--	62 ± 3	--
1.75	--	215 ± 41	--	354 ± 68
2	97 ± 10	315 ± 37	61 ± 6	234 ± 15
5	103 ± 7	300 ± 51	47 ± 3	237 ± 19

Microsomal protein = 50 µg; incubation volume = 0.1 ml.
Data are picomoles of 6-keto-$PGF_1\alpha$ or PGE_2/2 min/50 µg protein expressed as mean ± S.E.M. of duplicate incubations of separate microsomal fractions isolated from 4* or 3** hearts.

Table 2
Effect of Microsomal Protein Concentration on Bovine Coronary Arterial Prostacyclin Synthetase Activity

	Prostacyclin Synthetase			
	PGH_2 concentration			
protein (μg)	2.5 μM		10 μM	
	-GSH	+GSH	-GSH	+GSH
10	3 ± 1	8 ± 3	30 ± 7	23 ± 5
25	10 ± 4	20 ± 10	38 ± 6	102 ± 36
50	27 ± 7	88 ± 15	141 ± 30	224 ± 39
100	96 ± 8	133 ± 9	289 ± 81	380 ± 71
200	138 ± 16	148 ± 15	352 ± 92	608 ± 40

GSH = 2 mM; incubation volume = 0.1 ml.
Data are picomoles of 6-keto-$PGF_{1\alpha}$/2 min and are expressed as mean ± S.E.M. of duplicate incubations of separate microsomal fractions isolated from four hearts.

of GSH at two concentrations of PGH_2. The formation of PGE_2 in the presence of GSH (at 10 μM PGH_2) showed an initial increase which fell at higher GSH concentrations (Table 1). The activity of PGI_2 synthetase in the absence of GSH is similar to that previously reported for bovine coronary arterial microsomes (2). The increase in 6-keto-$PGF_{1\alpha}$ formation, which occurs over a narrow GSH amount range, is dependent on the concentration of microsomal protein (Table 2). Thromboxane B_2 formation was not observed indicating the absence of detectable thromboxane synthetase activity. Tranylcypromine (10mM), an inhibitor of PGI_2 synthetase (6), inhibited the formation of 6-keto-PGF_1 by 90%, both in the absence and presence of 2mM GSH (data not shown). The augmentation of PGI_2 synthetase activity by GSH was also observed in coronary arterial microsomes prepared from sheep, dog, and man (Table 3). The formation of PGE_2 was greater in the presence of GSH (at 10 μM PGH_2) for sheep and dog suggesting the presence of an active PGE_2 isomerase (Table 3).

The activity of PGI_2 synthetase was also modulated in a concentration related manner by dithiothreitol (DTT) and PGE_2 formation decreased as DTT concentration increased (Table 4).

DISCUSSION

These data indicate that coronary arterial microsomal PGI_2 synthetase activity declines with decreases in the concentration of the sulfydryl reducing reagents (antioxidants) GSH and DTT. The concentrations of GSH

Table 3

Microsomal Prostacyclin Synthetase and PGE_2 Isomerase Activities from Coronary Arteries of Sheep, Dog and Human

		Prostacyclin Synthetase		PGE_2 Isomerase	
		PGH_2 concentration		PGH_2 concentration	
Animal	Additions	2.5 μM	10 μM	2.5 μM	10 μM
Sheep*	No additions	24 ± 1	100 ± 2	85 ± 1	343 ± 2
	2 mM GSH	76 ± 3	158 ± 1	66 ± 3	414 ± 3
Dog**	No additions	30 ± 4	54 ± 1	56 ± 10	282 ± 34
	2 mM GSH	88 ± 8	114 ± 23	84 ± 8	408 ± 40
Human**	No additions	23	--	72	--
	2 mM GSH	70 ± 7	--	64 ± 1	--

Incubation volume = 0.1 ml

Data are picomoles of 6-keto-$PGF_1\alpha$ or PGE_2/2 min/50 μg protein and are expressed as mean ± S.E.M. of duplicate incubations of separate microsomal fractions isolated from 3 hearts,* 1 heart.**

employed are consistent with intracellular GSH concentration (9) and the modulation of 6-keto-$PGF_1\alpha$ formation occurs over a narrow GSH or DTT concentration range. These data suggest the possiblity that small physiologic decreases in intracellular GSH concentration may result in a change in redox state or sulfhydryl oxidation of PGI_2 synthetase and decrease the formation of PGI_2. A fall in coronary artery PGI_2 generation could affect vascular tone and vessel-platelet interaction which could lead to thromboembolic problems in large vessels or downstream in microvessels. The recent report (3) that the

Table 4

Effect of DTT Concentration on Activities of Bovine Coronary Arterial Prostacyclin Synthetase and PGE_2 Isomerase

	Prostacyclin Synthetase		PGE_2 Isomerase	
DTT	PGH_2 concentration		PGH_2 concentration	
(mM)	2.5 μM*	10 μM**	2.5 μM*	10 μM**
--	43 ± 11	74 ± 24	58 ± 5	252 ± 10
0.1	83 ± 13	80 ± 15	41 ± 4	236 ± 28
0.5	100 ± 18	130 ± 16	36 ± 7	219 ± 18
1	108 ± 25	135 ± 14	36 ± 9	226 ± 18
1.25	113 ± 8	263 ± 78	27 ± 3	166 ± 37
1.5	124 ± 8	--	30 ± 5	--
1.75	--	286 ± 23	--	192 ± 23
2	117 ± 13	301 ± 64	34 ± 6	162 ± 31
5	135 ± 9	--	30 ± 10	--

Microsomal protein = 50 μg; incubation volume = 0.1 ml.; DTT = dithiothreitol.

Data are picomoles of 6-keto-$PGF_1\alpha$ or PGE_2/2 min/50 μg protein expressed as mean ± S.E.M. of duplicate incubations of separate microsomal fractions isolated from 3** hearts.

antioxidant ascorbate increases the release of 6-keto-$PGF_{1\alpha}$ from aortic rings substantiates our findings and raises the possibility that prostacyclin synthetase activity in other vascular tissue may be modulated in a similar manner to that reported here. However, we have not observed this effect of GSH on microsomal PGI_2 synthetase of human saphenous vein (8), human (10) or bovine (unpublished results) intrapulmonary artery and vein.

Decreases in GSH concentration (antioxidant buffering capacity) may occur in response to the transformation of molecular oxygen into highly reactive superioxide anion, hyrodogen peroxide or hydroxyl radical. Formation of these reactive oxygen species could occur in polymorphonuclear leukocyte (PMN) infiltration during acute myocardial infarction (11). Additionally a reduction in PGI_2 formation could mediate or potentiate PMN infiltration as PGI_2 has been associated with inhibition of neutrophil chemotaxis, aggregation, lysosomal enzyme release, and superoxide anion production (12,13). These observations may partially explain the effect of PGI_2 infusion on reducing the area of infarction (13). PGI_2 formation by aorta of rats fed a diet deficient in the antioxidant Vitamin E has been correlated with an increase in aorta peroxide levels (14). Whether this is related to a decrease in PGI_2 synthetase activity subsequent to a decrease in the antioxidant buffering capacity due to Vitamin E deficiency or to direct inhibition of this enzyme by peroxides or both requires further investigation. Similar decreases in PGI_2 formation by atherosclerotic coronary arterial tissue have been correlated with production of lipid peroxides (12). Additionally, nitroglycerin is known to decrease GSH concentrations in various tissues including blood vessels (15). If coronary arterial vasodilatation in response to nitroglycerin is mediated at least in part by PGI_2 (16, 17), tolerance to the vasodilator effect of nitroglycerin might be attributed in part to a parallel fall in PGI_2 formation and GSH concentration.

Prostaglandin E_2 isomerase is a GSH requiring enzyme (1). The demonstration of the presence of an active PGE_2 isomerase is complicated as in the absence of GSH, PGH_2 spontaneously breaks down to PGE_2. Thus, in the

presence of GSH, PGE_2 formation is both enzymatic and nonenzymatic and the nonenzymatic component cannot be distinguished from the enzymatic component. The presence of an active PGE_2 isomerase is suggested by an increased PGE_2 formation in the presence of GSH. However, as the PGH_2 concentration is metabolically depleted in the presence of GSH, nonenzymatic conversion of PGH_2 to PGE_2 becomes less significant and it could be misleading to simply subtract the quantity of PGE_2 formed in the absence of GSH from that formed in the presence of GSH to obtain "net PGE_2" formation; such a figure is, however, useful and is employed (2) as an index of PGE_2 isomerase activity.

Demonstration of an active PGE_2 isomerase in the coronary arterial microsomes is complicated by the fact that in the presence of GSH the enzymatic formation of both PGI_2 and PGE_2 would be augmented and the non-enzymatic component of total PGE_2 formation would decrease due to a shunting of PGH_2 to PGI_2 formation. This is indicated by the fall in PGE_2 formation seen in the presence of DTT which can not serve as a co-factor for PGE_2 isomerase activity (1) but can modulate PGI_2 formation. Thus "net PGE_2" should not be employed as an index of PGE_2 isomerase activity for coronary arterial microsomes. However, as both GSH and DTT similarly affect PGI_2 formation the amount of PGE_2 formed in the presence of DTT (non-enzymatic formation) is more representative of non-enzymatic PGE_2 formation than is that formed in the absence of GSH. Thus the amount of PGE_2 formed in the presence of DTT (non-enzymatic) as presented on Table 4 should be subtracted from the amount of PGE_2 formed in the presence of GSH (enzymatic + non-enzymatic) as presented in Table 1. This difference more closely approximates PGE_2 isomerase activity. The data obtained in this study when so interpreted unmask the presence of an active PGE_2 isomerase in coronary arterial microsomes. This enzyme has been previously reported to be absent in the bovine coronary artery (2).

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