Enhanced effects of insulin and angiotensin II on intracellular pH and free cytosolic calcium in fibroblasts from microalbuminuric patients with non-insulin-dependent diabetes mellitus

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1. Whether an alteration in cell membrane cation transport after exposure to insulin and angiotensin II (two important growth promoters that have been shown to be involved in the pathogenesis of atherosclerosis and hypertension) is present in cells from non-insulin-dependent diabetes patients with microalbuminuria, a known risk factor for cardiovascular and renal disease, is unknown. We therefore examined intracellular pH and calcium changes after acute exposure to insulin and angiotensin II in cultured skin fibroblasts from eight non-insulin-dependent diabetes patients with and eight others without microalbuminuria and from a group of seven matched, normal control subjects.

2. Cultured fibroblasts were loaded with 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxy-methyl ester or fura 2-acetoxymethyl ester for continuous monitoring of intracellular pH and free calcium concentrations respectively.

3. In quiescent growth-arrested cells, both intracellular pH and free calcium concentrations were similar in the three groups of subjects. Acutely, insulin induced a gradual alkalinization in all groups of patients. The pH increase was significantly greater in non-insulin-dependent diabetes mellitus patients with microalbuminuria (\(\Delta\text{pH} +0.24 \pm 0.04\) pH units) than in normoalbuminuric patients with non-insulin-dependent diabetes mellitus (0.08 \(\pm\) 0.02; \(P<0.01\)) and normal control subjects (0.05 \(\pm\) 0.01; \(P<0.01\)). Although the alkalinizing effect of angiotensin II was smaller than that obtained by insulin, intracellular pH increase after angiotensin addition was more pronounced in non-insulin-dependent diabetes mellitus patients with microalbuminuria (\(\Delta\text{pH} +0.14 \pm 0.04\) pH units) than in those without (0.08 \(\pm\) 0.02; \(P<0.01\)) and in normal control subjects (0.02 \(\pm\) 0.02; \(P<0.01\)). That the increase in intracellular pH was mediated by the sodium–hydrogen antiport was demonstrated by its dependence on the presence of sodium in the medium and its inhibition by amiloride. Whereas insulin addition did not evoke any significant increase in intracellular free calcium levels in fibroblasts from the three groups studied, angiotensin II evoked a fast and transient rise in intracellular free calcium that was higher in fibroblasts from microalbuminuric patients with non-insulin-dependent diabetes mellitus than in cells from normoalbuminuric patients with non-insulin-dependent diabetes mellitus and control subjects. In the whole population of patients with non-insulin-dependent diabetes mellitus, the increase in intracellular pH after exposure to angiotensin II was positively correlated with intracellular free calcium increase (\(r = 0.53; P<0.05\)), suggesting a possible role of intracellular free calcium levels in the activation of the sodium–hydrogen antiport.

4. In conclusion, we have described an association between increased agonist-induced responsiveness of sodium–hydrogen antiport activity and the presence of microalbuminuria in patients with non-insulin-dependent diabetes mellitus. This increased responsiveness, persisting in cultured fibroblasts after several passages in vitro, suggests that in vitro phenotypic characteristics of fibroblasts are likely to be genetically determined and to be, at least in part, independent of the degree of metabolic control in vivo.

INTRODUCTION

The major cause of increased morbidity and premature mortality in patients with non-insulin-dependent diabetes mellitus (NIDDM) is cardiovascular disease [1, 2]. Several studies have shown that in NIDDM patients microalbuminuria is the
strongest predictor for risk of cardiovascular disease [3–6]. The mechanism responsible for accelerated development of atherosclerotic lesions in the arteries of these patients remains unknown.

In patients with insulin-dependent diabetes, a familial predisposition to hypertension and cardiovascular disease [7–9] appears to be an important determinant of susceptibility to renal disease and its cardiovascular complications. Studies of sodium–lithium countertransport, an intermediate phenotype of essential hypertension and its vascular complications [10, 11], have shown significant elevation in the rate of this cation transport system in insulin-dependent [12–14] and non-insulin-dependent diabetic patients [15, 16] with proteinuria or microalbuminuria and hypertension. The mechanism of sodium–lithium countertransport is in some respects similar to the ubiquitous physiological sodium–hydrogen (Na+/H+) antiport [17], a cell membrane exchanger involved in the regulation of intracellular pH, cell volume and growth [18] and sodium reabsorption in the proximal renal tubule [19]. An increase in Na+/H+ antiport activity has been described in leucocytes from insulin-dependent diabetic patients with elevated albumin excretion rate [20] as well as in patients with essential hypertension [21]. The persistence of this increased activity in long-term cultured skin fibroblasts [22, 23] and in immortalized lymphoblasts [24] from insulin-dependent diabetic patients with nephropathy suggest a likely intrinsic (possibly genetic) component in the pathogenesis of this altered membrane cation transport system. A similar alteration in Na+/H+ antiport activity after acid loading has also been observed in serially passaged fibroblasts derived from NIDDM patients with microalbuminuria and/or hypertension [25].

Recent interest has also been focused on the study of possible alterations of free cytosolic calcium in diabetes [26]. Substantial evidence has been gained in support of the role of an increased intracellular free calcium in both clinical and experimental hypertension [27, 28]. As contraction and growth of vascular smooth muscle cells are normally triggered by a rise in intracellular free calcium concentrations [29, 30], any abnormality in this ion handling may have important relevance in the pathogenesis of cardiovascular complications in NIDDM patients.

Insulin and angiotensin II (ANG II), two important growth promoters that have been involved in the pathogenesis of atherosclerosis and hypertension [31–34], exert their effect, at least in part, through the activation of Na+/H+ antiport and the mobilization of intracellular calcium [35, 36]. Whether an alteration in cell membrane cation transport after exposure to insulin and ANG II is present in cells from NIDDM patients with microalbuminuria and hypertension, two well-known risk factors for cardiovascular and renal disease, is unknown. We therefore examined intracellular pH and calcium changes after exposure to insulin and ANG II in cultured skin fibroblasts from NIDDM patients with and without microalbuminuria and from a group of matched normal control subjects.

SUBJECTS AND METHODS

Patients

Eight NIDDM patients with microalbuminuria were recruited consecutively from the outpatients clinic and were matched for diabetes duration, sex and body mass index (BMI) with eight normotensive NIDDM patients with normal albumin excretion rate (AER). All the diabetic patients included in this study fulfilled World Health Organization criteria for NIDDM [37] and were treated with sulphonylurea or metformin or both. Three out of eight microalbuminuric NIDDM patients had been treated for hypertension for at least 1 year. The other five microalbuminuric NIDDM patients were normotensive. The three patients taking antihypertensive drugs (one patient was taking angiotensin-converting enzyme inhibitors, the others furosemide) were asked to stop taking them at least 2 weeks before the study. Seven normal subjects without family history of hypertension or diabetes, well matched for age and sex, served as control subjects. All subjects were Caucasian and gave their informed consent to the study, which was approved by The Committee on Ethical Practice of University of Padua. Their demographic, clinical and biochemical features are shown in Table 1.

Microalbuminuria was defined as the presence of a persistent urinary AER between 20 and 200 mg/min in sterile urine. Hypertension was defined as systolic blood pressure higher than 140 mmHg and diastolic blood pressure higher than 90 mmHg or the use of known treatment for hypertension. Ophthalmoscopy through dilated pupils was carried out in all diabetic patients to assess the presence of retinopathy.

On the morning of the skin biopsy, height and weight were recorded without shoes and in light indoor clothing, and blood was taken for determination of glycosylated haemoglobin, serum total cholesterol and triacylglycerol concentrations and serum creatinine (Jaffe reaction rate method, Hitachi autoanalyser, BCL, U.K.). Glycosylated haemoglobin was measured by HPLC (normal range 4–6%). Serum total cholesterol and triacylglycerol concentrations were measured by enzymic assays (Boehringer Mannheim, Mannheim, Germany).

Arterial blood pressure was measured with a standard mercury sphygmomanometer, to the nearest 2 mmHg, in the dominant arm after at least 10 min rest in the supine position. Three timed overnight urine samples were collected for measure-
ment of urinary AER by radioimmunoassay [38] and the median value was used for calculation.

**Cell culture**

A skin biopsy was taken from the anterior surface of left forearm by excision under topical anaesthesia (ethyl chloride). Fibroblasts were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS). After the fourth passage, cells were harvested and stored in liquid nitrogen. For each experiment, fibroblasts were then thawed and grown as described above. All experiments were performed between passages 6 and 10.

**Measurement of intracellular pH**

After thawing, cells were seeded on coverslips, using about 2–3 × 10⁵ cells per coverslip. After at least 36 h growth in DMEM plus 10% FCS, upon achievement of morphological confluence, fibroblasts were rendered quiescent by 36 h serum withdrawal.

Intracellular pH was measured using the pH-sensitive dye 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) acetoxymethyl ester (AM) as previously described [25, 39]. Briefly, fibroblasts, loaded with BCECF-AM (10 μmol/l in DMEM without serum) at 37°C for 20 min, were inserted into a specially constructed thermostated cuvette in a Perkin–Elmer LS-50 luminescence spectrometer (Perkin-Elmer, Analytical Instruments, Norwalk, CT, U.S.A.). Intracellular pH in fibroblasts was determined in the Hepes-buffered saline containing (in mmol/l): 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 5 glucose, 20 Hepes/Tris, plus 1 g/l BSA (pH 7.4). The two excitation wavelengths were set at 490 and 435 nm with emission at 530 nm. Autofluorescence was subtracted from fluorescence readings before calculation of 490/435 ratios. The 490/435 ratios were subsequently converted to pH values at the end of each experiment by construction of a calibration curve to prevent bias from potential differences in position of the coverslip. This calibration of the fluorescent signal was achieved using high-concentration K⁺ buffers of various pH values containing 7 μmol/l nigericin [40]. Dye leakage was measured by removing cells from the cuvette after various times of incubation and was <1% over a 10 min interval. Because dye leakage could still potentially alter intracellular pH measurements, at the end of some experiments rapid lowering of extracellular pH with nitric acid caused no rapid change in fluorescent signal, indicating the absence of extracellular bound dye (data not shown).

After the determination of resting intracellular pH in fibroblasts for 10 min, cells were exposed to insulin (150 m-unit/l) or ANG II (0.1 μmol/l) and the effect of growth factor exposure on intracellular pH was recorded for the next 30 min. To verify whether the intracellular pH changes were mediated by the antipor, the same experiment was repeated in the presence of 1 mmol/l amiloride, an inhibitor of Na⁺/H⁺ antiport activity.

To measure intracellular buffering capacity, another coverslip with quiescent fibroblasts loaded with BCECF was exposed to 10 mmol/l ammonium chloride as described previously [25]. Buffer capacity at each intracellular pH value was calculated from (1) the equilibrium between NH₄⁺, NH₃ and pH in the extracellular medium as determined by the Henderson–Hasselbach relation using a pKₐ for NH₃ of 8.89 and (2) the formula Δ[NH₄⁺]/ΔpHᵢ, as described previously [41].

**Measurement of intracellular free calcium**

Cells were seeded onto coverslips and prepared as previously described. [Ca²⁺]ᵢ was measured by using

### Table 1. Clinical and biochemical features of NIDDM patients with and without microalbuminuria and of healthy control subjects. Data are given as means ± SEM, except for AER and triacylglycerol concentration, which are given as median and range. Statistical significance: *P < 0.05 compared with NIDDM without microalbuminuria and normal control subjects. fP < 0.05 compared with NIDDM with and without normoalbuminuria.

<table>
<thead>
<tr>
<th></th>
<th>NIDDM patients with microalbuminuria</th>
<th>NIDDM patients without microalbuminuria</th>
<th>Normal control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (M/F)</td>
<td>8 (4/4)</td>
<td>8 (3/5)</td>
<td>7 (3/4)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.5 ± 7.8</td>
<td>60.0 ± 4.5</td>
<td>57.7 ± 9</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>10.8 ± 4.1</td>
<td>10.1 ± 5.1</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.0 ± 7.3</td>
<td>28.2 ± 3.6</td>
<td>25.8 ± 4.7f</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.7 ± 1.8</td>
<td>9.1 ± 1.3</td>
<td>—</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.1 ± 2.4</td>
<td>6.1 ± 1.0</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>2.6 (1.0–6.9)</td>
<td>2.8 (0.6–9.9)</td>
<td>1.7 (0.6–2.4)f</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>153 ± 7*</td>
<td>133 ± 4</td>
<td>119 ± 3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>90 ± 3*</td>
<td>82 ± 3</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>AER (μg/min)</td>
<td>145 (55–280)</td>
<td>7 (2–18)</td>
<td>6 (2–12)</td>
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<td>Serum creatinine (mmol/l)</td>
<td>0.84 ± 0.09</td>
<td>0.82 ± 0.09</td>
<td>0.79 ± 0.02</td>
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<tr>
<td>Retinopathy (n)</td>
<td>7</td>
<td>3</td>
<td>—</td>
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the \([\text{Ca}^{2+}]\)-sensitive probe fura 2-AM. Fluorescence measurements were performed using a Perkin–Elmer LS-50 luminescence spectrometer. Briefly, fibroblasts were loaded for 30 min in standard saline containing 2 \(\mu\)mol/l fura 2-AM. After washing, the monolayer was superfused at 2 ml/min with standard saline using a peristaltic pump to prevent the accumulation of extracellular fura 2. Cells were excited at the wavelength pair 340/380 nm and the emission was monitored at 500 nm. Calibration of fluorescent signal was performed after each experiment as described previously [42].

All experiments were in triplicate, and all were performed by an operator masked to the identity of the subjects studied. For each individual experiment the mean of triplicate determinations was calculated. The inter-assay variations for intracellular pH and calcium determinations were below 2% and 6% respectively.

All chemicals were obtained from Sigma (St. Louis, MO, U.S.A.), except for BCECF-AM, which was purchased from Calbiochem (La Jolla, CA, U.S.A.) and for fura 2-AM from Boerhinger Mannheim Biochemica (Germany).

**Statistical analysis**

Data are given as means±SEM. Statistical calculation was performed using the Statistical Package for the Social Sciences (SPSS-PC). The steady-state values of intracellular pH and intracellular free calcium are the mean of at least 600 fluorimetric readings taken over a 5 min period. Differences between group means were tested by analysis of variance, and comparisons between groups were carried out using the Newman–Keuls test. The relationship between variables was tested with linear regression analysis. Skewed data (AER and plasma triacylglycerol concentrations) were logarithmically transformed.

**RESULTS**

NIDDM patients were well matched for sex, age, BMI, known duration of diabetes, glycosylated hæmoglobin and lipid plasma levels. Systolic and diastolic blood pressure levels were significantly higher in those with microalbuminuria. Normal control subjects were matched for age and sex with NIDDM patients but had a significantly lower BMI than diabetic patients (Table 1).

**Effect of insulin and ANG II on intracellular pH**

In 24 h serum-deprived, quiescent, non-proliferating cells, intracellular pH in the absence of bicarbonate was similar in NIDDM patients with and without microalbuminuria and in normal control subjects (7.09±0.03, 7.07±0.04 and 7.06±0.04 respectively) (Figs 1 and 2). When cells were exposed to insulin (150 m-unit/l), there was a gradual and significant alkalinization in the three groups of patients. A new steady state of intracellular pH was reached in approximately 10 min and persisted for the duration of the experiment (30 min) (Fig. 1). The pH increase was significantly greater in NIDDM patients with microalbuminuria (ApH +0.24±0.04 pH units) than in normoalbuminuric NIDDM patients (0.08±0.02; \(P<0.01\)) and normal control subjects (0.05±0.01; \(P<0.01\)) (Fig. 2).

Although the alkalinizing effect of ANG II was smaller than that obtained by insulin addition, ANG II addition caused an initial and transient decrease in intracellular pH, followed by a progressive and significant increase that was again more pronounced in NIDDM patients with microalbuminuria (ApH +0.14±0.04 pH units; \(P<0.01\)) than in those without (0.08±0.02) and in normal control subjects (0.02±0.02) (Figs 1 and 3). When insulin was tested in combination with ANG II, no synergistic responses were observed in any of the groups.
Intracellular pH and calcium in fibroblasts

Effect of insulin and ANG II on [Ca^{2+}].

Resting [Ca^{2+}] levels in cultured fibroblasts were similar in the three groups of subjects (72 ± 6 nmol/l in NIDDM patients with microalbuminuria compared with 60 ± 4 nmol/l in those without microalbuminuria and 71 ± 6 nmol/l in normal control subjects). Addition of ANG II evoked a fast and transient rise in [Ca^{2+}]; (Fig. 4). The kinetics of [Ca^{2+}] increase after angiotensin addition was similar in the three groups (time to peak about 30 s), but the maximum increase above resting level was higher in fibroblasts from microalbuminuric NIDDM patients (233 ± 14 nmol/l; P < 0.01) than in cells from normoalbuminuric NIDDM patients (170 ± 6) and control subjects (177 ± 18) (Fig. 4). That the ANG II effect on intracellular pH and [Ca^{2+}] was receptor mediated was confirmed by the complete inhibition of intracellular pH rise and [Ca^{2+}] spike by an ANG II receptor antagonist, saralasin, simultaneously applied in equimolar concentrations (data not shown). In contrast, insulin addition did not evoke any significant increase in [Ca^{2+}] in fibroblasts from the three groups studied (Fig. 4).

Correlations

In the whole population of NIDDM patients, the increase in intracellular pH after exposure to ANG II was positively correlated with [Ca^{2+}]; increase (r = 0.53; P < 0.05) (Fig. 5). No correlation was found between subjects’ age, known duration of diabetes, blood pressure levels, blood glucose levels and glycosylated haemoglobin and intracellular pH and [Ca^{2+}] changes after acute exposure to insulin and ANG II in any of the groups studied.

DISCUSSION

This study demonstrates that insulin and ANG II increased intracellular pH in long-term cultured skin fibroblasts and that this effect is greater in cells from microalbuminuric NIDDM patients than in those from normoalbuminuric NIDDM patients and control subjects. This effect was mediated by sodium–hydrogen antiport activation as suggested by its dependence on the presence of extracellular sodium and the inhibition induced by amiloride. As buffering power capacity was similar in all groups, these findings are consistent with an overactivity of Na^{+}/H^{+} antiport in these cells. Moreover, ANG II induced an increase in [Ca^{2+}], which was significantly greater in fibroblasts from microalbuminuric NIDDM patients than in those from normoalbuminuric NIDDM patients and control subjects. In contrast, insulin did not affect [Ca^{2+}] levels in any of the patients groups.

When fibroblasts were exposed to ANG II but not to insulin, a brief but significant acidification studied (data not shown). The increase in intracellular pH was dependent on the presence of sodium in the medium and was inhibited by amiloride, an inhibitor of the Na^{+}/H^{+} antiport (data not shown). The differences observed between the groups of patients were not due to different intracellular buffering capacity, which was similar in all groups (23 ± 1.2 mmol/l per pH unit in NIDDM patients with microalbuminuria, 24 ± 1.4 in those without microalbuminuria and 23 ± 1.5 in normal control subjects).
occurred. This is in agreement with previous studies which have shown that cell acidification requires an increase in [Ca^{2+}]; and is caused by H+ entry coupled to calcium efflux [43, 44].

Abnormalities in the activity of Na+/H+ antiport have been observed in a variety of blood cells 'ex vivo' [20, 21, 45, 46] from subjects with essential hypertension and from insulin-dependent diabetic patients with nephropathy. An accelerated intracellular pH recovery was also found in skeletal muscle 'in vivo' in a group of subjects with essential hypertension, suggesting that an enhanced antiport activity may be operating even in more physiological conditions [47]. The importance of intrinsic (possibly genetic) factors in determining this particular phenotype is underlined by the persistence of the kinetic abnormalities of Na+/H+ exchange in immortalized lymphoblasts and long-term cultured skin fibroblasts from insulin-dependent diabetic patients with nephropathy [22–24]. Recently an increased maximal velocity of Na^+/H^+ exchange was also found in fibroblasts from NIDDM patients with microalbuminuria or hypertension [25]. The similarity in the kinetic changes of the antiport between IDDM patients with nephropathy, NIDDM patients with microalbuminuria and those from non-diabetic subjects with essential hypertension [48], supports the contention that an inherited predisposition to essential hypertension increases the risk of diabetic renal disease. However, the method used in these studies evaluating the activity of the Na^+/H^+ antiport was based on the measurement of the initial rate of intracellular pH recovery from an acid load, and, in this experimental condition, transport activity was assessed at intracellular pH values well below physiological resting intracellular pH. In contrast, this study demonstrates that a significantly greater agonist-induced alkalinization, reflecting an increased activity of Na^+/H^+ antiport, is also present at more physiological intracellular pH values in cells from NIDDM patients with microalbuminuria compared with normoalbuminuric NIDDM patients and normal control subjects.

It is of note that in our study, unlike others, physiological doses of insulin were used, which can be observed in subjects with insulin resistance. Our data, which provide direct evidence for insulin stimulation of the Na^+/H^+ antiport in human fibroblasts, are in agreement with findings of insulin-stimulated Na^+/H^+ exchange in skeletal muscle and kidney proximal cells [36, 49, 50].

That ANG II effect on intracellular pH was receptor mediated was confirmed by the complete inhibition of intracellular pH rise by the ANG II receptor antagonist salarasin when simultaneously applied in equimolar concentrations. The evidence for functional and specific ANG II receptors in fibroblasts has been already established [51, 52].

The evaluation of [Ca^{2+}]_i changes after insulin and ANG II addition suggests the presence of different mechanisms of activation of Na^+/H^+ exchange. Whereas ANG II induces an [Ca^{2+}]_i increase, which was significantly greater in NIDDM
patients with than in those without microalbuminuria or normal control subjects, insulin failed to increase \([\text{Ca}^{2+}]\) in cells from all patient groups. This insulin failure to elicit an immediate rise in \([\text{Ca}^{2+}]\) has also been observed by other investigators [53]. The close relationship between the increment in intracellular pH and the rise in \([\text{Ca}^{2+}]\) after ANG II stimulation suggested a possible role of \([\text{Ca}^{2+}]\) in the activation of the Na\(^+/\)H\(^+\) antiport. It is known that ANG II elicits cellular responses by binding to specific high-affinity cell-surface receptors which, through the interaction with membrane-associated regulatory proteins, also termed G-proteins, activate phospholipase C followed by release of inositol triphosphate-sensitive intracellular calcium pools and stimulation of protein kinase C, which leads eventually to Na\(^+/\)H\(^+\) exchange activation [54]. On the other hand, insulin activates a distinct transmembrane signalling pathway, involving a tyrosine kinase activity [55]. When physiological levels of insulin were tested in combination with ANG II, no synergistic responses were observed, similarly to the data reported by Gesek and Schoolwerth [49] in isolated proximal tubules. Taken together, these results are consistent with the notion that the final step in Na\(^+/\)H\(^+\) exchange activation is mediated by an as yet unidentified serine protein kinase that can integrate signals from both receptor tyrosine kinases and G protein-coupled receptors [56].

Although blood pressure values were higher in NIDDM patients with microalbuminuria, a relationship between systolic or diastolic blood pressure and intracellular pH [\([\text{Ca}^{2+}]\)] was not observed in our patients. In particular, intracellular pH increase and [\([\text{Ca}^{2+}]\)] changes after agonist addition in the three hypertensive microalbuminuric NIDDM patients were similar to those observed in the other five normotensive microalbuminuric NIDDM patients. On the other hand, that microalbuminuria is directly related to an elevated Na\(^+/\)H\(^+\) antiport activity in NIDDM patients, independent of the presence of hypertension, was also recently shown in another study from our group [25].

Although glucose itself has been demonstrated to have an effect on Na\(^+/\)H\(^+\) antiport activity in leucocytes [57] and cultured vascular smooth muscle cells [58], it is unlikely that hyperglycaemia could explain the difference between the diabetic groups in that our groups of patients had similar metabolic control, as indicated by similar glycated haemoglobin levels, and, as far we can tell, they were exposed to the same duration and intensity of disease. However, we could not exclude the possibility of a different effect of prolonged hyperglycaemia in different individuals, but this again would imply an intrinsic difference.

The possibility exists that the differences in intracellular pH and [\([\text{Ca}^{2+}]\)] after agonist addition may reflect or contribute to the differences in cell growth rate observed in lymphoblasts from subjects with essential hypertension [48] and in cultured skin fibroblasts from IDDM patients with nephropathy [22].

In conclusion, we have described an association between increased agonist-induced responsiveness of Na\(^+/\)H\(^+\) antiport activity and the presence of microalbuminuria in NIDDM patients. This increased responsiveness, persisting in cultured fibroblasts after several passages in vitro, suggests that in vitro phenotypic characteristics of fibroblasts are likely to be genetically determined and to be, at least in part, independent of the degree of in vitro metabolic control. However, further studies comparing Na\(^+/\)H\(^+\) antiport activity in non-diabetic offspring of NIDDM patients with microalbuminuria with that of non-diabetic offspring of NIDDM patients without microalbuminuria will help to confirm the intrinsic nature of cation transport abnormalities described in this study. If the abnormalities in cell function, similar to that described in skin fibroblasts, apply to other cell types, such as vascular smooth muscle or mesangial cells, they could contribute to the pathogenesis of renal and cardiovascular complications in microalbuminuric NIDDM patients.

REFERENCES


Intracellular pH and calcium in fibroblasts


