Insulin does not regulate vascular smooth muscle Na^+ , K^+ -ATPase activity in rabbit aorta

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Summary. To determine whether insulin regulates vascular smooth muscle Na^+ , K^+ -ATPase activity and if impaired insulin stimulation of vascular smooth muscle Na^+ , K^+ -ATPase activity could be a cause of increased vascular reactivity to norepinephrine and angiotensin II in diabetic states, the effects of insulin on Na^+ , K^+ -ATPase activity were examined in normal rabbit aortic intima-media incubated with normal plasma glucose and *myo*-inositol levels for 30 min. Insulin at 100 $\mu\text{U}/\text{ml}$ (600 pmol/l) had no effect on Na^+ , K^+ -ATPase activity. At 250 $\mu\text{U}/\text{ml}$ it caused a $4.2 \pm 0.8\%$ increase, and at 500 $\mu\text{U}/\text{ml}$ insulin caused a $17.7 \pm 1.4\%$ increase in Na^+ , K^+ -ATPase activity that was completely inhibited by amiloride (1 mmol/l). Human insulin-like growth factor I (600 pmol/l) caused an $18.0 \pm 1.0\%$ increase in Na^+ , K^+ -ATPase activity that was inhibited by amiloride. Insulin does not regulate (stimulate) aortic vascular smooth muscle Na^+ , K^+ -ATPase activity. Supraphysiological insulin concentrations, probably acting through an insulin-like growth factor I receptor, stimulate Na^+/H^+ exchange in aortic vascular smooth muscle and

cause small secondary increases in Na^+ , K^+ -ATPase activity. In aortic intima-media incubated with normal plasma glucose and *myo*-inositol levels, endogenously released adenosine stimulates and maintains a component of resting Na^+ , K^+ -ATPase activity and stimulates acute increases in activity when norepinephrine (1 $\mu\text{mol}/\text{l}$) or angiotensin II (100 nmol/l) is added. These adenosine-stimulated components of Na^+ , K^+ -ATPase activity are selectively inhibited when the medium glucose is raised to 30 mmol/l during a 30-min equilibration and 30-min incubation. Insulin (100 $\mu\text{U}/\text{ml}$) added during the incubation had no effect on the alterations in Na^+ , K^+ -ATPase activity induced by glucose at an elevated plasma level. Impaired insulin stimulation of vascular smooth muscle Na^+ , K^+ -ATPase activity is not a possible cause for alterations in vascular reactivity in diabetes.

Key words: Na^+ - K^+ -ATPase, insulin, vascular smooth muscle, vasodilation, vasoconstriction.

Some factor or factors present in all diabetic states (e.g. hyperglycaemia, insulin deficiency or resistance) causes increased reactivity to norepinephrine and angiotensin II in specific arteries and arterioles. Thus the vasopressor responses to i.v. norepinephrine and angiotensin II are increased in diverse diabetic states, even when vascular complications, hyperinsulinaemia, or hypertension are absent. Increased contractile responses to these vasoconstrictors have been demonstrated in specific arteries and arterioles from rats and rabbits with diabetes induced by a beta-cell toxin [1–6]. Further, insulin treatment of streptozotocin diabetic rats is known to correct the increased reactivity of their aortae and mesenteric arteries to norepinephrine [7]. Decreased vascular smooth muscle (VSM) Na^+ , K^+ -ATPase activity is a cause for increased contractile responses to norepinephrine and angiotensin II and a postulated cause for essential hypertension [8, 9]. Whether insulin regulates (stimulates) Na^+ , K^+ -

ATPase activity in VSM, as it does in adipocytes and skeletal muscle, is unknown. We examined this question in normal rabbit aortic intima-media (AIM), a commonly used system for studies of VSM, to assess whether impaired insulin stimulation of VSM Na^+ , K^+ -ATPase activity is a possible cause for decreased VSM Na^+ , K^+ -ATPase activity and resulting alterations in vascular reactivity in diabetic states.

The mechanism by which hyperglycaemia decreases Na^+ , K^+ -ATPase activity in specific tissues and blood vessels has effects which are preventable in diabetic animals both by aldose reductase inhibitors and by raising the normal plasma *myo*-inositol (MI) levels approximately seven-fold [10–12]. This mechanism can be activated in vitro in normal rabbit AIM, and it was recently found to decrease VSM Na^+ , K^+ -ATPase activity by inhibiting a novel adenosine- Na^+ , K^+ -ATPase regulatory system, which plays a role in the modulation of the responses

Table 1. Effects of insulin and insulin-like growth factor I on Na^+ , K^+ -ATPase activity in rabbit aortic intima-media

Na^+ , K^+ -ATPase-mediated $^{86}\text{Rb}^+/\text{K}^+$ uptake ($\mu\text{mol} \cdot \text{g}^{-1} \cdot 20 \text{ min}^{-1}$)		Mean $\Delta \pm \text{SEM}$	<i>n</i>	<i>p</i>
Control	+ Insulin (100 $\mu\text{U}/\text{ml}$)			
2.19	2.20	$+ 0.01 \pm 0.01$	(8)	NS
Control	+ Insulin (250 $\mu\text{U}/\text{ml}$)			
2.25	2.36	$+ 0.11 \pm 0.01$	(9)	< 0.001
Control	+ Insulin (500 $\mu\text{U}/\text{ml}$)			
2.16	2.54	$+ 0.38 \pm 0.03$	(6)	< 0.001
Control + Amiloride	+ Insulin (500 $\mu\text{U}/\text{ml}$) + Amiloride			
2.13	2.16	$+ 0.03 \pm 0.03$	(6)	NS
Control	+ Insulin-like growth factor I (600 pmol/l)			
2.03	2.40	$+ 0.37 \pm 0.02$	(6)	< 0.001
Control + Amiloride	+ Insulin-like growth factor I (600 pmol/l) + Amiloride			
2.02	2.02	0.00 ± 0.01	(6)	NS

In each experiment paired aortic intima-media samples taken from a normal rabbit after an overnight fast were equilibrated and then incubated for 30 min in the absence or presence of insulin or insulin-like growth factor I in Krebs-Henseleit bicarbonate buffer containing normal rabbit plasma levels of glucose (30 mmol/l) and *myo*-inositol (70 $\mu\text{mol}/\text{l}$), and 9% dextran. In specific series of ex-

periments amiloride (1 mmol/l) was added to both paired samples. Na^+ , K^+ -ATPase-mediated $^{86}\text{Rb}^+/\text{K}^+$ uptake was determined during the final 20 min of the incubation, and the difference between the paired samples determined. Values are means and mean $\Delta \pm \text{SEM}$ between the paired samples. The mean $\Delta \pm \text{SEM}$ was analysed for significance by a paired Student's *t*-test

to norepinephrine and angiotensin II in AIM [13–15]. Adenosine is an autocoid and one of the endothelium-released vasodilators [16–18]. Stimulation of increased electrogenic Na^+ , K^+ -ATPase activity in VSM is one of the mechanisms by which endothelium-released vasodilators modulate vasoconstriction [18, 19]. In AIM incubated with normal plasma glucose and MI levels, endogenously released adenosine stimulates and maintains a distinct component of resting Na^+ , K^+ -ATPase activity and also stimulates acute increases in Na^+ , K^+ -ATPase activity when AIM is exposed to norepinephrine or angiotensin II to modulate the response [14, 15]. These normal adenosine-stimulated components of Na^+ , K^+ -ATPase activity are selectively inhibited within 60 min when the medium glucose is raised from a normal to a threshold elevated plasma level (10 mmol/l) or to 30 mmol/l [13–15]. Exposing AIM to an elevated glucose level induces increased polyol pathway activity that impairs MI transport at a normal plasma MI level; this inhibits the adenosine effect on Na^+ , K^+ -ATPase activity, because this effect is mediated through a receptor that stimulates phosphatidylinositol turnover (hydrolysis) in a discrete pool, which is maintained by a fraction of phosphatidylinositol synthesis that selectively requires MI transport to prevent its rapid inhibition [13, 14]. We also examined the effects of insulin on the alterations in Na^+ , K^+ -ATPase activity caused by exposing AIM to glucose in an elevated plasma level.

Materials and methods

Male, white New Zealand rabbits (2.0–2.5 kg) were fasted overnight, sedated with diazepam (2 mg/kg i.m.), and 90 min later anaesthetized with sodium pentobarbital (30 mg/kg i.v.) and decapitated. The

descending aorta was rapidly excised and used to prepare four tubular segments of AIM, each weighing approximately 30 mg; the method employed provides AIM that is free of adventitia and retains an intact endothelium with a normal transmission and scanning electron microscopic appearance [20, 21]. All of the media to which the aorta and AIM were exposed contained 9% clinical grade dextran (average *M_r* 70,000) to provide the oncotic pressure that is required to preserve an intact endothelium with a normal ultrastructural appearance in AIM and the normal pattern of energy metabolism in its constituent VSM cells [20, 21]. The aorta was dissected in Krebs-Henseleit bicarbonate buffer [22] (which has normal plasma concentrations of Na^+ and K^+), pH 7.4 at 37°C, that contained normal rabbit plasma levels of glucose (5 mmol/l) and MI (70 $\mu\text{mol}/\text{l}$) and was continuously bubbled with 5% $\text{CO}_2/95\% \text{O}_2$.

For each experiment the four AIM segments from a single aorta were used to provide two paired samples, each comprised of two individual AIM segments. The Na^+ , K^+ -ATPase-mediated $^{86}\text{Rb}^+/\text{K}^+$ uptake in the paired samples was compared during incubations in similar medium with insulin, insulin-like growth factor I, norepinephrine, or angiotensin II added to one of the paired samples. The method for the determination of Na^+ , K^+ -ATPase-mediated $^{86}\text{Rb}^+/\text{K}^+$ uptake in AIM has been previously reported in detail [13]. Briefly, the individual freshly prepared AIM segments were equilibrated for 30 min in 3.0 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4 at 37°C, containing 9% dextran, 70 $\mu\text{mol}/\text{l}$ MI, and glucose in a specified concentration, in a 10 ml Erlenmeyer flask continuously gassed with 5% CO_2/air , in a metabolic shaker set at 88 cycles per min. The individual AIM segments were then transferred to 3.0 ml of fresh medium of the same composition to which a tracer quantity of [^3H]sucrose had been added; ouabain (0.2 mmol/l) was added to one of two AIM segments in each paired sample, and the tissue was incubated as described for the equilibration. After the initial 10 min of the incubation a tracer quantity of [$^{86}\text{Rb}^+$]RbCl was added to each flask and the incubation continued for an additional 20 min. At the end of the incubation the individual AIM segments were rapidly removed, drained on filter paper, weighed, and transferred into 2 ml of Protosol in a glass liquid scintillation vial and digested for 16 h. The tissue digest was then decolorized with H_2O_2 , Econofluor 2 was added to the vial, and the sample counted for $^{86}\text{Rb}^+$ and ^3H in a liquid scintillation spectrometer with an external standard. A sample of the

Table 2. Effects of insulin (100 µU/ml), norepinephrine, and angiotensin II on Na⁺, K⁺-ATPase activity in aortic intima-media incubated with 30 mmol/l or 5 mmol/l glucose and a normal plasma myo-inositol level

Medium glucose	Na ⁺ , K ⁺ -ATPase-mediated ⁸⁶ Rb ⁺ /K ⁺ uptake (µmol · g ⁻¹ · 20 min ⁻¹)				
	Control	+ Insulin (100 µU/ml)	Mean Δ ± SEM	n	p
30 mmol/l	1.64	1.51	- 0.13 ± 0.32	(8)	NS
	Control + Insulin	+ Norepinephrine (1 µmol/l) + Insulin			
5 mmol/l	2.29	3.59	+ 1.30 ± 0.14	(6)	< 0.001
30 mmol/l	1.54	1.69	+ 0.15 ± 0.25	(6)	NS
	Control + Insulin	+ Angiotensin II (100 nmol/l) + Insulin			
5 mmol/l	2.31	3.39	+ 1.08 ± 0.12	(6)	< 0.001
30 mmol/l	1.31	1.50	+ 0.19 ± 0.12	(8)	NS

Paired aortic intima-media samples were equilibrated (30 min) and incubated (30 min) in Krebs-Henseleit bicarbonate buffer containing 70 mol/l myo-inositol, 9% dextran, and glucose at the concentration indicated. Insulin (100 µU/ml) was added at the outset of the incubation to one or both paired samples, as indicated. When norepinephrine or angiotensin II was added to one paired sample, it

was added to the outset of the final 20 min of the 30-min incubation. Na⁺, K⁺-ATPase-mediated ⁸⁶Rb⁺/K⁺ uptake was determined during the final 20 min of the incubation. Values are means and mean Δ ± SEM between the paired samples. The mean Δ ± SEM was analysed for significance by a paired Student's *t*-test

incubation medium was also counted and the medium [K⁺] determined. The ⁸⁶Rb⁺ counts recovered in the tissue digest were corrected for the ⁸⁶Rb⁺ counts present in the trapped extracellular fluid (incubation medium) in the tissue sample by means of the [³H]sucrose counts recovered in the tissue sample and the ratio of ⁸⁶Rb⁺ counts/³H counts in the incubation medium. The resulting estimate of intracellular ⁸⁶Rb⁺ counts was then expressed as µmol ⁸⁶Rb⁺/K⁺ uptake · g⁻¹ · 20 min⁻¹ by means of the ratio of the medium [K⁺] to the medium ⁸⁶Rb⁺ counts per unit volume. The difference between the ⁸⁶Rb⁺/K⁺ uptakes in the absence and presence of ouabain in the two AIM segments in each paired sample was used as an estimate of the Na⁺, K⁺-ATPase-mediated ⁸⁶Rb⁺/K⁺ uptake in that paired sample.

The sources of the materials used were: dextran, ouabain, angiotensin II, norepinephrine bitartrate (Sigma Chemical Co., St. Louis, Mo., USA); myo-inositol (Pfhanstiehl Labs. Inc., Waukegan, Ill., USA); human, recombinant insulin-like growth factor I (Boehringer-Mannheim Biochemicals, Indianapolis, Ind., USA); Protosol, Econofluor 2, [⁸⁶Rb⁺]RbCl, [³H]sucrose (New England Nuclear, Boston, Mass., USA). Pure porcine insulin was a gift from Eli Lilly and Co. (Indianapolis, Ind., USA).

Statistical analysis

The mean Δ ± SEM between the paired samples incubated in the absence and presence of the agonist tested in each group of paired experiments was analysed for significance by a Student's paired *t*-test analysis.

Results

Paired AIM samples taken from normal rabbits after an overnight fast were equilibrated and then incubated for 30 min in medium containing normal rabbit plasma concentrations of glucose (5 mmol/l) and MI (70 µmol/l) with insulin added to one sample, and the Na⁺, K⁺-ATPase-mediated ⁸⁶Rb⁺/K⁺ uptakes in the paired samples compared during the final 20 min of the incubation. Insulin at a concentration of 100 µU/ml (600 pmol/l) had no effect

on Na⁺, K⁺-ATPase activity (Table 1). Insulin at a concentration of 250 µU/ml caused a very small but significant increase in Na⁺, K⁺-ATPase activity (Table 1); the mean percentage increase ± SEM in the paired samples was only 4.2 ± 0.8%. Insulin at 500 µU/ml caused a greater, but still relatively small, increase in Na⁺, K⁺-ATPase activity (Table 1); the mean percentage increase ± SEM was 17.7 ± 1.4%. When amiloride (1 mmol/l), an inhibitor of the Na⁺, H⁺ exchanger [23], was added to the incubation medium, the addition of 500 µU/ml insulin had no significant effect on Na⁺, K⁺-ATPase activity (Table 1).

Human insulin-like growth factor I (600 pmol/l) caused a significant increase in Na⁺, K⁺-ATPase activity in AIM incubated with normal plasma levels of glucose and MI for 30 min (Table 1): the mean percentage increase ± SEM was 18.0 ± 1.0%. The magnitude of the increase in Na⁺, K⁺-ATPase-mediated ⁸⁶Rb⁺/K⁺ uptake evoked by 600 pmol/l insulin-like growth factor I was similar to that evoked by 3000 pmol/l (500 µU/ml) insulin (Table 1). When amiloride (1 mmol/l) was added to the incubation medium, insulin-like growth factor I (600 pmol/l) had no effect on Na⁺, K⁺-ATPase activity (Table 1).

Previous studies in paired AIM samples demonstrated that in medium containing MI at a normal plasma level (70 µmol/l) raising the medium glucose from 5 to 30 mmol/l during a 30-min equilibration and a 30-min incubation causes a significantly reduced rate of Na⁺, K⁺-ATPase-mediated ⁸⁶Rb⁺/K⁺ uptake during the final 20 min of the incubation [13, 14]. In paired AIM samples that were both equilibrated and incubated in medium containing 30 mmol/l glucose and 70 µmol/l MI, the addition of insulin (100 µU/ml) to one sample during the 30-min incubation had no effect on the reduced resting rate of Na⁺, K⁺-ATPase activity (Table 2).

Paired AIM samples were equilibrated and then incubated for 30 min in medium containing 5 mmol/l glucose and 70 µmol/l MI with 100 µU/ml insulin added to both

samples; under these conditions the addition of norepinephrine (1 $\mu\text{mol/l}$) or of angiotensin II (100 nmol/l) to one paired sample during the final 20 min of the incubation evoked a significant increase in Na^+ , K^+ -ATPase activity (Table 2). When these experiments were repeated but with the medium glucose raised from 5 to 30 mmol/l , the addition of norepinephrine (1 $\mu\text{mol/l}$) or of angiotensin II (100 nmol/l) evoked no significant change in Na^+ , K^+ -ATPase activity (Table 2).

Discussion

In most mammalian cells Na^+ , K^+ -ATPase activity is acutely regulated primarily or in part by the intracellular $[\text{Na}^+]$ and an increase in the normal intracellular $[\text{Na}^+]$ automatically increases Na^+ , K^+ -ATPase activity [24]. This accounts for the fact that in specific cell types insulin stimulation of a biological process that causes increased Na^+ influx can evoke a secondary increase in Na^+ , K^+ -ATPase activity [25]; such an insulin effect is distinct from insulin regulation of Na^+ , K^+ -ATPase activity per se and its demonstration does not identify a cell type in which insulin deficiency or resistance is a potential cause for a derangement in the regulation of Na^+ , K^+ -ATPase activity (or of intracellular $[\text{Na}^+]$). Insulin regulates (stimulates) Na^+ , K^+ -ATPase activity only in specific cell types which do not include all of the cell types in which insulin has acute metabolic effects. Thus insulin regulates Na^+ , K^+ -ATPase activity in adipocytes [26, 27] and skeletal muscle [28] but not in hepatocytes [25]. Insulin stimulates increased Na^+ , K^+ -ATPase-mediated $^{86}\text{Rb}^+/\text{K}^+$ uptake in rat adipocytes and soleus muscle that is readily demonstrable during a 20–25 min exposure to the hormone and does not result from an increase in intracellular $[\text{Na}^+]$ or from an increase in Na^+ , K^+ -ATPase molecules in the plasma membrane [26–28]. In adipocytes insulin has been found to stimulate Na^+ , K^+ -ATPase activity by decreasing the K_m 's for intracellular Na^+ of the α_2 and α_1 Na^+ , K^+ -ATPase isoforms in the cell and by increasing the $V_{\max}$ of the α_2 isoform, whose activity is most markedly affected by insulin [26]. Insulin causes half-maximal stimulation of Na^+ , K^+ -ATPase-mediated $^{86}\text{Rb}^+/\text{K}^+$ uptake in adipocytes at 33 $\mu\text{U/ml}$ [27].

We examined the effects of insulin on Na^+ , K^+ -ATPase-mediated $^{86}\text{Rb}^+/\text{K}^+$ uptake in normal rabbit AIM, one of the most extensively used systems for studies of hormonal and neurotransmitter effects in VSM [29]. VSM cells comprise most of the cell mass in AIM, and these VSM retain their normal histological relationship(s) with the endothelium, whose injury or removal rapidly alters the normal pattern of energy metabolism in the VSM cells [20, 21] and also alters their responses to some hormones and neurotransmitters [18]. The AIM preparation employed in this study retains an intact, ultrastructurally normal endothelium and its normal pattern of energy metabolism [20, 21]. When AIM from normal, overnight fasted rabbits is incubated with normal rabbit plasma levels of glucose (5 mmol/l) and MI (70 $\mu\text{mol/l}$) in Krebs-Henseleit bicarbonate buffer containing 9% dextran, the resting AIM maintains a stable rate of Na^+ , K^+ -ATPase-mediated

$^{86}\text{Rb}^+/\text{K}^+$ uptake that is comprised of two distinct components [13, 14]. Under these conditions, exposing AIM to insulin at a high physiological plasma level, 100 $\mu\text{U/ml}$, for 30 min had no effect on its Na^+ , K^+ -ATPase activity. At 250 $\mu\text{U/ml}$ insulin caused a very small increase in Na^+ , K^+ -ATPase activity ($4.2 \pm 0.8\%$). At 500 $\mu\text{U/ml}$ insulin caused a larger, but still small, increase in Na^+ , K^+ -ATPase activity ($17.7 \pm 1.4\%$) that was completely inhibited by amiloride (1 mmol/l), an inhibitor of the Na^+/H^+ exchange system [23]. It thus appears that insulin does not regulate (stimulate) VSM Na^+ , K^+ -ATPase activity in AIM, but that supraphysiological insulin concentrations stimulate Na^+/H^+ exchange in VSM and cause small secondary increases in Na^+ , K^+ -ATPase activity.

An insulin effect in an isolated tissue that is demonstrable only at supraphysiological concentrations may reflect an effect that results from insulin binding to an insulin-like growth factor I receptor [30]. Furthermore, many growth factors have been found to stimulate Na^+/H^+ exchange in their target cells [31]. Human, insulin-like growth factor I (600 pmol/l) was found to cause an increase in Na^+ , K^+ -ATPase activity in AIM incubated with normal plasma levels of glucose and MI, which was inhibited by amiloride. The effect of insulin-like growth factor I on Na^+ , K^+ -ATPase activity in AIM was demonstrable at a molar concentration at which insulin has no effect on Na^+ , K^+ -ATPase activity, and 600 pmol/l insulin-like growth factor I caused an increase in Na^+ , K^+ -ATPase activity similar to that caused by 3000 pmol/l (500 $\mu\text{U/ml}$) insulin. This suggests that supraphysiological insulin concentrations probably stimulate Na^+/H^+ exchange and cause secondary increases in Na^+ , K^+ -ATPase activity in VSM by cross-reacting with an insulin-like growth factor I receptor.

As noted previously, a mechanism has been identified in rabbit AIM by which hyperglycaemia could decrease Na^+ , K^+ -ATPase activity in resting VSM and also impair adenosine-mediated modulation of VSM contractile responses to norepinephrine and angiotensin II in specific vessels [13–15]. In normal AIM exposure to glucose at an elevated plasma level for a short period causes derangements in Na^+ , K^+ -ATPase activity by inducing increased polyol pathway activity that impairs MI transport at a normal plasma MI level and thereby inhibits the effector arm of a novel adenosine- Na^+ , K^+ -ATPase regulatory system [13, 14]. Insulin does not have acute effects on the major, commonly studied parameters of glucose metabolism in rabbit AIM that is free of adventitial adipocytes [32–34]. However, the possibility remained that insulin at physiological plasma levels might modify the effects of an elevated glucose level on Na^+ , K^+ -ATPase activity in AIM if insulin had some presently unknown effect on polyol pathway activity or MI transport in AIM. In AIM provided with medium MI at a normal plasma MI level, raising the medium glucose from 5 to 10 or 30 mmol/l during a 30-min equilibration and 30-min incubation selectively inhibits a distinct adenosine-stimulated component of resting Na^+ , K^+ -ATPase activity and the adenosine-stimulated increases in Na^+ , K^+ -ATPase activity that are normally evoked when AIM is exposed to norepinephrine

(1 $\mu\text{mol/l}$) or angiotensin II (100 nmol/l) in a concentration known to cause contraction in rabbit aorta. These alterations in Na^+ , K^+ -ATPase activity are prevented if an aldose reductase inhibitor is added or if the medium MI is raised seven-fold [13–15]. In AIM equilibrated and incubated in medium containing 30 mmol/l glucose and MI at a normal plasma level, adding insulin (100 $\mu\text{U/ml}$) during the 30-min incubation had no effect on the reduced resting rate of Na^+ , K^+ -ATPase activity and did not modify the complete inhibition of the increases in Na^+ , K^+ -ATPase activity that are normally evoked when AIM is stimulated by norepinephrine (1 $\mu\text{mol/l}$) or angiotensin II (100 nmol/l). It thus appears that the presence (or absence) of insulin at a high physiological plasma level does not modify the derangements in VSM Na^+ , K^+ -ATPase activity in AIM that result from exposure to glucose in an elevated plasma level.

Our observations provide no support for the frequent speculation that insulin deficiency or resistance in VSM would impair a normal insulin stimulation of VSM Na^+ , K^+ -ATPase activity and is a potential cause for decreased VSM Na^+ , K^+ -ATPase activity and resulting alterations in vascular reactivity in diabetic states [35]. Rather, our observations suggest that hyperglycaemia is probably the factor that induces increased reactivity to norepinephrine and angiotensin II in specific arteries and arterioles in diabetic states.

Insulin resistance per se in VSM has also been postulated to be a possible cause for hypertension and for the reported associations between various insulin-resistant states and hypertension [35]. This hypothesis is based on the premise that insulin resistance in VSM could cause the loss of normal insulin stimulatory effects on glucose utilization for energy provision or on Na^+ , K^+ -ATPase activity and thereby cause derangements in ionic regulation in VSM that increase the vasoconstrictive responses to norepinephrine and angiotensin II [35]. This hypothesis is untenable, because a body of evidence indicates that insulin does not regulate glucose metabolism in VSM [32–34] and because the observations in this report indicate that insulin does not regulate Na^+ , K^+ -ATPase activity in VSM.

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