Is the variation in the susceptibility of various species to atherosclerosis due to inborn differences in prostacyclin (PGI_2) formation?[1]

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Summary. Species exhibiting a higher susceptibility to the development of atherosclerosis have a reduced prostacyclin (PGI_2)-generation in the arterial wall, which differs in various parts of the vascular system. As the difference in PGI_2-formation in various diseases is a generalized vascular effect, the changes can be detected in all vessels. This is a very important point for diagnostic purposes in humans.

Prostacyclin (PGI_2) has been considered to be one of the key substances in early thrombosis and atherosclerosis. Apart from its antithrombotic activity[2], different reports about PGI_2-generation in atherosclerotic tissue have been published[3,4]. After the original discovery of PGI_2-formation in the rabbit aorta[2,5], it has been demonstrated that different arteries (and veins) of various other species generate PGI_2 too (an overview of the results reported in the literature is given in table 1).

Quantitative differences have been demonstrated in various species with respect to platelet aggregation after the addition of PGI_2[6]. The well-known species differences in susceptibility to atherosclerotic involvement[7] focused our attention on the question of whether there is a different rate of PGI_2-generation in the arteries of different species and whether this is related to the differences in proneness to develop the disease.

Material and methods. Vascular tissue (arteries and veins) from male and female humans, mini-pigs, sheep, rabbits, rats, mice and Syric golden hamsters of different ages was examined. The material was taken from the aorta (abdominal and thoracic), pulmonary artery and inferior cavic vein. Tissue with a wet weight of 5-55 mg was ivestigated for PGI_2-generation by its ability to inhibit the ADP-induced platelet aggregation. The bioassay procedure was performed as reported earlier[8] after the method originally described by Moncada et al.[2]. The inhibitory activity was expressed in pg PGI_2/mg tissue wet weight/min using a synthetic PGI_2-standard (kindly supplied by Dr John E. Pike, The Upjohn Company, Kalamazoo, Michigan, USA). The vascular tissue was morphologically examined by means of light (and in part scanning) microscopy.

Results. The prostacyclin generation in rodents' arteries is much more pronounced than in other species (table 2). The highest activity can be detected in the rat artery with high variations between different strains (in the rat for example from 45-180 pg/mg/min), followed by the rabbit and the other rodents, whereas the arteries of humans and swine produce statistically significant ($p < 0.01$) lower amounts of PGI_2.

There is no significant difference between the arterial and venous PGI_2-synthesizing activity in the species studied, except for the rat (table 3) where there is a statistically significant difference ($p < 0.0001$).

In various parts of the vascular system the PGI_2-formation is somewhat different (table 4). In mini pigs, e.g., the

Table 1. Overview of the literature reports of PGI_2-generation

Species	Vessels Artery (A), Vein (V)		Publications
Human	A	Aorta	Sinzinger et al.[18]
		Iliac	Sinzinger et al.[19]
		Coronary	Dusting et al.[20]
		Colic	Moncada et al.[21]
		Femoral	Silberbauer et al.[8]
		Gastric	Moncada et al.[21]
		Temporal	Sinzinger et al.[19]
	V	Intestinal	Moncada et al.[21]
		Forearm	Remuzzi et al.[15,21,22]
Pig/Minipig	A	Aorta	Moncada et al.[2]
	V	Pulmonary artery	Sinzinger et al.[24]
Rabbit	A	Aorta	Bunting et al.[25], Gryglewski et al.[5] Herman et al.[26], Hornstra et al.[27], Moncada et al.[2]
		Coeliac trunk	Bunting et al.[25]
		Mesenteric	Bunting et al.[25]
	V	Vena cava	Sinzinger et al.[19]
Rat	A	Aorta	Villa et al.[28], Hornstra et al.[27], Harrison et al.[29], Pace-Asciak et al.[30-32] Skidgel et al.[33]
		Tail, common iliac, renal, femoral, common carotid	Skidgel et al.[33]
	V	Femoral, renal, tail, external jugular	Skidgel et al.[33]
		Vena cava	Skidgel et al.[33], Sinzinger et al.[19]

Table 2. Abdominal aorta PGI_2-formation in different species

Species	Artery (abdominal aorta)		Number
	pg PGI_2/mg/min (±SEM)	Mean tissue wet weight (mg)	
Man	10±2	20	20
Rabbit	21±4	17	10
Rat	59±5	14	30
Mini pig	3±1	27	4
Syric golden hamster	9±4	18	7
Sheep	17±3	25	6
Mouse	38±4	4	4

Table 3. Arterial and venous PGI_2-formation in different species

Species	Artery (abdominal aorta)		Vein (inferior vena cava)	
	pg PGI_2/mg/min	Mean wet weight (mg)	pgPGI_2/mg/min	Mean wet weight (mg)
Man	10±2	20±3	8±3	22±4
Syric golden hamster	9±4	18±4	10±5	19±2
Rabbit	21±4	17±2	20±5	20±3
Rat	59±5	14±1	9±3	15±3

All values ± SEM.

Table 4. PGI_2-formation in the mini pig

Tissue		pg PGI_2/mg/min (±SEM)	Mean wet weight (mg)
♂	Thoracic aorta	4.88±1.94	52
	Abdominal aorta	2.64±1.12	44
	Pulmonary artery	6.25±0.84	47
♀	Thoracic aorta	7.65±2.19	43
	Abdominal aorta	5.55±1.90	51
	Pulmonary artery	6.49±0.77	48

n=4

Table 5. PGI_2-formation in rat abdominal aorta

pg PGI_2/mg/min (±SEM)	Animal weight (g)	n
111±39	150	10
147±41	260	10
174±63	330	10

highest values are obtained for the pulmonary artery, followed by the thoracic and abdominal aorta. Similar data can be obtained in all species.

In some species, there exist marked species differences in PGI_2-formation (e.g. in the mini pig, table 4). In most, the values for prostacyclin synthesis are higher in females than males. In addition, the prostacyclin formation is age-dependent, as demonstrated in rat aorta (table 5).

The morphological examination of the vascular tissue samples revealed that in none of the animals was there a detectable alteration such as atherosclerosis, fibrosis or other degenerative lesion.

To ascertain that the substance bioassayed was PGI_2, well-known properties, such as short half-life, heat- and pH-instability, as well as those summarized by other authors[8–10], were tested.

Discussion. Until now no general information has existed about normal PGI_2-synthesis in arteries and veins at different locations in various species. Only in the rat have these observations been reported[11]. Moncada et al.[12] found such a species difference for the relative potency of PGI_2 in inhibiting ADP-induced platelet aggregation in several species. Our finding, that species which are more susceptible to atherosclerois (human, pig) produce smaller amounts of PGI_2/mg tissue wet weight/min suggests that the higher parietal thrombotic risk[5] could enhance atherosclerosis. This would confirm data which showed a diminished activity in experimental[3,13], and human[14], atherosclerosis. Similar data were obtained from the arteries of stroke-resistant rats, which produced more prostacyclin than the arteries of stroke-prone spontaneously hypertensive rats[15]. In various species the lower PGI_2-activity in the abdominal aorta, compared with the thoracic part, could be interpreted in the same sense as well as the sex difference. The differences in wall composition and thickness do not seem to influence the data from various locations of the vascular tree (unpublished data).

It is very important to mention that an altered PGI_2-formation is detectable in all parts of the vascular system. That means that a different activity in the human forearm vein represents an altered prostacyclin formation in the vascular tree. This phenomenon has been shown to be a very useful parameter for detection of alterations in humans[16,17]. In general, the results demonstrate that the proneness of certain species to develop atherosclerosis is at least in part due to their arterial prostacyclin generation. The knowledge of location differences in the synthesis of PGI_2 might be of future diagnostic value, especially in human vascular tissue.

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2 S. Moncada, R.J. Gryglewski, S. Bunting and J.R. Vane, Prostaglandins *12*, 715 (1976).
3 A. Dembinska-Kiec, T. Gryglewska, A. Zmuda and R.J. Gryglewski, Prostaglandins *14*, 1025 (1977).
4 A. Zmuda, A. Dembinska-Kiec, A. Chytkowski and R.J. Gryglewski, Prostaglandins *14*, 1035 (1977).
5 R.J. Gryglewski, S. Bunting, S. Moncada, R.J. Flower and J.R. Vane, Prostaglandins *12*, 685 (1976).

6 S. Moncada, J.R. Vane and B.J.R. Whittle, J. Physiol. *273*, 2 (1977).
7 T. Roberts and H. Strauss, Comparative Atherosclerosis, 1972.
8 K. Silberbauer, H. Sinzinger and M. Winter, Lancet *1*, 1356 (1978).
9 S. Villa, A. Callioni and G. de Gaetano, Thromb. Res. *11*, 701 (1977).
10 S. Moncada, E.A. Higgs and J.R. Vane, Lancet *1*, 18 (1977).
11 R.A. Skidgel and M.F. Printz, Prostaglandins *16*, 1 (1978).
12 S. Moncada, A.G. Herman, E.A. Higgs and J.R. Vane, Thromb. Res. *12*, 367 (1977).
13 A. Szczeklik, Int. Atherosclerosis Conf., Milan 1977.
14 H. Sinzinger, K. Silberbauer and M. Winter, VASA *7*, 350 (1978).
15 M. Okuma, Y. Yamori, K. Ohta and H. Uchino, Prostaglandins *17*, 1 (1979).
16 G. Remuzzi, A.E. Cavenaghi, G. Mecca, M.B. Donati and G. de Gaetano, Lancet *2*, 1195 (1977).
17 G. Schernthaner, K. Silberbauer, H. Sinzinger, H. Piza-Katzer and M. Winter, New Engl. J. Med. *300*, 366 (1979).
18 H. Sinzinger, K. Silberbauer, M. Winter and W. Auerswald, Lancet *2*, 840 (1978).
19 H. Sinzinger, K. Silberbauer, O. Wagner, M. Winter and W. Auerswald, Atherogenese *3*, 123 (1978).
20 G.J. Dusting, S. Moncada and J.R. Vane, Prostaglandins *13*, 3 (1977).
21 S. Moncada and J.R. Vane, in: Biochemical Aspects of Prostaglandins and Thromboxanes, p. 155. Ed. Paoletti. Academic Press, New York 1977.
22 G. Remuzzi, R. Misiani, D. Marchesi, M. Livio, G. Mecca, G. de Gaetano and M.B. Donati, Lancet *1*, 871 (1978).
23 G. Remuzzi, M. Livio, R. Misiani, G. Mecca, G. de Gaetano and M.B. Donati, Atherogenese *4*, 9 (1979).
24 H. Sinzinger, P. Clopath, K. Silberbauer and M. Winter, Artery *5*, 448 (1979).
25 S. Bunting, R.J. Gryglewski, S. Moncada and J.R. Vane, Prostaglandins *12*, 897 (1976).
26 A.G. Herman, S. Moncada and J.R. Vane, Arch. int. Pharm. Ther. *227*, 162 (1977).
27 G. Hornstra, E. Haddeman and J.A. Don, Thromb. Res. *12*, 367 (1978).
28 S. Villa and G. de Gaetano, Prostaglandins *14*, 1117 (1977).
29 H.E. Harrison, A.H. Reece and M.J. Johnson, Life Sci. *23*, 351 (1978).
30 C.R. Pace-Asciak and M.C. Carrara, Prostaglandins *15*, 704 (1978).
31 C.R. Pace-Asciak, M.C. Carrara, G. Rangaraj and K.C. Nicolaou, Prostaglandins *15*, 1005 (1978).
32 C.R. Pace-Asciak, M.C. Carrara and K.C. Nicolaou, Prostaglandins *15*, 999 (1978).
33 R.A. Skidgel and M.F. Printz, Prostaglandins *16*, 1 (1978).

Esterase activity in the hepatopancreas of *Macrobrachium lamarrei* (Crustacea: Decapoda)[1]

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Summary. The activity of the hepatopancreatic esterase of the fresh water prawn *Macrobrachium lamarrei* was optimal at pH 7.4 and temperature 40 °C. The activity increased with the increase in incubation period and enzyme concentration. The Michaelis constant (K_m) of the enzyme was 2.1×10^{-3} M.

Very little work has been done on the properties of the digestive esterases of crustaceans, although almost every crustacean investigated so far has been shown to possess these enzymes[4]. As strong esterolytic activity was recorded in the hepatopancreatic extracts of *Macrobrachium lamarrei*[5], a study of the properties of the enzyme concerned (arylesterase; E.C. 3.2.1.2) was undertaken.

Material and methods. Animals were collected from the local river Gomati. The hepatopancreas was dissected out, weighed immediately and homogenized in cold distilled water using an all-glass homogenizer, and the homogenate was centrifuged at $3000 \times g$ for 15 min at 4 °C. The supernatant was used as the enzyme source. For enzyme assay the reaction mixture containing 0.2 ml of homogenate supernatant, 0.5 ml of buffer, 0.5 ml of substrate solution (β-naphthyl acetate, 5×10^{-4} M in distilled water) and 0.6 ml of distilled water was incubated at 37 °C (except for temperature experiments). After 30 min incubation, 0.5 ml of cold solution of fast blue B (0.45%) was added and the reaction was stopped by adding 0.5 ml of 40% trichloroactic acid. The esterase activity was estimated by the colorimetric method of Seligman et al.[6]. 1 unit of esterase activity was defined as the amount of enzyme liberating 1 µmole of β-naphthol at 37 °C in 1 h.

The enzyme activity was estimated at several pH-values ranging from 4.5 to 9.5 using different buffer systems (0.1 M sodium citrate-HCl buffer for pH 4.5–5.5; 0.1 M Sorensen's phosphate buffer for pH 5.5–7.0; 0.1 M veronal sodium-HCl buffer for pH 7.0–9.5). Effects of all other factors were studied at the pH at which optimal

pH and temperature optima of esterases from various crustaceans

Name of the animal	Substrate used	Optimum pH	Optimum temperature
Astacus fluviatilis[10]	Tributyrin	5.2–6.5	–
Thalamita crenata[11]	Tributyrin	6.97	–
Panulirus japonicus[9]	β-naphthyl acetate	6.9–8.0	30 °C
	β-naphthyl laurate	6.9–8.6	35 °C
	β-naphthyl stearate	5.3	35 °C
A. astacus and *Cambarus affinis*[12]	Phenyl acetate and Phenyl butyrate	8.5	–
Diogenes bicristimanus[8]	Amyl acetate and Olive oil	7.4	–
Streptocephalus dichotomus[13]	Milk fat	5.2–6.5	–
Paratelphusa masoniana[14]	Ethyl butyrate	6.2	–