Low O2-induced ATP release from erythrocytes of humans with type 2 diabetes is restored by physiological ratios of C-peptide and insulin

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Submitted 15 May 2014; accepted in final form 24 July 2014

Am J Physiol Regul Integr Comp Physiol 307: R862–R868, 2014. First published July 30, 2014; doi:10.1152/ajpregu.00206.2014.—ATP release from erythrocytes in response to reduced oxygen (O2) tension stimulates local vasodilation, enabling these cells to direct perfusion to areas in skeletal muscle in need of O2. Erythrocytes of humans with type 2 diabetes do not release ATP in response to low O2. Both C-peptide and insulin individually inhibit low O2-induced ATP release from healthy human erythrocytes, yet when coadministered at physiological concentrations and ratios, no inhibition is seen. Here, we determined: that 1) erythrocytes of healthy humans and humans with type 2 diabetes possess a C-peptide receptor (GPR146), 2) the combination of C-peptide and insulin at physiological ratios rescues low O2-induced ATP release from erythrocytes of humans with type 2 diabetes, 3) residual C-peptide levels reported in humans with type 2 diabetes are not adequate to rescue low O2-induced ATP release in the presence of 1 nM insulin, and 4) the effects of C-peptide and insulin are neither altered by increased glucose levels nor explained by changes in erythrocyte deformability. These results suggest that the addition of C-peptide to the treatment regimen for type 2 diabetes could have beneficial effects on tissue oxygenation, which would help to ameliorate the concomitant peripheral vascular disease.

Erythrocytes release oxygen (O2), as well as the potent vasodilator ATP, when exposed to low O2 tension, enabling these circulating cells to contribute to the efficient matching of O2 supply to metabolic need in skeletal muscle (5, 11, 13, 14, 30, 33, 43). This release of ATP stimulates local vasodilation, which is conducted upstream to feed arterioles, thereby directing perfusion to localized tissue areas lacking adequate oxygenation (11, 13, 43). However, erythrocytes from humans with type 2 diabetes exhibit an impairment in the ability to release ATP in response to reduced tissue O2 tension, which limits their participation in this important mechanism for the control of perfusion distribution (38, 42, 44).

Type 2 diabetes is associated with an increased incidence of vascular complications, including peripheral vascular disease and impaired wound healing (2, 15, 36). Impairment of low O2-induced ATP release from erythrocytes could contribute to these complications. In support of this hypothesis, it was reported that isolated skeletal muscle arterioles perfused with erythrocytes from healthy humans—cells that respond to low O2 with ATP release—dilate when exposed to reduced, extraluminal O2, a model of increased tissue O2 need (11, 43). In contrast, low O2-induced ATP release from erythrocytes from humans with type 2 diabetes is severely impaired (38, 42, 44), and these cells do not stimulate dilation of isolated, perfused skeletal muscle arterioles under similar conditions (42).

Whereas there is clearly an inherent defect in low O2-induced ATP release from erythrocytes of humans with type 2 diabetes, this can be exacerbated further by the treatment of the disease. Specifically, incubation of healthy human erythrocytes with insulin alone, at concentrations reported to be necessary to regulate blood glucose levels in individuals with insulin resistance, inhibits low O2-induced ATP release (19).

Although insulin alone is administered to humans with type 2 diabetes, in vivo, insulin is released from the pancreas, along with the connecting peptide (C-peptide) (46). The two peptides are released at equimolar concentrations, but due to differing clearance rates, circulating C-peptide levels exceed those of insulin (46). Although initially thought to be inert, recent studies have shown that C-peptide has biological activity (21, 22) and has been suggested to attenuate and perhaps reverse some diabetes-associated microvascular complications (22).

C-Peptide may also have important effects on erythrocytes. It has been shown in humans with type 1 diabetes that C-peptide improves erythrocyte deformability (18, 27). In addition, when coinubated with insulin at physiological ratios and concentrations, C-peptide has been shown to oppose the adverse effects of insulin on low O2-induced ATP release from healthy human erythrocytes (34). Although the signaling pathway(s) initiated by C-peptide are not well characterized, there is strong evidence that C-peptide stimulates a pertussis toxinsensitive G-protein-coupled receptor (GPCR) (21). Recently, GPR146, an orphan GPCR, was suggested to be a C-peptide receptor on KATOIII cells, based on the loss of C-peptide-stimulated cFos expression after small interfering RNA (siRNA) knockdown of GPR146 (50).

Here, we investigated the hypotheses: that 1) erythrocytes of healthy humans and humans with type 2 diabetes possess GPR146; 2) this receptor mediates the effects of C-peptide on ATP release from human erythrocytes; and 3) physiological ratios of C-peptide and insulin rescue the ability of erythrocytes from humans with type 2 diabetes to release ATP when exposed to reduced O2 tension.

METHODS

Isolation of human erythrocytes. Blood was obtained from healthy volunteers (n = 23) or individuals with type 2 diabetes (n = 25) via venipuncture, using a syringe containing heparin (500 U). Blood was centrifuged at 500 g at 4°C for 10 min, and the plasma, buffy coat, and
uppermost layer of erythrocytes were removed by aspiration. The remaining erythrocytes were resuspended and washed three times in a physiological buffer containing (in mM) 21.0 Tris, 4.7 KCl, 2.0 CaCl$_2$, 140.5 NaCl, 1.2 MgSO$_4$, 5.5 glucose, and 0.5% BSA with the pH adjusted to 7.4. After the final wash, the hematocrit of the isolated erythrocytes was measured. Erythrocytes were prepared on the day of use. Informed consent was obtained from all subjects, and the protocol for blood removal was approved by the Institutional Review Board of Saint Louis University.

**Measurement of ATP.** ATP was measured using the luciferin-luciferase assay. A 200-µl sample of the erythrocyte suspension (0.04% hematocrit) was injected into a cuvette containing 100 µl firefly lantern extract (10 mg/ml distilled water, FLE-250; Sigma, St. Louis, MO) and 100 µl D-luciferin solution (10 mg/20 ml distilled water; Research Products International, Mount Prospect, IL). The light emitted from the reaction with ATP was quantified using a luminometer (TD-20-20; Turner Designs, Sunnyvale, CA). A standard curve was generated for each experiment. ATP values were normalized to the amount released from 4 × 10$^8$ cells.

**Measurement of free hemoglobin.** To exclude the possibility that amounts of ATP measured were the result of erythrocyte lysis, samples used for measuring ATP were centrifuged at 500 g for 10 min at room temperature. The presence of hemoglobin in the supernatant was determined by measurement of light absorbance at 405 nm (23). Samples in which free hemoglobin increased were not included.

**Purification of erythrocyte membranes.** Washed erythrocytes were diluted 1:100 with ice-cold hypotonic buffer containing 5 mM Tris-HCl, 2 mM EDTA, pH 7.4, and stirred vigorously at 4°C for 20 min. The lysate was centrifuged at 23,000 g for 15 min at 4°C. The supernatant was removed and discarded. The pellet was washed with ice-cold buffer and centrifuged, and the procedure was repeated a second time. The membranes were frozen at −80°C. Membrane protein concentrations were determined using the BCA Protein Assay (Pierce, Thermo Fisher Scientific, Rockford, IL).

**Western analysis.** Erythrocyte membranes were suspended in buffer containing 150 mM NaCl, 50 mM Tris, pH 8.0, 1% Nonidet P-40, followed by centrifugation to remove insoluble debris. The resulting supernatant was loaded on a 4–20% gradient Tris-glycine precast gel (Bio-Rad Laboratories, Hercules, CA) at the same protein concentration. Electrophoresis at 100 V was carried out for 45 min in an SDS–Tris-glycine running buffer. Proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories) using ice-cold 1× Towbin’s transfer buffer [25 mM Tris, 192 mM glycine, 20% (vol/vol) methanol (pH 8.3)] for 1 h at 60 mA. The membrane was blocked overnight in Tris-buffered saline (TBS) with 0.5% BSA with agitation at 4°C. Primary antibody incubation was with a rabbit polyclonal antibody to GPR146 (G299; Assay Biotechnology, San Francisco, CA) at a 1:1,000 dilution for 1 h at room temperature with agitation. After being washed (3 × 10 min in TBS with 0.5% Tween 20), the blot was incubated with an anti-rabbit antibody (Jackson ImmunoResearch, West Grove, PA) at a 1:20,000 dilution for 1 h at room temperature. The blot was treated with Immobilon enhanced chemiluminescence reagent (Millipore, Billerica, MA) for 1 min and imaged with radiography film.

**Immunohistochemistry.** Formalin-fixed, paraffin-embedded renal tissue, obtained at autopsy from a human patient, was cut into 7 µm sections. Slides were deparaffinized in xylene and rehydrated, followed by antigen retrieval with proteinase K in Tris-EDTA, CaCl$_2$ buffer for 20 min at 37°C. Sections were then rinsed in TBS with 0.05% Tween 20. Slides were blocked for 30 min at room temperature in TBS with 0.1% cold fish-skin gelatin, 0.5% Triton X-100, 5% donkey serum, and 5% human serum. Sections were then incubated for 1 h at room temperature with a 1:200 dilution (final 0.005 mg/ml) of a rabbit polyclonal antibody to GPR146 (ab117104; Abcam, Cambridge, MA) or with a 1:200 dilution (final 0.0055 mg/ml) of whole rabbit IgG (Jackson ImmunoResearch). Slides were subsequently rinsed 3× in TBS and incubated for 45 min at room temperature with a donkey polyclonal antibody to rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch). Slides were washed in TBS ×3 and incubated with ImmPACT DAB (Vector Laboratories, Burlingame, CA), counterstained with Hematoxylin QS (Vector Labs), dehydrated, and mounted with coverslips. Light microscopy was performed on a Leica DM1000 (Leica Microsystems, Buffalo Grove, IL), fitted with a Canon EOS T1 camera (Canon USA, Melville, NY). Postprocessing for color balance (globally applied for white-point correction) and resizing (globally applied) was performed using Pixelmator (Vilnius, Lithuania).

**Determination of ATP release from erythrocytes in response to reduced O$_2$ tension in the presence and absence of C-peptide and insulin.** Isolated erythrocytes were diluted to a 20% hematocrit in tonometer buffer (4.7 mM KCl, 2.0 mM CaCl$_2$, 140.5 mM NaCl, 1.2 mM MgSO$_4$, 5.5 mM glucose, 24.8 mM NaHCO$_3$, 5.5 mM dextrose, 0.5% BSA, pH 7.4) at 37°C and equilibrated for 30 min in a thin-film tonometer (model DEQ1; Cameron Instruments, Guelph, Ontario, Canada) with gas containing 15% O$_2$, 6% carbon dioxide (CO$_2$), and balance nitrogen (N$_2$) (normoxia, partial pressure of O$_2$ (P$_{O2}$) = 110 ± 5 mmHg). ATP levels were then determined. The erythrocytes were then exposed to reduced O$_2$ with gas containing 0% O$_2$, 6% CO$_2$, and balance N$_2$ (low O$_2$; P$_{O2}$ = 15 ± 5 mmHg) for 10 min, with ATP levels again determined. The effect of a 20-min pretreatment of erythrocytes with insulin (1 nM; Humalog U-100; Eli Lilly, Indianapolis, IN) and C-peptide (0.4, 1, 4, or 6 nM; human; amino acid sequence 55–89; 97.4% pure by HPLC analysis; Sigma) on low O$_2$-induced ATP release was determined. In separate studies, the effect of coadministration of C-peptide and insulin at a 1:1 ratio on low O$_2$-induced ATP release was determined in the presence of high glucose (16.5 mM).

**Determination of ATP release from erythrocytes exposed to reduced O$_2$ tension in the presence of C-peptide or insulin after preincubation with an antibody to an external epitope of GPR146.** Isolated erythrocytes were diluted to a 20% hematocrit in tonometer buffer (described above) at 37°C and incubated with 10 µl or 30 µl GPR146 antibody (rabbit polyclonal; ab117104; 1 mg/ml; Abcam). These erythrocyte suspensions were equilibrated for 30 min in a thin-film tonometer with gas containing 15% O$_2$, 6% CO$_2$, and balance N$_2$ (normoxia, P$_{O2}$ = 107 ± 3 mmHg) before the addition of 1 nM C-peptide or 1 nM insulin. After 20 min, ATP levels were measured. The erythrocytes were then exposed to reduced O$_2$ with gas containing 0% O$_2$, 6% CO$_2$, and balance N$_2$ (low O$_2$; P$_{O2}$ = 16 ± 4 mmHg) for 10 min with ATP levels measured again.

**Measurement of erythrocyte deformability.** Erythrocyte deformability was measured using the St. George’s blood filterometer (Carri-Med, Dorking, UK) (39–41). This device develops a calibrated pressure gradient across a vertically mounted, 13-mm diameter polycarbonate filter (Nuclepore) with 9.53 mm exposed surface diameter and average pore size of 5 µm. Proximal to the filter, the inlet tube was filled with either buffer (in mM: 21.0 Tris(hydroxymethyl)amino methane, 4.7 KCl, 2.0 CaCl$_2$, 140.5 NaCl, 1.2 MgSO$_4$, 5.5 glucose, and 0.5% BSA, pH adjusted to 7.4 at 37°C) alone or buffer containing erythrocytes, diluted to a 10% hematocrit. The erythrocyte suspension or buffer alone was then passed through the calibrated filter, and the transit time was recorded. The ratio between the rate at which the erythrocyte suspension traversed the filter relative to that of buffer alone was used to determine the red (blood) cell transit time (RCTT). If average filter pore size and hematocrit are kept constant, then RCTT is a unitless index of the degree of deformability of the erythrocytes. A decrease in the RCTT indicates an increase in erythrocyte deformability. Measurements of erythrocyte deformability were made after a 20-min incubation with C-peptide (1 nM), insulin (1 nM), or a 1:1 ratio of C-peptide and insulin (1 nM each) or vehicle, saline.

**Statistical analysis.** Statistical significance was determined using an ANOVA. In the event that the F-ratio indicated that a change had occurred, a Fisher’s least significant difference test was performed to
identify individual differences between groups. Results are reported as
means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Characteristics of the subjects. Individuals with type 2 diabetes were identified by physicians at Saint Louis University in the Endocrinology Clinic. A patient history was collected for each individual, including a detailed listing of current medications and the patient’s age. The degree of glycemic control in humans with type 2 diabetes was determined by measurement of hemoglobin A1C (HbA1c) at the time of blood removal (A1CNow+ device; Bayer, Sunnyvale, CA). The patients were not fasted before blood withdrawal. The subjects studied were healthy human volunteers (n = 23; 15 women, 8 men) and humans with type 2 diabetes (n = 25; 12 women, 13 men) with a mean age of 36 yr (range 18–64 yr) and 59 yr (range 31–82 yr), respectively. The average HbA1c of humans with type 2 diabetes in this study was 8.6% (70 mmol/mol) with a range from 5.6% to 15.3% (38–144 mmol/mol). Patients with type 2 diabetes were treated with multiple drugs in various combinations, including aspirin (n = 13), angiotension-converting enzyme inhibitors or angiotension receptor blockers (n = 16), β-adrenergic receptor blockers (n = 8), oral hypoglycemic agents (n = 14), insulin (n = 18), lipid-lowering drugs (n = 13), calcium channel blockers (n = 4), diuretics (n = 5), and the phosphodiesterase 3 (PDE3) inhibitor, cilostazol (n = 2). The nature of the patient’s illness precluded discontinuation of medications. Record-keeping was in compliance with Health Insurance Portability and Accountability Act regulations.

Western analysis of a C-peptide receptor. With the use of an antibody directed against an intracellular domain of human GPR146, a band of ~36 kDa, consistent with the molecular weight of the receptor, was identified in membranes of healthy human erythrocytes, as well as in the human erythroleukemia cell line, TF-1, and the human gastric tumor cell line, KATOIII (positive controls; Fig. 1A) (50). A similar band was detected in membranes of erythrocytes of humans with type 2 diabetes with HbA1c levels ranging from 5.6 to 15.3 (Fig. 1B). Additionally, immunohistochemistry, using an antibody directed to an external epitope of GPR146, demonstrated immunoreactivity confined to erythrocytes in tissue sections of several organs, including human kidney (Fig. 1C). No reactivity was observed in an isotype-matched control (Fig. 1D).

Effect of preincubation with a GPR146 antibody on low O2-induced ATP release in the presence of C-peptide or insulin. Preincubation of healthy human erythrocytes with the GPR146 antibody used in immunohistochemistry studies, which is directed to an external epitope of the receptor, attenuated C-peptide-mediated inhibition of ATP release in response to low O2 in a concentration-dependent manner (Fig. 2A). In contrast, the antibody had no effect on insulin-induced inhibition of low O2-induced ATP release (Fig. 2B).

Effect of physiological ratios of C-peptide and insulin on low O2-induced ATP release from erythrocytes of humans with type 2 diabetes. As depicted in Fig. 3, coincubation of erythrocytes of humans with type 2 diabetes, with a 1:1 or 4:1 ratio of C-peptide to insulin, restored the ability of these cells to release ATP in response to low O2. However, at a ratio of 6:1 (6 nM C-peptide to 1 nM insulin), low O2-induced ATP release was inhibited, as was reported previously with healthy human erythrocytes (34). ATP levels at normoxia did not differ among groups.

Residual C-peptide in humans with type 2 diabetes is insufficient to prevent the adverse effect of insulin on low O2-induced ATP release from erythrocytes. Individuals with type 2 diabetes who require insulin therapy have been reported to have circulating C-peptide levels of ~0.4 nM (9, 10, 20). We
determined that 0.4 nM C-peptide is insufficient to restore low 
O2-induced ATP release from erythrocytes of humans with 
type 2 diabetes in the presence of 1 nM insulin, a concentration 
of insulin that may be required for the treatment of type 2 
diabetes (Fig. 4).

Effect of acute exposure to high glucose in the presence of 
C-peptide or insulin on low O2-induced ATP release from 
erythrocytes of humans with type 2 diabetes. Erythrocytes 
isolated from healthy subjects, type 2 diabetic patients, 
and type 1 diabetic patients were incubated with saline 
(control) or various ratios of C-peptide (1, 4, or 6 nM) to 
imulin (1 nM) for 20 min while exposed to a gas mixture 
containing 15% O2, 6% CO2, and balance N2 (normoxia). ATP 
release was determined during normoxia and after a 10-min 
exposure to 0% O2, 6% CO2, and balance N2 (low O2). Values 
are means ± SE. *P < 0.05, significant increase from normoxia; †P < 0.01, significant increase from normoxia.

Influence of C-peptide and/or insulin on deformability of 
erthrocytes. Incubation of erythrocytes from humans with 
type 1 diabetes with insulin or C-peptide has been shown to 
enhance erythrocyte deformability (12, 18). We determined 
that in our series of patients with type 2 diabetes, erythrocyte 
deformability was not decreased. Moreover, neither insulin nor 
C-peptide, either alone or in combination, had any effect on 
deformability of erythrocytes from healthy humans or humans 
with type 2 diabetes (Table 1).

DISCUSSION

The release of both O2 and the potent vasodilator ATP from 
erthrocytes exposed to reduced O2 tension contributes to 
the appropriate distribution of perfusion required for adequate 
tissue oxygenation in skeletal muscle (5, 11, 13, 43). 
Importantly, it has been reported that low O2-induced ATP release 
from erythrocytes of humans with type 2 diabetes is severely 
impaircd (38, 42, 44). Moreover, these cells do not stimulate 
dilation of isolated, perfused skeletal muscle arterioles exposed 
to reduced extraluminal O2, a model of increased tissue O2.
lineage, not only produced GPR146 protein (Fig. 1A) but also produced GPR146 mRNA (50). Finally, immunohistochemistry provided further evidence of the presence of GPR146 on human erythrocytes (Fig. 1, C and D). Although these data demonstrate the presence of GPR146 on erythrocytes, this does not establish a relationship between GPR146 and C-peptide-mediated effects on low O2-induced ATP release from erythrocytes.

To determine that GPR146 is responsible for C-peptide-mediated effects on low O2-induced ATP release from erythrocytes, we preincubated healthy human erythrocytes with an antibody directed to an external epitope of GPR146 with the intent of blocking the interaction between C-peptide and the receptor. Antibodies directed against an extracellular receptor domain to block activity have been used successfully with other receptors (6, 26, 31). Here, we show that preincubation of healthy human erythrocytes with the GPR146 antibody attenuates C-peptide-mediated inhibition of low O2-induced ATP release in a concentration-dependent manner (Fig. 2). Importantly, preincubation with the antibody had no effect on insulin-induced inhibition of low O2-induced ATP release, indicating that the antibody did not act in a nonspecific manner to stimulate low O2-induced ATP release. Taken together, these data provide strong evidence that GPR146 is a receptor responsible for C-peptide-mediated effects on low O2-induced ATP release from erythrocytes.

As reported previously, coincubation of healthy human erythrocytes with C-peptide and insulin at physiological ratios and concentrations does not interfere with low O2-induced ATP release (34). Here, we report that coincubation of erythrocytes from humans with type 2 diabetes with physiological ratios of 1:1 and 4:1 (C-peptide to insulin) rescues low O2-induced ATP release (Fig. 3). However, coincubation of erythrocytes from humans with type 2 diabetes with a 6:1 ratio of C-peptide to insulin does not. Thus, the maintenance of physiological ratios and concentrations of these peptides is critical for the rescue of low O2-induced ATP release from erythrocytes of humans with type 2 diabetes. It must be recognized that although the patients were on a variety of treatment regimens, including in two cases, the PDE3 inhibitor cilostazol, the inability to release ATP in response to low O2 and the rescue of that function by C-peptide were seen in all type 2 diabetes patients, regardless of their medications.

Although pancreatic release of insulin and C-peptide can be compromised severely in type 2 diabetes, some individuals

Table 1. Effect of either 1 nM C-peptide or 1 nM insulin or both peptides at a 1:1 ratio (1 nM) on deformability, as measured by RCTT

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>RCTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH control</td>
<td>16</td>
<td>9.00 ± 0.16</td>
</tr>
<tr>
<td>HH C-peptide</td>
<td>11</td>
<td>9.09 ± 0.23</td>
</tr>
<tr>
<td>HH insulin</td>
<td>5</td>
<td>8.76 ± 0.16</td>
</tr>
<tr>
<td>HH 1:1 (C-peptide + insulin)</td>
<td>5</td>
<td>8.40 ± 0.11</td>
</tr>
<tr>
<td>DM2 control</td>
<td>11</td>
<td>9.18 ± 0.18</td>
</tr>
<tr>
<td>DM2 C-peptide</td>
<td>6</td>
<td>9.59 ± 0.45</td>
</tr>
<tr>
<td>DM2 insulin</td>
<td>5</td>
<td>9.19 ± 0.43</td>
</tr>
<tr>
<td>DM2 1:1 (C-peptide + insulin)</td>
<td>5</td>
<td>8.70 ± 0.46</td>
</tr>
</tbody>
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RCTT, red (blood) cell transit time; HH, healthy human; DM2, type 2 diabetes. Values are means ± SE.
retain modest beta-cell activity, allowing for the production of low levels of C-peptide (9, 10, 20). In a study analyzing patient tertiles based on C-peptide levels, the incidence of retinopathy, nephropathy, and neuropathy decreased from the first (C-peptide \(\leq 0.47 \text{nM}\)) to the third tertile (C-peptide \(\geq 0.80 \text{nM}\)) (7). In humans with type 2 diabetes, several reports suggest that a basal level of C-peptide of \(\sim 0.4 \text{nM}\) is often present, with minimal increases occurring after meals due to some residual beta-cell activity (9, 10, 20). Here, we report that in the presence of 1 nM insulin—a concentration of insulin that may be present in insulin-treated humans with type 2 diabetes—0.4 nM C-peptide is insufficient to restore low O\(_2\)-induced ATP release from erythrocytes of humans with type 2 diabetes (Fig. 4). These data suggest a previously unrecognized mechanism contributing to microvascular complications in individuals with reduced C-peptide levels.

C-Peptide has been suggested to stimulate the uptake of glucose into erythrocytes, resulting in increased ATP release (28, 29). However, glucose entry into the erythrocyte is not the rate-limiting step in ATP formation via glycolysis (48). Moreover, increased ATP levels inhibit glycolysis in these cells due to end-product inhibition (3). Importantly, we have shown that erythrocytes of humans with type 2 diabetes with markedly increased HbA1c levels, indicating chronic increases in plasma glucose, do not exhibit differences in intracellular ATP when compared with healthy humans (42). Moreover, the impairment in low O\(_2\)-induced ATP release seen in erythrocytes of humans with type 2 diabetes is not related to HbA1c level (42). As shown in Fig. 5, elevated glucose concentration (16.5 mM, 300 mg/dl) had no effect on low O\(_2\)-induced ATP release from erythrocytes of healthy humans or humans with type 2 diabetes in the presence of a 1:1 ratio of C-peptide and insulin. Humans with type 1 diabetes are reported to have reduced erythrocyte deformability and increased blood viscosity attributed to a reduction in erythrocyte Na\(^+\)-K\(^+\)-ATPase activity (10, 25, 47). Interestingly, C-peptide has been reported to stimulate Na\(^+\)-K\(^+\)-ATPase activity in these erythrocytes, resulting in increased deformability (12, 16, 27). However, studies of the deformability of erythrocytes from humans with type 2 diabetes have yielded conflicting results (4, 8, 37, 49). This is important, because we and others have demonstrated that reduced erythrocyte deformability can inhibit low O\(_2\)-induced ATP release (45). Here, we examined the deformability of erythrocytes of healthy humans and humans with type 2 diabetes in the absence and presence of insulin and C-peptide. As depicted in Table 1, there were no differences in RCTT, a measure of erythrocyte deformability, among the various groups. Thus it is unlikely that restoration of low O\(_2\)-induced ATP release from erythrocytes of humans with type 2 diabetes by insulin and C-peptide is a direct result of altered erythrocyte deformability. Clearly, further study will be necessary to elucidate the signaling mechanisms involved in the rescue of this important physiological response.

Perspectives and Significance

Restoration of low O\(_2\)-induced ATP release from erythrocytes from humans with type 2 diabetes would re-establish an important contributor to the local control of perfusion distribution, resulting in the adequate delivery of O\(_2\) to skeletal muscle. This hypothesis is supported by reports that confusion of C-peptide with even a subphysiological concentration of insulin (1 \(\mu\text{U/ml}\)) induced concentration-dependent vasodilation in isolated skeletal muscle arterioles and returned coronary flow to normal levels in a type 1 diabetes rat model (24, 32). The results presented here suggest that the addition of C-peptide to the treatment regimen for type 2 diabetes could have beneficial effects on blood flow distribution in skeletal muscle (11, 34), which could be useful in the prevention and/or treatment of the peripheral vascular disease associated with type 2 diabetes.

ACKNOWLEDGMENTS

The authors thank J. L. Sprague for inspiration. The authors also thank E. A. Bowles of Saint Louis University for technical support and assistance in reviewing this manuscript.

GRANTS

Support for this study was provided by grants from the American Heart Association (13PRE16980043) and the American Diabetes Association (BS-150).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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C-PEPTIDE AND INSULIN RESTORE RBC ATP RELEASE


