Involvement of thromboxane and leukotriene in arachidonate induced coronary constriction in diabetic rats

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Summary. Vascular responsiveness to sodium arachidonate was examined in isolated perfused hearts from rats with streptozotocin-induced diabetes. In diabetic rats arachidonate induced a biphasic coronary vascular response characterized by initial vasoconstriction followed by prolonged vasodilation. Non-diabetic rats showed only a vasodilator response. The vasoconstrictor phase found in diabetic rats was abolished by ONO-3708, a selective thromboxane A₂ antagonist. Indomethacin partly inhibited the vasoconstrictor response, the residual response being abolished by a leukotriene antagonist,

ONO-1078. The vasodilator response, however, was completely abolished by indomethacin in both diabetic and non-diabetic rats. Furthermore, the coronary constrictor response to leukotriene D₄ was enhanced in diabetic compared to non-diabetic rats. These results suggest an involvement of leukotriene in the vasoconstrictor response to arachidonate in diabetic rats, especially when cyclooxygenase is inhibited.

Key words: Leukotriene, thromboxane A₂, arachidonic acid, vasoconstriction, coronary artery, diabetic rats.

Arachidonic acid (AA) is metabolized by two enzyme systems (cyclooxygenase and lipoxygenases), forming prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs) and other compounds [1]. The LTs, LTC₄ and LTD₄, have been shown to induce profound coronary vasoconstriction [2, 3]. The release of an LT-like substance from porcine coronary arteries after challenge with the calcium ionophore A23187 has been reported [4]. Recently, Piomelli et al. [5] showed that canine and human coronary vessels metabolized exogenous AA to LTC₄ and LTD₄.

Coronary heart disease is the major cause of death among diabetic patients [6]. Metabolites of AA may be involved in the alteration of coronary vascular function in diabetes. Studies comparing diabetic and normal animals showed either decreased [7–9] or increased [7, 10, 11] PG production of AA metabolites in blood vessels of diabetic animals. Gimeno et al. [12] suggested an involvement of lipoxygenase products in AA-evoked constriction of coronary arteries from diabetic dogs.

We investigated the involvement of LTC₄ and LTD₄ in the altered vascular reactivity to AA in diabetic rats. We examined the effects of selective TXA₂ and LT antagonists on the vascular responses of isolated perfused hearts from rats with streptozotocin-induced diabetes.

Materials and methods

Experimental animals

Diabetes was induced in male Wistar rats (weight 230–250 g) by i.v. injection of streptozotocin (35 mg/kg dissolved in citrate buffer at pH 4.5). The animals were used 10 weeks later. Diabetic rats markedly lost body weight (190 ± 3.4 g, $n=32$) as compared to age-matched normal rats (301 ± 19 g, $n=6$). Therefore weight-matched (192 ± 4.3 g, $n=17$), non-diabetic rats, obtained by restricting food intake, were used as controls.

A further group of animals was obtained by treating 5 of the diabetic rats with insulin, 4 U regular Insulin Lente (Novo, Bagsvaerd, Denmark) administered s.c. daily for 3 weeks, starting 7 weeks after the streptozotocin injection.

Blood collected when the rats were killed, was used for glucose determination by the o-toluidin boric acid method using a glucose test kit (Wako, Tokyo, Japan). The blood glucose level was significantly elevated in the diabetic group (30.8 ± 0.6 mmol/l, $n=32$, $p<0.01$) as compared to the control group (9.9 ± 0.3 mmol/l, $n=17$). The diabetic, insulin-treated rats had also normal blood glucose levels (10.1 ± 0.2 mmol/l, $n=5$) and their body weights were also normal (303 ± 8.3 g, $n=5$).

Perfused heart preparation

Hearts were quickly excised under anaesthesia with sodium pentobarbital (30 mg/kg, i.v.). All hearts were perfused by the Langendorff technique. A constant aortic pressure of 80 mmHg was used for an

Fig. 1. Typical tracings of the responses to sodium arachidonate (NaA) of isolated perfused hearts from diabetic and control rats (A), effects of indomethacin (B) and BW755C (C). NaA (5 µg) was infused for 1 min. Indomethacin (5 µmol/l) and BW755C (10 µmol/l) were applied 15 min before NaA infusion. PP: perfusion pressure

Fig. 2. Effects of indomethacin on arachidonate-induced vasoconstriction (upper panel) and vasodilation (lower panel) in isolated perfused hearts from diabetic (●) and control (○) rats. Solid and dotted lines represent the response to arachidonate before and after treatment with indomethacin (5 µmol/l), respectively. Indomethacin was applied 15 min before arachidonate infusion. Values are shown as mean ± SEM ($n=7$)

Fig. 3. Effects of ONO-3708 on arachidonate-induced vasoconstriction in isolated perfused hearts from diabetic rats. (A) Typical tracings of the response to sodium arachidonate (NaA) (5 µg) before and after treatment with ONO-3708 (0.5 µmol/l). PP: perfusion pressure. (B) Dose-response curves for NaA-induced vasoconstriction in the absence (○) and presence (●) of indomethacin (5 µmol/l). Solid and dotted lines represent the response to NaA before and after treatment with ONO-3708 (0.5 µmol/l), respectively. ONO-3708 or/and indomethacin was administered 15 min before NaA infusion. Values represent mean ± SEM ($n=5$)

initial 10 min, then the perfusion was switched to a constant flow of 12 ml/min. The perfusate employed was Krebs-Ringer solution aerated with a mixture of 95% O₂ plus 5% CO₂ and maintained at 37°C (Taiyo, Tokyo, Japan). The Krebs-Ringer solution contained NaCl 120, KCl 4.7, CaCl₂ 2.0, KH₂PO₄ 1.2, NaHCO₃ 25.0, MgSO₄ 1.2 and glucose 14.0 (mmol/l). Coronary perfusion pressure was monitored from a side-arm in the aortic inflow tract, connected to a pressure transducer (Nihon-Kohden, MPU-0.5, AP621G, Tokyo, Japan) and recorded on a thermal array recorder (Nihon-Kohden, WS-681G, Tokyo, Japan).

Experimental protocol and drugs

After an equilibration period of 30 min, sodium arachidonate (NaA) was infused into the perfusate in a volume of 100 µl for 1 min. PGs and LT analogues were injected as boluses in a volume of 3–5 µl. Dose-response curves were performed non-cumulatively while ventricular fibrillation was induced and maintained by electrical stimulation. In order to assess antagonist and inhibitor effects, these drugs were added 15 min prior to the NaA injections and they were kept in the perfusate continuously throughout each experiment.

The following drugs were used: sodium arachidonate, indomethacin, leukotriene D₄, prostaglandin E₂, prostaglandin I₂ and streptozotocin (Sigma Chemical Co., St. Louis, Mo, USA); 9,11-epithio-11,12-methano-thromboxane A₂ (STA₂), ONO-1078 (4-oxo-8-(p-

(4-phenylbutyloxy)benzoylamino)-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate) and ONO-3708 ((9,11),(11,12)-dideoxy-9 α , 11 α -dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epi-thromboxane A₂) (Ono Pharmaceutical Co, Osaka, Japan). BW755C was synthesized in our laboratory.

Statistical analysis

Results are expressed as mean ± SEM. Statistical evaluation was performed by Student's *t*-test and differences were considered significant for $p < 0.05$.

Results

Differing effects of NaA on diabetic and control rats

Basal perfusion pressure of diabetic rat hearts (84.3 ± 1.9 mmHg, $n=32$) was not significantly different, from that of control rat hearts (80.2 ± 1.9 mmHg, $n=17$). Typical records of the responses of perfusion pressure to exogenous NaA are shown in Figure 1.

NaA induced a biphasic coronary response characterized by initial vasoconstriction followed by prolonged vasodilation in diabetic rat hearts. Hearts from control rats showed only vasodilation in response to NaA. The dilator response to NaA was comparable in both groups (Fig. 2). The cyclooxygenase inhibitor indomethacin (5 $\mu\text{mol/l}$), completely abolished the vasodilator response in both diabetic and control rats. However, the vasoconstrictor phase observed in diabetic rats was only partly blunted by indomethacin. BW755C (10 $\mu\text{mol/l}$) which inhibits both cyclooxygenase and lipoxygenase, completely abolished both phases of the vascular response to NaA observed in diabetic animals (Fig. 1).

Effects of TXA_2 and LT antagonists on the contractile response to NaA in diabetic rats

Diabetic, but not control, rats showed a dose-dependent coronary constriction induced by NaA (Fig. 3). The vasoconstrictor phase was abolished by ONO-3708 (0.5 $\mu\text{mol/l}$), a selective TXA_2 antagonist [13], while the vasodilator phase was not affected. The residual vasoconstriction produced by NaA in the presence of indomethacin (5 $\mu\text{mol/l}$) was, however, not inhibited by ONO-3708.

ONO-1078 (1 $\mu\text{mol/l}$), a selective antagonist of LTC_4 and LTD_4 [14], did not alter the vasoconstricting effect of NaA in the absence of indomethacin. However, the residual vasoconstriction produced by NaA in the presence of indomethacin was inhibited by ONO-1078 (Fig. 4).

Effects of LTD_4 , TXA_2 , PGI_2 and PGE_2

Dose-response curves to LTD_4 , STA_2 , a stable TXA_2 analogue [15], PGI_2 , and PGE_2 are shown in Figure 5. The vasoconstrictor response to LTD_4 was significantly increased in diabetic animals compared with that of the control animals ($p < 0.05$). There were no significant differences between diabetic and control rats in vascular reactivity to STA_2 , PGI_2 and PGE_2 , indicating that the increased responsiveness to LTD_4 was specific.

The responses to STA_2 and LTD_4 were selectively inhibited by ONO-3708 (0.5 $\mu\text{mol/l}$) and ONO-1078 (1 $\mu\text{mol/l}$), respectively, in the two groups (not shown).

Effect of insulin treatment

In insulin-treated diabetic rats the vasoconstrictor phase was not observed and as with the hearts of control rats, only vasodilation was seen (Fig. 6). Treatment with insulin thus reversed the altered response to NaA found in diabetic rats.

Fig. 4. Effects of ONO-1078 on arachidonate-induced vasoconstriction in the absence (○) and presence (●) of indomethacin (5 $\mu\text{mol/l}$) in isolated perfused hearts from diabetic rats. Solid and dotted lines represent the response to sodium arachidonate (NaA) before and after treatment with ONO-1078 (1 $\mu\text{mol/l}$), respectively. ONO-1078 was administered 15 min before NaA infusion. Values represent mean $\pm$ SEM ($n = 7$)

Fig. 5. Dose-response curves for (A) leukotriene D_4 , (B) stable thromboxane A_2 analogue (STA_2), (C) prostaglandin I_2 and (D) prostaglandin E_2 in isolated perfused hearts from diabetic (●) and control (○) rats. Values represent mean $\pm$ SEM ($n = 5$). Asterisks indicate significant difference from control rats ($p < 0.05$)

Discussion

It is well established that the administration of AA into the coronary circulation is followed by an increased formation of PGs [16]. PGI_2 has been found to be the major metabolite of AA in the heart [17]. However, several investigators have reported an altered metabolism of vascular AA in diabetes mellitus [7–11]. In the present study, exogenous NaA produced coronary dilatation in non-diabetic rats. In contrast, untreated diabetic

Fig. 6. Effect of insulin treatment on the altered response to arachidonate in isolated perfused hearts from diabetic rats. Upper panel: vasoconstrictor phase. Lower panel: vasodilator phase. ●, non-treated diabetic rats ($n=7$); ○, insulin-treated diabetic rats ($n=5$). Values represent mean $\pm$ SEM

rats responded to NaA administration with a biphasic coronary response, characterized by an initial vasoconstriction followed by a slowly developing and long-lasting vasodilation. Again, only the vasodilator response to NaA was obtained in insulin-treated diabetic rats.

The initial vasoconstriction phase found in the diabetic rats was abolished by a selective TXA_2 antagonist, ONO-3708 [13]. ONO-3708 had little effect on vascular responses to other PGs and LTD_4 . These results indicate that TXA_2 may be responsible for the vasoconstriction by NaA in diabetic animals. According to the results of Roth et al. [7], aortic rings of diabetic rats showed an increased responsiveness to carbocyclic TXA_2 . However, the constrictor response to STA_2 , a stable TXA_2 -analogue, was not enhanced in coronary vessels of isolated perfused hearts from diabetic rats. This difference might indicate that vessels of different anatomical origin might be affected differently by the diabetic condition. Our findings indicate that coronary constriction by NaA in diabetic animals may be the result of increased TXA_2 generation in the vessels.

Indomethacin partly inhibited the vasoconstriction produced by NaA, while BW755C, which inhibits both cyclooxygenase and lipoxygenase(s) activities abolished the vasoconstriction. Similarly Gimeno et al. [12] showed that NaA-evoked constriction of coronary artery strips from diabetic dogs was abolished by the lipoxygenase inhibitor nordihydroguaiaretic acid but not by indomethacin and salicylic acid, compounds which act at the cyclooxygenase level. Therefore, we investigated the potential participation of lipoxygenase products in NaA-evoked constriction of coronary arteries in diabetic rat hearts.

The 5-lipoxygenase products LTC_4 and LTD_4 have been found to constrict coronary arteries [2, 18, 19]. Recently, Piomelli et al. [5] showed that canine and human coronary vessels metabolized exogenous AA to LTC_4 and LTD_4 . Therefore, we wondered whether LTC_4 and LTD_4 might participate in the vasoconstrictor effect of NaA in diabetic rats. We used ONO-1078, a newly synthesized selective antagonist of LTC_4 and LTD_4 [14] to address this problem. The vasoconstriction produced

by NaA in the presence of indomethacin was eliminated by ONO-1078, but not by ONO-3708, which suggested that LTs may play a role as a mediator of coronary constriction in diabetes when cyclooxygenase is inhibited. However, ONO-1078 did not affect the vasoconstrictive effect of NaA in the absence of indomethacin. Therefore, LTs may not be implicated in NaA-evoked constriction as long as the cyclooxygenase pathway is intact. This is also supported by the observation that the TXA_2 antagonist completely abolished the vasoconstrictor response under these conditions. However, the vascular responsiveness to LTD_4 was enhanced in the perfused hearts from diabetic animals. Therefore, the NaA-evoked coronary constriction seen in the presence of indomethacin appears to result from increases in both the generation of, and vascular reactivity to, LTs. The involvement of lipoxygenase-product, perhaps LTs, in NaA-evoked coronary constriction in diabetic dogs has been reported even in the absence of indomethacin [12]. In this species LTs appear to assume an even more prominent constrictor role under diabetic conditions.

The vasodilator response to NaA was not altered under diabetic conditions. Indomethacin completely abolished the vasodilation in both groups. Furthermore, there were no differences between the groups with respect to the vascular responses to prostanoid vasodilators (i.e. PGI_2 and PGE_2).

In conclusion, NaA-evoked coronary constriction found in diabetic rats was mediated by TXA_2 . However, when cyclooxygenase activity was inhibited, vasoconstriction still occurred, the LTs being responsible. This response may partly be attributed to increased vascular responsiveness to LTs in diabetic animals. Our results indicate that, in addition to TXA_2 , LTs may play a role in the altered response to AA in diabetes. These findings may have clinically important implications for diabetic patients treated with non-steroidal, anti-inflammatory drugs such as indomethacin. Further investigation into production of PGs and LTs is needed to clarify the causes of the altered AA response. It is possible that the alterations in vascular response to AA may be related to coronary artery dysfunction which is frequently found in patients with diabetes mellitus.

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