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Energy Metabolism in Neural Tissues *in vivo* at Rest and in Functionally Altered States

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2.1. INTRODUCTION

The biochemical pathways of energy metabolism in brain are in most respects like those of other tissues, but special conditions peculiar to the central nervous system produce obstacles to studies of intermediary metabolism of the brain *in vivo*. For example, the blood–brain barrier restricts the exchange of substrates and products between brain and blood, and, consequently, biochemical reactions normally operating within the brain tissues are often not detectable with traditional *in vivo* methods based on exchange of substrates and metabolites between the blood and brain. *In vitro* studies best serve to identify pathways of intermediary

metabolism, biochemical mechanisms, and potential rather than actual performance, but valid identification of the ultimate substrates and products of cerebral energy metabolism and their rates of utilization and production under conditions of normal brain function can be obtained only *in vivo*.

The first definitive studies of cerebral energy metabolism *in vivo* began with the development of the nitrous oxide method by Kety and Schmidt (1948). This method made it possible to determine the rates of cerebral blood flow (CBF) and oxygen consumption (CMRO₂). Originally designed for use in man it was also applicable in animals, and, furthermore, it could be used in the unanesthetized state, a particularly important requirement to study the normal functioning brain. The method was based on the indirect Fick principle which states that, when a chemically inert tracer (i.e. one that is neither consumed nor produced by a given tissue) is introduced into the circulation, the rate of change in its content in any tissue is equal to the difference between the rates at which it is brought to that tissue in the arterial blood and removed from it in the venous blood. The method originally employed low concentrations of nitrous oxide as the tracer but was subsequently adapted for use with inert radioactive gases, e.g., ⁸⁵Kr, ⁷⁹Kr, and ¹³³Xe. Inasmuch as application of the Fick principle requires sampling of both arterial and representative cerebral venous blood for determination of their tracer concentrations, cerebral arteriovenous differences for a variety of substrates and products of cerebral metabolism could also be measured and their rates of utilization or production by the brain calculated as the product of these arteriovenous differences and CBF.

Numerous studies with this method established that cerebral energy metabolism is extraordinarily active in the unanesthetized state (Clarke and Sokoloff, 1999). For example, in normal, conscious, young adult men the brain comprises only about 2% of total body weight, but it alone consumes about 20% of the total resting body oxygen consumption. Studies with the method also established that glucose is not only an essential but normally the almost exclusive substrate for the brain's energy metabolism. Under normal conditions the brain utilizes glucose in almost stoichiometric amounts with the oxygen required for the complete oxidation of glucose to CO₂ and H₂O (Table 2.1). For complete stoichiometry 6 mol of O₂ are consumed and 6 mol of CO₂ produced per mole of glucose utilized, i.e., a molar ratio (O₂/glucose ratio) of 6.0; the respiratory quotient (R.Q.) (ratio of CO₂ production to O₂ consumption) is then 1.0. Under normal conditions cerebral glucose utilization (CMR_{glc}) not only accounts for all the O₂ consumed, but exceeds the rate for complete stoichiometry with CMRO₂ by about 20%, and the measured O₂/glucose ratio is about 5.5 (Table 2.1). In ketotic states, such as those due to starvation, fat-feeding, diabetes, etc., the blood levels of the ketone bodies, D-β-hydroxybutyrate and acetoacetate, are elevated, and these can substitute partly but not completely for glucose as the substrate for the brain's

Table 2.1. Rates of and relationship between cerebral oxygen consumption and glucose utilization in normal, conscious, young adult men

Function	Rate
O ₂ consumption (μmol/100 g of tissue/min)	156
Glucose utilization (mmol/100 g of tissue/min)	31
O ₂ /glucose ratio (mol/mol)	5.5
Glucose equivalent of O ₂ consumption (μmol/100 g tissue/min) (assuming 6 mol O ₂ per mol of glucose)	26
Excess of total glucose utilized above glucose oxidized (mmol/100 g of tissue/min)	5
CO ₂ production (μmol/100 g of tissue/min)	156
Cerebral respiratory quotient (R.Q.)	0.97

oxidative metabolism (Owen *et al.*, 1967; Krebs *et al.*, 1971); in such cases the O_2 /glucose ratio rises above 6.0.

Numerous studies with the nitrous oxide method and its derivatives demonstrated that cerebral energy metabolism declines in parallel with reduced levels of consciousness. For example, $CMRO_2$ was moderately lower in conditions in which the sensorium was impaired but not to the level of unconsciousness, e.g., diabetic acidosis, moderate hypoglycemia, cerebral arteriosclerosis, etc., and markedly depressed in conditions with complete unconsciousness or coma, e.g., general anesthesia, diabetic coma, insulin coma, hepatic coma, and cerebral ischemia due to increased intracranial pressure caused by brain tumors (Kety, 1950; Clarke and Sokoloff, 1999). All these were, however, pathological conditions in which energy metabolism was probably reduced due to disrupted intracellular biochemical processes, insufficient substrate supply, or lesser demand for energy because of pathologically depressed neuronal functional activity. In physiological and pharmacological states with obviously altered mental functions, not necessarily altered levels of consciousness, such as in schizophrenia, LSD intoxication, mild alcoholic inebriation, sedation, mental exercise during solving of arithmetic problems, etc., no changes in $CMRO_2$ were found. The failure of the nitrous oxide method to detect a relationship between energy metabolic rate and functional activity in the brain was undoubtedly due to the fact that it measured only average rates of oxygen and glucose utilization in the brain as a whole and not in the specific regions of the brain where the functional activities were localized.

2.2. LOCAL CEREBRAL ENERGY METABOLISM

The capability to determine local rates of energy metabolism in specific regions of the brain *in vivo* first became available with development of the 2- $[^{14}C]$ deoxyglucose ($[^{14}C]$ DG) method (Sokoloff *et al.*, 1977). This method measures local rates of glucose utilization simultaneously in all regions of the nervous system. Furthermore, it is fully applicable to unanesthetized animals, thus avoiding the complications of alterations of neural functions and metabolism by anesthetic agents. Because of the close to stoichiometric relationship between oxygen and glucose utilization in brain, glucose utilization is generally as good a measure of cerebral energy metabolism as oxygen consumption. 2- $[^{14}C]$ Deoxyglucose (2- $[^{14}C]$ DG) was used because it is an analog of glucose with which it competes for blood–brain transport into the brain and for phosphorylation by hexokinase to their hexose-6-phosphate derivatives. Unlike glucose-6-phosphate, however, which is very rapidly metabolized further via the glycolytic and tricarboxylic pathways ultimately to H_2O and CO_2 , 2- $[^{14}C]$ DG-6-phosphate can be metabolized to some extent to 2- $[^{14}C]$ DG-1-phosphate, 2- $[^{14}C]$ DG-1-6-phosphate, and ^{14}C -labeled glycogen, but all the products of 2- $[^{14}C]$ DG phosphorylation remain trapped in the cells. Consequently, all the labeled products of 2- $[^{14}C]$ DG phosphorylation accumulate with time and are retained within the cells with negligible loss for considerable lengths of time. From a kinetic analysis of the behavior of glucose and tracer concentrations of 2- $[^{14}C]$ DG in brain an operational equation was derived with which to compute the local rates of CMR_{glc} from the measured times courses of arterial plasma glucose and 2- $[^{14}C]$ DG concentrations and the local tissue isotope concentration at the end of the experimental period, usually 30–45 min. Local ^{14}C concentrations in identifiable anatomical structures throughout the brain are determined by the use of quantitative autoradiography (Figure 2.1), a technique originally developed for measurement of local cerebral blood flow in animals with chemically inert, diffusible radioactive tracers (Landau *et al.*, 1955; Freygang and Sokoloff, 1958). The autoradiographs obtained with the 2- $[^{14}C]$ DG method can be converted by computerized image-processing techniques into color-coded quantitative images of the brain in which local rates of CMR_{glc} are encoded in the color scale. These processed autoradiographic images provide, therefore, the means to visualize local rates of glucose utilization throughout the brain exactly where they occur with a spatial resolution of 100–200 μm (Smith, 1983a).

Figure 2.1. ^{14}C DG autoradiographs of conscious rat brain and of calibrated ^{14}C methylmethacrylate standards as well as the calibration curve between optical density and tissue ^{14}C concentrations derived from densitometry of the standards.

Autoradiography is, of course, not applicable to man. To adapt the ^{14}C DG method for use in man it was necessary to introduce a γ -emitting radioactive label into 2-deoxyglucose to allow external detection of the tracer. This was done by attaching ^{18}F to the carbon in the 2-carbon position of 2-deoxyglucose to produce 2- ^{18}F fluoro-2-deoxy-D-glucose (^{18}F FDG), and the local tissue concentrations of label were determined by external scintillation counting with a single photon emission scanner in place of autoradiography (Reivich

et al., 1979). ^{18}F is a positron emitter, and the ^{18}FDG method was subsequently adapted further for use with positron emission tomography (PET) (Phelps *et al.*, 1979). PET provides better accuracy and spatial resolution than single photon detection although its spatial resolution in the mm range is still at least an order of magnitude below the 100–200 μm of the autoradiographic [^{14}C]DG method.

Applications of the [^{14}C]DG and ^{18}FDG methods in both animals and man showed that CMR_{glc} varies widely in the various structures of the brain. It is much higher in gray matter than in white matter and is generally reduced by anesthesia (Table 2.2) (Sokoloff *et al.*, 1977). It was also clearly established that neuronal functional activation stimulates energy metabolism in neural tissues just as it does in other tissues (Sokoloff, 1981). The magnitude of the increases in glucose utilization is quantitatively related to the intensity of functional activation. For example, retinal stimulation by randomly timed light flashes raises local CMR_{glc} in proportion to the logarithm of the light intensity in those structures of the brain that receive direct projections from the retina (Figure 2.2). (Miyaoka *et al.*, 1979; Sokoloff, 1981). Electrical stimulation of the cervical sympathetic trunk or sciatic nerve increases CMR_{glc} linearly with spike frequency in the

Table 2.2. Local rates of cerebral glucose utilization in rats in the conscious state and under light thiopental anesthesia (means $\pm$ SEM)

Structure	Conscious (6)	Anesthetized (8)	% Effect
<i>Gray Matter</i>			
Visual Cortex	111 $\pm$ 5	64 $\pm$ 3	–42
Auditory Cortex	157 $\pm$ 5	81 $\pm$ 3	–48
Parietal Cortex	107 $\pm$ 3	65 $\pm$ 2	–39
Sensory-motor Cortex	118 $\pm$ 3	67 $\pm$ 2	–43
Lateral Geniculate Nucleus	92 $\pm$ 2	53 $\pm$ 3	–42
Medial Geniculate Nucleus	126 $\pm$ 6	63 $\pm$ 3	–50
Thalamus: Lateral Nucleus	108 $\pm$ 3	58 $\pm$ 2	–46
Thalamus: Ventral Nucleus	98 $\pm$ 3	55 $\pm$ 1	–44
Hypothalamus	63 $\pm$ 3	43 $\pm$ 2	–32
Caudate-Putamen	111 $\pm$ 4	72 $\pm$ 3	–35
Hippocampus: Ammon's Horn	79 $\pm$ 1	56 $\pm$ 1	–29
Amygdala	56 $\pm$ 4	41 $\pm$ 2	–27
Cochlear Nucleus	124 $\pm$ 7	79 $\pm$ 5	–36
Nuclei of Lateral Lemniscus	114 $\pm$ 7	75 $\pm$ 4	–34
Inferior Colliculi	198 $\pm$ 7	131 $\pm$ 8	–34
Superior Colliculi	99 $\pm$ 3	59 $\pm$ 7	–40
Superior Olivary Nucleus	141 $\pm$ 5	104 $\pm$ 7	–26
Vestibular Nucleus	133 $\pm$ 4	81 $\pm$ 4	–39
Pontine Gray Matter	69 $\pm$ 3	46 $\pm$ 3	–33
Cerebellar Cortex	66 $\pm$ 2	44 $\pm$ 2	–33
Cerebellar Nuclei	106 $\pm$ 4	75 $\pm$ 4	–29
<i>White Matter</i>			
Corpus Callosum	42 $\pm$ 2	30 $\pm$ 2	–29
Genu of Corpus Callosum	35 $\pm$ 5	30 $\pm$ 2	–14
Internal Capsule	35 $\pm$ 5	29 $\pm$ 2	–17
Cerebellar White Matter	38 $\pm$ 2	29 $\pm$ 2	–24

Note: The effects of anesthesia were statistically significant in every structure ($p < 0.05$).
From Sokoloff *et al.* (1977).

Figure 2.2. Rates of glucose utilization as a function of intensity of retinal illumination with randomly timed light flashes in various layers of superior colliculus and lateral geniculate nucleus of dark adapted albino and pigmented rats. From Miyaoka *et al.* (1979).

superior cervical ganglion (Yarowsky *et al.*, 1983) and dorsal horn of the lumbar spinal cord, respectively (Figure 2.3) (Kadekaro *et al.*, 1985). The glucose consumed per action potential can be determined from the slope of the straight line and equals 0.4 nmol/g of tissue per spike in the superior cervical ganglion and 0.6 nmol/g per spike in the dorsal horn of the lumbar spinal cord (Figure 2.3).

Figure 2.3. Relationship between frequency of action potentials and glucose utilization in dorsal horn of lumbar spinal cord and in superior cervical ganglion during electrical stimulation of sciatic nerve or cervical sympathetic trunk at different frequencies, respectively. Derived from data of Kadekaro *et al.* (1985) (top) and Yarowsky *et al.* (1983) (bottom).

2.3. BIOCHEMICAL MECHANISMS OF FUNCTIONAL ACTIVATION OF ENERGY METABOLISM AT THE TISSUE LEVEL

Muscles move or support masses against gravitational forces; the heart pumps blood against a pressure head; and kidneys transport water and solutes against concentration and osmotic gradients. These functions involve clearly definable physical work that requires expenditure of energy. It was less obvious what energy-requiring work nervous tissues do when functionally activated. Inasmuch as CMR_{glc} increases more or less linearly with spike frequency (Figure 2.3), it was reasonable to presume that the increased energy metabolism was somehow related to the generation, propagation, and conduction of action potentials. Action potentials result from the opening of ion channels that lead to increased Na^+ influx and K^+ efflux in neurons; they do not themselves directly consume energy but draw upon the potential energy stored in the membrane potentials. As spike frequencies increase, there are greater ion displacements and larger rises in intracellular Na^+ ($[Na^+]_i$) and extracellular K^+ ($[K^+]_o$) concentrations and, therefore, the need for more electrogenic Na^+ , K^+ -ATPase activity to restore the ionic gradients across the membranes to their resting levels. This enzyme catalyzes the coupled transport of Na^+ out of and K^+ into the cells and consumes one molecule of ATP for every three Na^+ ion exported and two K^+ ions imported. Increased ATPase activity lowers intracellular ATP and raises ADP, AMP, phosphate acceptor, and inorganic phosphate concentrations, intracellular changes that stimulate glycolysis and electron transport and, therefore, energy metabolism. Many of the conditions of functional activation in which the $[^{14}C]DG$ method revealed local increases in CMR_{glc} have, in fact, been shown to be associated with elevated $[K^+]_o$ in the activated tissues, and increasing $[K^+]_o$ *in vivo* by applying K^+ directly to the cerebral cortex, a treatment known to depolarize cell membranes and to cause spreading cortical depression, which itself raises $[K^+]_o$ even further, profoundly stimulates cerebral cortical glucose utilization (Figure 2.4) (Shinohara *et al.*, 1979).

Figure 2.4. [^{14}C]Deoxyglucose autoradiographs showing changes in local CMR_{glc} during K^+ -induced spreading cortical depression and during recovery in the rat; greater optical densities indicate higher CMR_{glc} . In all cases the experimental side on the left was treated with topically applied KCl, and the control side on the right was similarly treated with equivalent concentrations of NaCl. **(A)** Autoradiographs of brain sections at various levels of cerebral cortex from conscious rat in which spreading cortical depression was produced and sustained on left side by 5 M KCl applied to surface of intact dura over parietal cortex every 15–20 min; right side treated comparably with NaCl. **(B)** Autoradiographs of brain sections at level of parietal cortex from three rats under barbiturate anesthesia. Top section is from normal, anesthetized rat; middle section is from similarly anesthetized rat in which 80 mM KCl in artificial cerebrospinal fluid was repeatedly applied directly to the surface of the left parietal cortex; bottom section is from similarly anesthetized rat studied immediately after return of cortical DC potential to normal after a single wave of spreading depression induced by a single application of 80 mM KCl to the parieto-occipital cortex of the left side. From Shinohara *et al.* (1979).

Table 2.3. Influence of sodium pump activity and neurosecretion on [^{14}C]deoxyglucose uptake in posterior pituitary *in vitro*^a

Condition	[^{14}C]Deoxyglucose uptake (Cpm/100 μg protein/15 min)
A. Dependence on activation of sodium pump	Electrical stimulation
Controls (4)	988 $\pm$ 19
+ Electrical stimulation at 10 Hz (4)	1272 $\pm$ 57 ^b
+ Electrical stimulation at 10 Hz + 10 μM ouabain (4)	1018 $\pm$ 51 ^c
B. Dependence on activation of sodium pump	Opening Na^+ channels
Controls (14)	1381 $\pm$ 50
+60 μM veratridine (14)	1891 $\pm$ 85 ^b
+6 μM tetrodotoxin (9)	1209 $\pm$ 84 ^c
+60 μM veratridine + 6 μM tetrodotoxin (8)	1551 $\pm$ 72 ^c
+10 μM ouabain (4)	1318 $\pm$ 57 ^c
+60 μM veratridine + 10 μM ouabain (4)	1218 $\pm$ 120 ^c
C. Independence from activation of secretion (Ca^{2+}-free medium)	
Controls (in Ca^{2+} -free medium) (6)	1142 $\pm$ 38
+60 μM veratridine (in Ca^{2+} -free medium) (6)	1681 $\pm$ 78 ^b

^a The values represent means $\pm$ SEM of results obtained in number of experiments indicated in parentheses.^b Indicates statistically significant difference from controls ($p < 0.001$).^c Indicates no statistical significant difference from controls. From Mata *et al.* (1980).

The possibility that activation of Na^+ , K^+ -ATPase activity by membrane-depolarizing stimulation mediates the coupling of energy metabolism to functional activity was examined directly in studies in which the rat neurohypophysis was electrically stimulated *in vitro* under conditions known to stimulate its secretion of vasopressin (Mata *et al.*, 1980). Such electrical stimulation raised [^{14}C]DG phosphorylation, indicating increased glucose utilization, and this stimulation was completely blocked by addition of ouabain, a specific inhibitor of Na^+ , K^+ -ATPase (Table 2.3). Addition of veratridine, an agent that depolarizes cell membranes by opening voltage-dependent Na^+ channels and allows Na^+ entry into the cells, also markedly stimulated [^{14}C]DG phosphorylation, and this stimulation too was eliminated by ouabain, as well as by tetrodotoxin, a known blocker of voltage-dependent Na^+ channels (Table 2.3). The stimulation of [^{14}C]DG phosphorylation was not related to increased vasopressin secretion; the neurohypophysis *in vitro* cannot be stimulated to secrete hormone in Ca^{++} -free medium, but even in such a medium veratridine stimulated [^{14}C]DG phosphorylation (Table 2.3).

It appears then that the extra energy metabolism associated with electrical and functional activation in neural tissue is not used directly in the generation and propagation of action potentials. The energy is used rather to restore ionic gradients and resting membrane potentials that are partially degraded by the action potentials and also to some extent for other processes associated with or secondary to action potentials, such as neurotransmitter release, reuptake, and metabolism, exocytosis, endocytosis, etc.

2.4. LOCALIZATION OF FUNCTIONALLY ACTIVATED ENERGY METABOLISM AT THE TISSUE LEVEL

Traditional electrophysiology focuses largely on electrical recordings from neurons, and it was, therefore, originally assumed that the increases in local CMR_{glc} associated with functional activation observed with the [^{14}C]DG method were primarily in perikarya. Careful comparisons between the autoradiographs produced by the [^{14}C]DG method and the stained brain sections from which they were prepared did not, however,

Figure 2.5. [^{14}C]Deoxyglucose autoradiograph of normal, conscious rat brain. Greater optical density reflects greater CMR_{glc} . Note high rate in molecular layer of hippocampus.

support this assumption. In the autoradiographs higher rates of glucose utilization are reflected in increasing darkness in the films (Figure 2.1). It was noted in the autoradiographs that the most metabolically active region in the hippocampus, which was at first thought to represent the cell-rich pyramidal cell layer, was actually the molecular layer, a region predominantly rich in synapses and poor in cell bodies (Figure 2.5).

In the striate cortex of the normal monkey CMR_{glc} is highest in a sub-layer of Layer IV that is not particularly rich in cellular elements; it is the layer where axonal terminals of the afferent geniculocalcarine pathway synapse with dendrites of neurons situated in other laminae of the visual cortex (Figure 2.6A, C) (Kennedy *et al.*, 1976). It is in this neuropil-rich layer that CMR_{glc} is most reduced when visual input is interrupted (Figure 2.6B).

The hypothalamo-neurohypophyseal pathway originates in cell bodies in the supraoptic and paraventricular nuclei in the hypothalamus and terminates in the neurohypophysis, 40 % of which consists of axonal terminals of the afferent tract (Nordmann, 1977). Osmotic stimulation by salt-loading is known to activate this pathway to stimulate vasopressin release from the neurohypophysis. Salt-loading was found also to stimulate local CMR_{glc} markedly in the neurohypophysis but to have no apparent effect on CMR_{glc} in the supraoptic and paraventricular nuclei (Figure 2.7) (Schwartz *et al.*, 1979). In contrast, hypotension produced by α -adrenergic blockade with phenoxybenzamine or hemorrhage was found to stimulate local CMR_{glc} markedly in both these nuclei (Figure 2.7). The difference is that osmotic stimulation acts directly on the cell bodies to activate the hypothalamo-neurohypophyseal pathway whereas hypotension exerts its effects on the cell bodies in these nuclei indirectly via afferent inputs from the nucleus tractus solitarius in the brain stem that are part of the neural pathways of the baroreceptor reflexes. Results such as these suggested that *it is mainly the energy metabolism in regions rich in neuropil and synapses and not cell bodies that is linked to functional activity.*

To compare directly the effects of functional activation on local CMR_{glc} in perikarya and nerve terminals simultaneously in the same pathway, Kadarkar *et al.* (1985) stimulated the sciatic nerve at different frequencies and examined the effects on CMR_{glc} in both the dorsal root ganglia and the dorsal horn of corresponding segments of the lumbar spinal cord. The advantage of this system is that the body of the dorsal root ganglion contains perikarya devoid of neural processes, thus allowing examination of cell bodies free of nerve terminals simultaneously with the nerve terminals of the same pathway in the dorsal horn of the spinal cord. The results confirmed that it is CMR_{glc} in the region of the nerve terminals and not in the cell bodies that is linked to the functional activity. Glucose utilization increased linearly with the

Figure 2.6. [^{14}C]Deoxyglucose autoradiographs demonstrating effects of bilateral and monocular visual occlusion on local CMR_{glc} in striate cortex of conscious monkey. Greater optical density indicates greater CMR_{glc} . (A) Striate cortex from animal with both eyes open. Note heterogeneity in laminae; the darkest lamina corresponds to Layer IV. (B) Striate cortex from monkey with both eyes patched. Note general reduction in density and almost complete disappearance of laminar heterogeneity, especially in Layer IV. (C) Striate cortex from animal with only right eye patched. Left half of autoradiograph corresponds to left hemisphere contralateral to occluded eye. Note alternating dark and light columns traversing the full thickness of the striate cortex which represent the ocular dominance columns. The dark bands represent the columns for the open eye; the light bands represent the columns for the patched eye and demonstrate the reduced glucose utilization resulting from reduced visual input. The arrows point to regions of bilateral asymmetry; these are the loci of representation of the blind spots of the visual fields. From Kennedy *et al.* (1976).

increasing frequency of stimulation of the sciatic nerve in the dorsal horn of the lumbar spinal cord, but it did not budge from its baseline levels in the cell bodies of the same pathway in the dorsal root ganglia (Figure 2.8). The lack of a metabolic response to electrical stimulation in the perikarya in the dorsal root ganglion may be surprising, but there is evidence that the soma membrane of neurons is not very excitable and not very productive of action potentials because of a relative paucity of voltage-gated Na^+ channels. Smith (1983b), using patch clamp electrodes, obtained evidence that soma and dendrites of spinal cord neurons and soma of dorsal root ganglion cells cultured *in vitro* do not generate action potentials. Freygang

Figure 2.7. Effects of activation of hypothalamo-neurohypophyseal pathway by salt-loading or hypotension on local cerebral glucose utilization in the conscious rat. (A) Histological sections of brain stained with cresyl violet (Nissl) and pituitary stained with toluidine blue demonstrating positions of supraoptic nucleus (SON), paraventricular nucleus (PVN), posterior pituitary (PP), and anterior pituitary (AP). (B) $[^{14}\text{C}]$ Deoxyglucose autoradiographs of brain and pituitary from normal control rat drinking only water. (C) $[^{14}\text{C}]$ Deoxyglucose autoradiographs from rat given 2% (w/v) NaCl in drinking water for 5 days. Note selective marked increase in density in posterior hypophysis, indicating increased glucose utilization. (D) $[^{14}\text{C}]$ Deoxyglucose autoradiographs from rat made hypotensive by administration of 20 mg/kg of phenoxybenzamine 45–60 min prior to administration of the $[^{14}\text{C}]$ deoxyglucose. Note selective increases in labeling of supraoptic and paraventricular nuclei and posterior pituitary. From Schwartz *et al.* (1979).

Figure 2.8. Frequency-dependent effects of electrical stimulation of sciatic nerve on local CMR_{glc} in dorsal root ganglion and dorsal horn of lumbar spinal cord. Error bars represent SEM. From Kadekaro *et al.* (1985).

(1958) and Freygang and Frank (1959) had previously concluded from analyses of extracellular potentials recorded from single spinal motor neurons and single neurons in the lateral geniculate nucleus that the soma-dendritic membrane can be driven synaptically to produce post-synaptic potentials but not propagating action potentials. Conduction through the cell body is mainly by electrotonic conduction rather than by action potentials. If action potentials do indeed mediate the coupling of energy metabolism to functional activity but are absent in the perikarya, then increased glucose metabolism due to functional activation is not to be expected in the perikarya. Some energy metabolism does, of course, proceed in cell bodies even at rest, but it is probably used more for vegetative, biosynthetic, and transport processes needed to maintain cellular structural and functional integrity rather than for processes directly related to functional activity. Some energy-consuming processes within the cell bodies that are related to intracellular signal transduction must also be activated by functional activation, but apparently their requirements are too small to be detected in the overall energy consumption of the cell.

The finding that rates of energy metabolism are linked to spike frequency in the terminal zones of the afferent pathways in the neuropil and not in cell bodies in the pathway should help to resolve questions about metabolic responses associated with functional excitation and inhibition. Glucose utilization has sometimes been observed to increase in structures in which electrophysiological evidence indicated inhibition of neuronal activity, thus raising questions whether active inhibition required energy just like excitation. It appears, however, that it is the spike activity in the afferent nerve terminals that correlates with the energy consumption, and this activity is the same whether the terminals are releasing excitatory or inhibitory neurotransmitters. The energy metabolism of the post-synaptic cell bodies, whether activated or inhibited, is not significantly altered, and to determine which has occurred, it is necessary to look downstream at the next synapses in the projection zones of those neurons. Glucose utilization is depressed in the projection zones of inhibited neurons and increased in the projection zones of excited neurons.

2.5. CELLULAR LOCALIZATION OF FUNCTIONAL ACTIVATION OF ENERGY METABOLISM

The neuropil, in which functionally activated energy metabolism is localized, contains not only neuronal elements, e.g., axonal terminals, dendritic processes, and synapses, but astrocytic processes as well. Because stimulation of local CMR_{glc} is linked to Na^+ , K^+ -ATPase activity and the frequency of action potentials which are associated with increased Na^+ influx and K^+ efflux, some of the increased energy metabolism must be used to restore the ionic gradients in the neuronal elements where the action potentials are produced. Astrocytic processes envelop the synapses and could also be involved in the metabolic response. Astrocytes are believed to regulate $[\text{K}^+]_o$ either by passive diffusion (Orkand *et al.*, 1966; Medzihradsky *et al.*, 1971) and/or active transport (Henn *et al.*, 1972) following increases in $[\text{K}^+]_o$ resulting from neuronal excitation (Hertz, 1977; Erecinska and Silver, 1994), and studies with tissue slices (Yarowsky *et al.*, 1986; Badar-Goffer *et al.*, 1992) or cultured cells (Cummins *et al.*, 1979a; Cummins *et al.*, 1979b; Brookes and Yarowsky, 1985; Hertz and Peng, 1992; Peng *et al.*, 1994) have indicated that energy is consumed in the process. The spatial resolution of the autoradiographic ^{14}C DG method is limited to 100–200 μm (Smith, 1983a) which is inadequate to identify the specific cellular or subcellular elements in neuropil that contribute to the increases in CMR_{glc} during functional activation.

2.5.1. Effects of Increased Extracellular K^+ and Intracellular Na^+ Concentrations

To approach this question, studies have been carried out *in vitro* with cells in culture. Changes in the extracellular medium expected to result from increased spike activity *in vivo* were simulated *in vitro*, and their effects on glucose metabolism of cultured neurons and astroglia examined. Spike activity in axonal terminals leads to increased extracellular K^+ and intracellular Na^+ concentrations and release of neurotransmitters. Therefore, the effects of elevated $[\text{K}^+]_o$, $[\text{Na}^+]_i$, and the extracellular concentration of glutamate, the most prevalent excitatory neurotransmitter in brain, on ^{14}C DG phosphorylation to ^{14}C DG-6-phosphate, a measure of glucose utilization, were examined in cultured neurons and astroglia (Takahashi *et al.*, 1995).

Increasing $[\text{K}^+]_o$ in the range of 5.4–56 mM stimulated ^{14}C DG phosphorylation in both neuronal and mixed neuronal-astroglial cultures (Figure 2.9) (Takahashi *et al.*, 1995), and these increases were prevented by ouabain, implicating Na^+ , K^+ -ATPase in the mechanism of the effect. Raising $[\text{K}^+]_o$ in the same range had no such effect in the cultured astroglia. In fact, the highest levels of $[\text{K}^+]_o$ examined tended to inhibit ^{14}C DG phosphorylation in astroglia (Figure 2.9). On the other hand, promoting Na^+ entry into the cells and increasing $[\text{Na}^+]_i$ either by opening membrane voltage-gated Na^+ channels with veratridine or by adding the Na^+ ionophore monensin markedly stimulated ^{14}C DG phosphorylation in astroglia, but only if Na^+ were present in the extracellular medium (Figure 2.10) (Brookes and Yarowsky, 1985; Takahashi *et al.*, 1995). Tetrodotoxin, which blocks voltage-dependent Na^+ channels, did not alter baseline rates of ^{14}C DG phosphorylation in astroglia, and, as expected, prevented the stimulation by veratridine and had no effect on the stimulation by monensin (Figure 2.10). Inhibiting Na^+ , K^+ -ATPase activity with ouabain lowered the basal rate of ^{14}C DG phosphorylation, completely blocked the stimulation by veratridine, and partially suppressed the stimulation by monensin (Figure 2.10). The absence of a tetrodotoxin effect on the monensin stimulation can be explained by the fact that monensin-induced Na^+ entry is not through voltage-dependent Na^+ channels and is, therefore, insensitive to blockade by tetrodotoxin. The less than complete blockade of the monensin stimulation by ouabain can also be explained by the fact that monensin is a Na^+/H^+ exchanger that exchanges extracellular Na^+ for intracellular H^+ . It, therefore, raises Na^+ and lowers H^+ concentrations in the cells, both ionic changes that can enhance glucose metabolism, the

Figure 2.9. Effects of $[K^+]_o$ on rates of $[^{14}C]$ DG phosphorylation in cultured neurons, mixed neuronal-astroglia cultures, and astroglia cultures. Values are means $\pm$ SEM obtained from quadruplicate wells. Numbers above bars indicate duration of incubation. * $p < 0.05$, ** $p < 0.01$ compared to the 5.4 mM $[K^+]_o$ (Dunnett's test for multiple comparisons). Representative of at least three such experiments for each condition. From Takahashi *et al.* (1995).

former by ouabain-sensitive stimulation of Na^+ , K^+ -ATPase activity, and the latter by ouabain-insensitive stimulation of phosphofructokinase activity due to increased intracellular pH.

2.5.2. Effects of Glutamate on Glucose Utilization in Astroglia

Glutamate, the most prevalent excitatory neurotransmitter in the brain, is toxic to neurons, and must, therefore, be rapidly cleared from the extracellular space when it is released at glutamatergic synapses. Astrocytes, which have processes that envelop the synapses, carry out this function. They take up the glutamate from the extracellular space and convert it to glutamine, both steps that involve the consumption of energy. Addition of glutamate to the medium stimulates glucose utilization by astroglial cultures (Pellerin and Magistretti, 1994; Takahashi *et al.*, 1995). This stimulation is Na^+ dependent, ouabain sensitive, unaffected by tetrodotoxin, and insensitive to NMDA and non-NMDA glutamate receptor antagonists (Figure 2.11). DL-Threo- β -hydroxyaspartate, which competes with glutamate for uptake by

Figure 2.10. Effects of veratridine (upper) and monensin (lower) on [^{14}C]DG phosphorylation in astroglia (21-day old culture, no dbcAMP) and the effects of ouabain, tetrodotoxin (TTX), and Na^+ -free medium on the veratridine and monensin effects. Values are means $\pm$ SEM obtained from measurements of quadruplicate wells in three experiments. Numbers above bars indicate percent difference from each control. Representative of in three different astroglial preparations. ** $p < 0.01$; *** $p < 0.001$; n.s., not statistically significant from each control (grouped t test). From Takahashi *et al.* (1995).

the Na^+ /glutamate co-transport system (Flott and Seifert, 1991), itself stimulates glucose utilization, and glutamate no longer stimulates in its presence. The hydroxyaspartate stimulation is also blocked by ouabain, absent in Na^+ -free medium, and unaffected by tetrodotoxin and inhibitors of NMDA or non-NMDA receptors. These results show that the glutamate stimulation of glucose utilization by astroglia does not involve NMDA and non-NMDA glutamate receptors or voltage-dependent Na^+ channels in the astroglia. It is due instead to the activation of Na^+ , K^+ -ATPase activity by the increase in $[\text{Na}^+]_i$ that results from the coupled uptake of Na^+ with glutamate into the astroglia. This uptake is mediated by a Na^+ /glutamate co-transporter that co-transport 2–3 Na^+ ions with each glutamate molecule. In addition, much of the glutamate taken up by the astroglia is converted by glutamine synthetase to glutamine, a reaction consuming one ATP molecule for each molecule of glutamine formed. Inasmuch as it takes one molecule of ATP to pump out

Figure 2.11. Effects of glutamate on [^{14}C]DG phosphorylation in astroglia and the effects of DL-2-amino-5-phosphonovaleric acid (APV), CNQX, ouabain, tetrodotoxin (TTX), DL-threo- β -hydroxyaspartic acid (THA), and Na^+ -free medium on the glutamate effects. Values are means $\pm$ SEM obtained from quadruplicate wells, in three experiments, each with a different astroglial preparation. Numbers above bars indicate percent differences from each control. *** $p < 0.001$; n.s., not statistically significant, from each control group (grouped t test). From Takahashi *et al.* (1995). Similar results were reported by Pellerin and Magistretti (1994).

the 2–3 Na^+ ions co-transported inward with each molecule of glutamate taken up, and one molecule of ATP is consumed in converting the molecule to glutamine, it appears that the net gain of two molecules of ATP from the glycolysis of one molecule of glucose is consumed in the processing of one molecule of glutamate by astroglia.

2.6. SUMMARY

The main energy-consuming work of functionally activated neural tissues is related to the generation and propagation of action potentials and the disposition of the neurotransmitter molecules that are released at the synapses. Normally, the energy required by neural tissues is almost entirely derived from glucose metabolism which increases almost linearly with spike frequency when tissue is functionally activated. Spikes result from Na^+ influx and K^+ efflux in the neuronal elements where they are generated, and the greater the spike frequency the greater the ionic displacements. Increased extracellular K^+ and intracellular Na^+ concentrations stimulate Na^+ , K^+ -ATPase activity to restore the ionic gradients to their normal resting levels, and energy metabolism is then increased to resynthesize the ATP consumed in that process.

Functional activation of glucose utilization is essentially confined to neuropil which contains axonal, dendritic, and astrocytic processes. Obviously, some of the increased energy metabolism must occur in the

axonal terminals and dendrites where the spikes are generated. Functional activation of energy metabolism is not seen in perikarya, presumably because they have few voltage-dependent Na^+ channels and, therefore, generate few if any action potentials.

Astrocytes contribute to the increased energy metabolism in neuropil during functional activation, but by other mechanisms. Astrocytic membranes can be depolarized by increased $[\text{K}^+]_i$, but they do not produce action potentials that would allow the Na^+ influx into the cells needed to stimulate Na^+ , K^+ -ATPase activity. Action potentials in axonal terminals, however, are associated with release of neurotransmitters. Glutamate is the most prevalent excitatory neurotransmitter in brain, but it is also a neurotoxin, and its extracellular concentration must be kept low by astrocytic reuptake via Na^+ -dependent glutamate transporters that transport 2–3 Na^+ ions with each glutamate molecule into the cell. The increased $[\text{Na}^+]_i$ then results in stimulation of Na^+ , K^+ -ATPase activity and energy metabolism. In addition, glutamate taken up by astroglia is converted there to glutamine, an energy-requiring reaction that also consumes ATP.

Astrocytic processes surround the capillaries that bring glucose to the brain, and some if not all the glucose must traverse them before reaching the neurons. Evidence has been accumulating that astrocytes first metabolize glucose to lactate which they then export to the neurons for oxidation to CO_2 and H_2O (Magistretti and Pellerin, 1996; Tsacopoulos and Magistretti, 1996). This would mean that glucose metabolism is compartmentalized between astroglia and neuronal elements, glycolysis in the astroglia and oxidation of the lactate/pyruvate products by the neurons. Glycolysis yields two molecules of ATP and two molecules of pyruvate/lactate per molecule of glucose glycolyzed. The two molecules of ATP produced in the astroglia are then consumed in the processing of each molecule of glutamate, suggesting that there may be a close quantitative relationship between glucose consumption and glutamate processing in the astroglia (Sibson *et al.*, 1998). The oxidation of the two molecules of pyruvate/lactate produced from glycolysis of one molecule of glucose leads to the generation of 36 additional molecules of ATP which is consumed mainly for the restoration of the ionic gradients and the membrane potentials degraded by the action potentials.

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