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Contractile Activity and Prostacyclin Generation in Isolated Coronary Arteries from Diabetic Dogs

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Summary. The present study was aimed at determining the generation of 'prostacyclin (PGI_2)-like-material' in coronary arteries from normal and diabetic (pancreatectomized) dogs as well as the contractile responses to prostacyclin of preparations from normal, diabetic and insulin-treated diabetic animals. PGI_2 produced a dose-dependent relaxation of coronary arteries from normal dogs. In contrast, those from diabetic animals were not relaxed; indeed, at low concentrations PGI_2 failed to evoke any effect but at higher ones it induced a distinct contraction. In arteries from diabetic animals treated with insulin, PGI_2 induced a biphasic contractile effect, which lay between that of normal controls and untreated diabetics. In addition the basal generation of 'PGI_2-like-material' by coronary arteries was significantly higher in the diabetic (141 $\pm$0.2 pg/mg, mean $\pm$SEM) than in normal dogs (59 $\pm$0.2 pg/mg). The present experiments demonstrate that the generation of 'PGI_2-like-substance' is significantly increased in coronary arteries from diabetic dogs, but the same vessels are unable to respond to added authentic PGI_2 with relaxation; on the contrary they react with a distinct positive contractile response.

Key words: Isolated coronary arteries, diabetes, prostacyclin, PGI_2 generation, coronary constriction, coronary relaxation, pancreatectomized dogs.

Prostacyclin (PGI_2) is a labile prostaglandin which can be synthesized by several vascular tissues [1], including coronary vessels [2–4]. PGI_2 is a potent inhibitor of platelet aggregation [1, 5] and is also able to relax a variety of isolated arteries [2, 6–9].

Alterations in vascular function and increased platelet aggregation have been suggested as factors involved in the vasculopathy that accompanies diabetes [10, 11]. In addition it has been reported that vascular smooth muscle from diabetic animals shows changes in reactivity to vasoconstrictor agents [12].

In view of these findings it was decided to investigate the possible importance of PGI_2 in the coronar-arteries of diabetic dogs. Firstly the reactivity to PGI_2 of isolated normal, diabetic and insulin-treated diabetic dog coronary vessels was examined, and secondly the generation of 'PGI_2-like-material' in vitro by coronary arteries from diabetic and normal dogs was determined.

Subjects and Methods

Animals

Coronary arteries were obtained from dogs weighing between 9 and 20 kg. Three different groups of animals were used: a) normal (n = 6); b) totally pancreatectomized (n = 6) and c) totally pancreatectomized injected with insulin (n = 3). Experiments were performed 5–7 days after the operation and approximately 16 h following the last meal [13]. The pancreatectomized dogs were fed with raw beef heart ad libitum, and 70 g of raw pancreas daily [13]. Normal and insulin-treated dogs were fed with the same food. Pancreatectomized animals not treated with insulin developed hyperglycaemia and ketosis.

Some of the totally pancreatectomized dogs were given soluble insulin (E. Lilly) in doses of 0.5 to 1.0 unit $\cdot$ kg^{-1} $\cdot$ day^{-1} at 0800 h for 5 days, but he hormone was discontinued 2 h before sacrifice. Blood glucose was determined at the time of sacrifice (1000 h) by the Somogyi method. As previously reported mean blood glucose concentration ($\pm$ SEM) was normal in control dogs (5.7 $\pm$0.5 mmol/l, n = 6) and significantly elevated in pancreatectomized animals (18.4 $\pm$1.3 mmol/l, n = 6) [13]. Dogs were anaesthetised with pentobarbital (25–30 mg/kg, IV) and the beating heart was rapidly removed from the chest [14].

Coronary Artery Preparations

After excision of the heart, the aortic root and its attached left anterior descending and circumflex arteries were dissected en bloc, completely cleaned of surrounding (non-arterial) tissue and immediately immersed in Krebs-Ringer-bicarbonate (KRB) solution at pH 7.4, composed as reported elsewhere [15]. A coronary artery

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Table 1. Basal resting tension or initial pre-load of the isolated coronary arteries after equilibration for 90 min

Experimental animals	Basal resting tension (mg)
Normal dogs	627 ± 25 (n = 9)
Pancreatectomized dogs	598 ± 17 (n = 12)
Pancreatectomized dogs receiving insulin	619 ± 20 (n = 5)

Values are expressed as mean ± SEM. They represent the basal tensions recorded before the addition of drugs. n = number of strips tested

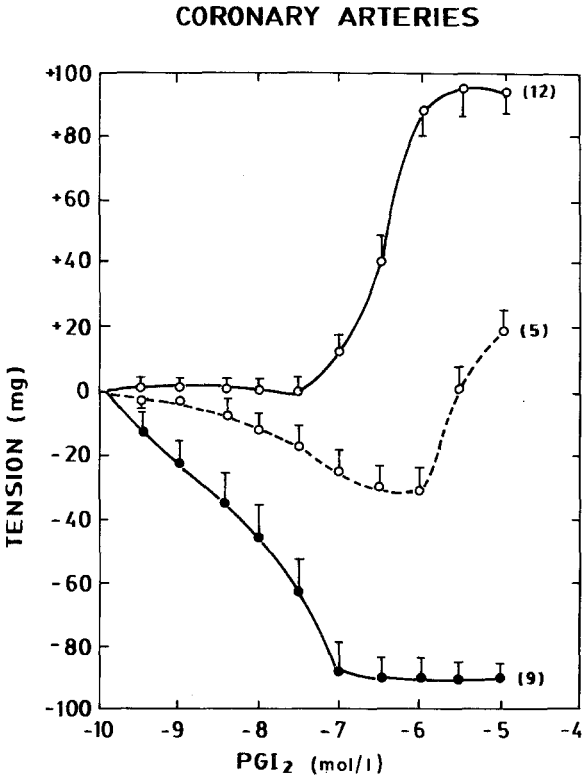

Fig. 1. Cumulative dose-response curves of PGI_2 on the tension developed by isolated coronary arterial strips from: ●—● normal dogs; ○—○ diabetic dogs untreated with insulin; and ○---○ diabetic dogs treated with insulin. Points and bars represent mean ± SEM, respectively. Figures in parentheses indicate the number of preparations

from each dog was cut in segments and one to four strips were used for the measurement of contractile activity and others to test their platelet anti-aggregatory capacity.

Tonic Changes of Isolated Coronary Arteries

Arterial strips 2 cm long, which ranged between 20 and 26 mg in blotted wet weight, were cut helically and mounted in a muscle chamber containing 20 ml KRB solution, gassed with 5% CO_2 in oxygen and kept at 37 °C and pH 7.4. The contractile activity was recorded as previously reported [14, 16]. Briefly one end of the strips was attached to a glass holder and the other was connected by means of a thread to a force transducer coupled to an oscillographic recorder via a carrier preamplifier.

Under an initial preload of 1 g the strips were allowed to equilibrate for 90 min. After this equilibration arteries underwent a stress relaxation and tension stabilized around 600 mg. At 90 min there were no significant differences in basal resting tone between the three groups (Table 1). The tonic changes of the vessels elicited by experimental additions were expressed as mg of tension above or below the basal resting tensions or initial preloads (0 mg in the figures).

Cumulative dose-response curves for PGI_2 were constructed for untreated control coronary arteries as well as for those previously exposed to haloperidol or phentolamine. A stock solution was made daily, using Tris buffer at pH 9.3. All PGI_2 solutions were kept at 0–4 °C and the drug was added directly to the muscle chamber every 5 min. Haloperidol 10^{-7} mol/l (Haldol, McNeil) or phentolamine 10^{-5} mol/l (Regitine, Ciba) were delivered into the tissue bath 30 min before PGI_2.

Determination of 'PGI$_2$-Like-Material'

Strips of coronary arteries (20–25 mg dry weight) were incubated for 20 min in 200 µl of KRB at room temperature and at pH 8.0 ± 0.1. The supernatant from each reaction mixture was removed and 50 µl of the incubate were placed in the cuvette of an aggregometer containing platelet-rich plasma from blood donors. The addition was made 45 s before adding adenosine diphosphate at 5 µmol/l as the aggregating agent. Light transmission through the sample was recorded as a measurement of aggregation. The inhibition of aggregation by the tissue supernatant was quantitated against the anti-aggregatory effect of standardized prostacyclin dissolved in Tris buffer 0.05 mol/l (pH 9.0) on the day of the experiment. The amount of 'PGI_2-like-material' generated by strips of coronary arteries was calculated and expressed as PGI_2-equivalence in pg/mg dry weight. When anti-aggregatory activity was detected in the samples they were retested after incubation for 30 min at 37 °C. All measurements of all the groups were made in the same platelet-rich plasma. Further details have been reported elsewhere [16, 17]. It must be stressed that despite the limitations of bioassay, it is extremely useful in many experimental conditions, particularly when the aim is to follow changes in the concentration of prostaglandins rather than measuring absolute levels.

Statistics

Results were compared using Student's 't' test. Means were considered significantly different when p = 0.05 or less. Results are presented as mean ± SEM.

Results

Effects of PGI_2 on the Basal Tone of Isolated Dog Coronary Arteries

Figure 1 shows the cumulative dose-response curves of PGI_2 versus the tension developed by isolated coronary arteries from normal, pancreatectomized and insulin-treated pancreatectomized dogs. It can be seen that PGI_2 produced a dose-dependent decrement of basal tone of isolated coronary arteries from normal dogs, whereas in coronary preparations from diabetic animals PGI_2 at low concentrations (from 10^{-9} up to 5×10^{-8} mol/l) had no effect and at higher ones (10^{-7} to 10^{-5} mol/l) evoked an enhancement

Table 2. Influence of phentolamine or haloperidol on the effect of PGI_2 on isolated coronary arteries from normal and diabetic dogs

	Normal dogs			Diabetic dogs		
	PGI_2 (mol/l)					
	10^{-8}	10^{-7}	10^{-6}	10^{-8}	10^{-7}	10^{-6}
No drug	−45 ± 12	−89 ± 8	−96 ± 6	0 ± 0	+14 ± 1	+87 ± 9
Phentolamine (10^{-5} mol/l)	−42 ± 10	−88 ± 9	−99 ± 7	0 ± 0	+12 ± 1	+92 ± 8
Haloperidol (10^{-7} mol/l)	−47 ± 14	−86 ± 11	−94 ± 5	0 ± 0	+15 ± 2	+95 ± 7

Values expressed as mg of tension above and below the basal resting tone. Results expressed as mean ± SEM ($n = 5$ in each group). Phentolamine and haloperidol did not modify the basal tone

of basal tone proportional to the dose. In preparations from insulin-treated diabetic dogs PGI_2 elicited a biphasic effect: relaxation at low concentrations and stimulation at higher ones. Both influences lay between those observed in untreated pancreatectomized and normal control animals. Figure 2 shows direct oscillographic tracings of the effects of PGI_2 on coronary arteries from normal and pancreatectomized diabetic dogs.

Preincubation for 30 min with phentolamine (10^{-5} mol/l) or haloperidol (10^{-7} mol/l) failed to block the stimulating effect of PGI_2 on isolated coronary arteries from diabetic dogs (Table 2).

Generation of 'PGI$_2$-Like-Material' by Isolated Dog Coronary Arteries

Table 3 shows the comparative production of 'PGI_2-like-material' by coronary arteries isolated from normal and diabetic dogs. The basal release of 'PGI_2-like-substance' by coronary strips from diabetic dogs was significantly greater than in preparations from normal dogs ($p = 0.001$).

Table 3. Release of 'PGI_2-like material' by coronary arteries isolated from normal and diabetic dogs

Condition	'PGI_2-like material'[a]
Normal	59 ± 0.2 ($n = 6$)
Diabetic	141 ± 2.5 ($n = 5$)

[a] Values expressed as pg/mg dry weight (mean ± SEM); n = number of strips tested.
'PGI_2-like material' was characterized by the anti-aggregatory capacity against ADP-induced aggregation of platelet rich plasma detected after 20 min at room temperature, and absence of anti-aggregation with samples incubated for 30 min at 37 °C

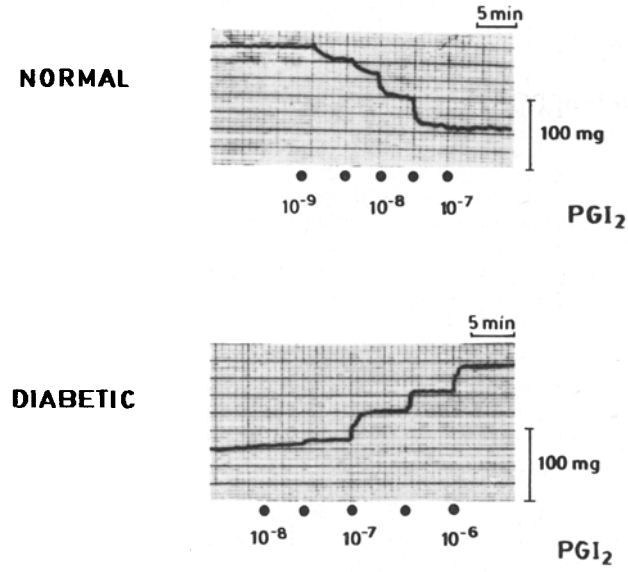

Fig. 2. Direct tracings showing the contractile response to PGI_2 (mol/l) of coronary artery strips isolated from a normal and a diabetic dog

Discussion

The present study shows that coronary arteries isolated from diabetic dogs exhibit a contractile response to PGI_2 different from that of preparations from normal animals. Furthermore, isolated coronary arteries from diabetic animals generate an increased amount of 'PGI_2-like-material' compared with controls.

Prostacyclin produced a dose-dependent relaxation of coronary arteries isolated from normal dogs, this being in keeping with reports of studies in vivo [18–20]. However, isolated coronary arteries from diabetic dogs were unable to respond with relaxation following PGI_2. Indeed, at low concentrations prostacyclin failed to evoke any effect, whereas at higher ones it induced a distinct contraction.

An alteration in the vascular contractile effects of noradrenaline and 5-hydroxytryptamine has recently been shown in diabetic rats. The effect of noradrenaline was greatly increased in diabetic aorta while the response to 5-hydroxytryptamine was significantly decreased [12]. In addition, it has been demonstrated that the stimulating action of PGI_2 may be linked to the release of noradrenaline and to an alpha adrenoceptive mechanism [21–23]. The fact that in isolated coronary arteries from the diabetic dogs the vasoconstrictor influence of PGI_2 was not blocked either by phentolamine or by haloperidol suggests that this ef-

fect does not result from an enhancement in prostacyclin-induced release of neurotransmitters.

In diabetic animals treated with insulin a concentration-dependent biphasic effect of PGI_2 (relaxation and contraction) was observed. However, both responses were attenuated and lay between those of normal controls and untreated diabetics.

To test whether diabetes has a direct effect on the release of prostacyclin, we have investigated the 'PGI_2-like-material' generated by isolated diabetic dog coronary arteries. A significantly higher production of 'PGI_2-like-substance' was observed in the diabetic condition than in controls. This is in agreement with findings in perfused hearts of acutely diabetic rats [24]. However, there are several reports describing decreased prostacyclin production in diabetes [25, 26]. Experimental animals lose considerable amounts of body fat during the first few days following induction of diabetes and it is possible that the availability of substrates for prostaglandin synthesis may therefore be increased during this period. This could possibly explain the differences in PGI_2 production between the different reports, since in both the present study and in the experiments already quoted [24] animals were used within a few days after the induction of diabetes.

In view of the aforementioned results the exact role of PGI_2 in coronary vascular complications of diabetes remains unclear.

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