Inactivation of Corticotropin-Releasing Hormone–Induced Insulinotropic Role by High-Altitude Hypoxia

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We have shown that hypoxia reduces plasma insulin, which correlates with corticotropin-releasing hormone (CRH) receptor 1 (CRHR1) in rats, but the mechanism remains unclear. Here, we report that hypobaric hypoxia at an altitude of 5,000 m for 8 h enhances rat plasma CRH, corticosterone, and glucose levels, whereas the plasma insulin and pancreatic ATP/ADP ratio is reduced. In islets cultured under normoxia, CRH stimulated insulin release in a glucose- and CRH-level-dependent manner by activating CRHR1 and thus the cAMP-dependent protein kinase pathway and calcium influx through L-type channels. In islets cultured under hypoxia, however, the insulinotropic effect of CRH was inactivated due to reduced ATP and cAMP and coincident loss of intracellular calcium oscillations. Serum and glucocorticoid-inducible kinase 1 (SGK1) also played an inhibitory role. In human volunteers rapidly ascended to 3,860 m, plasma CRH and glucose levels increased without a detectable change in plasma insulin. By contrast, volunteers with acute mountain sickness (AMS) exhibited a marked decrease in HOMA insulin sensitivity (HOMA-IS) and enhanced plasma CRH. In conclusion, hypoxia may attenuate the CRH-insulinotropic effect by reducing cellular ATP/ADP ratio, cAMP and calcium influx, and upregulated SGK1. Hypoxia may not affect HOMA-IS in healthy volunteers but reduces it in AMS volunteers.

To enjoy social activities, millions of people travel to high altitudes every year. High-altitude hypoxia often induces dysfunction and illness, particularly acute mountain sickness (AMS) (1). During the construction of the Qinghai-Tibet railway (at altitudes of 3,000–5,000 m) in China, >100,000 construction workers were involved, and 51% of them developed AMS (2). Moreover, since the railway began service, >10 million travelers have visited the Tibet region in 2012, of whom 31% developed AMS despite traveling with an oxygen supply on the train (3). Increasing evidence in both humans and animals suggests that exposure to either high-altitude or hypobaric hypoxia influences plasma insulin levels and glucose homeostasis, depending on the oxygen level and duration of exposure (4–9). We previously showed that subacute hypoxia at an altitude of 5,000 m for 5 days reduces plasma insulin in rats, and this effect is blocked by a corticotropin-releasing hormone (CRH) receptor 1 (CRHR1) antagonist in vivo (10). However, the underlying mechanisms have not been clearly addressed.

Insulin, the unique hypoglycemic hormone, plays a crucial role in maintaining glucose sensing in pancreatic β-cells and regulating glucose uptake in a variety of tissues and cells during health and disease (11,12). Apart from glucose, many neural and endocrine hormones regulate pancreatic insulin release (13). In particular, CRH is the key regulator of the hypothalamic-pituitary-adrenal (HPA) axis; is activated by a variety of stressors, including hypoxia; and mediates a variety of neural and endocrine dysfunction and illness, particularly acute mountain sickness (AMS) (1). During the construction of the Qinghai-Tibet railway (at altitudes of 3,000–5,000 m) in China, >100,000 construction workers were involved, and 51% of them developed AMS (2). Moreover, since the railway began service, >10 million travelers have visited the Tibet region in 2012, of whom 31% developed AMS despite traveling with an oxygen supply on the train (3). Increasing evidence in both humans and animals suggests that exposure to either high-altitude or hypobaric hypoxia influences plasma insulin levels and glucose homeostasis, depending on the oxygen level and duration of exposure (4–9). We previously showed that subacute hypoxia at an altitude of 5,000 m for 5 days reduces plasma insulin in rats, and this effect is blocked by a corticotropin-releasing hormone (CRH) receptor 1 (CRHR1) antagonist in vivo (10). However, the underlying mechanisms have not been clearly addressed.

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response to stress (14). Studies have shown that CRHR1 exists in human, mouse, and rat islets (15,16) and that CRH enhances calcium influx (17), increases insulin content, and elevates insulin secretion in a glucose-dependent manner in cultured islets (15,16,18). Furthermore, CRH modulates development, proliferation, and antiapoptosis in islets (15,16,19). These findings suggest that CRH and CRHR1 play significant roles in regulating insulin release under normal conditions. We previously showed that hypobaric hypoxia results in upregulated CRH in the paraventricular nucleus and corticosterone (CORT) in the plasma of rats (20,21), which was associated with reduced plasma insulin (reversed by a CRHR1 antagonist) and glucose levels in vivo (10).

In the current study, we address the mechanisms by which CRHR1 mediates insulin secretion and glucose homeostasis during hypoxia. To achieve this goal, we completed comparative studies on rats under hypobaric hypoxia and on humans following a rapid ascent to the Tibetan plateau. Under hypoxia, the cell metabolic state switches from aerobic metabolism toward anaerobic glycolysis, which may lead to reduced ATP production and plasma insulin levels (10). In the present article, the data suggest that a fall in the ATP/ADP ratio and loss of cAMP signaling capacity during hypoxia attenuate voltage-gated calcium influx and, thus, inactivate insulinotropic action of CRH.

RESEARCH DESIGN AND METHODS

Animals
Male Sprague-Dawley rats weighing 200–220 g were purchased from the Laboratory Animal Center of Zhejiang Province, China (certification no. SCXK2008-0033), and maintained in a 12-h light/dark cycle (lights on at 0600 h) at 20 ± 2°C with food and water ad libitum. Rats were adapted for 1 week before experiments. All animal experiments were approved by the Animal Care and Use Committee of the School of Medicine, Zhejiang University.

Islet Isolation
Pancreatic islets were isolated by collagenase digestion from rats as previously described (22). Intact islets were cultured in RPMI 1640 medium (containing 8.3 mmol/L glucose) supplemented with 10% FBS, 10 mmol/L HEPES, and penicillin/streptomycin (Invitrogen, Carlsbad, CA) at 37°C under 5% CO2 and 21% O2 for overnight recovery before experiments.

Human Hypoxia Exposure
Sixty-seven healthy male volunteers (18–23 years old) were recruited in Chengdu, China, at 540 m altitude, as basal lowland control subjects. They were informed about the objectives of the study and agreed to the experimental protocols. All studies were approved by the Ethics Committee of the Third Military Medical University. The volunteers’ oxygen saturation (SpO2) was determined with a finger pulse oximeter. Fasting blood samples were collected at 0700–0800 h before breakfast in Chengdu. Each day, starting 2 days before the flight to Rikaze, China, at 3,860 m altitude, volunteers were given a Rhodiola capsule (Z10980020, Tibet Nuodikang Medicine, Lhasa, China) to improve endurance and resistance to hypoxia. On the third morning at Rikaze, SpO2 was measured, and fasting blood samples were collected again. The AMS score was obtained using the Lake Louise Score ≥ 3 (23). Plasma was obtained by centrifugation as soon as possible and stored at −80°C until use.

Hypoxia Exposure of Animals and Isolated Islets
The rats in the hypoxia group were placed in a hypobaric chamber (FLYDWC-50-IIC; AVIC Guizhou Fenglei Aviation Armament Co., Ltd, Guizhou, China) and exposed to hypoxia of 2,000 m altitude (79.97 kPa, equivalent to 16.0% O2 at sea level) or 5,000 m altitude (54.02 kPa, 10.8% O2). The chamber was opened daily for 30 min to clean and replenish food and water during the 5 days of exposure. The normoxia group was placed in an identical chamber at sea level (100.08 kPa, 20.9% O2). Rats received intraperitoneal injections of the CRHR1 antagonist cp-154,526 (30 mg/kg) (donated by Pfizer, Groton, CT), the glucocorticoid receptor (GR)-specific antagonist RU486 (50 mg/kg) (Tocris, Bristol, U.K.), or vehicle 30 min before exposure. After exposure, rats were killed by decapitation at 1300–1400 h, and trunk blood was collected. Plasma was obtained by centrifugation and stored at −80°C. The liver and pancreas were immediately removed, frozen in liquid nitrogen, and stored at −80°C until use. Isolated islets in the hypoxia group were incubated in 5% CO2 and various O2 conditions delivered by the hypoxia chamber (ProOx model P110 and ProCO2 model P120 systems; BioSpherix, Lacona, NY) (24).

Insulin Secretion
Size-matched islets were washed and preincubated for 1 h in RPMI 1640 medium containing 2.8 mmol/L glucose, 10 mmol/L HEPES, and 0.1% BSA. Ten islets per well were then incubated in testing RPMI 1640 medium containing 10 mmol/L HEPES and 0.1% BSA with the indicated glucose and drugs under hypoxia or normoxia. At the end of the experiments, the testing medium was collected for insulin measurement using a rat insulin enzyme immunosorbent assay kit (Mercodia, Uppsala, Sweden), whereas islets were placed in lysis buffer for quantitative PCR (qPCR) assay.

Calcium Imaging
Islets were washed and loaded with 5 µmol/L Fluo-4 AM (Molecular Probes, Eugene, OR) in Krebs-Ringer bicarbonate HEPES buffer comprising (in mmol/L) 129 NaCl, 4.7 KCl, 1.2 KH2PO4, 5.0 NaHCO3, 2.0 CaCl2, 1.2 MgSO4, 10 HEPES, and 0.1% BSA at pH 7.4 containing 5.6 mmol/L glucose for 1 h at 37°C. Calcium-free conditions were achieved by use of calcium-free Krebs-Ringer bicarbonate HEPES buffer containing 2 mmol/L EGTA. Intact islets were immobilized with a wide-bore glass suction pipette under a Nikon TE2000 inverted microscope with a Yokogawa spinning disk confocal system (PerkinElmer,
Wellesley, MA). Calcium images were captured at 3-s intervals and three different depths with 488-nm excitation and 505–530-nm emission. At the end of each experiment, 0.5 mmol/L tolbutamide, a KATP channel inhibitor (Sigma, St. Louis, MO), was added to present the functional β-cells (25). As a rule, cells in islets were defined as β-cells if fluorescence signals were markedly increased in response to tolbutamide. The change of fluorescence intensity (ΔF) was calculated as a percentage of the basal level (F0, background subtracted), and frequency was calculated as events per minute. Methods for assays of plasma hormones and metabolism (glucose, lactate, pyruvate, ATP, ADP, and AMP), drug treatment, cAMP assay, qPCR, immunofluorescence staining, and Rhodiola rosea treatment are described in the Supplementary Data.

Statistical Analysis

All data are presented as mean ± SD and were analyzed using Student unpaired two-tailed t test and one-way ANOVA with Tukey post hoc test (GraphPad Prism 6 software). The paired t test was used in calcium imaging and human data analyses. P < 0.05 was considered significant.

RESULTS

Acute Hypobaric Hypoxia Affects Insulin and Glucose Levels in Rat Plasma Through CRHR1

To investigate the effect of hypoxic stress on plasma glucose, insulin, CRH, and CORT, rats were exposed to hypoxia of 2,000 or 5,000 m altitude for 8 h. Hypoxia of 5,000 m but not of 2,000 m decreased plasma insulin and increased plasma glucose, CRH, CORT, and HOMA insulin sensitivity (HOMA-IS) (Fig. 1A–E). All changes were reversed by treatment with a CRHR1 antagonist (cp-154,526) (Fig. 1A–E). Therefore, after activation of the HPA axis, CRHR1 is likely involved in the regulation of insulin release and glucose metabolism under acute hypoxia.

Acute or Subacute Hypobaric Hypoxia Reduces ATP Level in Rat Pancreas

To determine how hypobaric hypoxia influences ATP homeostasis in the pancreas, the lactate/pyruvate, ATP/ADP, and ATP/AMP ratios were assessed in both the pancreas and the liver of rats under 5,000 m hypoxia. After 8 h of hypoxia, rat pancreas exhibited an elevated lactate/pyruvate ratio, lowered ATP/ADP ratio, and elevated AMP/ATP ratio (Fig. 2A, C, and E). The lactate/pyruvate ratio was also elevated in the liver (Fig. 2B) but without detectable falls in the ATP/ADP or AMP/ATP ratio (Fig. 2D and F). However, after subacute hypoxia for 5 days, the lactate/pyruvate ratio was not changed (Fig. 2A and B), even though the ATP/ADP ratio decreased and AMP/ATP ratio increased in both pancreas and liver (Fig. 2C–F). These changes indicate that acute or subacute hypoxia switches glucose metabolism to anaerobic glycolysis and thus reduces ATP production in the pancreas.

CRH Stimulates and Hypoxia Suppresses Insulin Release

To investigate the insulinotropic action of CRH and the effects of hypoxia on this process, isolated islets were exposed to hypoxia and rotenone, an inhibitor of mitochondrial respiratory chain complex I. Treatment of islets with CRH (10 pmol/L–10 nmol/L) for 1 h induced dose-dependent increases in insulin secretion at 5.6 or 11.1 mmol/L glucose but not at 2.8 mmol/L glucose (Fig. 3A). The CRH-induced maximum magnitude of insulin at 11.1 mmol/L glucose (1.7-fold) was larger than at 5.6 mmol/L glucose (1.4-fold) (Fig. 3A). The insulinotropic action of CRH was persistently shown after 1, 12, and 24 h incubation at 5.6 mmol/L glucose, and this augmentation was completely abolished by pretreatment with 1 μmol/L cp-154,526, a CRHR1 antagonist (Fig. 3B). This CRH-insulinotropic action was blocked by cAMP-dependent protein kinase (PKA) inhibitors (H89 and Rp-8-Br-cAMPS, 10 μmol/L) but not by inhibitors of protein kinase C (Go 6983, 10 μmol/L) or phospholipase C (U-73122, 10 μmol/L) (Fig. 3C). Moreover, this insulinotropic effect of CRH depended on extracellular calcium and was blocked by an L-type calcium channel inhibitor (nifedipine, 10 μmol/L) (Fig. 3C).

Hypoxia reduced the CRH-insulinotropic action in a manner that was inversely related to O2 supply. Under

![Figure 1](https://example.com/figure1.png)

**Figure 1**—CRHR1 is involved in the regulation of rat plasma insulin and glucose under hypoxia of 5,000 m altitude for 8 h. Hypoxia of 5,000 m significantly decreased plasma insulin (A) and increased plasma glucose (B) and HOMA-IS (C); these effects were blocked by pretreatment with a CRHR1 antagonist. CRHR1 antagonist administration reversed the hypoxia-induced high CRH (D) and high CORT (E) levels in plasma. n = 7 in each group. *P < 0.05, **P < 0.001 vs. control group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. cp group. cp, cp-154,526.
Hypoxia Inactivates Insulinotropic Role of CRH

5% O2, CRH (0.01–1 nmol/L) failed to stimulate insulin secretion at 5.6 mmol/L glucose (Fig. 3D), whereas 1% O2 not only suppressed the insulinotropic action of CRH, but also reduced basal insulin secretion (Fig. 3D). To mimic the inhibition of oxidative phosphorylation and ATP production in the absence of hypoxia, rotenone was used to inhibit complex I of the mitochondrial electron transport chain in islets. Rotenone treatment (0.1–10 nmol/L) gradually inhibited insulin secretion. Rotenone (0.1 nmol/L) suppressed the augmentation of insulin release by 0.1 nmol/L CRH, whereas 1 or 10 nmol/L rotenone completely abolished 1 nmol/L CRH-stimulated insulin release (Fig. 3E).

Of note, qPCR results indicated an increase in Chrhl mRNA in islets under 5% O2 hypoxia but not under 1 nmol/L rotenone-induced ATP deficiency (Fig. 3F). These findings indicate that although hypoxia increases Chrhl expression (Fig. 3F), the insulinotropic action of CRH was attenuated by hypoxia due to insufficient supplies of O2 and ATP.

CRH-Insulinotropic Effect Is Inactivated by Gradually Reducing cAMP, ATP, and Calcium Influx in Islets

To determine the requirement for ATP as a substrate for the CRHR1-activated insulin release pathway, the cAMP level, ATP/ADP ratio, and calcium oscillations in islets were assessed. CRH (0.1–1 nmol/L) significantly enhanced the accumulation of cAMP in normoxia, and 1 nmol/L CRH increased the cAMP levels at 0.1 or 1 nmol/L rotenone, whereas the basal cAMP levels were decreased and not stimulated by CRH at 10 nmol/L rotenone (Fig. 4A). Furthermore, 1 nmol/L CRH markedly elevated the ATP/ADP ratio under normoxia. This elevation was abolished by pretreatment with 10 nmol/L rotenone (Fig. 4B), which increased the AMP/ATP ratio in a concentration-dependent manner (0.1–10 nmol/L) (Fig. 4D). Moreover, 1 and 10 nmol/L rotenone suppressed the basal ATP/ADP ratio (Fig. 4B), and the CRH-induced increases in ATP/ADP ratio were blocked by the CRHR1 antagonist with and without 1 nmol/L rotenone (Fig. 4C).

CRH 1 nmol/L markedly increased the frequency of calcium oscillations in identified β-cells (Fig. 4E and F), and this increase was reversed by pretreatment with the CRHR1 antagonist (1 μmol/L) and the PKA inhibitor (10 μmol/L Rp-8-Br-cAMPs) (Fig. 4B). In calcium-free conditions, calcium oscillations were not induced by 1 nmol/L CRH or 1 μmol/L forskolin (Fig. 4I). In the rotenone-induced ATP-deficient condition, the frequency of calcium oscillations was unaltered by CRH (Fig. 4E and G) but was still increased by forskolin (Fig. 4E and H). These findings strongly suggest that a deficiency of ATP and reduced ATP/ADP ratio limit cAMP production and thus lead to defective calcium oscillations in islets in a manner that may contribute to the inactivation of the CRH-insulinotropic action.

Hypoxia Inhibits Insulin Release by Both Reducing ATP and Increasing CORT in Islets

To examine the effects of glucocorticoid and intracellular ATP on insulin secretion under hypoxia, insulin secretion and GR target gene mRNA levels were assessed in isolated rat islets. Dexamethasone (DEX) concentration-dependent decreased insulin secretion under normoxia and 5% O2 and severely inhibited it under 1% O2 (Fig. 5A). Reduction of insulin release correlated with increased DEX and rotenone (ATP reduction) concentration (Fig. 5B). DEX elevated serum and glucocorticoid-inducible kinase 1 (Sgk1) mRNA and inhibited Glut2 and Chrhl mRNA expression under normoxia (Fig. 5C). These changes were not affected by ATP deficiency (1 nmol/L rotenone) or hypoxia (5% O2) despite that hypoxia increased the Chrhl mRNA level (Fig. 5C). These data suggest that both hypoxia-stimulated glucocorticoid release and a reduced ATP supply inhibit insulin release from pancreatic islets.

GR Mediates Inhibition of Insulin Release Under Hypobaric Hypoxia

To test the role of glucocorticoid in insulin secretion and glucose homeostasis under hypoxia in vivo, a GR antagonist (RU486) was used in rats under hypoxia of 5,000 m altitude for 8 h. RU486 pretreatment reversed the hypoxia-induced hyperglycemia and low plasma insulin (Fig. 6A). Immunofluorescence also showed a higher SGK1 signal in the nuclei of islet β-cells under hypoxia. The
proportion of SGK1-positive β-cells increased under hypoxia, and this was blocked by RU486 (Fig. 6B and C). These data suggest that hypoxia-evoked increases of SGK1 contribute to the inhibitory role of GR in insulin release in pancreatic islets.

High-Altitude Hypoxia Affects Human Plasma Insulin and Glucose Homeostasis

To determine the regulatory effect of high-altitude hypoxia on circulating hormones and plasma glucose in humans, 67 volunteers rapidly ascended to 3,860 m altitude in Rikaze and stayed for 2 days. On the morning of day 3, plasma glucose, insulin, CRH, and cortisol levels were analyzed. High-altitude hypoxia induced a dramatic decrease in SpO2 and increase in plasma CRH (Fig. 7A and D) but exerted no detectable effect on plasma insulin (Fig. 7B). Despite unaffected plasma insulin levels, plasma glucose was significantly elevated relative to control (Fig. 7C). Further analysis showed that plasma glucose increased in volunteers with AMS (12 of 67) but not in those without AMS (55 of 67) (Fig. 7F). HOMA-IS declined only in AMS volunteers (Fig. 7G), who had a higher plasma CRH compared with those without AMS (Fig. 7D and H). Plasma cortisol was decreased under hypoxia in all volunteers (Fig. 7E).
DISCUSSION

We addressed a different regulatory effect of CRH and glucocorticoids on hypoxia-reduced insulin levels. Under hypoxia, CRH-stimulated insulin release was abolished due to hypoxia-reduced cellular ATP and cAMP levels, and a consequent inhibition of calcium influx in isolated rat islets, despite that hypoxia-activated CORT, still inhibited insulin release (Fig. 8). In humans, rapid ascent to high altitude acutely elevated plasma glucose without altering plasma insulin. Furthermore, AMS volunteers exhibited reduced HOMA-IS and higher plasma CRH compared with non-AMS volunteers.

CRH, a critical stress peptide, plays a key role in regulating the HPA axis and adjustments in neural, endocrine, metabolic, and glucose homeostasis to various stressors, including hypoxia (14,20,26,27). Consistent with
our previous study (10), we show that acute hypobaric hypoxia induces marked increases in plasma CRH, which mediates reduced insulin and increased glucose in rat plasma (Fig. 1). Insulin, the hypoglycemic hormone, is only secreted from pancreatic islet β-cells and is mainly regulated by glucose and neuroendocrine inputs (11,13). There have been reports that CRHR1 is expressed in human, mouse, and rat islets (15,16), which upon activation by CRH, increases calcium influx (17) and stimulates insulin secretion in a glucose-dependent manner (15,16,18). The current study demonstrates a 24-h persistent insulino-tropic role of CRH under normoxia, depending on both glucose and CRHR1 signaling pathways, namely PKA and L-type calcium channel-mediated calcium influx (Fig. 3A–C). However, under hypoxia, this effect of CRH was inactivated (Fig. 3D) despite Crhr1 mRNA being upregulated in

**Figure 5**—DEX inhibits insulin secretion under normoxic, hypoxic, and ATP-deficient conditions in isolated rat islets. DEX inhibited 24-h insulin secretion under normoxia (21% O₂) and hypoxia (5% and 1% O₂) (A). Inhibition of insulin release by DEX was negatively correlated with ATP level (0.1–10 nmol/L rotenone) (B). DEX elevated Sgk1 mRNA and inhibited Glut2 and Crhr1 mRNA expression under normoxia, and these changes were not affected by ATP deficiency (1 nmol/L rotenone) or hypoxia (5% O₂) (C). Hypoxia induced Crhr1 mRNA expression. n = 3–4 in each group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. each control group; $P < 0.05, $$$P < 0.001 vs. vehicle treatment group.

**Figure 6**—GR mediates hypoxia-induced plasma insulin reduction. GR antagonist RU486 administration reversed the increased glucose and decreased insulin in rat plasma induced by hypoxia (5,000 m, 8 h) (A). Immunofluorescence of SGK1 (red), insulin (green), and nuclei (DAPI blue) showed a high SGK1 signal (B) and an elevated percentage of SGK1-positive cells (C) in pancreatic islets under hypoxia; these increases were reversed by RU486. In A, n = 7–8 rats in each group. In B and C, n = 15–20 islets from five to seven rat pancreata. Scale bar = 100 μm; dotted line, islet region. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control group; ###P < 0.01, ####P < 0.001 vs. hypoxia group. (A high-quality color representation of this figure is available in the online issue.)
islets (Fig. 3F). These findings suggest that the insulino-
tropic effect of CRH depends on sufficient O₂ supply, and
this view is supported by previous reports of attenuated
glucose-stimulated insulin secretion with stable basal insu-
lin levels under hypoxia of 6.7% (28) and 3% O₂ (29).

It is well documented that decreased O₂ supply
switches cell respiration from aerobic metabolism toward
anaerobic glycolysis. Consistent with this view, ATP pro-
duction was deficient in rat pancreas under 5,000 m hyp-
oxia (Fig. 2C). Islet β-cells express low levels of lactate
dehydrogenase (30); thus, islets may have a lower capacity
for anaerobic glycolysis and ATP production. The ATP/
ADP ratio of islet β-cells, therefore, may decrease more
dramatically than that of pancreas under hypoxia. ATP is
not only an energy molecule but also an important signal-
ing molecule in islet β-cells, controlling insulin secretion.
Under physiological conditions, insulin secretion is tightly
correlated with the ATP/ADP ratio in islet β-cells, al-
though it is still controversial whether increased ATP or
decreased ADP contributes to the alteration of ATP/ADP
ratio upon glucose stimulation (31,32). Other nutrients,
including amino acids and free fatty acids, also have the
capacity to modify the ATP/ADP ratio and insulin secre-
tion (11). Therefore, islets may be more sensitive to ATP
deficiency under hypoxia than other cells. Here, rotenone-
induced ATP deficiency abolished the stimulatory role of
CRH on insulin secretion (Fig. 3E), as did 5% O₂ (Fig. 3D).
Under normoxia, CRH increased cAMP production by
stimulating G-protein binding to CRHR1 and thus acti-
vating adenylyl cyclase (AC), but cAMP production was
lowered (not enhanced) when islets were deficient in ATP
(Fig. 4A). This reduction in cAMP likely contributes to the
suppression of CRHR1 signaling and might result from
a low ATP/ADP ratio under hypoxia and consequent
reductions in substrate supply for AC-dependent cAMP
production. Hypoxia or ATP deficiency also induced an
increased AMP/ATP ratio (Figs. 2E and 4D), resulting in
inhibited AC activity through binding to the intracellular
P site (33,34). Otherwise, a low ATP/ADP ratio under ATP

Figure 7—High-altitude hypoxia results in changes of insulin and glucose homeostasis, and these are correlated with AMS in humans. Compared with low altitude (540 m), high altitude (3,860 m) decreased SpO₂ (A) and caused no change in plasma insulin (B), increased plasma glucose (C) and CRH (D), and decreased plasma cortisol (E). Plasma glucose was elevated in volunteers with AMS (F), whereas their HOMA-IS was reduced (G). The increase in plasma CRH was significantly higher in volunteers with AMS than in those without (H). n = 67 (12 with AMS, 55 without). *P < 0.05, **P < 0.01, ***P < 0.001 vs. low altitude; $P < 0.05 AMS vs. no AMS.

Figure 8—Proposed inhibitory regulation of insulin release by hypoxia-activated CRH and CORT. Hypoxia switches from aerobic to an-
aerobic glycolysis, resulting in low ATP/ADP and high AMP/ATP ratios, which inhibit cAMP production and calcium oscillations in
rat islets. This consequently inactivates the insulino tropic role of
CRH, although CRHR1 is triggered by upregulated plasma CRH.
Meanwhile, hypoxia-stimulated plasma CORT inhibits insulin secre-
tion through upregulated Sgk1 mRNA; Cnr1 mRNA is also inhibited
by CORT. (A high-quality color representation of this figure is avail-
able in the online issue.)
deficiency (Fig. 4B and C) might limit the CRHR1 signaling pathway by gating of KATP channels, closure of L-type calcium channels, reduction of calcium oscillations (Fig. 4E), and consequent reductions in insulin secretion (Fig. 3E). We conclude, therefore, that the insulinotropic role of CRH is inactivated by a fall in ATP production under hypoxia, consequent reductions in the cAMP level and ATP/ADP ratio, and, thus, inhibition of calcium oscillations in islets (Fig. 8). Consequently, high plasma CRH during hypoxia may not exert the expected insulinotropic effects.

Acute (Fig. 1) or subacute (10,26) hypoxia increases not only plasma CRH but also CORT through activation of the HPA axis, raising the possibility that hypoxia-stimulated CORT is important in insulin regulation. Glucocorticoids coordinate various stress responses and glucose homeostasis (35,36), and DEX is known to inhibit insulin secretion in isolated islets (37,38). We found the same inhibitory effect of DEX on insulin secretion in cultured islets (Fig. 5A and B) associated with upregulated Sgk1 and downregulated Glut2 and Crhr1 mRNA expression under ATP-deficient or hypoxic conditions (Fig. 5C). These data suggest that CORT, activated by hypoxia, may initiate a discrete inhibitory pathway from CRH and correlate with the O2 and ATP supply. The current in vivo study shows that acute hypoxia-induced high CORT indeed contributes to the low plasma insulin and high plasma glucose mediated by GR (Fig. 6A) through high Sgk1 expression in islets (Fig. 6B and C).

In humans, high-altitude hypoxia exposure for 2 days significantly reduced SpO2 (Fig. 7A) and increased plasma CRH (Fig. 7D). To reduce the responsiveness to hypoxia, all volunteers took a Rhodiola capsule (extracted from R. rosea), a traditional Chinese herb to improve resistance to AMS. Of note, R. rosea can reduce hypoxia-induced high plasma CRH and CORT in rats (Supplementary Fig. 1). Consistent with the antistress effect in previous reports (39–41), R. rosea may thus contribute to low plasma cortisol at high altitude (Fig. 7E). In the current study, cortisol remained low, but high plasma CRH levels were still evoked by hypoxia in human volunteers. Under these conditions, the raised plasma CRH level fails to stimulate insulin release during high-altitude hypoxia. These results greatly support the conclusion derived from animal experiments that hypoxia increases CRH and inactivates its insulinotropic role by inhibiting the CRHR1 signaling pathway.

In the current study, acute hypoxia (5,000 m, 8 h) induced low plasma insulin, high plasma glucose, and increased insulin sensitivity in rats (Fig. 1A–C). Other studies individually showed a gradual decrease in insulin level of rats during acute hypoxia for 1–4 days (6) or hyperglycemia in mice less than 1 day of hypoxia (7). We found that the CRH-induced high CORT mainly decreased insulin release and elevated blood glucose. High CORT directly elevates blood glucose through decreased glycogen synthesis and glucose uptake in muscle (35). In liver, the current study shows that a high anaerobic glycolysis rate and glycogen storage maintain stable liver ATP production and support hyperglycemia under acute hypoxia (Fig. 2D). After 5 days of hypoxia, low insulin modulated by CRHR1 still occurs in rats. However, decreased blood glucose is not affected by CRHR1 (10). Low insulin level and hypoglycemia also occur in mice after 4 weeks of hypoxia (8) and likely result from adaptations to hypoxia that consequently reduce the anaerobic glycolysis rate and ATP/ADP ratio in liver (Fig. 2D), despite that hepatic glycogen is sufficient and CORT remains high (10). Therefore, CRHR1 regulation of blood glucose likely depends on high hepatic metabolism. In man, 3,860 m hypoxia for 2 days only increased plasma glucose and affected insulin sensitivity in the AMS group (Fig. 7C and G) in association with low cortisol (Fig. 7E) induced by R. rosea. Other studies have reported high cortisol, glycemia, and insulin levels in man in the first 1–2 days of exposure to 4,300 m hypoxia without any drugs (4,5). The sustained hyperglycemia with different insulin and cortisol levels in acute hypoxia may imply that blood glucose is regulated by greater integration of systems, including, for example, the autonomic nervous system. In short, a greater increase of CRH in the AMS group may activate the sympathetic nervous system (42) and induce hyperglycemia (43). Furthermore, 5–10 days of adaptation to hypoxia returns hyperglycemia to basal levels in healthy people (5). Moreover chronic hypoxic exposure reduces fasting insulin and improves insulin resistance in type 2 diabetic patients (44–46) and decreases the insulin dosage in type 1 diabetic patients (47,48). Of note, lower insulin levels or reduced insulin signaling is beneficial for health and longevity (49,50). It is therefore tempting to speculate that fast travel to high altitude may benefit both healthy people and diabetic patients in terms of glucose-insulin metabolic control.

In conclusion, we propose a dynamic modulation of a CRH-insulinotropic role in pancreatic islets, which depends on CRH, glucose levels, and O2 availability. Under normoxia, the CRH-insulinotropic role increases with raised glucose levels but is inactivated under hypoxia due to reductions in ATP, cAMP, and calcium influx into islets, even though plasma CRH and islet Crhr1 are upregulated. Additionally, hypoxia-stimulated CORT inhibits insulin release through activated Sgk1 and in an O2- and ATP-dependent manner. Consistent with these findings, rapid ascent to high altitude does not affect HOMA-IS in healthy volunteers but reduces HOMA-IS in AMS volunteers who usually exhibit high plasma CRH.

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