Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat

(cell bodies/axon terminals/deoxy[^{14}C]glucose)

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ABSTRACT   Electrical stimulation of the proximal stump of the transected sciatic nerve produces a frequency-dependent activation of glucose utilization, measured with the autoradiographic deoxy[^{14}C]glucose method, in the dorsal horn of the spinal cord but produces no change in glucose utilization in the dorsal root ganglion cells. These results suggest that axon terminals and not the cell bodies are the sites of enhanced metabolic activity during increased functional activity of this pathway.

Applications of the quantitative autoradiographic deoxyglucose method have demonstrated a close relationship between local functional activity and rates of glucose utilization in nervous tissue (1, 2), and the method has been extensively used to map functional neural pathways on the basis of evoked metabolic responses. The rate of local glucose utilization is, at least in part, regulated by spike frequency (3). It has not been clearly established, however, in which cellular elements (e.g., cell body, initial segment, axon terminals, or dendrites) the increase in glucose utilization occurs during increased impulse activity. *In vivo* studies in vertebrates have indicated that areas rich in neuropil have the most intense metabolic activity as compared to areas rich in cell bodies. For example, in the striate cortex of the monkey layer IV, the locus of termination of the geniculocalcarine pathway, has higher rates of glucose utilization than the cell-rich layers II and III (4). Bilateral visual occlusion lowers the rates of glucose consumption in the striate cortex as a whole, but the greatest reduction occurs in layer IV (4). It has also been found in rats that functional stimulation of the hypothalamo-neurohypophysial system by salt loading enhances glucose utilization in the terminals of this pathway in the pituitary neural lobe but not in the cell bodies of the tract in the paraventricular and supraoptic nuclei (5, 6). On the other hand, *in vitro* studies in the molluscan nervous system have indicated that neuronal cell bodies do increase the uptake of [^{3}H]deoxyglucose in response to increased electrical activity (7, 8).

The present experiments were undertaken to examine with the quantitative autoradiographic deoxy[^{14}C]glucose method (2) whether cell bodies increase their rates of glucose utilization in response to increased afferent input. The dorsal root ganglion of rats was chosen as a simpler model for these studies because the sensory neurons in the ganglia do not possess dendritic processes and are spatially separated from any regions with neuropil. Glucose utilization was measured in the cell bodies of the dorsal root ganglion and in the dorsal horn of spinal cord, the site of nerve terminals of the pathway, in response to electrical stimulation of the sciatic nerve. The results demonstrate a frequency-dependent activation of glucose utilization in the dorsal horn of the spinal cord but no metabolic change in the cell bodies of the dorsal root ganglion. These results suggest that the terminals and not the cell bodies are the sites of enhanced metabolic activity during increased afferent activity in this pathway.

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. Adult male Sprague–Dawley rats were purchased from Taconic Farms (Germantown, NY). The experiments were carried out on animals weighing 300–400 g. Prior to the experiment, the animals were allowed water and Purina Laboratory Chow *ad libitum* and were kept in a controlled environment with alternate 12-hr light and dark cycles.

Preparation of the Animals. On the day of the experiment the animals were anesthetized by an intraperitoneal injection of 45 mg of sodium pentobarbital per kg of body weight, and polyethylene catheters were inserted into one femoral artery and vein. The sciatic nerves on both sides were exposed, tied, and transected bilaterally at the level of the gluteus muscles. A wire loop was inserted around the skin incision on each side and drawn to make pools in which paraffin oil, used to prevent desiccation of the nerves, could be retained. Anesthesia was subsequently maintained by intravenous administration of sodium pentobarbital as needed.

Electrical Stimulation. The proximal portion of one transected sciatic nerve was placed on bipolar platinum electrodes and stimulated via a stimulus isolation unit with pulses 2 msec in duration at a current intensity of 200–400 μA and at a frequency of 5 Hz (n = 4), 10 Hz (n = 4), or 15 Hz (n = 4). The current intensity was continuously monitored on an oscilloscope. The stimulating cathode was positioned closest to the dorsal root ganglion. The effectiveness of the electrical stimulation was monitored by observation of the reflex contraction of the gluteus muscles. Electrical stimulation was begun 5 min before the initiation of the 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 125-μCi/kg pulse of 2-deoxy-[1-^{14}C]glucose. Timed arterial blood samples were drawn throughout the following 45 min, and the plasma was assayed for 2-deoxy[^{14}C]glucose and glucose concentrations as pre-

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viously described (2). At approximately 45 min after the pulse of 2-deoxy[^{14}C]glucose the animals were killed by an intravenous injection of a lethal dose of sodium pentobarbital. The dorsal root ganglia at the fourth, fifth, and sixth lumbar levels attached to the conus medullaris were dissected out on both sides, mounted in frozen embedding medium (M-1 embedding matrix, Lipshaw Manufacturing, Detroit, MI) in small aluminum foil containers and were frozen in isopentane chilled to −45°C with dry ice. The spinal cord at the lumbar level was dissected out and also frozen in the isopentane. The dorsal root ganglia and the spinal cord were cut into 20-μm sections in a cryostat maintained at −20°C to −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 previously described (2). The ^{14}C concentrations in the tissues were determined by densitometric analysis of the autoradiographs of the sections of spinal cord and dorsal root ganglia and the calibrated 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 2-deoxy[^{14}C]glucose and glucose concentrations by the operational equation of the deoxyglucose method (2).

Physiological Status. The physiological status of the animals was assessed during the experimental period by monitoring of the mean arterial blood pressure and arterial pH, P_{O_2}, and P_{CO_2}. Body temperature was maintained at 37°C by means of an electrical heating pad.

RESULTS

Physiological Variables. Arterial blood pH and P_{O_2} were slightly below (7.39 ± 0.03 and 81 ± 2 torr, respectively, means ± SEM, $n = 9$; 1 torr = 133 Pa = 1 mm Hg) and P_{CO_2} slightly higher (43 ± 1 torr, mean ± SEM, $n = 9$) than the values observed in normal conscious animals examined concurrently in this laboratory (7.42 ± 0.01, 86 ± 3 torr, and 37 ± 1 torr, means ± SEM, $n = 8$, for pH, P_{O_2}, and P_{CO_2}, respectively). Sodium pentobarbital anesthesia caused a fall in mean arterial blood pressure from a normal mean level of 128 ± 3 mm Hg, mean ± SEM, $n = 8$, to 105 ± 3 mm Hg, $n = 12$).

Effects of Electrical Stimulation of Sciatic Nerve on Glucose Utilization in the Spinal Cord and Dorsal Root Ganglia. Electrical stimulation of one sciatic nerve increased glucose utilization in the terminal field of the afferent projection of the sciatic nerve in the ipsilateral dorsal horn of the spinal cord compared to that of the control unstimulated side (Table 1, Fig. 1 *Left*). The increase in glucose utilization was statistically significant at all the frequencies of stimulation ($P < 0.002$) (Table 1), and the magnitude of the metabolic activation was frequency dependent over the range of frequencies, 5–15 Hz, studied (Fig. 2). The product–moment coefficient of correlation between the difference in rates of glucose utili-

zation in the stimulated and unstimulated sides and the frequency of stimulation was 0.89 ($P < 0.0001$). The rate of glucose utilization in the cell bodies of the dorsal root ganglion ipsilateral to the stimulated sciatic nerve, however, remained the same as that of the nonstimulated side over the full range of frequencies examined (Table 1, Fig. 1 *Right*). Electrical stimulation had no frequency-dependent effects on glucose utilization in the dorsal root ganglion or the dorsal horn of the spinal cord contralateral to the side of stimulation (Table 1), and the control values for glucose utilization in the spinal cord were similar to those reported by Crosby *et al.* (10) for pentobarbital-anesthetized rats.

DISCUSSION

The results of the present study demonstrate that electrical stimulation of the sciatic nerve results in a frequency-dependent increase in glucose utilization in the region of the afferent terminals in the dorsal horn of the spinal cord but not in the cell bodies of the dorsal root ganglion. The increased glucose utilization in the dorsal horn of the spinal cord in response to increased impulse activity is probably related to the activation of the Na^+,K^+-ATPase needed to restore the resting ionic distribution across the nerve membrane after the action potential. Mata *et al.* (11) have demonstrated that activation of deoxy[^{14}C]glucose uptake in slices of pituitary neural lobe by electrical stimulation *in vitro* is blocked by ouabain, an inhibitor of the Na^+,K^+-ATPase. The fact that the cell bodies in the dorsal root ganglion do not increase their rates of glucose utilization after electrical stimulation of the sciatic nerve raises the question of whether the cell bodies in the ganglion generate action potentials. Using a patch electrode, Smith (12) obtained electrophysiological evidence that the soma and dendrites of spinal cord neurons and the soma of dorsal root ganglion cells of mouse grown in tissue culture do not actively generate action potentials. Similar results have been obtained in other systems. By an analysis of extracellular potentials from single spinal motorneurons and single neurons in the lateral geniculate nucleus, Freygang (13) and Freygang and Frank (14) presented evidence that most of the soma-dendritic membrane can be excited synaptically to produce postsynaptic potentials but does not produce propagating action potentials. The absence of changes in glucose utilization in the dorsal root ganglion cells during stimulation of the sciatic nerve is to be expected if, in fact, these cell bodies do not fire action potentials.

The results reported here are, in some respects, similar to those of previous work which showed that osmotic stimulation of the hypothalamoneurohypophysial system resulted in increased glucose utilization in the terminals of the pathway in the posterior pituitary but not in the cell bodies of origin of this neural pathway (5, 6). On the other hand, Yarowsky *et al.* (15) have observed that antidromic stimulation of the external carotid nerve enhances glucose utilization only in the caudal region of the superior cervical ganglion below the origin of the external carotid nerve, a region corresponding to the area in the ganglion that has been shown by the horse-

Table 1. Effects of unilateral electrical stimulation of sciatic nerve on rates of glucose utilization in dorsal horn of spinal cord and cell bodies of dorsal root ganglion

Frequency of stimulation, Hz	No. of experiments	Glucose utilization, μmol/100 g per min			
		Dorsal horn of spinal cord		Dorsal root ganglion	
		Control side	Experimental side	Control side	Experimental side
5	4	36 ± 1	56 ± 2*	16 ± 1	18 ± 1
10	4	37 ± 3	75 ± 6*	17 ± 1	16 ± 1
15	4	39 ± 2	90 ± 2*	20 ± 1	19 ± 1

Values are means ± SEM of the number of animals indicated.
*Statistically significant difference compared with control; $P < 0.002$ as determined by paired t test.
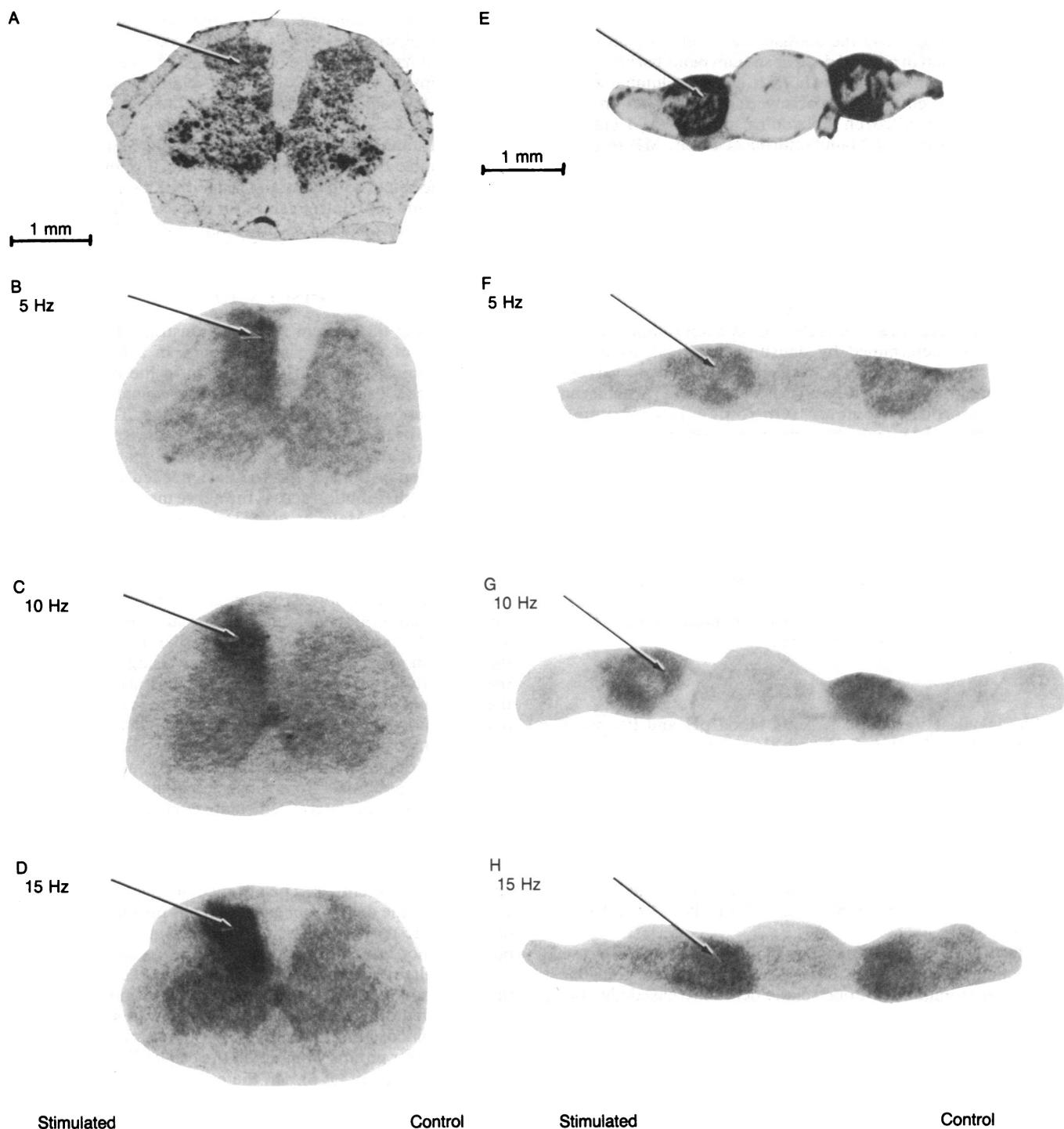
FIG. 1.   Glucose utilization in the spinal cord (Left) and dorsal root ganglion (Right) during electrical stimulation of the sciatic nerve. A and E are cresyl violet-stained frontal sections. In the spinal cord the arrows indicate the stimulated dorsal horn. (B–D) Deoxy[^{14}C]glucose autoradiographs of spinal cord sections stimulated at the indicated frequencies. (Right) Arrows indicate the stimulated dorsal root ganglia. (F–H) Deoxy[^{14}C]glucose autoradiographs of dorsal root ganglion sections during electrical stimulation.

radish peroxidase technique and extracellular recordings (16, 17) to contain the postganglionic neurons that give rise to the axons of the external carotid nerve. This observation indicates that an increased rate of glucose utilization associated with impulse activity can occur in postsynaptic elements, but the identification of the specific postsynaptic elements (i.e., perikaryon, dendritic processes, initial segment) in which glucose utilization is activated has not been possible because of the limited spatial resolution of the deoxy[^{14}C]glucose method.

From the results of the present studies in a clearly defined and delineated system, it is apparent that functional activation of a sensory pathway by sciatic nerve stimulation increases glucose utilization in the terminal zone of the pathway in the dorsal horn of the spinal cord but not in the cell bodies of the pathway in the dorsal root ganglion. Whether this is a general phenomenon applicable to other pathways with other cell types or peculiar to a pathway with unipolar or pseudounipolar cells, like those in the dorsal root ganglion, must be resolved by further studies.
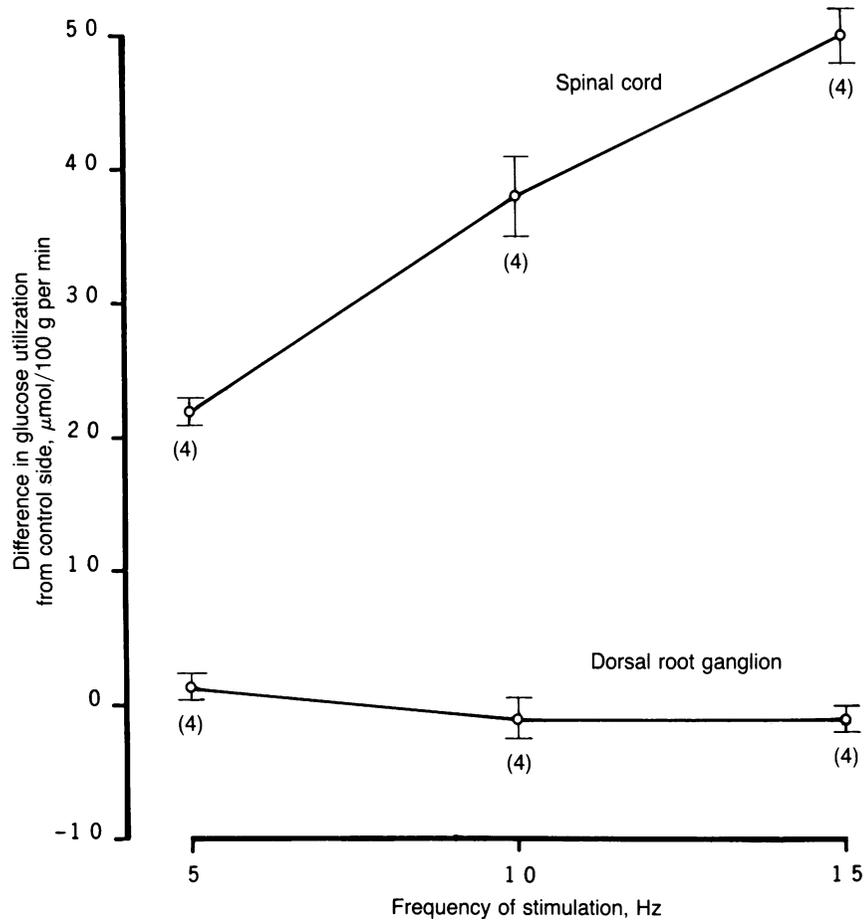
FIG. 2. Changes in glucose utilization from those of control unstimulated side as a function of frequency of electrical stimulation of the sciatic nerve in the dorsal horn of spinal cord and dorsal root ganglion. Results are means ± SEM for the numbers of animals in parentheses. The product–moment coefficients of correlation between change in glucose utilization and frequency of stimulation for the dorsal horn of the spinal cord and dorsal root ganglion are 0.89 ($P < 0.0001$) and -0.56 ($P > 0.05$), respectively.

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