Intravenous administration of amino acids during anesthesia stimulates muscle protein synthesis and heat accumulation in the body

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Yamaoka, Ippei, Masako Doi, Mitsuo Nakayama, Akane Ozeki, Shinji Mochizuki, Kunio Sugahara, and Fumiaki Yoshizawa. Intravenous administration of amino acids during anesthesia stimulates muscle protein synthesis and heat accumulation in the body. Am J Physiol Endocrinol Metab 290: E882–E888, 2006. First published December 13, 2005; doi:10.1152/ajpendo.00333.2005.—The present study was conducted to determine the contribution of muscle protein synthesis to the prevention of anesthesia-induced hypothermia by intravenous administration of an amino acid (AA) mixture. We examined the changes of intraperitoneal temperature (Tcore) and the rates of protein synthesis (K_s) and the phosphorylation states of translation initiation regulators and their upstream signaling components in skeletal muscle in conscious (Nor) or propofol-anesthetized (Ane) rats after a 3-h intravenous administration of a balanced AA mixture or saline (Sal). Compared with Sal administration, the AA mixture administration markedly attenuated the decrease in Tcore in rats during anesthesia, whereas Tcore in the Nor-AA group became slightly elevated during treatment. Stimulation of muscle protein synthesis resulting from AA administration was observed in each case, although K_s remained lower in the Ane-AA group than in the Nor-Sal group. AA administration during anesthesia significantly increased insulin concentrations to levels ~6-fold greater than in the Nor-AA group and enhanced phosphorylation of eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and ribosomal protein S6 protein kinase relative to all other groups and treatments. The alterations in the Ane-AA group were accompanied by hyperphosphorylation of protein kinase B and the mammalian target of rapamycin (mTOR). These results suggest that administration of an AA mixture during anesthesia stimulates muscle protein synthesis via insulin-mTOR-dependent activation of translation initiation regulators caused by markedly elevated insulin and, thereby, facilitates thermal accumulation in the body.

thermogenesis; translation initiation; insulin; hypothermia

HYPOTERMIA CAUSED BY THE ADMINISTRATION of anesthetics occurs commonly during surgery and results from decreased heat production, increased heat loss, and lack of hypothalamic thermoregulation (38). It has been frequently reported that intravenous infusion of an amino acid (AA) mixture increases heat production, which leads to prevention of hypothermia during surgery (26, 35–37). However, the mechanism behind the stimulation of thermogenesis by AA administration during anesthesia remains unknown.

Extra-splanchnic oxygen consumption accounts for ~73 to 75% of the total oxidative metabolism seen in a whole human body given an AA mixture during surgery (37). In alert subjects, intravenous administration of an AA mixture not only increases oxygen uptake but also augments blood flow in extra-splanchnic tissues (7). Although we can infer from these observations that thermogenesis resulting from AA administration occurs mainly in extra-splanchnic organs capable of varying oxygen metabolism, we cannot find reports showing where the rise in energy expenditure occurs and affects heat accumulation in the body.

The AA, as a nutrient, has been shown (14) to be especially effective at increasing energy expenditure in either the degradable pathway (gluconeogenesis and ureagenesis) or the non-oxidative disposal pathway (protein synthesis). In particular, the elevation of protein synthesis may be widely accepted as the most likely explanation for enhanced nutrient-induced thermogenesis (NIT) resulting from AA administration under anesthesia. This explanation is supported by the finding that the AA-induced rise in NIT values is not observed in puromycin (an inhibitor of protein synthesis)-treated rats (43). Also, increases in energy expenditure seen during AA administration are dose dependent and are correlated to AA-induced protein synthesis (21). However, we know of no examination that shows whether AA administration under anesthesia stimulates protein synthesis in some tissues.

The most important stage where nutritional changes play a major role in protein synthesis in skeletal muscle is translation initiation where, as frequently reviewed (25, 27, 29, 39), the translation initiation factors eukaryotic initiation factor (eIF) 4E-binding protein-1 (4E-BP1) and ribosomal protein S6 protein kinase (S6K1) play a central role through the mammalian target of rapamycin (mTOR) pathway. Enhancement of these elements of the translation initiation cascade by intravenous or oral AA administration is thought to require both stimulation by AA and insulin (4, 18, 44, 45, 47).

The present study was conducted to determine the contribution of muscle protein synthesis to the prevention of hypothermia that results from AA administration in rats during anesthesia. First, we examined the rate of protein synthesis (K_s) in skeletal muscle of rats in which a balanced AA mixture was infused intravenously either during propofol anesthesia or in the conscious state. To clarify how the treatment with AA and anesthetics is involved in this process, we measured the phosphorylation states of protein participating in translation initiation control. Furthermore, to elucidate the primary stimuli involved in the stimulation of protein synthesis, we examined the phosphorylation state of protein kinase B (PKB) and mTOR and evaluated plasma insulin and AA concentrations.

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METHODS

Animals. Male Sprague-Dawley rats from Charles River Japan (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. They were fed a standard diet and water ad libitum. The following surgical and experimental procedures were approved by the Committee on the Care and Use of Laboratory Animals of Otsuka Pharmaceutical Factory.

Seven days before the experiment, under pentobarbital sodium anesthesia (50 mg/kg), the rats were chronically implanted with electrodes and a transmitter to monitor physiological indexes related to the state of anesthesia. These consisted of an electroencephalogram (EEG) with round-tip miniature screws over the right frontal and left occipital cortices, an electromyogram (EMG) with two stainless-steel wires in the neck muscle, and an intraperitoneal temperature (Tcore) transmitter (TA10TA-F40; Data Science International, St. Paul, MN) in the peritoneal cavity via an abdominal incision. On the day before the experiment, a silicon catheter was inserted into the jugular vein and threaded 2.5 cm proximally from the tip in the rats under diethyl ether anesthesia. This catheter was joined to two plastic tubes via a plastic Y connector, enabling the injection of different solutions from two different pumps. Saline (Sal) was infused continuously at a rate of 1 ml/h per rat via one vinyl tube to prevent blood coagulation; the other tube was filled with Sal and sealed hermetically. Food was withheld from rats for 18 h, but rats were allowed free access to water.

Infusion protocol. An infusion of the test solution (an AA mixture or Sal) and anesthesia were started simultaneously and continued for 180 min (0–180 min). During the treatment, the conscious (Nor) rats in the Nor-Sal group received a fat emulsion preparation (10% soybean oil; Intralipos, Otsuka Pharmaceutical Factory) and anesthesia were started simultaneously and continued for 180 min. The rats in the Nor-AA group received a fat emulsion preparation and a balanced AA mixture (Amiparen, Otsuka Pharmaceutical Factory). The anesthetized (Ane) rats in the Ane-Sal group were anesthetized with a continuous infusion of propofol (1% Diprivan; Zeneca, Milan, Italy) and Sal, and rats in the Ane-AA group received propofol and an AA mixture, all via the same method as the Nor-Sal group. Each test solution was infused at a rate of 14 ml·kg⁻¹·h⁻¹. The fat emulsion preparation and propofol were administered via a bolus injection of >5 s (1.5 ml/kg), and this was followed by a sequential infusion at rates of 4.5 ml·kg⁻¹·h⁻¹ (0–30 min) and 2.25 ml·kg⁻¹·h⁻¹ (30–180 min).

Measurement of intraperitoneal temperature. The rats were connected by a thin cable to an electrical swivel for monitoring EEG and EMG activity, and the rats were placed in the plastic cage that was located on the receiver for temperature transmitter. From 1 h before the start of the test solution infusion to the end of the treatment, the output signal from the transmitter was collected by an antenna in the receiver and then digitized by a Cambridge Electronic Design (CED) 1401 data processor connected to a personal computer (PC-9801NX; NEC, Tokyo, Japan) and stored on a hard disk. Online data analysis was carried out using a Spike 2 analyzing program (CED). Tcore was continuously sampled every second and was averaged over a 1 min period at 30-min intervals, from 30 min before the start of the test solution infusion to the end of the treatment.

Tissue preparation. At the end of the experiment, pentobarbital sodium (50 mg/kg) was administered intraperitoneally, and blood injection through a catheter, and blood was collected from the abdominal artery and centrifuged at 1,800 g for 20 min at 4°C to obtain the plasma. Skeletal muscle (gastrocnemius muscle) was removed and rinsed in ice-cold saline. Tissues were immediately weighed and homogenized in seven volumes of buffer A [in mM: 20 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (pH 7.4), 100 KCl, 0.2 EDTA, 2 ethylene glycol-bis-(β-aminoethyether)-N,N,N’,N’-tetraacetic acid, 1 dithiothreitol, 50 NaF, 50 β-glycerophosphate, 0.1 phenylmethylsulfonyl fluoride, 1 benzamidine, and 0.5 sodium vanadate] with a Polytron homogenizer. The homogenates were centrifuged at 10,000 g for 10 min at 4°C. The resulting supernatant was used to examine the phosphorylation of 4E-BP1, S6 protein kinase (S6K1), PKB, and mTOR, as described below.

Measurement of protein synthesis in skeletal muscle. In a separate experiment series, with the animal model treated as described above, the fractional rate of protein synthesis was measured by a flooding-dose method of phenylalanine (19) during the last 10 min of the treatment. The rats were injected with 1 ml/100 g body wt of a solution of [U-2,6-3H]phenylalanine (150 mmol/l), containing 3.70 GBq/l via the implanted catheter that was used to administer the test solution. After that, the catheter was immediately connected with the plastic Y connector, and the infusion was resumed. Ten minutes after that, the rats were injected with pentobarbital sodium (50 mg/kg) followed immediately by excision of the gastrocnemius muscles, which were quickly frozen in liquid N₂ by the clamping method. In all rats the periods between injection of radioisotope and clamping of each tissue were accurately recorded. After fractionation to purified protein and homogenate supernatant, the specific radioactivity of phenylalanine was determined. The fractional rate of protein synthesis (the percentage of protein mass synthesized in minutes) was calculated as $K_s (%) = (Sb/Sa) \times 1,440/t \times 100$, where Sb is the specific radioactivity of the protein-bound phenylalanine, Sa is the specific radioactivity of free tissue phenylalanine, and t is the time of labeling in minutes.

Assessment of phosphorylation state in 4E-BP1. For analysis of the phosphorylation state in 4E-BP1, which is a heat-stable protein, one aliquot of the 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 supernatant was mixed with an equal volume of 2× SDS sample buffer (2 ml of 0.5 M Tris, pH 6.8, 2 ml of glycerol, 2 ml of 10% SDS, 0.2 ml of β-melecaptoethanol, 0.4 ml of a 4% solution of bromophenol blue, and 1.4 ml of water to a final volume of 8 ml), reheated for 3 min at 100°C, and subjected to electrophoresis on a 15% polyacrylamide gel. After electrophoresis, the proteins were transferred electrophoretically onto polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with polyclonal antibodies specific for 4E-BP1 (cat. no. 6936, Santa Cruz Biotechnology). The blots were then developed by use of an enhanced chemiluminescense (ECL) Western blotting kit according to the manufacturer’s instructions. The film was scanned (EPSON GT-9500) and analyzed with National Institutes of Health Image 1.61 software. The 4E-BP1 resolves into multiple electrophoretic forms during SDS-PAGE, depending on which and how many sites are phosphorylated, with the more slowly migrating forms representing more highly phosphorylated 4E-BP1.

Assessment of phosphorylation state in S6K1, PKB, and mTOR. 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. The samples were subjected to electrophoresis on a 7.5% polyacrylamide gel for S6K1, a 12.5% gel for PKB, or a 7.5% gel for mTOR. Proteins were electrophoretically transferred to PVDF membranes. The blots were incubated with primary antibodies to total S6K1 (cat. no. 230, Santa Cruz Biotechnology), phosphorylated (Ser473; cat. no. 9271, Cell Signaling Technology) 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). The blots were then developed by use of an ECL Western blotting kit according to the manufacturer’s instructions. Films were scanned and quantitated as described in determination of the phosphorylation state of 4E-BP1. As discussed for 4E-BP1, resolution of S6K1 on SDS polyacrylamide gels results in the separation of the protein into multiple isoelectric forms. The slowest-migrating forms represent hyperphosphorylated forms of the protein, and the fastest migrating forms represent hypo- or dephosphorylated forms of the protein.

Measurement of plasma concentrations of insulin and free AAs. Plasma insulin concentrations were analyzed by the use of a commercial radioimmunoassay kit for rat insulin (DiaSorin, Stillwater, MN).
For determination of plasma concentrations of free AAs, plasma was combined with an equal volume of deproteinizing agent (6% sulfosalicylic acid) derivatized with phenylisothiocyanate and followed by high-performance liquid chromatography analysis.

**Statistical method.** Data for each group are represented as means ± SE. Statistical evaluation of the data was performed by use of a two-way ANOVA followed by the Tukey-Kramer test to determine treatment effect. Differences between the groups were considered significant when \( P < 0.05 \).

**RESULTS**

**Changes in Tcore.** To examine whether intravenous administration of an AA mixture affects body temperature differently during states of anesthesia (Ane) and consciousness (Nor), we first measured the Tcore of rats during a 3-h infusion of an AA mixture or Sal together with either the anesthetic propofol (consisting of fat emulsion) or a fat emulsion (Fig. 1A). The baseline values of Tcore did not differ among the groups. Tcore in the Nor-AA group increased at 1 h after the onset of infusion, showing the significant difference compared with baseline through the infusion periods \( (P < 0.01; \text{paired } t\text{-test}) \). After a 3-h administration of test solutions, Tcore in the Nor-AA group increased from baseline by 0.45°C. On the other hand, Tcore in the Nor-Sal group did not change during the infusion. Although Tcore in both Ane groups fell dramatically during the infusion, the reduction of Tcore from baseline was 4.6 and 3.3°C in the Sal and AA groups, respectively. At the end of the infusion, Tcore was significantly higher in the Ane-AA group than in the Ane-Sal group.

**Protein synthesis in skeletal muscle.** We next examined the \( K_s \) in skeletal muscle of rats infused for 3 h with an AA mixture or Sal during states of anesthesia and consciousness to examine whether AA administration affects muscle protein synthesis differently during the states of anesthesia and consciousness (Fig. 1B). The \( K_s \) in skeletal muscle was less in the anesthetized rats than in the normal rats. The \( K_s \) was 44% less in the Ane-Sal group than in the Nor-Sal group and 56% less in the Ane-AA group than in the Nor-AA group. The \( K_s \) in skeletal muscle was 24% greater in the Nor-AA group \( (6.8%/\text{day}) \) than in the Nor-Sal group. Under anesthesia, when muscle protein synthesis is markedly repressed, the \( K_s \) was 57% greater in the Ane-AA group \( (3.8%/\text{day}) \) than in the Ane-Sal group. However, the \( K_s \) remained lower in the Ane-AA group than in the Nor-Sal group.

**Plasma concentrations of insulin and free AAs.** We have no information regarding how administration of an AA mixture to rats under anesthesia affects plasma insulin and free AA levels. Therefore, we measured plasma insulin and free AA concentrations in rats infused for 3 h with Sal or AA under the states of anesthesia and consciousness. Plasma insulin levels did not significantly differ between the Nor-Sal group and the Ane-Sal group (Fig. 1C). In contrast, plasma insulin concentrations in the Ane-AA group were highest among the treatment groups,
of all AA concentrations in both AA groups were increased; the respective free AA levels in rats given Sal. Total amounts shown in Table 1, the treatment with anesthetic did not affect about sixfold greater compared with the Nor-AA group. As shown in Table 1, the treatment with anesthetic did not affect the respective free AA levels in rats given Sal. Total amounts of all AA concentrations in both AA groups were increased; the AA concentrations were greater in the Ane-AA group than in the Nor-AA group. The observations of the individual plasma AA levels in the AA-infused groups showed that plasma concentrations of all AAs excluding tryptophan, asparagine, glutamine, and serine were higher in the Ane-AA group than in the Nor-AA group.

Phosphorylation of several components of translation initiation cascade. The mechanism through which AA administration regulates the stimulation of muscle protein synthesis under anesthesia remains to be determined. As shown in Fig. 2A, immunoblots of 4E-BP1 after SDS-PAGE exhibit three electrophoretic forms, termed α, β, and γ by the differences in their states of protein phosphorylation. The most highly phosphorylated form, the γ-form, dissociates itself with eIF4E and increases the availability of eIF4E required for the formation of eIF4G-eIF4E complex (41). The proportion of 4E-BP1 in the γ-form did not differ between the Nor-Sal group and the Ane-Sal group. In contrast, greater change in electrophoretic mobility of 4E-BP1 (~6-fold) to the γ-form in the Nor-AA group was shown when compared with the Nor-Sal group. Moreover, we confirmed that the infusion of the AA mixture in rats under anesthesia dramatically accelerated the increase in the amount of 4E-BP1 in the γ-form by 40% compared with those in the conscious state.

We also examined the proportion of the phosphorylated forms of S6K1 in skeletal muscle (Fig. 2B) because the phosphorylation forms of S6K1 are associated with increased activity of the protein for phosphorylating the downstream

Table 1. Plasma AA concentrations

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Nor</th>
<th>Ane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion Solutions</td>
<td>Saline</td>
<td>AAs</td>
</tr>
<tr>
<td>Arginine</td>
<td>147±4</td>
<td>771±37*</td>
</tr>
<tr>
<td>Histidine</td>
<td>52±1</td>
<td>194±3*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>85±4</td>
<td>655±19*</td>
</tr>
<tr>
<td>Leucine</td>
<td>125±3</td>
<td>1,267±34*</td>
</tr>
<tr>
<td>Lysine</td>
<td>419±22</td>
<td>1,412±93*</td>
</tr>
<tr>
<td>Methionine</td>
<td>53±1</td>
<td>265±10*</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>69±1</td>
<td>225±5*</td>
</tr>
<tr>
<td>Threonine</td>
<td>199±6</td>
<td>647±20*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>55±2</td>
<td>167±4*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>75±3</td>
<td>153±5*</td>
</tr>
<tr>
<td>Valine</td>
<td>148±4</td>
<td>1,353±26*</td>
</tr>
<tr>
<td>Alanine</td>
<td>246±13</td>
<td>543±10*</td>
</tr>
<tr>
<td>Asparagine</td>
<td>51±2</td>
<td>21±1*</td>
</tr>
<tr>
<td>Aspartate</td>
<td>3±0</td>
<td>20±1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>82±4</td>
<td>248±17*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>580±13</td>
<td>977±24*</td>
</tr>
<tr>
<td>Glycine</td>
<td>348±15</td>
<td>491±13*</td>
</tr>
<tr>
<td>Serine</td>
<td>229±7</td>
<td>294±5</td>
</tr>
<tr>
<td>Total AAs</td>
<td>2,967±64</td>
<td>9,705±176*</td>
</tr>
</tbody>
</table>

Values are means ± SE in μM, n = 8. Nor, conscious; Ane, anesthesia; Sal, saline; AA, amino acid. */†Significant differences compared with the Nor-Sal group or the Nor-AA group, respectively.

A

B

C

D

Fig. 2. Effects of treatment with anesthetic and AA on phosphorylation states of 4E-binding protein-1 (4E-BP1; A), S6 protein kinase (S6K1; B), PKB (C), and mammalian target of rapamycin (mTOR; D) in skeletal muscle. A–D: top: representative immunoblots; bottom: densitometric analysis of immunoblots as described in METHODS. The proportions of 4E-BP1 in the hyperphosphorylated γ-form in the Nor-AA group was shown when compared with the Nor-Sal group. Moreover, we confirmed that the infusion of the AA mixture in rats under anesthesia dramatically accelerated the increase in the amount of 4E-BP1 in the γ-form by 40% compared with those in the conscious state.

We also examined the proportion of the phosphorylated forms of S6K1 in skeletal muscle (Fig. 2B) because the phosphorylation forms of S6K1 are associated with increased activity of the protein for phosphorylating the downstream

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targets (9). Like 4E-BP1, in the Ane-AA group the proportion of phosphorylated forms of S6K1 was dramatically increased compared with all other groups. Rats in the Nor-AA group also showed significant increases in the phosphorylation forms of S6K1 compared with rats in the Nor-Sal group.

We examined phosphorylation of PKB on Ser^{473} in skeletal muscle because the phosphorylation of PKB on Ser^{473} is associated with increased activity of the protein for phosphorylating downstream targets (2). In the Nor-AA group, the phosphorylation of PKB on Ser^{473} was markedly enhanced compared with that in both Sal groups (Fig. 2C). The phosphorylation of PKB on Ser^{473} in the Ane-AA group was further enhanced, and the amounts of the phosphorylated PKB were 53% higher in the Ane-AA group than in the Nor-AA group. However, no significant difference in the phosphorylation of PKB on Ser^{473} was found between the Nor-Sal group and the Ane-Sal group. Any change in PKB content was not observed under any experimental conditions.

mTOR is activated through the phosphorylation at Ser^{2448} by PKB in the insulin-signaling pathway (33, 34) and phosphorylates 4E-BP1 and S6K1 (20, 22). Administration of the AA mixture dramatically phosphorylated the residue of mTOR at Ser^{2448} compared with the two Sal treatment groups (Fig. 2D). The phosphorylation of mTOR was further enhanced by the simultaneous treatment with AA and anesthetic. However, the degree of phosphorylation of mTOR on Ser^{2448} did not differ between the two Sal groups. Neither the treatment with AA nor the treatment with anesthetic altered mTOR content in the muscle of rats examined.

**DISCUSSION**

The present study was conducted to elucidate the contribution of protein synthesis, after intravenous administration of an AA mixture, to the prevention of hypothermia during anesthesia. Using propofol-treated rats, we confirmed that administration of an AA mixture during anesthesia attenuated the marked decrease of Tcore during anesthesia. A significant increase in Tcore was also observed in the Nor-AA group. As speculated in several reports (39, 40), this difference possibly indicates that the feedback regulation in the central nervous system to prevent the temperature of intracranial blood from exceeding a certain value may control the heat production by the AA administration in the conscious state (23) more strictly than in the state under anesthesia (38). According to a recent report (31), even in conscious subjects the AA administration adjusts thermoregulatory defense thresholds upward and increases the resting core temperature.

The present study demonstrated that administration of an AA mixture prominently elevated plasma insulin levels and stimulated protein synthesis in skeletal muscle, even in rats under anesthesia when protein synthesis was markedly depressed. The increase of insulin at supraphysiological blood concentrations or the correction of insulin deficiency, both of which were accomplished by the exogenous addition of insulin, contributed to activate the stimulation of protein synthesis in skeletal muscle (14, 15, 17, 24, 42, 44). These several lines of evidence supported the suggestion that the marked increases in plasma insulin levels seen in the Ane-AA group contributed to the improvement of anabolic response under anesthesia. However, muscle $K_s$ in the Ane-AA group remained lower than in the Nor-Sal group, implying that some systemic alteration during anesthesia limits the effect of insulin on muscle protein synthesis. As a factor, the decline in body temperature itself might play a significant role in lowering the $K_s$ in the Ane-AA group, as is suggested by the finding that a drop of 2°C in body temperature results in an ~20% inhibition of $K_s$ in skeletal muscle and liver of rats (8). The fall in metabolic rate caused by the administration of anesthetics may be also relevant to the negative effect on the protein synthesis due to the fall in metabolic rate, because the treatment with anesthetics depresses metabolic rate 20 to 30% (38) and the amount of oxygen consumption correlates with the $K_s$ (21). Moreover, the decrease in blood flow caused by the hypotension and the diminished heart rate during propofol anesthesia (10) may reduce $K_s$ in the Ane-AA group because muscle protein synthesis is positively correlated with blood flow (5).

The observations shown in the present study suggest that the synergistic increase in insulin levels caused by the administration of an AA mixture and the anesthetic possibly results in the activation of insulin signaling to the translation initiation factors through mTOR in skeletal muscle under conditions of anesthesia and that the phase of translation initiation plays an important role in stimulating muscle protein synthesis by acute nutritional changes during anesthesia as well as in the conscious state (25, 27, 29, 39). However, the phosphorylations of these translation factors are higher in the Ane-AA group than in either of the conscious groups, whereas muscle $K_s$ is less in the Ane-AA group than in either of the conscious groups, implying the existence of other rate-limiting factors in the stimulation of protein synthesis by the treatment with an AA mixture and anesthetic. One potential mechanism is that, unless the delivery of Met-tRNA$_{Met}$ to the 40S ribosomal subunit mediated by eIF2 is also appropriately maintained to the delivery of 7-methyl-GTP-capped mRNAs to the 40S ribosomal subunit, the stimulation of muscle protein synthesis by the administration of AA may not be adequately induced during anesthesia. Note that in the brain of the hibernating animal, in which hypothermia is accompanied by decreased protein synthesis, the eIF2 $\alpha$-phosphorylated form, which decreases the eIF2B availability, is increased (16). However, an obvious relationship between the stimulation of protein synthesis by acute nutritional change and phosphorylation of eIF2$\alpha$ or the activity of eIF2B has not been shown in previous in vivo studies (3, 28, 46). Hence, further studies are needed to clarify what the limiting component is in the stimulation of muscle protein synthesis by AA administration during anesthesia.

The finding of synergistic elevation in insulin levels when an AA mixture and anesthetics were coinfused, first demonstrated by the present study, is very intriguing. It is well known that GABA$_A$ agonists such as the propofol, used in the present study (13), have reduced the sympathetic tone, decreased the release of norepinephrine (10–12), and, furthermore, inhibited norepinephrine uptake in synaptic transmission at a clinically relevant concentration (40). The reduction in sympathetic tone leads to the exertion of inhibitory effects on insulin release from $\beta$-cells via the $\alpha_2$-adrenergic pathway (32). In addition, various AAs were highly effective in stimulating insulin secretion (6, 30). Integrating these findings with our present data, we suggest that anesthetics can augment the insulin secretion affected by AA administration in the absence of neuronal-adrenal feedback inhibition. This view is supported by the
observation that postprandial plasma insulin levels were increased in quadriplegic patients with complete cervical cord lesions when compared with those in healthy subjects (1).

Note that the observations in this study did not explain completely the mechanisms underlying the prevention of hypothermia by AA administration under anesthesia. Administration of AA under anesthesia contributes not only increased heat production but also a raised threshold for thermoregulatory vasoconstriction (26). How these alterations caused by AA administration are integrated remains unknown, but a role for AA administration in the stimulation of accumulation of heat is likely to gain wide acceptance.

In summary, the present data suggest that the stimulation of protein synthesis in skeletal muscle contributes in some way to the attenuation of anesthesia-induced hypothermia by AA administration. We further demonstrated that administration of both AA and the anesthetic exerts a synergic effect on the phosphorylation of S6K1 and 4E-BP1 via insulin-mTOR signaling, suggesting that an elevation of translation initiation factor eIF4E is a major mediator of AA-induced thermogenesis under anesthesia. These responses are likely to be triggered by the markedly elevated insulin levels that result from administering AA in rats under anesthesia compared with those in the conscious state.

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