

ENHANCED Na-K ATPase ACTIVITY IN THE AORTA MAY EXPLAIN THE UNALTERED CONTRACTILE RESPONSES TO KCl IN DIABETES MELLITUS

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Abstract : Sodium-potassium ATPase activity and transmembrane calcium influx in the aortic smooth muscle from control and diabetic rats were assessed indirectly through the measurement of KCl relaxation and contractile responses to CaCl_2 in attempts to explain the contractile responses to KCl following streptozotocin-induced diabetes mellitus. There were no significant changes in the maximum contractile responses of the aortas from 4 and 12 week diabetic rats to KCl even when significant increases in calcium influx were demonstrable. On the other hand, the diabetic aortas were significantly ($P<0.05$) more sensitive to KCl-induced relaxations than the controls. This provides an indirect evidence for increased activity of the sodium-potassium ATPase enzyme in the aortas from streptozotocin diabetic rats. This may, atleast in part, explain the inability of KCl to produce greater than normal contractions of the aortas from diabetic rats.

Key words : KCl contraction

aorta

sodium-potassium ATPase

streptozotocin-induced diabetes

INTRODUCTION

It has been suggested that some of the cardiovascular disturbances which are known to occur in patients with diabetes mellitus are a consequence of alterations in the reactivity of blood vessels to neurotransmitters and circulating hormones (1, 2). This has encouraged studies using blood vessels from animals made diabetic with either streptozotocin or alloxan.

However, the results have been inconsistent. For KCl, enhanced (3), unaltered (4) and depressed (5, 6, 7, 8), responses have been reported in different vascular preparations. The discrepancies in these results are difficult to explain but might be due to differences in metabolic changes at the time of experimentation.

Potassium causes contraction of vascular smooth muscle by depolarizing the membrane and promoting influx of calcium via voltage-dependent calcium

channels (9). Thus, any metabolic change that affects its ability to depolarize vascular smooth muscle membranes or transmembrane calcium influx would affect its contractile responses. In the present study, some membrane-based activities capable of influencing contractile responses to KCl have been assessed. These include, sodium-potassium ATPase activity and transmembrane calcium influx, both of which have been indirectly (or physiologically) measured.

METHODS

Male Wistar rats initially weighing 130-140 g and aged 6-10 weeks were used for this study. They were divided into control and diabetic groups, organized in such a way that all the animals had come up to same age before sacrifice. Each test animal was made diabetic by intraperitoneal injection of streptozotocin (60 mg/kg body weight) in citrate buffer (pH 4.5). The control animals received the citrate buffer alone. All animals had free access to food and water and were

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monitored daily for the development of glycosuria using Uristix reagent strips (Ames Division, Miles Laboratories, England).

Four and 12 weeks after induction of diabetes, rats were sacrificed by stunning and the aorta quickly dissected, freed of connective tissues and put into a beaker containing normal physiological salt solution (NPSS) of the following composition (mM/l) : NaCl, 119.0; KCl, 4.7; CaCl₂, 1.6; MgSO₄.7H₂O, 1.2; NaHPO₄, 1.2; NaHCO₃, 14.9; and glucose, 11.0 of pH 7.4. The solution was continuously oxygenated with 95% O₂ and 5% CO₂ gas mixture at temperature of 37°C. The aorta was cut into rings of about 3 mm in length each of which was suspended between two rectangular stainless wires in a 10 ml organ bath containing NPSS and oxygenated as described above. Each ring was connected to an isometric force displacement transducer (FT.03C) which was in turn, connected to a Grass Model 7D polygraph for recording of tension. All tissues were allowed to equilibrate for 90 minutes under a resting tension of 1 g prior to the commencement of experiments.

Concentration-response tests to KCl : After the equilibration period, concentration - response tests to KCl were conducted by cumulative addition of the salt (10-100 mM) into the fluid bathing the tissues; higher concentration being added only after the effect of the previous one had reached a plateau. These were conducted on control and diabetic aortic rings. Changes in tension in the various preparations were expressed as percentages of the maximum tension in the control EC₅₀ of KCl for the various preparations were also calculated as a measure of their sensitivities.

Concentration-response tests to CaCl₂ : These were conducted (to indirectly assess transmembrane calcium influx in the presence of KCl) as follows :

The aortic rings were initially contracted with noradrenaline (10⁻⁵ M) and when contractions were stabilised, the tissues were rinsed with calcium-free solution containing Ethylene Glycol-bis (P-aminoethyl ether) N, N', N'-tetraacetic acid (EGTA). Fifteen minutes after, the tissues were re-stimulated with noradrenaline (10⁻⁵M) and rinsed again with the

calcium-free EGTA solution to ensure complete depletion of calcium releaseable from the intracellular compartment as previously described (10,11). Thereafter, application of noradrenaline produced no contraction. The tissues were then left in this medium for a total of 30 minutes from the time of the first rinsing. At the end of this time, the bathing medium was changed to a calcium-free solution without EGTA but containing KCl (100 mM) and the tissues left in this medium for 5 minutes before the commencement of cumulative addition of CaCl₂ (2.5 × 10⁻⁵ to 2.5 × 10⁻²M). This protocol was adopted for all aortic preparations from control and diabetic rats of the two durations (4 and 12 weeks). Change in tension for each concentration of calcium chloride was expressed as percentage of the tension developed in response to Noradrenaline (10⁻⁵M) at the beginning of each procedure.

ATPase activity : This was assessed indirectly, by potassium-induced relaxation as previously described (17).

The aortic rings were initially exposed to a potassium-free solution for 30 minutes. The potassium-free solution was prepared by omitting KCl from the NPSS, with equipmolar substitution with NaCl. Thereafter, the rings were contracted with noradrenaline (10⁻⁷M). When the contractions were stabilized, KCl (0.01-7 mM) was applied cumulatively into the bathing medium, and concentration-dependent relaxations were recorded. The relaxation to each concentration of KCl was expressed as percentage decrease in initial tension induced by noradrenaline. IC₅₀ values of KCl for the various preparations were also compared.

Statistics : Data are presented as mean ± S.E. Comparison between control and each diabetic group was made with the student's unpaired t test. P values less than 0.05 were considered statistically significant. n denotes number of animals in each group.

RESULTS

Contractile responses to KCl : KCl produced concentration-dependent contractions of all vessel preparations from both diabetic and non-diabetic

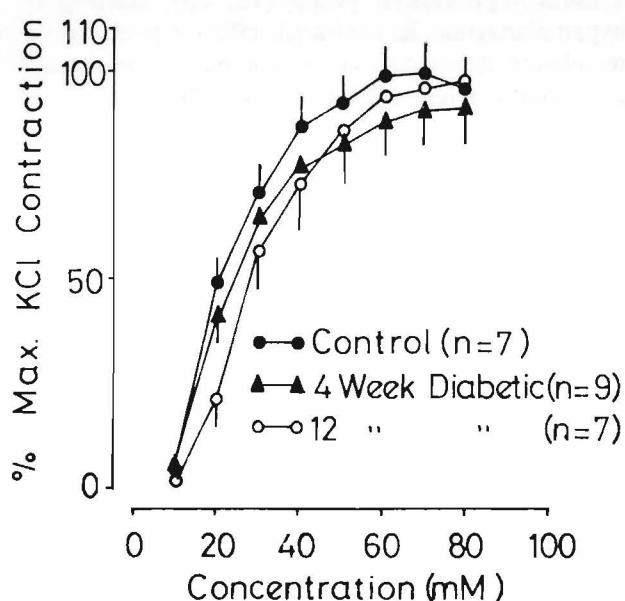


Fig. 1: Contractile responses of aortic rings (from control and diabetic rats) to KCl. KCl (10 - 100 mM) was applied cumulatively and contractile responses recorded in the aortae from control, and 4 and 12 week diabetic rats. 'n' represents the number of animals used in each group.

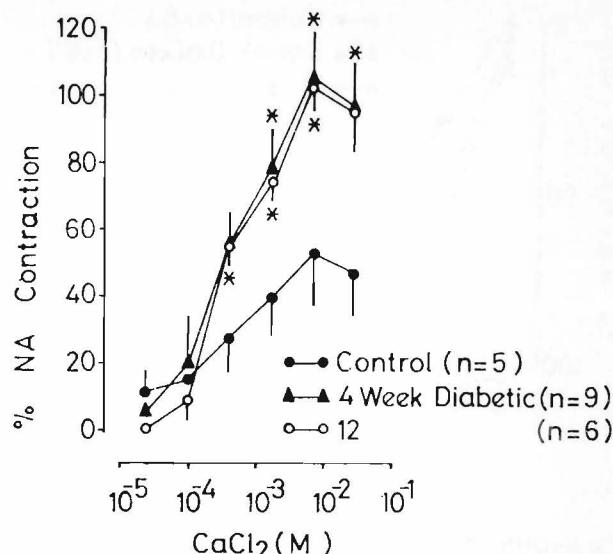
(control). The curves for the diabetics were shifted to the right (Fig. 1) and the EC_{50} value of KCl was significantly ($P<0.001$) greater for the 12 - week diabetic preparations than the controls (Table I). However, the maximum contractile responses to KCl were similar for all groups.

TABLE I: EC_{50} values (mean \pm SE) of KCl for the contraction of aortic rings from control and diabetic rats.

		EC_{50} (mM)
Control	(n = 7)	21.29 \pm 1.39
4 week diabetic	(n = 9)	22.29 \pm 1.64
12 week diabetic	(n = 7)	28.57 \pm 1.04**

**P<0.01, significantly greater than control.

Contractile responses to $CaCl_2$: The maximum contractile responses to cumulative addition of $CaCl_2$ in the presence of KCl (100 mM) were significantly ($P<0.05$) increased in the two diabetic durations (4 and 12 weeks) when compared with controls (Fig. 2).



* P<0.05 Significantly greater than control

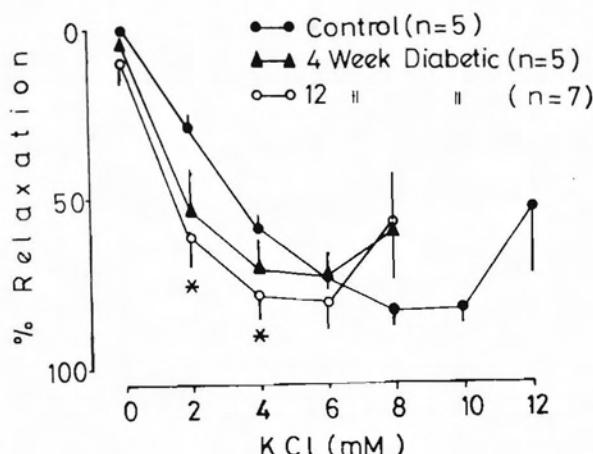
Fig. 2: Contractile responses of aortic rings (from control and diabetic rats) to $CaCl_2$. $CaCl_2$ was applied cumulatively in the presence of KCl (100 mM) and contractile responses recorded in aortae from control and 4 and 12 week diabetic rats! 'n' represents the number of animals used in each group and, *represents significantly ($P<0.05$) greater contraction than control.

ATPase activity : Low concentrations of KCl (0.01-7 mM) produced concentration-dependent relaxations of aortic rings preincubated in K^+ -free PSS from both control and diabetic rats. The diabetic rings were significantly ($P<0.05$) more responsive than the controls (Fig. 3). In addition, the IC_{50} values of KCl were significantly ($P<0.01 - 0.001$) less for the diabetic preparations than the controls (Table II).

TABLE II: IC_{50} values (mean \pm SE) of KCl for the relaxation of aortic rings from control and diabetic rats.

		EC_{50} (mM)
Control	(n = 5)	1.82 \pm 0.2
4 week diabetic	(n = 5)	0.83 \pm 0.22**
12 week diabetic	(n = 7)	0.59 \pm 0.8***

P<0.01, * P<0.001, significantly less than control.



* p<0.05 significantly greater than control

Fig. 3 : Relaxation responses of Aortic rings (from control and diabetic rats) to KCl. Following pre-incubation in K⁺ free PSS and contraction with Noradrenaline (10⁻⁷M), low concentrations of KCl (0.01-12 M) produced concentration-dependent relaxations of aortic rings from control and 4 and 12 week diabetic rats. 'n' represents the number of animals in each group and, *represents significantly (P < 0.05) greater relaxation than control.

DISCUSSION

Extracellular concentration of potassium ion has biphasic action on the responses of vascular smooth muscle. At low concentrations, it produces relaxation (12), while at higher concentrations, contractions are observed (11). Each of these effects is produced by a different mechanism; while relaxation to KCl is mediated via alteration of the activity of

sodium - potassium pump (12, 13), leading to hyperpolarization, its contractile effect is produced via membrane depolarization which opens the voltage dependent calcium channels (VDC) (9). The greater relaxation to KCl observed in the diabetic aorta in the present study, probably reflects an increase in the activity of the sodium-potassium ATPase enzyme in the streptozotocin diabetic rat aorta. Similar observation for this enzyme has been made in the kidney of diabetic rats (14).

The present observation might be relevant in explaining the unaltered contractile responses to KCl in the diabetic aortic rings (Fig. 1). Similar observation on KCl depolarization in the aorta from diabetic rats has been made (4) and significant decrease in response has also been reported (5).

Our results with CaCl₂ (Fig. 2) show that calcium influx is significantly enhanced in diabetes. This is in agreement with the reports of Agrawal and McNeil (15) and Kamata et al (16). Thus, the inability of KCl to produce greater contractile responses in the diabetic rat aorta in the present study could not be explained by a decrease in transmembrane calcium influx, but probably, by membrane resistance to KCl depolarization. Such a membrane resistance may be a consequence of increased sodium-potassium ATPase activity as observed here.

In conclusion, the present study has provided an indirect evidence for enhanced activity of the sodium-potassium ATPase in the aorta of streptozotocin diabetic rat; a situation that may be responsible atleast, in part, for the membrane resistance to KCl depolarization in the diabetes.

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