Increased Contractility to Noradrenaline and Normal Endothelial Function in Mesenteric Small Arteries from the Goto-Kakizaki Rat Model of Type 2 Diabetes

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Abstract: Type 2 diabetes is associated with many circulatory manifestations, including alteration in endothelial function and hypertension. In this study we investigate the morphology and contractile response as well as the endothelial function of resistance arteries from the spontaneously diabetic Goto-Kakizaki (GK) rat, a model of lean type 2 diabetes expressing glucose intolerance. METHODS: Isolated mesenteric small arteries were investigated under isometric conditions in a wire myograph system using noradrenaline (NA) and the endothelium-dependent vasorelaxant acetylcholine (ACh). Media thickness was measured and media lumen ratio calculated. RESULTS: No apparent morphological difference was noted between the arteries from GK rats and control Wistar (CW) rats. When exposed to the maximal NA concentration used (30 μM), arteries from GK rats developed significantly more tension than arteries from CW rats. In the presence of indomethacin (a specific blocker of the COX synthase) and of L-NAME (an inhibitor of eNOS), the response to NA was still significantly greater in GK rat arteries. Under control conditions, arteries from both groups showed intact relaxation to ACh. After incubation with indomethacin and L-NAME, both groups showed a non-NO nonprostaglandin-dependent relaxation to ACh. This relaxation could be blocked by a combination of apamin and charybdotoxin. CONCLUSION: This study shows that mesenteric small arteries from the diabetic GK rat have increased contractile response to NA, along with a normal endothelial function and unaltered morphology.

Key words: mesenteric small arteries, Goto-Kakizaki rat, acetylcholine, noradrenaline, type 2 diabetes.

Diabetes mellitus is associated with a wide range of circulatory manifestations, including alterations in endothelial function, nephropathy, neuropathy, and retinopathy [1, 2]. This is commonly known as vascular dysfunction and is closely associated with an increased risk of cardiovascular diseases [3]. Resistance arteries are arteries with an inner diameter <400 μm and are considered essential to flow distribution [4]. Increasing evidence indicates that in diabetic patients, the resistance arteries develop a marked endothelial dysfunction characterized by impaired endothelium-dependent relaxation and/or altered contraction of vasoconstrictors. A decrease in the endothelium-dependent relaxant properties of the resistance arteries have been shown to correlate with patients’ overall risk of cardiovascular diseases [5, 6].

The endothelium-dependent relaxation of resistance arteries is mediated through several pathways. The three most important are (i) release of nitric oxide (NO) from the endothelial cells, (ii) production of prostaglandins (the most potent vasodilator being prostacyclin), and (iii) the non-NO, nonprostaglandin-dependent endothelium-derived hyperpolarizing factor (EDHF) type of relaxation [7]. The EDHF pathway is generally considered to be of major importance in resistance arteries, whereas its contribution to relaxation in conduit arteries is only slight. For the most part, the three pathways act in parallel but contribute differently in different vascular beds. Thus if one or more of the pathways are defective, the other pathways will compensate or the relaxant properties of the arteries will decrease [8]. The latter will lead to a decrease in blood flow and subsequently a decrease in the supply of oxygen and nutrition to the tissue, as observed in diabetes leading to microvascular complications and diabetic angiopathy [1].

To elucidate the role of these individual pathways, we can use selective pharmacological tools. The NO pathway is blocked by L-NAME, which inhibits eNOS synthase, and the prostaglandin-dependent pathway is blocked via inhibition of the COX synthase using in-
domethacin. The EDHF-type relaxation is dependent on the small- and intermediate-conductance calcium-activated potassium channels, SK_Ca and IK_Ca [9], and can be blocked by a combination of apamin and charybdotoxin (ChTX) [10].

The Goto-Kakizaki (GK) rat is a model of lean type 2 diabetes, originally derived by selective and repetitive in-breeding of glucose-intolerant Wistar rats [11]. Pathological symptoms develop spontaneously as early as 4 weeks after birth and include hyperglycemia, glucose intolerance along with mild hyperinsulinemia, and some reports have included mild hypertension [12–15]. These traits are consistent in humans with low body weights (BMI < 18.5) suffering from type 2 diabetes, also referred to as lean type 2 diabetes [16].

The present study investigates the vascular responses in mesenteric small arteries from the GK rat and compares these to similar arteries from control Wistar (CW) rats. We have investigated the morphology along with the vascular properties, using noradrenaline (NA), and the relaxant properties of mesenteric small arteries, using the endothelium-dependent vasodilator acetylcholine (ACh). Our data suggest that mesenteric small arteries from GK rats have an unaltered endothelial function and increased contractility when compared to age-matched CW rats.

METHODS

Twenty-four male GK-rats were divided into two groups, A and B. Group A was used for NA experiments and group B for ACh experiments. The mesenteric small arteries from GK rats were compared to arteries from age-matched male CW rats (Wistar Hannover GALAS; HanTac:WH). All rats were obtained waiting 2–3 min at each concentration before measuring. To evaluate the contributions of NO, prostaglandins, and the EDHF-like relaxation, we repeated the experimental protocol, the arteries were stimulated twice with 10 μM NA and 123 mM KPSS for 2 min to test their viability; any vessel that failed to contract to more than 125% of the force developed at L_{100} was discarded. All compounds were added directly to the bath, and all experiments were performed in the same glucose concentration to ensure that responses were compared under similar conditions.

Protocols. Group A: To evaluate the contractile properties of the vessels, we made a cumulative NA concentration-response curve (0.1–30 μM NA), increasing the concentration every 2 min to allow the force to reach a steady state. After washing the vessels in PSS, we incubated them for 30 min with 3 μM indomethacin (a blocker of the prostaglandin-producing cyclooxygenase, COX), and the concentration-response curve was repeated. The vessels were then washed with PSS and depolarized for 2 min with 123 mM KPSS in the presence of indomethacin. Vessels were then washed with PSS and incubated for 30 min with 100 μM L-NAME (N^S-nitro-L-arginine methyl ester, an inhibitor of the nitric oxide synthase, eNOS), together with indomethacin before the NA concentration-response curve was repeated.

Group B: After testing the viability of the vessels, we added NA to preconstrict the vessel to 80% of the maximal force. On top of the preconstriction, a cumulative ACh concentration response curve (0.01–30 μM ACh) was obtained waiting 2–3 min at each concentration before measuring. To evaluate the contributions of NO, prostaglandins, and the EDHF-like relaxation, we repeated the cumulative ACh concentration response curve 4 times: (i) with 3 μM indomethacin present, to evaluate the effect of endogenous-derived prostaglandins; (ii) with indomethacin and 100 μM L-NAME present, to evaluate the effect of NO; (iii) with indomethacin and 0.5 μM apamin (a small-conductance Ca^{2+}-activated K^+ channel inhibi-
tor) plus 0.1 μM ChTX (large and intermediate conductance K\textsubscript{Ca} channel inhibitor) present, to evaluate the NO contribution; (4) with indomethacin and L-NAME present, together with apamin and ChTX.

Time controls were performed for both groups to ensure that the vessels were viable throughout the experiment. The incubation time before each experiment was 30 min.

**Calculations.** Isometric wall tension was calculated as force development divided by twice the segment length and expressed in mM/mm (Tension \[mN\]/2 × segment length [mm]). EC\textsubscript{20} values were calculated by nonlinear regression of sigmoidal slope using GraphPad Prism (GraphPad Software Inc., CA, USA). The EC\textsubscript{20} values reported are the mean EC\textsubscript{50}.

The relaxation to ACh was normalized to the NA-induced preconstriction ([Tension\textsubscript{ACCh}/Tension\textsubscript{preconstriction}] × 100) and is given as percent relaxation.

All calculations were done with GraphPad Prism 4.01 (GraphPad Software Inc., CA, USA).

**Materials and Chemicals.** The composition of PSS was (in mM) 119 NaCl, 4.7 KCl, 1.18 KH\textsubscript{2}PO\textsubscript{4}, 1.17 MgSO\textsubscript{4}, 25 NaHCO\textsubscript{3}, 1.6 CaCl\textsubscript{2}, 0.026 EDTA, and 5.5 mM glucose. KPSS was PSS in which Na\textsuperscript{+} was substituted by an equimolar concentration of K\textsuperscript{+} to reach 123 mM K\textsuperscript{+}. Acetylcholine, noradrenaline, L-NAME, apamin, charybdotoxin, and indomethacin were all obtained from Sigma Chemical (St. Louis, MO, USA).

**Statistics.** All data are presented as mean ± SEM with a significance level of \(P < 0.05\); \(n\) is the number of animals. The curves were evaluated by two-way ANOVA for repeated measures followed by a Bonferroni posttest. EC\textsubscript{50}, maximal force, and maximal relaxation were compared using unpaired Student’s \(t\)-test. All statistical analyses were made with GraphPad Prism 4.01 (GraphPad Software Inc., CA, USA).

**RESULTS**

**Animal characteristics.** Blood values and morphological characteristics are presented in Table 1. After an 8 h fasting period, the plasma glucose levels were significantly higher in 16-week-old GK rats than in age-matched CW rats, whereas there were no differences in blood pressure. At the time of experiments (22 weeks), the heart weights of GK rats were higher than in age-matched CW rats, and the body weights were lower.

**Vessel morphology.** The normalized lumen diameters of the arteries from GK and CW rats were, in group A, (243 ± 9 μm and 232 ± 11 μm \([n = 9 \text{ and } 8]\), and in group B, (233 ± 7 μm and 229 ± 5 μm \([n = 9 \text{ and } 8]\)), NS, GK, and CW rat arteries, respectively. There was no difference in lumen diameter between the two groups. Neither were any morphological differences found in media thickness or in media lumen ratio between vessels (Table 1).

**NA contraction.** When obtaining the cumulative NA concentration-response curve, the maximal response to NA was significantly higher in GK rats compared to CW rats (5.4 ± 0.4 N/m vs. 4.2 ± 0.3 N/m, \(n = 8\); GK and CW, respectively, \(P < 0.03\)) (Fig. 1). There was no difference in EC\textsubscript{50} (2.9 ± 0.1 μM and 2.4 ± 0.1 μM, \(n = 8\); GK and CW, respectively, \(P > 0.05\)). Arteries from both groups showed vasomotion (a rhythmic oscillation of vascular tone; for definition, see [18]), with similar frequencies (0.21 ± 0.01 Hz and 0.22 ± 0.01 Hz, \(n = 8\); GK and CW, respectively, \(P > 0.05\) and amplitude (3.08 ± 0.3 mN and 3.7 ± 0.5 mN, \(n = 8\); GK and CW respectively, \(P > 0.05\)).

An addition of the COX inhibitor indomethacin had no effect on the NA sensitivity of either group, and GK rats still developed significantly more tension than CW rats. However, the addition of indomethacin did reduce the maximal contraction in arteries from GK rats (from 5.4 ± 0.4 to 4.2 ± 0.3 N/m, \(n = 8\), \(P < 0.02\)), but not in arteries from CW rats (from 4.2 ± 0.3 to 3.3 ± 0.4 N/m, \(n = 8\), \(P > 0.05\)) (Fig. 1B). This reduction was not significantly different between GK and CW arteries (\(P = 0.38\)). Depolarizing the vessels by 123 mM KPSS in the presence of indomethacin caused similar contractions in GK and CW rats (Table 1).

Simultaneous incubation with indomethacin and L-NAME had no effect on EC\textsubscript{50} or maximal contraction in the two groups. Thus in the presence of the two blockers, arteries from GK rats were still developing a significantly higher wall tension than arteries from CW rats (5.0 ± 0.4 vs. 3.8 ± 0.4 N/m; GK and CW, respectively, \(n = 8\), \(P < 0.04\)) (Fig. 1C).

**Acetylcholine.** The cumulative ACh concentration-response curves on NA-preconstricted mesenteric small arteries showed intact endothelial-dependent relaxing responses in both GK and CW arteries under control conditions (95 ± 1% and 94 ±...
± 2% relaxation, \( n = 8, 7; \) GK and CW rats, respectively) with similar EC\(_{50}\) values (0.22 ± 0.03 μM and 0.19 ± 0.04 μM; GK and CW rats, respectively) (Fig. 2). Incubation with indomethacin had no significant effect on the maximal relaxing response (95 ± 3% and 94 ± 1% relaxation, \( n = 7, 5; \) GK and CW rats, respectively) or on EC\(_{50}\) values.

To evaluate the vasorelaxing contribution of NO, vessels were incubated with L-NAME together with indomethacin. In both groups, this caused a decrease in the maximal relaxing response (to 61 ± 11% and 78 ± 7% relaxation, \( n = 8; \) GK and CW, respectively) and concomitantly.

**Fig. 1.** A: Original trace of force development to cumulative noradrenaline concentrations in a mesenteric small artery from a GK rat. Arrows indicate time of increasing concentration with 0.5 Log steps from 0.1 μmol/l to 30 μmol/l. B: Development of tension in isolated mesenteric small arteries from GK (black square) and CW (black triangle) rats in response to cumulative norepinephrine concentrations under control conditions and after incubation with 3 μmol/l Indomethacin (open symbols). C: Development of tension in isolated mesenteric small arteries from GK (black square) and CW (black triangle) rats in response to cumulative noradrenaline concentrations under control conditions and after incubation with 10 μmol/l L-NAME + 3 μmol/l Indomethacin (open symbols). The data are presented as mean ± SEM; they represent two arteries from each of 8 GK and 9 CW rats, \(*P < 0.05\) (Student’s t-test).

**Fig. 2.** A: Original trace of relaxation to cumulative acetylcholine concentrations in a noradrenaline-preconstricted mesenteric small artery from a GK rat. Arrows indicate time of increasing concentration in 0.5 Log steps, from 0.01–30 μmol/l ACh. B: The relaxation of isolated mesenteric small arteries from GK (black square) and CW (black triangle) rats in response to cumulative concentrations of acetylcholine under control conditions and after incubation with 100 μmol/l L-NAME + 3 μmol/l Indomethacin (open square and triangles). The circles represent the response after further addition of 0.5 μmol/l apamin and 0.1 μmol/l charybdoxin (open: GK, black: CW). The vessels were preconstricted with NA to 80% of maximal contraction. The data are presented as mean ± SEM; they represent two arteries from each of the 8 GK and 9 CW rats, \(*P < 0.05\) (Student’s t-test).
tantly rightward shifting of the concentration response curve with increasing EC₅₀ values (GK: from 0.22 ± 0.03 μM to 0.62 ± 0.08 μM M; and CW: from 0.19 ± 0.04 μM to 0.88 ± 0.07 μM, P < 0.01) (Fig. 2). There was no difference between the mean EC₅₀ of the two groups after treatment.

After incubation with apamin and ChTX, together with indomethacin and L-NAME, the relaxing response was completely abolished (–13 ± 1% and –4 ± 3% relaxation, n = 7, 8; GK and CW, respectively) (Fig. 2).

**DISCUSSION**

In the present study we used the GK rat model of lean type 2 diabetes and studied small mesenteric arteries. The arteries used were small enough to be regarded as resistance arteries used were small enough to be regarded as resistance arteries and are therefore considered to be of importance for the regulation of flow distribution.

The aim of this study was to investigate potential differences in the vascular responses when treated with exogenous NA and ACh. The main observation was that the major endothelial-dependent relaxing pathways seem intact, though the contractile response to NA was increased, despite the unaltered morphology of the arteries.

It has previously been shown that the GK rat model developed hypertension when fed a high-salt diet [19, 20]. However, some controversy exists on whether the GK rats developed hypertension when fed a normal diet. The body weights of GK rats at 22 weeks were lower than the weights of the control rats, which also was consistent with previous findings on 22-week-old [15] and 36-week-old [27] rats, and in accordance with telemetric measurements on 14–18-week-old [23, 24], 20–26-week-old [25], and 20–35-week-old GK rats [26]. However, other studies have found elevated blood pressures in the GK rats at 12 weeks, but not at 36 weeks [27] or during 8 through 24 weeks [28]. The reason for these discrepancies is unknown.

The increases in heart weight that we observed in GK rats have not been described before, but it has previously been shown that GK rats have decreased heart rates [29]. The body weights of GK rats at 22 weeks were lower than the weights of the control rats, which also was consistent with previous findings on 22-week-old [15] and 36-week-old GK rats [27].

We observed a marked increase in tension development in vessels from the GK rat with increasing EC₅₀ values (GK: from 0.22 ± 0.03 μM to 0.62 ± 0.08 μM M; and CW: from 0.19 ± 0.04 μM to 0.88 ± 0.07 μM, P < 0.01) (Fig. 2). There was no difference between the mean EC₅₀ of the two groups after treatment.

Another possibility is that the media thickness is increased in blood vessels from diabetic rats. This possibility is supported by the observation of an increased media thickness (and media-to-lumen ratio) in mesenteric small arteries from 18-week-old nonhypertensive GK rats [33]. However, in the study by Sachidanandam et al. [33], the vessel morphology was not assessed under mechanically well-defined conditions. We therefore repeated these measurements under well defined conditions and we observed no difference in media thickness or media-to-lumen ratio. We therefore conclude that the increased contractility to NA is not caused by an increased media thickness. This conclusion is strongly supported by the similar response to depolarization with K-PSS, which would not be expected if the media thickness were increased.

The preserved ACh-mediated endothelial-dependent relaxation of mesenteric small arteries from GK rats that we found in this study confirms previous studies of these arteries [23, 24]. However, studies of the endothelial-dependent relaxation of other vascular beds from the GK rat show a variance in the response between different vascular beds from the same model. In the superior mesenteric arteries from GK rats, the ACh-mediated relaxation appears to be attenuated [15, 19, 20], as well as in aortic segments [27, 34] and in the basilar arteries [23, 35]. The reduction we find in the maximal tension of GK arteries in the presence of indomethacin could indicate that a vasoconstricting prostaglandin might also be of importance, as previously reported by Kimura et al. [30]. However, the effect of indomethacin was not significantly different between the two groups, and the difference in contractile response of GK arteries persisted after indomethacin treatment, making it unlikely that the prostaglandin pathway is a major mechanism explaining the different contractility. In the presence of both indomethacin and L-NAME, we found no significant decrease of the contractility in either group. It is therefore unlikely that a change in both the prostaglandin pathway and the NO pathway is responsible for the enhanced contractility. On these grounds we conclude that a change at the receptor level or beyond is most likely responsible for the increased contractility to NA.

Selective blocking of the prostaglandin-mediated relaxation did not alter the relaxant properties of the arteries. This suggests that the endothelial-dependent relaxing pathways in these arteries are not importantly dependent on vasorelaxing prostaglandins.

Another important candidate in endothelial-mediated relaxation is NO. The L-NAME sensitive response is a reflection of the NO-mediated relaxation and was not differ-
ent between the two groups, indicating unaltered NO-mediated relaxation of the GK arteries.

The third major endothelial-dependent relaxing pathway is the non-NO nonprostaglandin-dependent relaxation, which was found to be similar in the mesenteric small arteries from both the GK and the CW rats. This relaxation could be blocked by a combination of apamin and ChTX, which is consistent with the response being an EDHF-type relaxation [10]. However, electrophysiologically measurements would be required to conclusively state that it is EDHF. A preserved non-NO nonprostaglandin-dependent relaxation in these arteries contradicts other findings on similar arteries from other diabetic rat models, such as the Zucker Diabetic rat [36, 37], the Otsuka Long-Evans Tokushima Fatty rat [38], and the streptozotocin (STZ) rat [39, 40], where a reduced EDHF-type response has been reported.

CONCLUSION

The study shows increased contractility in response to NA, but not to high potassium, and demonstrates that the increased response to NA cannot be explained by an altered morphology of mesenteric small arteries from the type 2 diabetic GK rat. Furthermore, we confirmed previous findings of a preserved ACh-mediated endothelial-dependent relaxation in these arteries. We extended these observations by showing that the non-NO and nonprostaglandin, but apamin- and ChTX-sensitive (EDHF-type response) parts of the ACh sensitive relaxing responses are preserved.

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