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Frequency-dependent activation of glucose utilization in the superior cervical ganglion by electrical stimulation of cervical sympathetic trunk

(deoxy[^{14}C]glucose/energy metabolism)

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ABSTRACT **Electrical stimulation of the distal stump of the transected cervical sympathetic trunk produces a frequency-dependent activation of glucose utilization, measured by the deoxy[^{14}C]glucose method, in the superior cervical ganglion of the urethane-anesthetized rat. The frequency dependence falls between 0–15 Hz; at 20 Hz the activation of glucose utilization is no greater than at 15 Hz. Deafferentation of the superior cervical ganglion by transection of the cervical sympathetic trunk does not diminish the rate of glucose utilization in the ganglion in the urethane-anesthetized rat. These results indicate that the rate of energy metabolism in an innervated neural structure is, at least in part, regulated by the impulse frequency of the electrical input to the structure, and this regulation may be an essential component of the mechanism of the coupling of metabolic activity to functional activity in the nervous system.**

The autoradiographic deoxyglucose method was designed to measure the rates of glucose utilization simultaneously in all of the anatomical and functional components of the nervous system in conscious animals (1). Its use has demonstrated a close relationship between local functional activity and glucose utilization in nervous tissue (2), and it has been extensively applied to identify structures in the nervous system with altered functional activity in experimentally induced physiological, pharmacological, and pathophysiological states (2–4). Its use as a tool to map functional neural pathways is based on the principle of an evoked metabolic response. Conditions that increase the functional activity of a neural structure lead to an enhancement of its rate of glucose utilization; decreased functional activity depresses this rate. An essential feature of the deoxyglucose method is its use of quantitative autoradiography to localize the rates of glucose utilization to specific structures in the nervous system (1). The metabolic effects of altered functional activity are often so pronounced that the direction and location of the changes in metabolic rate can be directly visualized in the autoradiographs (2–4).

Studies in sensory systems, particularly the visual system, have demonstrated a quantitative relationship between the functional activity and the rate of glucose utilization in the neural tissues. In the rat the rate of glucose utilization in the regions of the brain to which the retina projects is linearly related to the frequency of retinal stimulation by light flashes (5). In the dark-adapted rat the rates of glucose utilization in the superficial layer of the superior colliculus and the lateral geniculate nucleus are directly proportional to the logarithm of the intensity of retinal illumination by randomly spaced flashes of light, at least to the point of saturation (3, 6). These results raise the question of the mechanism of the coupling between functional and metabolic activities. Presumably the metabolic activation in the structures of the brain is elicited, or at least initiated, by electrical signals transmitted from the stimulated retina to its projection areas.

The present studies were carried out to examine the influence of electrical activity in an afferent pathway on the rate of glucose utilization of the neural structure innervated by it. Specifically, the influence of spike frequency was examined. The experiments were carried out on the superior cervical ganglion of the urethane-anesthetized rat because of the ease with which the afferent nerves to this structure can be identified, isolated, and electrically stimulated and the physiological activation of the ganglion monitored in the target organs innervated by its efferent nerves. The results demonstrate a frequency-dependent activation of glucose utilization in the superior cervical ganglion by electrical stimulation of the cervical sympathetic trunk in the physiological range of spike frequency in this neural system.

MATERIALS AND METHODS

Chemicals. 2-Deoxy-D-[1-^{14}C]glucose (specific activity = 50–55 mCi/mmol; 1 Ci = 3.7×10^{10} Bq) was purchased from New England Nuclear. Calibrated [^{14}C]toluene, used for internal standardization in the measurement of plasma deoxy[^{14}C]glucose concentrations, was also obtained from New England Nuclear.

Animals. Normal adult male Sprague–Dawley rats were purchased from Taconic Farms (Germantown, NY). The experiments were carried out on animals weighing 270–430 g. The animals were allowed water ad lib but were fasted for 15–18 hr prior to the experiment.

Preparation of the Animals. The surgical preparation of the animals and the experiments were carried out under urethane anesthesia. Urethane was chosen as the anesthetic agent to minimize the possibility of blockade of ganglionic transmission by the anesthesia (7). The animals were anesthesized by the intraperitoneal injection of 1 g of urethane per kg of body weight. Catheters were inserted into one femoral artery and vein, and the superior cervical ganglia and cervical sympathetic trunks were exposed bilaterally. Anesthesia was subsequently maintained by the intermittent administration of urethane via the venous catheter as needed. The cervical sympathetic trunks

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were dissected free from the common carotid arteries and the vagus nerves. In 33 animals in which the effects of electrical stimulation of the afferent pathway were examined, the cervical sympathetic trunk on both sides was transected about 1 cm from the caudal pole of the superior cervical ganglion, and a small segment of the nerve was removed. Transection of the cervical trunk at this distance from the ganglion does not disturb the blood supply to the ganglion (8). In 11 animals used as controls to determine the effects of deafferentation *per se*, the cervical sympathetic trunks were similarly exposed bilaterally but transected on only one side with the other left intact.

Electrical Stimulation. The distal portion of one transected cervical sympathetic trunk was placed on bipolar platinum electrodes in a mineral oil bath and stimulated via a stimulus isolation unit with pulses of 0.5 msec in duration at a current intensity of 250 μA and at a frequency of 5, 10, 15, or 20 Hz. The current intensity was measured with an analog milliammeter (Hewlett–Packard model 428B). The stimulating cathode was positioned proximal to the ganglion. The effectiveness of the electrical stimulation was monitored by observation of the physiological responses in the ipsilateral eye—i.e., mydriasis, exophthalmos, and widening of the palpebral fissure. The electrical stimulation was begun 5 min before the onset of the period of measurement of glucose utilization and was continued until approximately 5 min before the termination of the measurement.

Measurement of Glucose Utilization. The period of measurement of glucose utilization was initiated by the intravenous administration of a pulse of 125 μCi of deoxy[^{14}C]glucose (specific activity = 50–55 mCi/mmol) per kg. Timed arterial blood samples were then drawn at appropriately spaced intervals to determine the time courses of the deoxy[^{14}C]glucose and glucose concentrations in the plasma during the following 45 min. The blood samples were immediately centrifuged in a Beckman Microfuge B to separate the plasma, which was then stored on ice until analyzed for the deoxy[^{14}C]glucose and glucose concentrations. Deoxy[^{14}C]glucose concentration was determined by liquid scintillation spectrometry of measured volumes of plasma with internal standardization with calibrated [^{14}C]toluene. Plasma glucose concentration was measured in a Beckman Glucose Analyzer 2. At approximately 45 min after the pulse of deoxy[^{14}C]glucose the animal was killed by intravenous injection of sodium pentobarbital. Both superior cervical ganglia were dissected out, desheathed, and mounted in frozen embedding medium (M-1 Embedding Matrix, Lipshaw Manufacturing, Detroit, MI) in a small aluminum foil container. The ganglia were then stored in plastic bags at −70°C until sectioned. Sections 20 μm in thickness were cut along the longitudinal axis of the ganglia in a cryostat (Hacker Instrument, Fairfield, NJ) maintained at −22°C. The sections were picked up and thaw-mounted on glass coverslips, dried on a hot plate at 60°C, and autoradiographed along with calibrated [^{14}C]methylmethacrylate standards as described (1). The ^{14}C concentrations in the ganglia were determined by densitometric analysis of the autoradiographs of the sections of ganglia and the plastic standards by means of a Photoscan P-1000 microdensitometer (Optronics International, Chelmsford, MA) and the image-processing system described by Goochee *et al.* (9). Glucose utilization was calculated from the tissue concentration of ^{14}C and the time courses of the arterial plasma deoxy[^{14}C]glucose and glucose concentrations by the operational equation of the deoxyglucose method (1). The rate constants and "lumped constant" required by the equation were those previously determined for gray matter of rat brain (1).

Two similar series of experiments were carried out, in which the electrical stimulation and measurement of glucose utilization in the superior cervical ganglion were initiated approximately 1$^1/_2$ hr in one series or 3–4 hr in the other series after the onset of the urethane anesthesia. The two periods of anesthesia were examined separately because it has been found by Ito *et al.* (10) that urethane causes a transient stimulation of glucose utilization in the superior cervical ganglion, probably indirectly via adrenomedullary mechanisms, which disappears after a period of time (unpublished data).

Physiological Status. The physiological status of the animals was monitored during the experimental period by measurement of mean arterial blood pressure and arterial pCO_2, pH, and pO_2. Body temperature was maintained at 37°C by means of an electric heating pad.

RESULTS

Physiological Variables. Blood pH, pCO_2, and pO_2 were maintained within normal levels during the urethane anesthesia. However, at the doses used, urethane caused a marked fall in mean arterial blood pressure from a normal mean level of approximately 130 mm of Hg to 80 mm of Hg and a moderate bradycardia from a normal mean heart rate of 482 to one of 342 beats per min. The changes in cardiovascular variables were sustained throughout the experimental period.

Effects of Deafferentation on Glucose Utilization of Superior Cervical Ganglion. The rate of glucose utilization in the superior cervical ganglion of the normal conscious rat is approximately 20 μmol/100 g per min (10). Urethane anesthesia stimulates this rate, but the stimulation is prevented by prior adrenalectomy (10) or becomes attenuated with continued anesthesia (unpublished data). In the present studies the mean (±SEM) rate of glucose utilization in the superior cervical ganglia innervated by intact cervical sympathetic trunks was 38 ± 2 μmol/100 g per min in the animals studied 1$^1/_2$ hr after the onset of urethane anesthesia (Table 1), considerably above the level reported in normal conscious rats (10), but it fell to a mean (±SEM) of 25 ± 1 μmol/100 g per min in the animals studied 3–4 hr after the onset of the anesthesia. In both series there

Table 1. Effects of orthodromic stimulation on rates of glucose utilization in the rat superior cervical ganglion

Stimulation frequency, Hz	Experiments, no.	Glucose utilization in ganglia, μmol/100 g per min*	
		Control side	Experimental side
Duration of urethane anesthesia: 1$^1/_2$ hr			
0	7	(38 ± 2)	36 ± 2
5	3	38 ± 3	63 ± 5†
10	7	40 ± 2	67 ± 1‡
15	3	38 ± 1	74 ± 3‡
20	5	38 ± 4	70 ± 2‡
Duration of urethane anesthesia: 3–4 hr			
0	4	(25 ± 1)	27 ± 1
5	3	26 ± 0.4	38 ± 3†
10	6	28 ± 2	52 ± 4‡
15	4	32 ± 3	71 ± 8‡
20	2	22 ± 1	57 ± 5‡

Parentheses indicate that the cervical sympathetic trunk was intact but unstimulated on the control side; on the experimental side the cervical sympathetic trunk was transected but also unstimulated. In all other experiments the cervical sympathetic trunks were transected bilaterally and electrically stimulated on the experimental side at the indicated frequency; the control side was left unstimulated. P values were determined by a paired t test.

*Values are shown as mean ± SEM.

†$P < 0.05$.

‡$P < 0.02$.

were no significant differences between the rates of glucose utilization in the ganglia with intact or with transected but unstimulated cervical sympathetic trunks (Table 1). Therefore, acute deafferentation *per se* does not appear to alter the rate of glucose utilization in the superior cervical ganglion of the urethane-anesthetized rat.

Effects of Orthodromic Stimulation on Glucose Utilization of Superior Cervical Ganglion. The effects of electrical stimulation on glucose utilization of the superior cervical ganglion were examined in rats with bilateral deafferentation under urethane anesthesia for approximately $1^1/_2$ or 3–4 hr. The distal stump of one transected cervical sympathetic trunk was stimulated, and the efficacy of the stimulation was confirmed by mydriasis, exophthalmos, and widening of the palpebral fissure of the ipsilateral eye. The deafferented and unstimulated superior cervical ganglion of the other side served as the control. In both groups of animals there was a frequency-dependent stimulation of glucose utilization in the stimulated superior cervical ganglion above that of the control side in the frequency range of 0–15 Hz (Table 1). At a frequency of 20 Hz, there was no further augmentation of glucose utilization above that at 15 Hz (Table 1). The stimulated glucose utilization was heterogeneously distributed throughout the ganglia; therefore, the data in Table 1 represent the average rates of glucose utilization

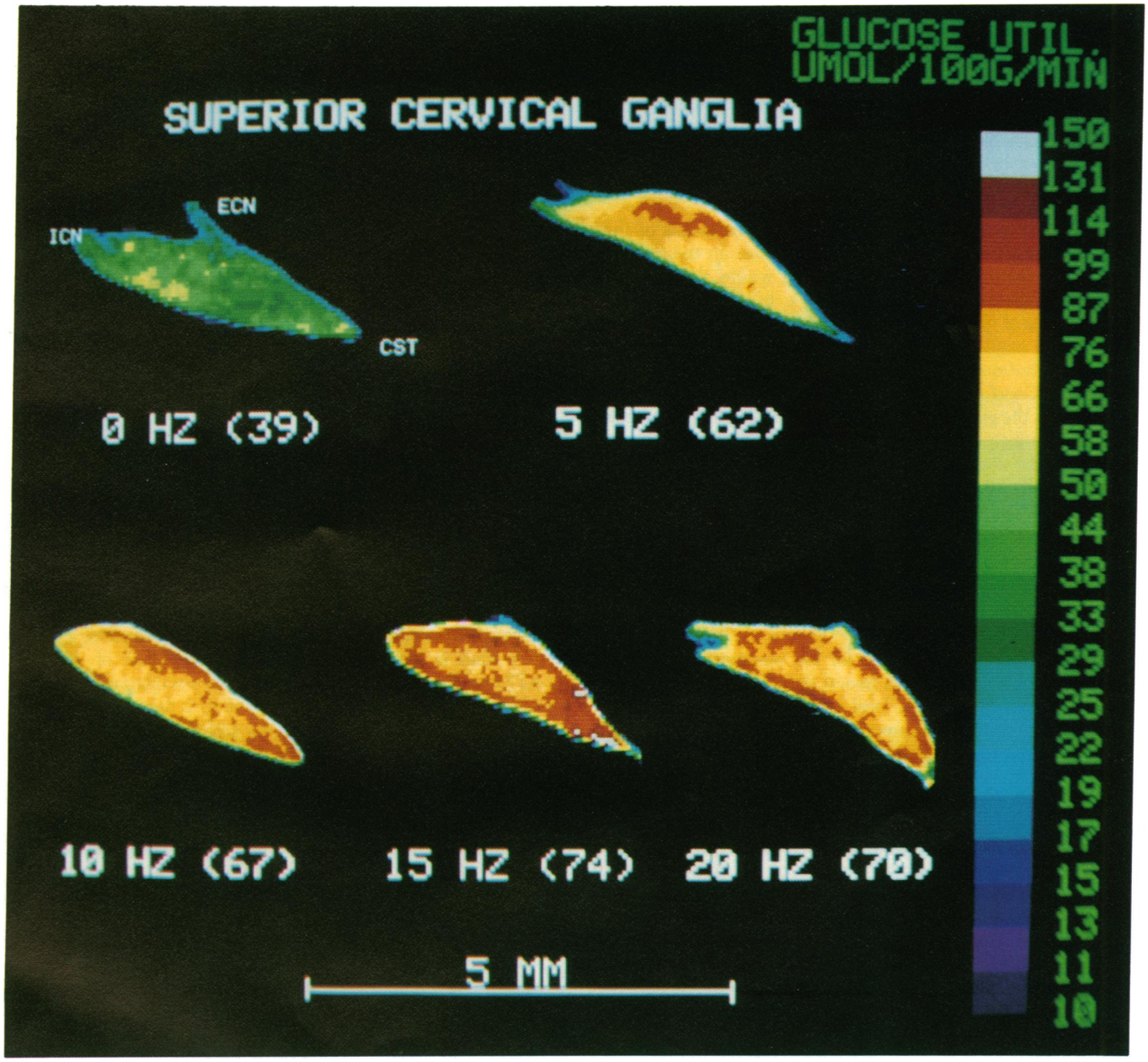

FIG. 1. Quantitative color-coded transforms of autoradiographs of superior cervical ganglia into quantitative metabolic maps representative of the results obtained at each stimulation frequency $1^1/_2$ hr after onset of urethane anesthesia. The absolute rates of glucose utilization are encoded in the color according to the accompanying calibration scale. The numbers in parentheses are the mean rates of glucose utilization obtained in the entire group of ganglia studied at that frequency of stimulation (see Table 1). The regions of entrance or exit of the cervical sympathetic trunk, internal carotid nerve, and external carotid nerve in the ganglion are indicated by CST, ICN, and ECN, respectively, placed next to the autoradiographic image of the control ganglion. The image-processing and color-coding of the autoradiographs were carried out as described by Goochee *et al.* (9).

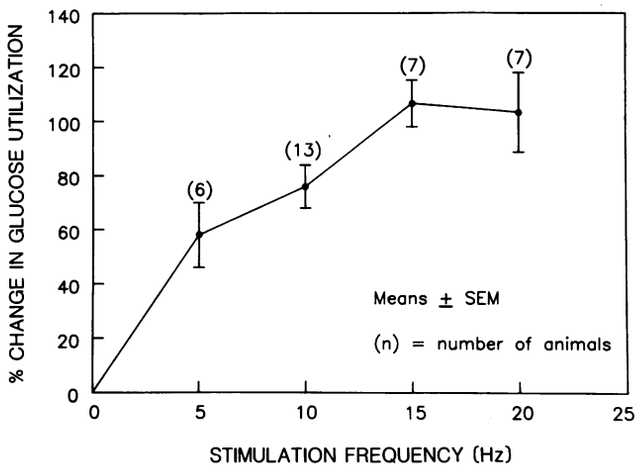

FIG. 2. Relationship between frequency of electrical stimulation of cervical sympathetic trunk and the percent increase in the rate of glucose utilization in the superior cervical ganglion above that of the control ganglion on the other side. The values represent the means ± SEM of the individual percent effects.

in the ganglia taken as a whole. Representative results of the effects of the different stimulation frequencies on the rates of glucose utilization and their distribution within the superior cervical ganglia are illustrated in Fig. 1, in which typical autoradiographs of ganglia have been transformed into color-coded quantitative maps of the distribution of the absolute rates of glucose utilization encoded in the color scale according to the method of Goochee *et al.* (9).

Although the absolute rates of glucose utilization of the ganglia and the increments due to stimulation were lower in the animals under urethane anesthesia for 3–4 hr than in those under anesthesia for $1^1/_2$ hr (Table 1), the fractional increases due to the electrical stimulation were similar in the two groups. Therefore, the data from the two groups were pooled to define the percent increase in glucose utilization of the ganglion as a function of stimulation frequency (Fig. 2).

DISCUSSION

The deoxy[^{14}C]glucose method for the measurement of local rates of glucose utilization in neural tissue has demonstrated a close relationship between local functional and metabolic activities in the central nervous system (2–4). Functional activity in neural tissues is generally considered to be reflected by the electrical activity of the tissue. The present studies demonstrate directly a relationship between electrical activity and energy metabolism in neural tissue. Electrical stimulation of the cervical sympathetic trunk at intensities sufficient to activate both the pre- and postsynaptic elements of the superior cervical ganglion in the rat has been found to stimulate glucose utilization in the ganglion, and the metabolic activation is frequency-dependent in the physiological range of impulse activity in this neural system. Electrical stimulation at frequencies between 0 and 15 Hz evokes progressively larger increases of glucose utilization in the ganglion. With stimulation at a frequency of 20 Hz there is no further increase in glucose utilization above that at 15 Hz. The failure to sustain a progressively increasing rate of glucose utilization at a stimulation frequency of 20 Hz is consistent with the results of electrophysiological studies; continuous orthodromic stimulation of the superior cervical ganglion at frequencies above 15 Hz has been found to lead to progressive diminution of the amplitude of the postganglionic compound action potential because of the failure of neurotransmitter release to follow the impulse rate in the cervical sympathetic trunk and also because of a decreased amplitude of the preganglionic compound action potential (11–13).

The rate of impulse activity in the cervical sympathetic trunk is normally very low in the anesthetized state, at most 1–2 Hz, with many nerve fibers either silent or only intermittently active (14, 15). In deafferented superior cervical ganglia, as used in the present studies, and in excised ganglia studied *in vitro*, neurons exhibit little spontaneous firing (16, 17). In intact preparations studied *in vivo*, neurons of the superior cervical ganglion generally show low firing rates of approximately 4–8 Hz, with a high percentage of silent or only intermittently active cells (18), although higher firing rates can be seen for short intervals during asphyxia or hemorrhage (19, 20). The range of impulse activities, 0–15 Hz, over which frequency-dependent activation of glucose utilization was observed in the present studies falls, therefore, within the normal physiological range. The failure to observe a reduction in glucose utilization after deafferentation of the superior cervical ganglion probably reflects the normally low-impulse activity in the intact cervical sympathetic trunk and the neurons of the ganglion in the anesthetized state.

Previous studies with excised superior cervical ganglia studied *in vitro* have demonstrated frequency-dependent activation of energy metabolism by electrical stimulation (7, 14, 15, 21–23), and the maximal increase in oxygen consumption was also found to occur with a stimulation frequency of 15 Hz (15). The present results demonstrate that the same relationship exists *in vivo* and suggest that frequency of impulse activity may mediate, at least in part, the coupling of energy metabolism to functional activity (3). Electrical stimulation of the afferents to sympathetic ganglia has been found to increase extracellular K^+ concentration in the ganglia (15, 24). Each compound action potential is normally associated with a sharp transient rise in extracellular K^+ concentration, which then rapidly falls and transiently undershoots before returning to the normal level (24); ouabain slows the decline in extracellular K^+ concentration after the impulse and eliminates the undershoot. Continuous stimulation at a frequency of 6 Hz produces a sustained increase in extracellular K^+ concentration (24). Mata *et al.* (25) have demonstrated an activation of glucose utilization in posterior pituitary slices by electrical stimulation *in vitro*, and this activation is blocked by ouabain, an inhibitor of Na^+,K^+-ATPase. Therefore, it is likely that the stimulation of glucose utilization by enhanced electrical activity is due to the ionic currents associated with spike activity and synaptic potentials and the consequent activation of the Na^+,K^+-ATPase activity to restore the ionic gradients to the resting levels.

Electrical stimulation of the cervical sympathetic trunk increases the electrical activity of presynaptic terminals and postsynaptic cell bodies in the superior cervical ganglion. The level of resolution of the deoxy[^{14}C]glucose method used in the present studies is inadequate to identify the structural elements in which the glucose utilization is activated. Stimulation of the hypothalamo-hypophysial system by salt-loading has been found to activate glucose utilization only in the terminals of the hypothalamo-hypophysial tract in the posterior pituitary and not in the cell bodies of origin of the tract in the paraventricular and supraoptic nuclei (26). There is considerable other evidence that glucose utilization is enhanced during increased functional activity mainly in axonal terminals or axo-dendritic processes rather than in cell bodies (3, 26). However, metabolism in the perikarya of the superior cervical ganglion may also be activated by electrical stimulation (unpublished data). Antidromic stimulation of the external carotid nerve leads to enhancement of glucose utilization in the region of distribution of the cell bodies of origin of the efferent nerve within the gan-

glion (11, 27, 28). Therefore, it is likely that glucose utilization is enhanced in both the terminal processes and the postsynaptic cell bodies by orthodromic stimulation of the superior cervical ganglia.

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