Neural control of glucose uptake by skeletal muscle after central administration of NMDA

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Lang, Charles H., Mohammad Ajmal, and Andrew G. S. Baillie. Neural control of glucose uptake by skeletal muscle after central administration of NMDA. Am. J. Physiol. 268 (Regulatory Integrative Comp. Physiol. 37): R492-R497, 1995.—Intracerebroventricular injection of N-methyl-D-aspartate (NMDA) produces hyperglycemia and increases whole body glucose uptake. The purpose of the present study was to determine which tissues are responsible for the elevated rate of glucose disposal. NMDA was injected intracerebroventricularly, and the glucose metabolic rate (R\textsubscript{g}) was determined for individual tissues 20–60 min later using 2-deoxy-D-[U-\textsuperscript{14}C]glucose. NMDA decreased R\textsubscript{g} in skin, ileum, lung, and liver (30–35%) compared with time-matched control animals. In contrast, R\textsubscript{g} in skeletal muscle and heart was increased 150–160%. This increased R\textsubscript{g} was not due to an elevation in plasma insulin concentrations. In subsequent studies, the sciatic nerve in one leg was cut 4 h before injection of NMDA. NMDA increased R\textsubscript{g} in the gastrocnemius (149%) and soleus (220%) in the innervated leg. However, R\textsubscript{g} was not increased after NMDA in contralateral muscles from the denervated limb. Data from a third series of experiments indicated that the NMDA-induced increase in R\textsubscript{g} by innervated muscle and its abolition in the denervated muscle were not due to changes in muscle blood flow. The results of the present study indicate that 1) central administration of NMDA increases whole body glucose uptake by preferentially stimulating glucose uptake by skeletal muscle, and 2) the enhanced glucose uptake by muscle is neurally mediated and independent of changes in either the plasma insulin concentration or regional blood flow.

MATERIALS AND METHODS

Animal preparation. Male Sprague-Dawley rats (350–375 g; Charles River, Wilmington, MA) were used in all experiments. Animals were anesthetized with an intraperitoneal injection of ketamine and xylazine (90 mg/kg and 9 mg/kg, respectively) and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). A cannula was inserted unilaterally into the lateral ventricle of the brain, as previously described (11, 16). After surgery, animals were placed in individual cages, provided with food and water ad libitum, and allowed to recover for 4–5 days. Proper positioning of the cannula was verified at the end of the experiment by intracerebroventricular injection of trypan blue. All experiments were approved by the Animal Care and Use Committee of the State University of New York at Stony Brook. These studies adhered to the National Institutes of Health guidelines for the use of experimental animals.

In vivo glucose uptake by individual tissues. In the first series of experiments, animals had catheters implanted in the right jugular vein and left carotid artery with use of aseptic techniques (10, 12, 13). Animals were then fasted overnight but allowed free access to water. Rats were injected intracerebroventricularly with either NMDA (6 nmol, Sigma Chemical, St. Louis, MO) or water. We have previously determined that this dose of NMDA rapidly increases the rate of whole body glucose utilization (16). Twenty minutes after the injection of NMDA, a tracer amount of 2-deoxy-D-[U-\textsuperscript{14}C]glucose (2-[U-\textsuperscript{14}C]DG), 6 \textmuCi/rat; Amersham, Arlington Heights, IL) was injected intravenously (10, 12, 13). Serial arterial blood samples (500 \textmuL) were withdrawn, plasma was deproteinized with perchloric acid (PCA), and radioactivity was determined (Wal...
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lac LSC 1409, Gaithersburg, MD). An aliquot of the PCA supernatant from each sample was neutralized and the glucose concentration determined. At the end of the 40-min in vivo labeling period, a large blood sample (2 ml) was obtained to determine the plasma concentration of various hormones. Animals were then anesthetized with pentobarbital sodium and exsanguinated, and selected tissues were excised to determine the intracellular accumulation of phosphorylated 2-[U-14C]DG.

A representative piece of each tissue was quickly immersed in ice-cold 0.5 N PCA, homogenized, and centrifuged at 3,000 g for 15 min. The supernatant was neutralized with 5 N KOH and centrifuged. The resulting neutral extract was assayed for total radioactivity. Subsequently, a Somogyi extraction [Ba(OH)2-ZnSO4] was performed to precipitate phosphorylated 2-[U-14C]DG, and the radioactivity remaining in the supernatant (free 2-[U-14C]DG) was counted. The concentration of phosphorylated 2-[U-14C]DG in tissues was calculated as the difference between total radioactivity of the neutral extract and the radioactivity remaining after the Somogyi treatment (10, 12, 13). The glucose metabolic rate (Rg; mmol·min⁻¹·g wet wt⁻¹) for each tissue examined was calculated on the basis of in vivo accumulation of phosphorylated 2-[U-14C]DG by a respective tissue, the integrated 2-[U-14C]DG-to-glucose ratio in plasma during the 40-min labeling period, and the lumped constant (12, 13). Organ glucose utilization rates were calculated as the product of organ weight (g/100 g body weight) and tissue Rg values (mmol·min⁻¹·g tissue⁻¹) (12, 13). The peripheral glucose utilization rate was calculated as the algebraic sum of the individual rates for all of the soft tissues examined.

Arterial blood samples for the determination of plasma hormone levels were collected in chilled syringes containing aprotinin (500 KIU/ml), and plasma was stored at -70°C until assayed. Immunoreactive insulin, glucagon, and corticosterone were measured by radioimmunoassay (DPC, Los Angeles, CA).

A second series of experiments was performed to determine whether the changes in glucose uptake by skeletal muscle were dependent on the muscle being innervated. These rats were surgically prepared as described above. In addition, on the morning of each experiment, rats were lightly anesthetized with ether, and a 5-mm section of the sciatic nerve was incised and made visible but not cut. An incision was made and the nerve was visualized but not cut. Animals were conscious and moving in their cages 5–10 min after the denervation procedure was completed. All experiments were performed 4 h after denervation.

Cardiac output (CO) and regional blood flow determinations. In this experimental series, each animal had a catheter implanted in the left ventricle via the right carotid artery. The position of the catheter tip was demonstrated by the left ventricular pressure pulse, and its location in the ventricle was verified by postmortem examination. A catheter was also placed in the caudal artery of the tail. After an overnight fast, a unilateral sciatic nerve transection was performed on each rat. Thirty minutes after intracerebroventricular injection of NMDA, microspheres labeled with 14C (15 μm in diameter; DuPont-New England Nuclear, Boston, MA) were injected into the left ventricle for determination of CO and regional blood flow (12). The microspheres were slowly injected into the left ventricle, and the catheter was flushed with sterile saline. A reference blood sample was withdrawn from the caudal artery over a 1-min period at a constant rate of 0.34 ml/min. At the conclusion of each experiment, rats were anesthetized with pentobarbital and exsanguinated. Tissues were excised, weighed, and counted for radioactivity (model 1272, LKB, Gaithersburg, MD). Mean arterial blood pressure (MABP) and heart rate (HR) were determined via the caudal artery catheter before the injection of microspheres (model 79E, Grass, Quincy, MA).

CO and regional distribution of blood flow were calculated by methods similar to that of Rudolph and Heymann (19). Total peripheral resistance (TPR) and stroke volume (SV) were calculated (MABP/CO and CO/HR, respectively).

Statistics. All data are presented as means ± SE. The number of animals per group is indicated in the legends to the figures and tables. Data were analyzed using either Student’s t-test or analysis of variance followed by the Newman-Keuls test to determine treatment effect. Statistical significance was set at P < 0.05.

RESULTS

Regional glucose uptake. The intracerebroventricular injection of NMDA resulted in a mild hyperglycemia (29%, Table 1). It also increased plasma corticosterone levels (93%) but did not significantly alter circulating concentrations of either insulin or glucagon (Table 1). These changes are consistent with those previously reported (16). The calculated rate of total peripheral glucose disposal in control rats, based on the 2-DG uptake values for individual tissues, was 4.47 ± 0.36 μmol·min⁻¹·100 g body wt⁻¹ in control rats. This value was increased 41% in rats injected intracerebroventricularly with NMDA (6.28 ± 0.38 μmol·min⁻¹·100 g body wt⁻¹; P < 0.05). However, this increase in whole body glucose uptake was not distributed evenly among the various tissues in the body. A sample of hindlimb skeletal muscle, which is a mixture of red and white fiber types, and heart demonstrated a 150–160% increase in Rg (Fig. 1). In contrast, Rg in skin, ileum, lung, and liver actually decreased 30–35% when determined 20–60 min after intracerebroventricular injection of NMDA (Fig. 2). In the other tissues examined, including kidney, epidymidal fat, diaphragm, spleen, and whole brain, NMDA did not significantly alter tissue Rg (Fig. 3). In both control and NMDA-treated rats, Rg in muscle, intestine, and skin accounted for ~90% of the total whole body glucose disposal. As a result of the central injection of NMDA, however, there was a redistribution of glucose uptake, away from the intestine and skin and toward skeletal muscle (Fig. 4). After NMDA administration, glucose uptake by skeletal muscle alone was estimated to account for almost 70% of the rate of glucose disposal by the whole animal.

Table 1. Metabolic characteristics produced by icv injection of NMDA

<table>
<thead>
<tr>
<th>Control</th>
<th>NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, μmol/ml</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>160 ± 12</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>255 ± 24</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 rats/group. Arterial blood was collected 60 min after injection of N-methyl-d-aspartate (NMDA; 1 μg/rat icv), and plasma was obtained for determination of reported metabolic variables. *P < 0.05 vs. time-matched control rats injected intracerebroventricularly (icv) with water (5 μl).
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Fig. 1. In vivo glucose metabolic rate (Rg) in skeletal muscle (A) and heart (B) after intracerebroventricular (icv) injection of N-methyl-D-aspartate (NMDA). NMDA (6 nmol) was injected icv, and 2-[U-14C]-deoxy-D-glucose (2-DG) uptake was determined 20–60 min later. Control animals were injected with an equal volume (5 μl) of sterile water. Skeletal muscle represents a combination of hindlimb muscles. Values are means ± SE; n = 7 rats/group. *P < 0.05 vs. time-matched control values.

Neural control of muscle glucose uptake. In separate experiments the importance of innervation to the enhanced glucose uptake in skeletal muscle was examined. In these experiments, cutting the sciatic nerve eliminates neural input to the muscles of the lower hindlimb; however, the adductor longus muscle, which was also examined, lies proximal to the point of denervation and hence remains innervated. In control rats, denervation did not alter Rg of the gastrocnemius, soleus, or adductor longus compared with the innervated muscles from the contralateral leg of the same animal (Fig. 5). Central injection of NMDA increased Rg in the innervated gastrocnemius (149%), soleus (220%), and adductor longus (24%). In contrast, no increase in Rg was observed in the gastrocnemius and soleus muscles after denervation. The increased Rg in the adductor longus was the same in both legs.

Fig. 2. Rg in skin (A), ileum (B), lung (C), and liver (D) after icv injection of NMDA. Values are means ± SE; n = 7 rats/group. *P < 0.05 vs. time-matched control values.

Hemodynamics and regional blood flow. NMDA produced minimal changes in hemodynamics when determined 30 min after intracerebroventricular injection (Table 2). No changes in MABP, cardiac index, SV, or TPR were detected in NMDA-treated rats compared with time-matched control animals. NMDA-treated rats did demonstrate a 15% elevation in HR.

Although NMDA did not significantly alter cardiac index, it did substantially influence regional blood flow. Table 3 illustrates that NMDA increased blood flow to the small intestine (89%), cecum (141%), spleen (32%), and adrenal gland (56%). As a result of these changes, the calculated hepatic portal blood flow was elevated

Fig. 3. Rg in kidney (A), spleen (B), fat (C), diaphragm (D), and brain tissue (E) after icv injection of NMDA. Values are means ± SE; n = 7 rats/group.

Fig. 4. Contribution of individual tissue Rg to whole body glucose disposal in control (open bars) and NMDA-treated rats (hatched bars). Glucose uptake for skeletal muscle and intestine was calculated on basis of Rg in gastrocnemius muscle and ileum, respectively, as previously described (10, 12, 13). Values are means ± SE; n = 7 rats/group. *P < 0.05 vs. time-matched control values.
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Fig. 5. Rg in gastrocnemius (A), soleus (B), and adductor longus muscles (C) in control rats and animals injected icv with NMDA. In both groups of animals, sciatic nerve was sectioned on 1 side (i.e., denervated; solid bars) and left intact in contralateral leg (i.e., innervated; open bars). Values are means ± SE; n = 7 rats/group. ∗P < 0.05 vs. values obtained from innervated muscle from same treatment group. + P < 0.05 vs. time-matched control values from same leg (e.g., innervated vs. innervated or denervated vs. denervated).

Table 2. Hemodynamic effects of icv injection of NMDA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mmHg</td>
<td>110 ± 3</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>391 ± 13</td>
<td>448 ± 8*</td>
</tr>
<tr>
<td>CI, ml·min⁻¹·kg⁻¹</td>
<td>329 ± 13</td>
<td>334 ± 34</td>
</tr>
<tr>
<td>SV, ml/beat</td>
<td>0.34 ± 0.02</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>TPR, mmHg·ml⁻¹·min</td>
<td>0.85 ± 0.06</td>
<td>0.79 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7/group. Values were determined 30 min after icv injection of NMDA. MABP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; SV, stroke volume; TPR, total peripheral resistance. ∗P < 0.05 vs. control value.

Table 3. Regional blood flow after icv injection of NMDA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>0.15 ± 0.02</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.93 ± 0.08</td>
<td>0.91 ± 0.07</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.67 ± 0.06</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.61 ± 0.05</td>
<td>1.15 ± 0.09*</td>
</tr>
<tr>
<td>Cecum</td>
<td>1.08 ± 0.11</td>
<td>2.61 ± 0.31*</td>
</tr>
<tr>
<td>Colon</td>
<td>0.70 ± 0.05</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td>Fat</td>
<td>0.19 ± 0.02</td>
<td>0.13 ± 0.02*</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.16 ± 0.12</td>
<td>1.53 ± 0.09*</td>
</tr>
<tr>
<td>Kidney, right</td>
<td>2.09 ± 0.19</td>
<td>2.75 ± 0.35</td>
</tr>
<tr>
<td>Kidney, left</td>
<td>2.09 ± 0.21</td>
<td>2.81 ± 0.32</td>
</tr>
<tr>
<td>Adrenal</td>
<td>3.01 ± 0.24</td>
<td>4.71 ± 0.36*</td>
</tr>
<tr>
<td>Heart</td>
<td>6.53 ± 0.43</td>
<td>5.63 ± 0.49</td>
</tr>
<tr>
<td>Lung</td>
<td>1.40 ± 0.24</td>
<td>0.76 ± 0.15*</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>0.96 ± 0.10</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>Brain</td>
<td>0.96 ± 0.08</td>
<td>0.89 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7/group. Blood flow data are expressed as ml·min⁻¹·g tissue⁻¹. Lung includes bronchial artery and arteriovenous shunts. Left and right renal blood flows were determined to verify adequacy of microsphere mixing (13, 19). ∗P < 0.05 vs. control value.

DISCUSSION

The NMDA-induced hyperglycemia, although relatively mild, would be expected to increase glucose uptake in most tissues as a result of mass action (1, 2, 10, 12, 13). In contrast, the mild insulinopenia produced by NMDA would tend to impair glucose uptake by insulin-sensitive tissues, such as muscle and adipose tissue (12, 13). The present study indicates that central administration of NMDA profoundly alters regional glucose uptake in an unexpected manner. Despite the hyperglycemia, glucose uptake by several tissues (skin, ileum, lung, and whole brain) was reduced by 10.2 ± 20.3% on June 30, 2017 http://ajpregu.physiology.org/ Downloaded from

70% compared with control values (Fig. 6B). NMDA also produced a dramatic threefold elevation in hepatic arterial blood flow (Fig. 6A). Collectively, these changes more than doubled the total hepatic blood flow (Fig. 6C). Two of the tissues examined, epididymal fat and lung, demonstrated a significant reduction in blood flow after injection of NMDA (32 and 45%, respectively; Table 3). Blood flow to the other tissues examined (including skin, pancreas, stomach, colon, kidney, heart, diaphragm, and whole brain) was not altered 30 min after the intracerebroventricular injection of NMDA.

Blood flow was also determined in animals after unilateral sectioning of the sciatic nerve. In both control and NMDA-treated rats, blood flow to the denervated gastrocnemius and soleus muscle was reduced (Fig. 7). However, NMDA did not significantly alter blood flow to the gastrocnemius, soleus, or adductor longus regardless of whether the muscle was innervated or denervated.

Intravenous injection of NMDA. In separate animals, NMDA was injected intravenously at the same dose as that administered intracerebroventricularly (6 nmol). This low dose of NMDA did not result in detectable changes in either regional glucose uptake or blood flow in the tissues examined (data not shown).
Glucose uptake by skeletal muscle and heart was selectively enhanced by NMDA. Although hyperglycemia may account for a small part of the increased NIMGU, it was not of sufficient magnitude to explain the majority of the enhanced glucose uptake by muscle (12, 13). The increased glucose uptake in skeletal muscle was clearly dependent on neural innervation, because acute denervation before NMDA prevented the stimulation of glucose uptake by the gastrocnemius and soleus. Ablation of the NMDA-induced increase in glucose uptake could not be explained by changes in regional blood flow in the denervated muscles. From these data, it is not possible to determine whether denervation influenced the NMDA-induced increase in glucose uptake by altering nerve activity to the muscle and/or muscle responsiveness to a nonneural stimulus. The latter possibility is known to occur and is exemplified by the development of insulin resistance in denervated muscle (25). Whether denervation also impairs NIMGU has not been investigated. Regardless of the exact mechanism, intracerebroventricular injection of NMDA altered regional glucose disposal, resulting in a redistribution of glucose toward skeletal muscle and away from skin and intestine (and, to a lesser extent, other tissues). This redistribution is similar to that seen after intracerebroventricular injection of the cholinergic agonist carbachol (11) but unlike that observed in other stress conditions produced by diabetes, infection, or endotoxemia (10, 12, 13, 15).

An increase in glucose uptake by skeletal muscle and heart has been observed after electrical or chemical stimulation of the ventral medial hypothalamus (VMH) (21, 22) and in the heart after stimulation of efferent cardiopulmonary nerves (24). It is important to note that the study by Sudo et al. (22) was performed in anesthetized animals, a procedure that eliminates or minimizes muscle contractile activity. Hence, these data suggest that, although the present study was performed in conscious animals, a change in muscle activity induced by NMDA was not the cause of the increased glucose uptake in skeletal muscle. The same study by Sudo et al. also demonstrated that VMH stimulation increased glucose uptake in brown adipose tissue and that the increase was prevented by prior denervation. This increased glucose uptake appears to be due to an increase in the functional activity of the membrane-bound glucose transporters and not to changes in either the number or affinity of those transporters (23). These findings are in contrast to those observed after insulin treatment, in which the number of membrane-bound transporters is increased (8, 23), and further illustrate that VMH stimulation and insulin enhance glucose transport by different mechanisms. Although NMDA-type receptors are present throughout the brain, the VMH has the highest regional density of such receptors within the hypothalamus (14). Therefore it is possible that NMDA injected into the lateral ventricle exerted its effect by interacting with receptors located in the VMH. Although regional blood flow measurements were performed primarily to determine whether NMDA and/or denervation altered blood flow to skeletal muscle, blood flow to other organs was simultaneously assessed.
Whole body hemodynamics were not significantly altered 30 min after NMDA administration, except for the presence of tachycardia. Although NMDA did decrease blood flow to adipose tissue, the most striking observation was the increased total hepatic blood flow. Elevations in hepatic portal flow resulting from increased blood flow to the small intestine, cecum, and spleen were detected, as well as a threefold elevation in hepatic arterial blood flow. The increased total hepatic blood flow in conjunction with hyperlactacidemia undoubtedly contributes to the NMDA-induced elevation in hepatic glucose production previously reported (16).

**Perspectives**

Previously, the large majority of studies aimed at elucidating the mechanisms for the various metabolic responses to trauma or stress have focused on blood-borne mediators, such as glucoregulatory hormones and cytokines. The results of the present study, however, indicate that under one specific condition (i.e., NMDA injection) and in one specific tissue (i.e., skeletal muscle), at least one component of the stress response (i.e., increased glucose uptake) is dependent on neural innervation and independent of changes in circulating mediators of glucose metabolism and regional blood flow alterations. Although this study illustrates the potential for the central nervous system to directly regulate glucose metabolism in peripheral tissues, additional studies are needed to determine whether this mechanism is operational in other tissues, whether it functions during different stress and traumatic conditions, and whether its role can be expanded to other metabolic pathways. This regulatory mechanism would appear to be most likely involved in mediating the changes in glucose metabolism observed after brain injury, cerebral ischemia, and epilepsy (9, 18), where elevated levels of glutamate and other EAs have been found in the brain (5, 6). It is, moreover, conceivable that such a mechanism would permit pharmacological manipulation of the metabolic response to trauma by selective EAA antagonists.

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