Biochemical and Biophysical Research Communications 386 (2009) 252–256

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Insulin mediates the linkage acceleration of muscle protein synthesis, thermogenesis, and heat storage by amino acids

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ARTICLE INFO

Article history:
Received 2 June 2009
Available online 10 June 2009

Keywords:
Body temperature
Translation initiation
Anesthesia
Somatostatin
Hypothermia
Nutrient
Rat

ABSTRACT

Amino acid (AA) administration can stimulate heat accumulation in the body, as especially found under anesthetic conditions. To test our hypothesis that marked rise in plasma insulin concentrations following AA administration plays an important role in the heat storage, we intravenously administered either a balanced AA mixture or saline over 3 h, both with and without a primed-constant infusion of somatostatin in propofol-anesthetized rats. Rats on AA but lacking marked rise in plasma insulin by somatostatin treatment failed to show: attenuation of fall in core body temperature; partial increases in oxygen consumption; and stimulated muscle protein synthesis. Furthermore, the AA's stimulatory effects on phosphorylation of mTOR, 4E-BP1, and S6K1 were partially blocked by somatostatin. Our findings strongly suggest that the marked rise in insulin following AA administration promote translation initiation activities and stimulate muscle protein synthesis, which facilitates heat accumulation in the body.

Introduction

Hypothermia commonly occurs during surgery due to anesthesia-caused impairment of the thermoregulatory responses [1]. Even mild intraoperative hypothermia can lead to major postoperative complications [2–5]. Intravenous administration of an amino acid (AA) mixture can warm patients from within and prevents intraoperative hypothermia [6], but the precise mechanisms remain unknown.

AA administration is particularly effective in increasing energy expenditure by either degradative pathways or nonoxidative disposal pathways (protein synthesis) [7]. Since skeletal muscle accounts for the largest proportion of the body mass, a rise in muscle protein synthesis should cause a substantial increase in heat production. AA-induced increases in muscle protein synthesis are indeed accompanied by an attenuation of the core body temperature (Tcore) decreases in anesthetized rats [8]. However, no direct evidence exists that substantiates a causal relationship between the AA administration-induced muscle protein synthesis and heat accumulation in the body. Furthermore, factors regulating these physiological responses remain unknown.

We previously confirmed that AA administration causes marked elevation of the plasma insulin levels and phosphorylation of translation initiation components in anesthetized rats as compared to that seen in conscious controls on AA [8]. Exogenous administration of insulin can stimulate muscle protein synthesis [9–11]. In the insulin-induced stimulation of muscle protein synthesis, modulation of translation initiation plays an important role [12–14]. These results suggest that the marked increases in insulin after the AA administration promote translation initiation activities and stimulate muscle protein synthesis, which facilitates heat accumulation in the body.

We therefore made biochemical and physiological analyses using propofol-anesthetized rats given a balanced AA mixture combined with or without somatostatin (which is an inhibitor of pancreatic hormones) to clarify the physiological role of the increased plasma insulin following AA administration during anesthesia in the heat accumulation.

Materials and methods

Animals and surgery. Male Sprague–Dawley rats (Charles River Japan Inc., Yokohama, Japan), weighing 250–310 g, were maintained under conditions of constant humidity and temperature (22 ± 2 °C) on a 12:12-h light–dark cycle. Rats were fed a standard diet and given water ad libitum. The Committee on the Care and Use of Laboratory Animals of Otsuka Pharmaceutical Factory, Inc., approved all of the surgical and experimental procedures. In order to monitor the intraperitoneal temperature in the peritoneal

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0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.06.031

cavity, seven days before the experiment, rats were chronically implanted with transmitters (TA10TA-F40, Data Sciences International, St. Paul, MN) via an abdominal incision under sodium pentobarbital anesthesia (50 mg/kg). On the day before the experiment, a silicon catheter was inserted under diethyl-ether anesthesia into the jugular vein and threaded proximally 2.5 cm from the tip. To prevent blood coagulation, saline (SAL) was continuously infused via implanted catheters at a rate of 1 mL/h per rat. Food was then withheld for 18 h, although rats were allowed free access to water.

Determination of somatostatin dosage. In order to determine the dosage of somatostatin required to suppress plasma insulin to the levels similar to those seen in the SAL group, rats received either an intravenous bolus injection of vehicle (1350 μL/kg; with equal volumes of SAL used in all of the somatostatin treatment groups), or a somatostatin dose of 60, 180, or 540 μg/kg (Somatostatin 68-1-10, American Peptide Company, Inc., Sunnyvale, CA) via the implanted catheter. Subsequently, all rats were given the anesthetic agent, propofol, (Diprivan® 1%, AstraZeneca, Osaka, Japan) over 5 s in a bolus volume of 1.5 mL/kg via the catheter. The catheter was then immediately joined to two plastic tubes via a Y connector. Rats were then received 0, 180, 540, or 1620 μg/kg/h somatostatin dissolved in a balanced AA mixture (Amiparen®, Otsuka Pharmaceutical Factory, Inc.) at a rate of 14 mL/kg/h via a single vinyl tube for three hours, respectively. The lowest somatostatin dose (60 μg/kg + 180 μg/kg/h) used during the primed-constant infusion was selected based on a previous study [15]. Using sequential infusion rates of 4.5 mL/kg/h (0–60 min) and 2.25 mL/kg/h (60–180 min), rats were simultaneously given propofol via the other tube. Blood samples (300 μL) were taken just before the infusion of propofol, and then at 1 and 3 h after the infusion.

Infusion protocol and core body temperature monitoring. Propofol anesthesia was immediately induced in the non-somatostatin and somatostatin treatment groups after the intravenous bolus injections of 1350 μL/kg SAL or 540 μg/kg somatostatin dissolved in 0.4 μg/μL, respectively. This was followed for 3 h by the simultaneous and continuous infusion of the test solutions and the anesthetic, as described above. The SAL and AA groups were only given the saline or the AA mixtures, while the SAL-somatostatin and AA-somatostatin groups were given saline or the AA mixture containing 116 μg/mL somatostatin, respectively.

The rats were placed in a plastic cage that was positioned on a receiver (model RPC-1, Data Sciences International). Tcore information was sent by the telemetry transmitter (TA10TA-F40, Data Sciences International) to the receiver via a radio signal, and the information from the receiver was then relayed to an automated data acquisition system (Dataquest A.R.T.™, Data Sciences International). Values for Tcore were averaged every hour except during the period just before the infusion (0.5 h).

Tissue preparation. At the end of the experiment, under sodium pentobarbital anesthesia (50 mg/kg, i.v.) blood was collected and skeletal muscle (gastrocnemius muscle) was removed, immediately weighed and homogenized and the supernatants were obtained as has been described [8].

Measurement of whole body oxygen consumption. In a separate series of experiments, VO_2 in each group was measured in a metabolic chamber (30 × 30 × 20 cm, an air flow rate of 2 L/min, a measurement period of 15 s) using a computerized system (ARCO-1000, Arco System, Chiba, Japan). VO_2 values were corrected for differences in body surface area, and are expressed as mL/min/kg$^{0.75}$ [16]. VO_2 values were averaged every hour except during the period just before the infusion (0.5 h).

Measurement of protein synthesis in skeletal muscle. In a separate series of experiments, the fractional rate of protein synthesis (Ks) was measured using the flooding-dose method with phenylalanine [17] during the last 10 min of the treatment, as has been described [8]. The values were expressed as the percent of the mean of the values of the SAL group.

Assessment of phosphorylation state of 4E-BP1, S6K1, PKB and mTOR. For analysis of the phosphorylation state of eukaryotic factor 4E-binding protein-1 (4E-BP1), one aliquot of supernatant was heated for 10 min at 100 °C, cooled to room temperature, and centrifuged at 10,000*g* for 30 min at 4 °C. The preparation and the other aliquot of the supernatant was combined with 2× SDS sample buffer in equal proportions, heated for 3 min at 100 °C, and then cooled to room temperature. Each supernatant was subjected to immunoblot analysis using a polyclonal antibody specific for 4E-BP1 (Cat. No. 6936, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), total ribosomal protein S6 kinase (S6K1, Cat. No. 230, Santa Cruz Biotechnology Inc.), phosphorylated (Ser^{473}: Cat. No. 9271, Cell Signaling Technology, Beverly, MA) and total PKB (Cat. No. 9272, Cell Signaling Technology), or phosphorylated (Ser2448: Cat. No. 2971, Cell Signaling Technology) and total mTOR (Cat. No. 2972, Cell Signaling Technology), as has been previously described [8]. There were no changes in the PKB or mTOR content observed under any experimental conditions. The amount of phosphorylated PKB or mTOR was then normalized for the total amount of PKB or mTOR, respectively.

Measurement of plasma insulin. Plasma insulin was determined using a commercial ELISA kit for rat insulin (Shibayagi, Gumma, Japan).

Statistical methods. Values for each group are presented as means ± SEM. Differences among the groups were analyzed by two-way ANOVA or two-way repeated measures ANOVA, and when appropriate, followed by a Tukey's post hoc test. Statistical significance was set at $p < 0.05$.

Results

Determination of somatostatin dosage

We examined the dosage levels where somatostatin attenuates increases in plasma insulin levels irrespective of the AA administered during anesthesia. The groups that received a primed-constant infusion of somatostatin (180 μg/kg + 540 μg/kg/h or 540 μg/kg + 1620 μg/kg/h) along with the AA mixture exhibited a marked decrease in plasma insulin levels, as compared to the group receiving the AA mixture alone (Fig. 1). In subsequent exper-

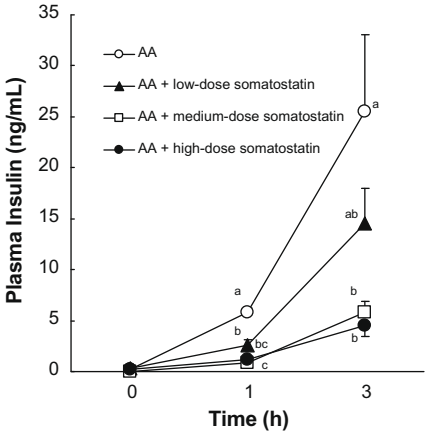

Fig. 1. Dose response analysis with somatostatin on plasma insulin concentrations. Plasma insulin concentrations at baseline, 1 and 3 h after onset of infusion were measured in rats infused for 3 h with either a balanced AA mixture (open circles) or AA mixture with somatostatin (bolus [μg/kg] + continuous [μg/kg/h], closed triangles; 60 + 180, open squares; 180 + 540, closed circles; 540 + 1620). Values are means ± SE, $n = 4–5$. Means not sharing a superscript are significantly different ($p < 0.05$).