Effects on prostaglandins $F_{2\alpha}$, I_2, and indomethacin on isolated coronary arteries from healthy and alloxan-diabetic dogs

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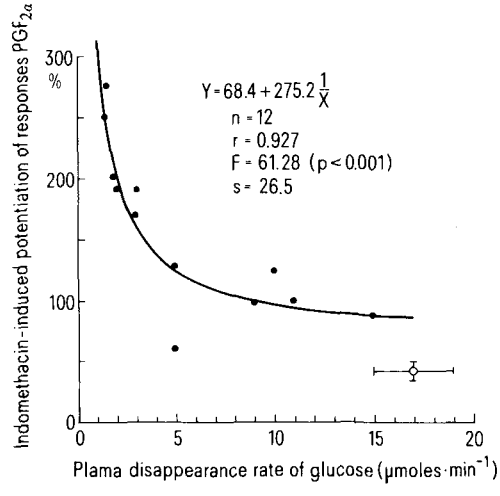
Summary. Contractile responses of isolated coronary arteries from healthy and alloxan-diabetic dogs to prostaglandin $F_{2\alpha}$ were enhanced by indomethacin and inhibited by prostaglandin I_2. The potentiation by indomethacin was more prominent in diabetic vessels than in normal arteries.

Patients with diabetes mellitus have an increased susceptibility to atherosclerosis and thrombotic complications[1,2]. In addition to accelerated atherosclerosis, evidence has been accumulated for the occurrence of cardiac disorders in diabetic patients with an intact vascular system[3,4]. It is well known that platelet aggregation and coronary vascular tone is influenced by prostaglandins, the latter in particular under hypoxic conditions[5-8]. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) enhances, whereas prostaglandin I_2 (PGI_2) reduces coronary muscle tone in many species including the dog[8-12]. In the hearts of diabetic rats prostaglandin release was found to be higher than in control animals, and the release of prostaglandins could been prevented by indomethacin[13,14]. It has also been documented that nmolar concentrations of $PGF_{2\alpha}$ increased the release of PGI_2 in perfused rat hearts[15]. The effects of $PGF_{2\alpha}$, PGI_2, and indomethacin were, therefore, investigated on the tone of isolated coronary arteries on healthy and alloxan-diabetic dogs.

Methods. Healthy mongrel dogs of both sexes, weighing 15-30 kg, 2-3 years of age, were selected for the study. The dogs had no clinical evidence of disease in 4 weeks of observation before the study began. All dogs received the same diet consisting of 15% fat, 25% protein and 60% carbohydrate. At the beginning of the experiment urine samples collected over 24 h were tested for glucose[16] and acetone[17]. Fasting venous blood samples were also taken for glucose[16] and urea nitrogen[18]. The plasma disappearance rate of glucose[19] was determined by i.v. challenge using 1 g/kg glucose in the unanesthetized state. After determination of the basal values 12 dogs were made diabetic without ketosis using alloxan tetrahydrate (Merck) 120 mg/kg i.v. 7 dogs served as controls. The in vitro investigation was performed 3 months after the induction of diabetes. Before exposure and dissection of the circumflex coronary artery under pentobarbital (Nembutal, Serva, 30 mg/kg) anesthesia, all chemical variables were redetermined. The circumflex coronary artery was freed from fat and myocardium, and cut helically into strips (40 × 2 mm). The tissue was suspended under 0.75 g initial tension in Krebs bicarbonate solution. The bathing fluid was aerated with 5% CO_2 in O_2, and maintained at 37 °C. Changes in tone were measured isometrically by means of a microdynamometer (Isometric transducer, Type DY-3, Ugo Basile). After 90 min of equilibration dose-response curves for $PGF_{2\alpha}$ (0.8-28 µmoles) were obtained before and after 20 min incubation with indomethacin (3 µmoles). To study the relaxant effect of PGI_2 the strips were exposed to 3 µmoles $PGF_{2\alpha}$ which produced a sustained concentration. When the tone reached a stable level, PGI_2 was added to the bath in increasing concentrations (0.03-0.48 µmoles). Dose-response curves for PGI_2 were constructed, and the concentration producing 50% relaxation was calculated.

PGI_2 (Chinoin) was dissolved in Tris buffer (pH 8.2), and diluted with Krebs bicarbonate solution. Stock solution of PGF_2 (Chinoin) was prepared using 95% ethanol, and diluted with saline. Indomethacin (Merck) was dissolved in



Correlation between plasma disappearance rate of glucose and indomethacin-induced percent potentiation of responses to $PGF_{2\alpha}$. The open circle indicates mean value of controls; bars relate to SEM. The solid circles indicate the individual values of alloxan-diabetic animals.

Metabolic state of dogs and the effect of prostaglandin $F_{2\alpha}$, I_2, and indomethacin on coronary arteries from healthy and alloxan-diabetic dogs

	Metabolic state of dogs				Contractile responses to 1 µmole prostaglandin $F_{2\alpha}$		Concentration of prostaglandin I_2 producing 50% relaxation
	Plasma disappearance rate of glucose (µmoles/min)	Plasma glucose (mmoles/l)	Glucose excretion (mmoles/day)	Body weight (kg)	No indomethacin (mg)	3 µmoles indomethacin (mg)	(nmoles/l)
Control (n = 7)	17 ± 2	5.3 ± 0.3	0 ± 0	19 ± 2	99 ± 23	166 ± 25[c]	65.4 ± 9.8
Before alloxan (n = 12)	18 ± 1	5.0 ± 0.2	0 ± 0	20 ± 1	-	-	-
After alloxan (n = 12)	7 ± 1[2a,2b]	13.2 ± 1.2[2a,2b]	133 ± 26[2a,2b]	15 ± 1[2a,2b]	75 ± 12	295 ± 44[a,c]	85.0 ± 7.0

Legend: Data are expressed as mean ± SEM. Significance, referred to control values, is indicated by: [a] $p < 0.05$; [2a] $p < 0.001$; significance, referred to values obtained before alloxan treatment, is indicated by: [b] $p < 0.05$; [2b] $p < 0.001$; significance, referred to values obtained before indomethacin treatment, is indicated by: [c] $p < 0.05$.

ethanol, and diluted with Krebs bicarbonate solution. The results were evaluated statistically using Student's paired and unpaired t-tests and regression analysis.

Results and discussion. The plasma disappearance rate of glucose decreased, and the fasting plasma glucose level rose considerably and lastingly under the influence of alloxan treatment. Urinary glucose excretion increased to a corresponding degree, but no acetone was excreted. Decrease of body weight by about 25% followed alloxan treatment, while blood urea nitrogen did not change significantly (table).

$PGF_{2\alpha}$ (1–30 µmoles) produced a similar, concentration-dependent increase in the tone of coronary strips both from healthy and alloxan-diabetic dogs. Indomethacin (3 µmoles) enhanced considerably the contractile response to $PGF_{2\alpha}$ in both groups of arteries, but the enhancing effect of indomethacin was significantly greater in alloxan-diabetic vessels than in normal ones (table). A close, inverse correlation was found between the plasma disappearance rate of glucose and the indomethacin-induced percent potentiation of response to $PGF_{2\alpha}$ (figure).

PGI_2 did not alter or only slightly diminished the basic tone of coronary strips, but when the arteries were precontracted by $PGF_{2\alpha}$, PGI_2 (0.03–0.43 µmoles) produced a similar and marked, concentration-dependent relaxation in arteries from healthy and alloxan-diabetic animals (table). To explain the enhancing effect of indomethacin on the contractile responses to $PGF_{2\alpha}$ it may be assumed that $PGF_{2\alpha}$ releases PGI_2 in coronary strips in the same way as in rat heart[15]. In this case, indomethacin would reduce PGI_2 release[13,14], and the diminished release of PGI_2 would counteract the contractile effect of $PGF_{2\alpha}$ to a lesser extent. It is kown that PGI_2 release[14] and coronary blood flow[13] are higher in diabetic hearts. Consequently, it may be supposed that in diabetic coronary arteries $PGF_{2\alpha}$ exerts a more prominent PGI_2 release, and after indomethacin treatment it exerts a more pronounced contractile effect. On the other hand, the relaxant effect of PGI_2 on strips from diabetic dogs was similar to that on strips from healthy animals when PGI_2 was added to the bath. This finding is evidence against an enhanced responsiveness of diabetic coronary arteries to PGI_2. To the extent that it is permissible to extrapolate from in vitro studies to the more complex situation in vivo, an unfavourable effect of indomethacin on the coronary circulation of diabetic animals would be predicted.

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Galactose and leucine transport in the developing rat small intestine

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Summary. Jejunal transport of galactose and leucine was studied in 9–45-day-old rats by means of the everted sac technique. Maximum transport was observed in 9–10-day-old rats, but then decreased until the 22nd day, and remained unchanged from then on. There were no remarkable differences in the pattern of galactose and leucine transport over this period.

Intestinal ability to transport non-electrolytes changes with age. In the rabbit[2], mouse[3] and human[4], the active transport systems develop before birth, In the chick, maximum transport capacity for galactose occurs soon after hatching[4] and in the guinea-pig maximum transport for α-methyl glucoside and many aminoacids is detected the first day after birth[5]. In rats, Younoszai and Linch[6] observed that the maximum absorption of glucose was at 21–23 days after birth. These experiments were done in vivo with no distinction between the active and diffusional components of sugar absorption.

The active transport of galactose and leucine in the jejunum of the rat at various age periods was studied. Our results indicate that the maximum transport of both substrates occurs before the time of weaning.

Methods. Male Wistar rats 9–42 days old were used. After weaning animals were fed a standard rat chow (U.A.R., A03) and water ad libitum. Rats were starved for 8 h (9–21-day-old, suckling animals) or 16 h (22–45-day-old) before the experiment. The study was done on everted sacs of mid jejunum, as described by Wilson and Wiseman[7]. The pieces of small intestine were removed under urethane anesthesia. Sacs were filled with Krebs-Henseleit bicarbonate buffer[8] and incubated in 10 ml of the same at 37 °C for 45 min. The mucosal solution was continuously gassed with 95% oxygen and 5% carbon dioxide. D-galactose (5 mM) and ^{14}C-