Advances in Neurochemistry

Volume 4

Edited by

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PREFACE

This series has been directed at providing scientists possessing considerable biochemical background with specialized reviews of neurobiological interest. Some have dealt with completed bodies of research, while others consist of extensive reports of research in progress, judged to be of current interest to the active researcher. We have selected recognized scientists and allowed them freedom to reflect and speculate in the field in which they have achieved prominence. We note with sadness the passing of Dr. Jordi Folch-Pi, who served as an advisory editor when the series was initiated. He played a central role in the development of neurochemistry, as well as the creation of professional societies and journals. He will be remembered fondly by all those whose lives he touched.

The editors acknowledge the cooperation of the Upjohn Company in the preparation of the color plate included in this volume. We also acknowledge the skillful editorial assistance of Dr. Kenneth C. Leskawa. We are pleased to honor the retirement of Dr. E. Martin Gál, a former advisory editor of *Advances*, with the inclusion of a chapter by him in this volume.

B. W. Agranoff
M. H. Aprison

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CHAPTER 1

THE RADIOACTIVE DEOXYGLUCOSE METHOD

THEORY, PROCEDURE, AND APPLICATIONS FOR THE MEASUREMENT OF LOCAL GLUCOSE UTILIZATION IN THE CENTRAL NERVOUS SYSTEM

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1. INTRODUCTION

The brain is a complex, heterogeneous organ composed of many anatomical and functional components with markedly different levels of functional activity that vary independently with time and function. Other tissues are generally far more homogeneous with most of their cells functioning similarly and synchro-

Abbreviations used in this chapter: DG, 2-deoxyglucose; DG-6-P, 2-deoxyglucose-6-phosphate; G-6-P, glucose-6-phosphate

nously in response to a common stimulus or regulatory influence. The central nervous system, however, consists of innumerable subunits each integrated into its own set of functional pathways and networks and subserving only one or a few of the many activities in which the nervous system participates. Understanding how the nervous system functions requires knowledge not only of the mechanisms of excitation and inhibition but even more so of their precise localization in the nervous system and the relationships of neural subunits to specific functions.

Historically, studies of the central nervous system have concentrated heavily on localization of function and mapping of pathways related to specific functions. These have been carried out neuroanatomically and histologically with staining and degeneration techniques, behaviorally with ablation and stimulation techniques, electrophysiologically with electrical recording and evoked electrical responses, and histochemically with a variety of techniques, including fluorescent and immunofluorescent methods and autoradiography of orthograde and retrograde axoplasmic flow. Many of these conventional methods suffer from a sampling problem. They generally permit examination of only one potential pathway at a time, and only positive results are interpretable. Furthermore, the demonstration of a pathway reveals only a potential for function; it does not reveal its significance in normal function.

Tissues that do physical and/or chemical work, such as heart, kidney, and skeletal muscle, exhibit a close relationship between energy metabolism and functional activity. From the measurement of energy metabolism it is possible to estimate the level of functional activity. The existence of a similar relationship in the tissues of the central nervous system has been more difficult to prove, partly because of uncertainty about the nature of the work associated with nervous functional activity, but mainly because of the difficulty in assessing the levels of functional and metabolic activities in the same functional component of the brain at the same time. Much of our present knowledge of cerebral energy metabolism *in vivo* has been obtained by means of the nitrous oxide technique of Kety and Schmidt (1948*a*) and its modifications (Scheinberg and Stead, 1949; Lassen and Munck, 1955; Eklöf *et al.*, 1973; Gjedde *et al.*, 1975), which measure the average rates of energy metabolism in the brain as a whole. These methods have demonstrated changes in cerebral metabolic rate in association with gross or diffuse alterations of cerebral function and/or structure, such as, for example, those that occur during postnatal development, aging, senility, anesthesia, disorders of consciousness, and convulsive states (Kety, 1950, 1957; Lassen, 1959; Sokoloff, 1960, 1976). However, these methods have not detected changes in cerebral metabolic rate in a number of conditions with, perhaps, more subtle alterations in cerebral functional activity, e.g., deep slow-wave sleep, performance of mental arithmetic, sedation and tranquilization, schizophrenia, and LSD-induced psychosis (Kety, 1950; Las-

sen, 1959; Sokoloff, 1969). It is possible that there are no changes in cerebral energy metabolism in these conditions. The apparent lack of change could also be explained by either a redistribution of local levels of functional and metabolic activity, without significant change in the average of the brain as a whole, or the restriction of altered metabolic activity to regions too small to be detected in measurements of the brain as a whole. What has clearly been needed is a method that measures the rates of energy metabolism in specific discrete regions of the brain in normal and altered states of functional activity.

Kety and associates (Landau *et al.*, 1955; Freygang and Sokoloff, 1958; Kety, 1960; Reivich *et al.*, 1969) developed a quantitative autoradiographic technique to measure the local tissue concentrations of chemically inert, diffusible, radioactive tracers, which they used to determine the rates of blood flow simultaneously in all the structural components visible and identifiable in autoradiographs of serial sections of the brain. The application of this quantitative autoradiographic technique to the determination of local cerebral metabolic rate has proved to be more difficult because of the inherently greater complexity of the problem and the unsuitability of the labeled species of the normal substrates of cerebral energy metabolism, oxygen and glucose. The radioisotopes of oxygen have too short a physical half-life. Both oxygen and glucose are too rapidly converted to carbon dioxide (CO_2), which is then cleared from the cerebral tissues too rapidly. Sacks (1957), for example, has found in man significant losses of $^{14}CO_2$ from the brain within 2 min after the onset of an intravenous infusion of [^{14}C]glucose, labeled either uniformly or in the C-1, C-2, or C-6 position. These limitations of [^{14}C]glucose have been avoided by the use of 2-deoxy-D-[^{14}C]glucose, a labeled analogue of glucose with special properties that make it particularly appropriate for this application (Sokoloff *et al.*, 1977). It is metabolized through part of the pathway of glucose metabolism at a definable rate relative to that of glucose. Unlike glucose, however, its product, [^{14}C]deoxyglucose-6-phosphate, is essentially trapped in the tissues, thus allowing the application of the quantitative autoradiographic technique. The use of radioactive 2-deoxyglucose to trace glucose utilization and the autoradiographic technique to achieve regional localization has recently led to the development of a method that measures the rates of glucose utilization simultaneously in all components of the central nervous system in the normal conscious state and during experimental physiological, pharmacological, and pathological conditions (Sokoloff *et al.*, 1977). Because the procedure is so designed that the concentrations of radioactivity in the tissues during autoradiography are more or less proportional to the rates of glucose utilization, the autoradiographs provide pictorial representations of the relative rates of glucose utilization in all the cerebral structures visualized. Numerous studies with this method have established that there is a close relationship between functional activity and energy metabolism in the central nervous system (Sokoloff,

1977; Plum *et al.*, 1976). The method has become a potent new tool for mapping functional neural pathways on the basis of evoked metabolic responses.

2. THEORETICAL BASIS OF THE RADIOACTIVE 2-DEOXYGLUCOSE METHOD

2.1. Biochemical Properties of 2-Deoxyglucose in Brain

2-Deoxy-D-glucose differs from glucose only in the replacement of the hydroxyl group on the second carbon atom by a hydrogen atom. The remainder of the molecule is indistinguishable from that of glucose. It is metabolized qualitatively exactly like glucose until a point in the glycolytic pathway is reached where its anomalous structure prevents its further metabolism. Thus, deoxyglucose is transported between blood and brain tissues by the same saturable carrier that transports glucose (Bidder, 1968; Bachelard, 1971; Oldendorf, 1971; Horton *et al.*, 1973). In the tissues it competes with glucose for hexokinase, which phosphorylates both substrates to their respective hexose-6-phosphates (Sols and Crane, 1954). It is at this point in the biochemical pathway that the further metabolism of the two compounds diverges.

Glucose-6-phosphate is converted to fructose-6-phosphate by phosphohexoseisomerase and metabolized further via the glycolytic pathway and tricarboxylic acid cycle. 2-Deoxyglucose-6-phosphate cannot be isomerized to fructose-6-phosphate because of the lack of a hydroxyl group on its second carbon atom; therefore, its metabolism ceases at this point in the pathway (Sols and Crane, 1954; Wick *et al.*, 1957; Tower, 1958; Bachelard *et al.*, 1971; Horton *et al.*, 1973). Although not a substrate for further metabolism, deoxyglucose-6-phosphate does have an affinity for the phosphohexoseisomerase and, when present in sufficiently high concentrations, can competitively inhibit glucose-6-phosphate metabolism at this point (Wick *et al.*, 1957; Tower, 1958; Horton *et al.*, 1973). Indeed, it is probably mainly by this competitive inhibition at the phosphohexoseisomerase step that pharmacological doses of deoxyglucose lead to an inhibition of glycolysis and produce a clinical syndrome like that of hypoglycemic coma (Tower, 1958; Landau and Lubs, 1958; Horton *et al.*, 1973; Meldrum and Horton, 1973); inhibition at the hexokinase step, either competitively by deoxyglucose or by depletion of adenosine triphosphate (ATP), may also be contributory (Tower, 1958; Horton *et al.*, 1973).

There are alternative pathways of glucose-6-phosphate metabolism, but these do not appear to have significant influence on the fate of deoxyglucose-6-

phosphate in brain. Glucose-6-phosphate can be oxidized by glucose-6-phosphate dehydrogenase, the first step in the hexosemonophosphate shunt, but deoxyglucose-6-phosphate does not appear to be a substrate for this enzyme (Sols and Crane, 1954; Tower, 1958; Horton *et al.*, 1973). Glucose-6-phosphate can also be hydrolyzed back to free glucose by glucose-6-phosphatase. The activity of this enzyme has been reported to be very low in mammalian brain (Hers and DeDuve, 1950; Hers, 1957; Raggi *et al.*, 1960; Prasannan and Subrahmanyam, 1968), but its possible influence in the deoxyglucose technique will be considered in greater detail in Section 4.3.

2.2. Description of Theoretical Model

The theoretical basis of the [^{14}C]deoxyglucose technique is derived from the analysis of a model of the biochemical behavior of deoxyglucose in brain. This model is diagrammatically illustrated in Figure 1. According to the model, [^{14}C]deoxyglucose and glucose in the plasma share and compete for a common carrier in the blood–brain barrier for transport from plasma to brain. [^{14}C]Deoxyglucose and glucose, transported into a homogeneous tissue, enter a common precursor pool in which they compete either for the carrier for trans-

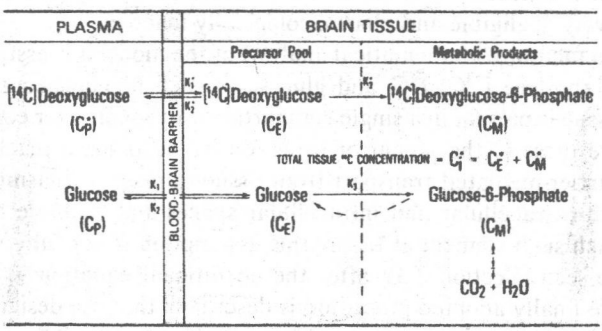

FIGURE 1. Diagrammatic representation of the theoretical model. C_t^* represents the total ^{14}C concentration in a single homogeneous tissue of the brain. C_p^* and C_p represent the concentrations of [^{14}C]-DG and glucose in the arterial plasma, respectively; C_E^* and C_E represent their respective concentrations in the tissue pools that serve as substrates for hexokinase. C_M^* represents the concentration of [^{14}C]-DG-6-P in the tissue. The constants k_1^*, k_2^*, and k_3^* represent the rate constants for carrier-mediated transport of [^{14}C]-DG from plasma to tissue and back from tissue to plasma, and for phosphorylation by hexokinase, respectively. The constants k_1, k_2, and k_3 are the equivalent rate constants for glucose. [^{14}C]-DG and glucose share and compete for the carrier, which transports both between plasma and tissue, and for hexokinase, which phosphorylates them to their respective hexose-6-phosphates. The dashed arrow represents the possibility of G-6-P hydrolysis by glucose-6-phosphatase activity. (From Sokoloff *et al.*, 1977.)

port back from brain to plasma or for the enzyme, hexokinase, which phosphorylates them to [^{14}C]deoxyglucose-6-phosphate ([^{14}C]-DG-6-P) and glucose-6-phosphate (G-6-P), respectively. The hexokinase reaction is essentially irreversible, and inasmuch as [^{14}C]-DG-6-P is not a suitable substrate for any other enzymes known to be present in significant amounts, it is trapped and accumulates as it is formed. On the other hand, G-6-P does not accumulate but is eventually metabolized further to CO_2 and water. The model allows for the possibility that a fraction of the G-6-P is hydrolyzed back to free glucose by glucose-6-phosphatase activity (broken arrow in Figure 1).

The essential features of the model are founded on these experimentally established biochemical properties of deoxyglucose (DG) in cerebral metabolism. The application of this model to the quantification of local cerebral glucose utilization is dependent, however, on the validity of some additional assumptions and/or conditions:

1. The model is applicable only to a localized region of tissue that is homogeneous with respect to the following: rate of blood flow; rates of transport of [^{14}C]-DG and glucose between plasma and tissue; concentrations of [^{14}C]-DG, glucose, [^{14}C]-DG-6-P, and G-6-P; and rate of glucose utilization.
2. The [^{14}C]-DG and [^{14}C]-DG-6-P are present in tracer amounts, i.e., their molecular concentrations in blood and/or tissues are quantitatively negligible and pharmacologically inactive.
3. To facilitate mathematical analysis of the model it is assumed that all of the free [^{14}C]-DG and glucose in each homogeneous element of tissue is present in a single compartment in which their concentrations are those of the precursor pools for the hexokinase reaction and the carrier-mediated transport from tissue to plasma. Inasmuch as there are extracellular and intracellular spaces and multiple cell types in each such element of tissue, this assumption is not fully valid. It will be seen (Section 2.3), after the operational equation is derived and the finally adopted procedure is described, that the design of the procedure serves to minimize, if not eliminate, possible errors arising from invalidity of this assumption.
4. Carbohydrate metabolism in the brain is in a steady state. The plasma glucose concentration, the rate of local cerebral glucose utilization, and the concentrations of the intermediates of the glycolytic pathway remain constant throughout the period of measurement.
5. The capillary plasma concentrations of [^{14}C]-DG and glucose are approximately equal to or bear a constant relationship to their arterial plasma concentrations. Experiments in this laboratory have demonstrated that the cerebral extraction ratio of [^{14}C]-DG is normally very low, approximately 5%; the mean capillary plasma concentration can-

not differ, therefore, by more than 5% from the arterial plasma level. In the case of glucose a constant relationship between arterial and capillary concentrations is implicit in the assumption of steady state conditions for glucose delivery and metabolism.

2.3. Mathematical Analysis of Model

At any time following the introduction of [^{14}C]-DG into the blood, C_i^*, the total content of ^{14}C per unit mass of any tissue, i, is equal to the sum of the concentrations of the free [^{14}C]-DG in the precursor pool in the tissue, C_E^*, and its product, [^{14}C]-DG-6-P, C_M^*, in that tissue (Figure 1). Therefore,

$$C_i^* = C_E^* + C_M^* \tag{1}$$

and the derivative of equation (1) with respect to time, t, is

$$dC_i^*/dt = dC_E^*/dt + dC_M^*/dt \tag{2}$$

The rate of change of the free [^{14}C]-DG concentration in the tissue, dC_E^*/dt, is equal to the difference between the rates of its transport into the tissue from the plasma and its loss from the tissue by transport back to the plasma or by hexokinase-catalyzed phosphorylation to [^{14}C]-DG-6-P. This relationship can be described by the equation

$$dC_E^*/dt = k_1^* C_P^* - k_2^* C_E^* - k_3^* C_E^* \tag{3}$$

where C_P^* equals the concentration of [^{14}C]-DG in the arterial plasma, and k_1^*, k_2^*, and k_3^* are the rate constants for the transport of [^{14}C]-DG from plasma to brain tissue, for the transport of free [^{14}C]-DG back from tissue to plasma, and for the phosphorylation of [^{14}C]-DG in the tissue, respectively. The term in which each rate constant appears represents the rate of the process to which it applies.

It should be noted that C_P^* actually represents mean capillary rather than arterial plasma concentration. Capillary concentration, however, is not readily measured. Inasmuch as the difference between arterial and cerebral venous [^{14}C]-DG concentrations is generally less than 5% of the arterial level, the mean capillary plasma concentration can reasonably closely be approximated by the arterial plasma concentration. Furthermore, as will be seen in this section, any potential error associated with this approximation is partially counteracted by a corresponding approximation made for the plasma glucose concentration.

The assumption of first-order rate constants, k_1^*, k_2^*, and k_3^*, in the math-

ematical description of saturable processes, such as carrier-mediated transport and enzyme-catalyzed reactions, might appear to be questionable. With saturable processes first-order kinetics apply to only a narrow range of the lowest substrate concentrations. The basic requirements and assumptions of the model presented above provide conditions, however, in which k_1^*, k_2^*, and k_3^* behave as true first-order rate constants. For example, [^{14}C]-DG and glucose compete for the same carrier for transport from plasma into brain. The rate of inward transport of [^{14}C]-DG can, therefore, be described by the classical Michaelis–Menten equation, modified for the influence of the presence of the competitive substrate, glucose (Dixon and Webb, 1964). Thus,

$$v_i^* = \frac{V_M^* C_P^*}{K_M^*(1 + C_P/K_M) + C_P^*} \tag{4}$$

where v_i^* represents the rate of inward transport of [^{14}C]-DG, V_M^* represents the maximal velocity of [^{14}C]-DG transport, K_M^* and K_M are the apparent Michaelis–Menten constants of the carrier for [^{14}C]-DG and glucose, respectively, and C_P^* and C_P are the plasma concentrations of [^{14}C]-DG and glucose, respectively.

The model, however, requires that the [^{14}C]-DG be administered in tracer amounts and that tracer theory apply. C_P^* can therefore be considered to be negligible compared to K_M^* $(1 + C_P/K_M)$ and thus

$$v_i^* = \left[\frac{V_M^*}{K_M^*(1 + C_P/K_M)}\right] C_P^* \tag{5}$$

In equation (3) it is assumed that

$$v_i^* = k_1^* C_P^* \tag{6}$$

Equating equations (5) and (6),

$$k_1^* = \frac{V_M^*}{K_M^*(1 + C_P/K_M)} \tag{7}$$

The model also requires a steady state of cerebral glucose utilization and a constant arterial plasma glucose concentration, i.e., a constant C_P. It is apparent then that within the constraints imposed by the model k_1^* is a constant independent of the plasma [^{14}C]-DG concentration and, therefore, a true first-order rate constant.

By comparable analyses k_2^* and k_3^* can be similarly defined and shown to

be true rate constants as used in equation (3). Equation (3) can therefore be integrated and solved for C_E^* as a function of time as follows:

$$C_E^*(T) = k_1^* e^{-(k_2^*+k_3^*)T} \int_0^T C_p^* e^{(k_2^*+k_3^*)t} dt \tag{8}$$

where T is any given time elapsed following the introduction of the [^{14}C]-DG into the circulation.

The tissue concentration of [^{14}C]-DG-6-P as a function of time can also be mathematically described. dC_M^*/dt equals the rate of formation and accumulation of [^{14}C]-DG-6-P per unit mass of tissue. Thus

$$dC_M^*/dt = k_3^* C_E^* \tag{9}$$

Substituting for C_E^* its equivalent function defined in equation (8) and integrating and solving for C_M^*,

$$C_M^*(\tau) = k_1^* k_3^* \int_0^\tau \left[e^{-(k_2^*+k_3^*)T} \int_0^T C_p^* e^{(k_2^*+k_3^*)t} dt \right] dT \tag{10}$$

where τ is any given time elapsed following the introduction of the [^{14}C]-DG into the circulation.

The functions for C_E^* and C_M^* defined in equations (8) and (10), respectively, can now be substituted for these variables in equation (1) to obtain the following equation:

$$C_t^*(\tau) = k_1^* e^{-(k_2^*+k_3^*)\tau} \int_0^\tau C_p^* e^{(k_2^*+k_3^*)t} dt + k_1^* k_3^* \int_0^\tau \left[e^{(k_2^*+k_3^*)T} \int_0^T C_p^* e^{(k_2^*+k_3^*)t} dt \right] dT \tag{11}$$

Equation (11) defines the total tissue concentration of ^{14}C as a function of time in terms of the history of the plasma concentration from zero time to any given time, τ, and the rate constants k_1^*, k_2^*, and k_3^*. The application of this equation to the determination of the rate constants will be described.

The behavior of glucose is similar to that of [^{14}C]-DG, but its mathematical description is simpler because of the assumptions of a constant arterial plasma glucose concentration and a steady state of glucose uptake and metabolism in brain. Thus,

$$dC_E/dt = k_1 C_P - k_2 C_E - k_3 C_E \tag{12}$$