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Research report

Dynamic changes in glucose metabolism accompanying the expression of the neural phenotype after differentiation in PC12 cells

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Abstract

To assess what properties of glucose metabolism are most closely related to expression of the neural phenotype, some parameters of glucose metabolism in PC12 cells before (tumor-type) and after differentiation (neuron-type) were investigated. Neuron-type cells exhibited a 2.7-fold higher level of [^{3}H]DG retention than tumor-type cells, accompanied by a higher glucose transport rate and higher levels of hexokinase activity. [^{14}C]CO_2 production from [U-^{14}C]glucose in neuron-type was also more than four-times greater than that in tumor-type cells. The levels of [^{14}C]carbon in macromolecules from [^{14}C]glucose in neuron-type cells were also much higher (10.6-fold) than those in tumor-type cells, and the levels of incorporation of [^{14}C]carbon were almost as high as those of [^{14}C]CO_2. From the metabolite analysis, amino acids appeared to be the major compounds converted from glucose. On the other hand, the uptakes of [^{35}S]methionine–[^{35}S]cysteine and [^{3}H]uridine in neuron-type cells were lower than those in tumor-type cells. Following depolarization with 50 mM potassium, [^{14}C]CO_2 production increased, but the retention of [^{14}C]carbon was not changed in neuron-type cells. The largest change accompanied by acquisition of the neural phenotype was carbon incorporation into the macromolecules derived from glucose. This property may be important for the expression of the neural phenotype as well as the higher levels of both glucose uptake and oxygen consumption.

Theme: Development and regeneration

Topic: Nutritional and prenatal factors

Keywords: Glucose metabolism; Oxidative phosphorylation; Glucose transport; PC12; Neuron; Depolarization

1. Introduction

High levels of glucose metabolism are one of the characteristics of cells in brain tissue [6]. This is thought to be because glucose is almost the only substrate used for energy supply in the nervous system under normal conditions. In addition, glucose metabolism in the nervous system has several unique features. For example, the rate-limiting step of glucose metabolism has been reported to be the phosphorylation of glucose in both the brain and neurons [1,2,26], although that in muscle and tumor cells has been reported to be the transport step [7,24]. Oxygen consumption, which is coupled to the turnover of the tricarboxylic acid (TCA) cycle in efficient energy production from glucose, is also as high in brain cells as in muscle during strenuous exercise [22]. From the perspective of metabolites of glucose, the synthesis of neurotransmitters is thought to be distinctive in the brain [20,25]. From the above, neurons can be understood to have many distinctive features in glucose metabolism, and these features appear to be strongly related to expression of the neural phenotype. Conversely, because there are many unique features of glucose metabolism in neurons and the nervous system, it is difficult to determine which property or properties are necessary for expression of the neural phenotype.

The clonal cell line PC12, derived from a rat pheochromocytoma, undergoes differentiation following treatment with nerve growth factor (NGF) from a tumor cell (tumor-type) to a neuron-like cell phenotype (neuron-type). The neuron-type cell is known to express a variety of properties

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shared by neurons including the overall phenotype and has been generally used as a neuron model [10,17]. By comparison of glucose metabolism in PC12 before and after differentiation, the properties of glucose metabolism related to expression of the neural phenotype can be determined.

In the present study, to elicit the specific properties of glucose metabolism necessary for expression of the neural phenotype, some parameters of glucose metabolism in PC12 cells before and after differentiation were systematically investigated, including (1) glucose metabolic activity ([^{3}H]2-deoxyglucose retention), (2) the activity of glucose transport and hexokinase, (3) aerobic glycolysis (lactate production), (4) oxidative phosphorylation of glucose ([^{14}C]CO_2 production derived from [U-^{14}C]glucose), (5) the production of cellular constituents (the incorporation of [U-^{14}C]glucose into macromolecules), (6) the use of carbon derived from glucose in neuron-type cells (metabolite analysis), and (7) the effects of depolarization on glucose uptake and the utilization of glucose. Glucose metabolism related to the neural phenotype will be discussed herein based on these results.

2. Materials and methods

2.1. Materials

PC12 cells, human malignant melanoma cells (A375), human lung carcinoma cells (A549), human colon adenocarcinoma cells (LS180), and human fibroblasts were purchased from Dai-Nippon Seiyaku (Osaka, Japan), 2.5S NGF, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and heat-inactivated horse serum (HS) were purchased from Gibco BRL (Tokyo, Japan), Minimum essential medium (MEM; glucose concentration: 1000 mg/l) was purchased from Nissui Seiyaku (Tokyo, Japan); and ATP, NADP, glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and hexokinase (HK; EC 2.7.1.1) were purchased from Boehringer Mannheim (Tokyo, Japan). 2-6-[^{3}H]2-Deoxy-D-glucose (specific activity 51.0 Ci/mmol), [U-^{14}C]glucose (306 mCi/mmol), [^{35}S]methionine, cysteine mixture (Redivue PRO-MIX; 1000 Ci/mmol), and [^{3}H]uridine (38.0 Ci/mmol) were obtained from Amersham (Tokyo, Japan). All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Cell culture

PC12 cells were maintained in culture dishes (100 mm in diameter) coated with rat tail collagen I (Falcon, Tokyo, Japan) supplemented with DMEM containing 10% HS and 5% FBS. PC12 cells cultured with DMEM containing 2.5S NGF at a concentration of 100 ng/ml for 7 days were used as differentiated PC12 cells (neuron-type). A375, A549, and LS180 cell lines were maintained using the procedure recommended by the retailer. Fibroblasts were cultured in the medium obtained with the cells.

2.3. [^{3}H]2-Deoxy-D-glucose retention

PC12 cells were detached from culture dishes with 1 mM EDTA-phosphate-buffered saline (PBS), and the other cell lines were detached with trypsin-EDTA (0.05% trypsin; 0.2% EDTA). Cells were collected gently by centrifugation (100 *g*, 5 min) and used for the experiments described below. Aliquots of $1.5 \cdot 10^5$ of each cell line were suspended in 500 μl of MEM containing 2 μCi/ml of [^{3}H]2-deoxy-D-glucose (DG), and then incubated for 60 min at 37°C. MEM supplemented with 100 ng/ml of NGF was used for the neuron-type cells. Immediately after incubation, the medium was removed by centrifugation (600 *g*, 1 min, 4°C) with a microcentrifuge. This medium was stored at −20°C until the lactate assay. Pellets were washed twice with ice-cold PBS. Lysis was performed with 500 μl of 0.1 M NaOH, and then whole-cell lysate was used for measurement of the radioactivity. Lysates were mixed with ACS II (Amersham) and counted with a liquid scintillation counter (LSC-5100; Aloka, Tokyo, Japan). At the same time, cell protein content was measured using a BCA protein assay kit (Pierce, Rockford, IL, USA).

2.4. Glucose transport rate

The glucose transport rates in both types of PC12 cells were estimated by monitoring cytochalasin B-inhibitable DG uptake during short-term incubation (1–5 min), which is widely used to measure the glucose transport rate [14,27]. The experiment was performed basically in the same way as the DG retention experiment, except with the addition of 50 μM cytochalasin B to the medium. Glucose transport rates were calculated by the subtraction of the DG retention in the medium without cytochalasin B from that with cytochalasin B.

2.5. Hexokinase activity

Cells were detached as described above, suspended, and lysed with ice-cold hypotonic (10 mM) Tris–HCl buffer (pH 7.6) at a final concentration of $5 \cdot 10^6$ cells/ml. Lysates were homogenized with a Dounce homogenizer, then centrifuged to remove nuclei (600 *g*, 10 min, 4°C), and the supernatants were assayed.

Hexokinase activity was measured by the method of Vinuela et al. [23] with some modifications. Briefly, 2.5 ml of 0.05 M triethanolamine–HCl buffer (pH 7.5), 50 μl of 0.3 M $MgCl_2$, 50 μl of 15 mM NADP, 200 μl of 75 mM ATP (pH 7.0), and 6 μl of G6PDH were mixed and preincubated at 20°C for 5 min. The cell homogenate was

added to the mixture and the reaction was started. After 5 and 15 min, 100-μl aliquots of the reaction mixture were sampled and absorption was measured at 340 nm. The differences in absorbance between 5 and 15 min were calculated, and hexokinase activity was determined from the standard curve. The protein content of the supernatants was measured using a BCA protein assay kit.

2.6. Lactate production

The medium obtained in the DG retention study, stored at −20°C, was thawed at room temperature and heated 15 min at 80°C. The samples were centrifuged (3000 *g*, 5 min) and the supernatants were assayed. Lactate content was measured with an F-kit (L-lactate; Boehringer Mannheim), following the protocol from the manufacturer, and the protein content, measured in the DG retention study, was used for this experiment.

2.7. Lactate dehydrogenase

Cells were detached as described above, suspended, and lysed with ice-cold hypotonic (10 mM) Tris–HCl buffer (pH 8.0) at a final concentration of $5 \cdot 10^6$ cells/ml. Lysates were homogenized with a Dounce homogenizer, then centrifuged (3000 *g*, 5 min, 4°C), and the supernatants were assayed. The protein content of the supernatants was measured using a BCA protein assay kit.

Total lactate dehydrogenase (LDH) activity was measured with an assay kit for clinical use (Wako, Osaka, Japan). The procedure was based on the manufacturer's protocol, and the protein content was measured using a BCA protein assay kit.

LDH isozyme was assayed by cellulose acetate electrophoresis (100 V, 75 min) with Tris–barbital buffer (pH 8.6, ionic strength $\mu = 0.06$). After electrophoresis, the cellulose acetate membrane was stained with the reagent mixture containing L-lactate, NAD+, 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and phenazine methosulfate (PMS). The concentrations of the isozymes were determined using a conventional scanner (Sharp, Osaka, Japan) and a personal computer (Apple Computer, Tokyo, Japan) with NIH Image ver. 1.6 software.

2.8. Effects of high K^+ and/or ouabain on DG retention

To depolarize the neuron-type PC12 cells, we used 50 mM KCl–MEM and reduced the NaCl content to 72 mM to adjust the osmolarity. To estimate the use of glucose to maintain membrane potential, 5 mM ouabain, a blocker of Na^+–K^+-ATPase, was used.

The DG retention study was performed as briefly described with the exception of the incubation medium and incubation time (20 min). Ouabain treatment was performed from time 0 of the DG retention experiment.

2.9. Oxidative phosphorylation of ^{14}C-glucose

PC12 cells cultured on collagen-coated 100-mm dishes were placed in an air-tight chamber that we had made. MEM containing [U-^{14}C]glucose (10 μCi/ml) was added, and dishes were kept at 37°C for 1 h with a constant airflow (5% CO_2–95% air; 10 ml/min). $^{14}CO_2$ was trapped in 15 ml of the amine-based absorber Carbo-Sorb E (Packard, Tokyo, Japan). After a 1-h incubation, 1 ml of perchloric acid (3.7 M) was hermetically added to the dishes with a syringe to acidify the medium. $^{14}CO_2$ in the medium and cells was liberated and collected by an additional 20-min incubation under a stream of air. Next, 10 ml of Carbo-Sorb E containing trapped $^{14}CO_2$ was transferred to vials containing 10 ml of Permafluor E+ (Packard) and counted for radioactivity with a liquid scintillation counter. The perchloric acid-treated cell residue was collected with the cell scraper and washed twice with PBS, and the remaining radioactivity was counted as ^{14}C radioactivity in the macromolecules. This fraction is referred to hereafter as the ^{14}C in perchloric acid-treated cell residue. The cell residue was also used for determining the protein content. Because of the addition of perchloric acid to cells, the standard curve of protein content under the influence of perchloric acid was prepared with the BCA protein assay kit and bovine serum albumin.

The effects of 50 mM KCl on [U-^{14}C]glucose metabolism were also investigated by the same procedure as those described above except for the use of KCl and ouabain treatment.

2.10. Metabolite analysis of [U-^{14}C]glucose in neuron-type PC12 cells

Neuron-type PC12 cells were incubated with 5 ml of 50 μCi [U-^{14}C]glucose for 60 min, and after two washes with ice-cold PBS, 11.4 ml of methanol–chloroform–water (2:1:0.8) was added to the cells. Following the Bligh and Dyer extraction method [4], the radioactivity in the organic layer, as the lipid-soluble fraction, and in the water layer, as the water-soluble fraction, was measured. The organic and the water layers were concentrated at room temperature to approximately 100 μl under reduced pressure. A 5-μl portion of the concentrated organic layer was spotted on a silica gel G plate (Whatman, Tokyo, Japan), and lipids were separated by thin-layer chromatography (TLC) using a solvent system consisting of chloroform–methanol–aqueous ammonium (210:90:7.5) to sort the lipids [5]. The lipids were identified by comparison with known standards. In the solvent system, the lipids were separated as follows: phosphatidic acid [rate of flow (R_F)= 0.06±0.003], phosphatidylserine–phosphatidylinositol (R_F=0.12±0.03), phosphatidylcholine (R_F=0.41±0.05),

Table 1
Comparison of various parameters in glucose metabolism between tumor-type and neuron-type PC12 cells[a]

Assay	Tumor-type	Neuron-type	Ratio[b]	Unit
Glucose metabolism				
2-Deoxyglucose retention (60 min)	1110±88	3026±269*	2.7	(dpm/μg protein/h)
CB-inhibitable DG transport (1 min)	15.4	37.4	2.4	(dpm/μg protein/min)
Hexokinase activity	0.09±0.004	0.22±0.006*	2.4	(mU/mg protein)
Lactate production	1.22±0.04	1.59±0.02*	1.3	(μmol/μg protein/h)
LDH activity	44.1±0.6	30.4±2.0	0.7	(unit/μg protein)
LDH isozyme pattern	$M_4>M_3H$	$M_4>M_3H$		
$^{14}CO_2$ production from ^{14}C-glucose	25.7±1.2	110±3.5*	4.3	(dpm/μg protein/h)
^{14}C in perchloric acid-treated cell residue	9.0±1.0	95.7±3.5*	10.6	(dpm/μg protein/h)
Substrate uptake for synthesis of protein and RNA				
^{35}S-methionine uptake	20 892±1318	5835±475*	0.3	(dpm/μg protein/4 h)
^{35}S activity incorporated to $M_r>5000$	19 902±2223	4466±506*	0.5	(dpm/μg protein/4 h)
^{3}H-uridine uptake	40 998±1079	8955±197*	0.2	(dpm/μg protein/4 h)
^{3}H activity incorporated to $M_r>5000$	31 459±3721	2779±790*	0.1	(dpm/μg protein/4 h)

[a] Values are means±S.D. ($n=4$), except CB-inhibitable DG transport, which was calculated with the slope in Fig. 2.
[b] Ratio represents the ratio of the values in neuron-type compared to those in tumor-type cells.
*$P<0.001$, compared to those in tumor type.

phosphatidylethanolamine ($R_F=0.50\pm0.06$), and neutral lipids near the solvent front. The lipids were detected by iodine vapor. An aliquot (7.5 μl) of the concentrated water layer was spotted on chromatography paper (anion exchanger, DE 81, Whatman). Using a solvent system containing water-saturated phenol–0.025 M sodium dihydrogenphosphate (10:0.5), which is a modification of the solvent system of Stark et al. [20], the water-soluble products, including glucose and amino acids, were separated as follows: glucose (near the solvent front), and amino acids ($R_F=0.88\pm0.05$). Amino acids were detected by spraying ninhydrin. To identify the glucose, [U-^{14}C]glucose was developed on the same system. The plates and papers were placed on an imaging plate (BAS-MP 2040S; Fuji Photo Film, Tokyo, Japan), and the exposed plate was scanned with a bio-imaging analyzer (BAS-1500; Fuji Photo Film) to detect radioactivity.

2.11. [^{35}S]Methionine–[^{35}S]cysteine and [^{3}H]uridine uptake and incorporation into a macromolecule

Aliquots of $1.5\cdot10^5$ cells were incubated in MEM (containing 100 ng/ml of NGF for differentiated PC12 cells) containing 5 μCi of [^{35}S]methionine and a [^{35}S]cysteine mixture (Redivue PRO-MIX; Amersham) or 5 μCi of [^{3}H]uridine for 4 h. After incubation, cells were collected by centrifugation (600 *g*, 1 min) and washed twice with PBS, and a sample of the cells was used to measure the uptake of these tracers. Cells were then lysed with hypotonic buffer (0.5% SDS, 10 mM Tris–HCl, pH 7.5), and a portion of the lysate was used for the measurement of protein content and radioactivity (shown as uptake), as described above. Lysates were filtered with Ultrafree (C3LCC; Millipore, Tokyo, Japan), and macromolecules ($M_r>5000$) were collected. After drying, the radioactivity remaining on the filters was counted with a liquid scintillation counter.

2.12. Data analysis

Data are reported basically as means and S.D. The data were compared to the control data, and statistical analyses were performed with the Student's two-tailed *t*-test. Significance for all tests was assumed at the level of $P<0.05$.

3. Results

All data are summarized in Tables 1–3. In Table 1, the differences in glucose metabolism between tumor- and

Table 2
Changes in glucose metabolism in neuron-type PC12 cells by depolarization[a]

	DG retention (dpm/μg protein/h)	$^{14}CO_2$ production (dpm/μg protein/h)	^{14}C in perchloric acid-treated cell residue (dpm/μg protein)
Control	984±99	87.8±6.3	106.0±3.7
50 mM KCl	1258±118*	123.7±6.8*	111.5±1.5**
5 mM Ouabain	853±73**	–	–
50 mM KCl+5 mM ouabain	945±114**	–	–

[a] Values are means±S.D. ($n=4$).
*$P<0.001$, compared to that in control; ** no significant difference to control.

Table 3
Metabolite analysis of [U-^{14}C]glucose in neuron-type PC12 cells[a]

	% of total radioactivity
(A) Water-soluble fraction	83.7
(B) Lipid-soluble fraction	15.7
(A) Water-soluble fraction	
^{14}C-glucose	55.3
Amino acids	45.7
(B) Lipid-soluble fraction	
Phosphatidic acid	0.82
Phophatidyl inositol/serine	2.89
Sphingomyelin	16.0
Phosphatidyl ethanolamine	5.51
Neutral lipids	68.7

[a] Values are means (n=3).

neuron-type cells are indicated. The ratios refer to the values for the neuron-type PC12 cells compared to those for the tumor-type cells. The effects of depolarization by 50 mM KCl on glucose metabolism in neuron-type PC12 cells are indicated in Table 2. Table 3 shows the results of the metabolite analysis of [U-^{14}C]glucose in neuron-type PC12 cells.

DG retention in three other tumor cell lines and fibroblasts was examined as a reference. Neuron-type PC12 cells showed a 2.7-fold greater level of DG retention than tumor-type cells, and all cell lines examined in this study showed a lower retention of DG than neuron-type PC12 cells (Fig. 1). Increased glucose transport and glucose phosphorylation were thought to be important supporting factors for the high retention of DG in neuron-type cells.

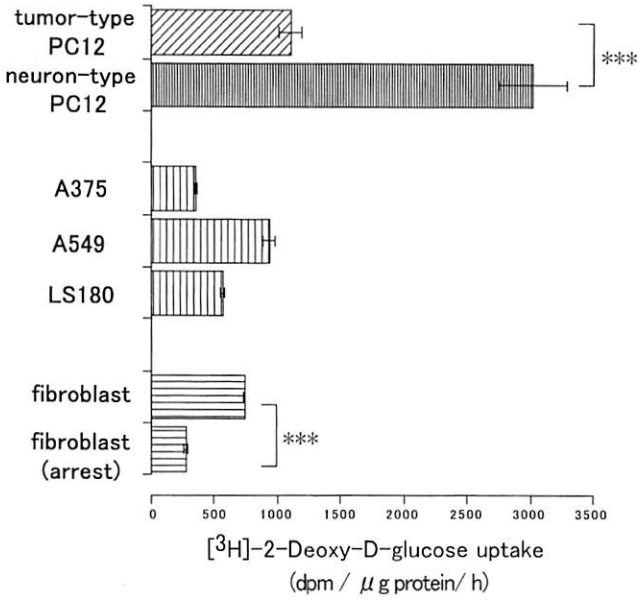

Fig. 1. [^{3}H]2-Deoxy-D-glucose retention in PC12 cells and some cultured cell lines. Results represent means±S.D. from four independent experiments. ***P<0.001, compared with tumor-type PC12 cells by the Student's two-tailed t-test.

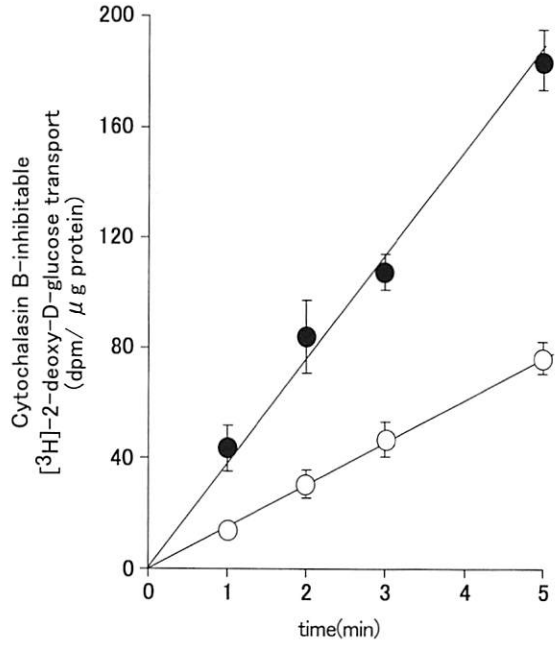

Fig. 2. Time course of cytochalasin B-inhibitable [^{3}H]2-deoxy-D-glucose transport in tumor-type (open symbol) and neuron-type (closed symbol) PC12 cells. Results represent means±S.D. from four independent experiments.

To monitor these two processes, the glucose transport rate and hexokinase activity were investigated. Both glucose transport (Fig. 2) and hexokinase activity in neuron-type PC12 cells occurred at 2.4-fold higher levels than in tumor-type cells (Table 1).

Lactate production in neuron-type cells was higher than that in tumor-type cells, but the ratio in lactate production between neuron- and tumor-types was not as high as that in DG retention. LDH activity in neuron-type cells, which produces lactate from pyruvate, was lower than that in tumor-type cells. LDH isozyme patterns were also examined, and only M_4 and M_3H types were observed in both cell types. To examine the activity of glucose metabolism coupled with oxygen, [^{14}C]CO_2 production from [U-^{14}C]glucose was investigated. The levels of [^{14}C]CO_2 production for 1 h were greatly higher than those in tumor-type cells. To compare the incorporation of carbon atoms into cellular constituents derived from glucose in both cell types, ^{14}C radioactivity incorporated into macromolecules was investigated following the addition of [U-^{14}C]glucose in the same experiments for [^{14}C]CO_2. In perchloric acid-treated cell residue of neuron-type cells, almost the same amount of ^{14}C radioactivity as $^{14}CO_2$ in neuron-type cells was observed, and the value was over 10-times higher than that in tumor-type cells. In addition, to investigate in detail the use of carbon derived from glucose in the neuron-type cells, metabolite analysis of [U-^{14}C]glucose was performed (Table 3). Over 80% of the ^{14}C radioactivity was observed in the water-soluble fraction, but almost 50% of the radioactivity in the water-

soluble fraction was non-metabolite [U-^{14}C]glucose. Accordingly, 30% of the metabolites were lipid and the residue was made up of amino acids. In the lipid fraction, the largest constituent was neutral lipids. In contrast, the levels of uptake of [^{35}S]methionine–[^{35}S]cysteine and [^{3}H]uridine in neuron-type cells were much lower than those in tumor-type PC12 cells.

The maintenance of membrane potential in neurons is thought to require a great deal of energy expenditure. To estimate the glucose retention necessary for the maintenance of membrane potential in neuron-type PC12 cells, DG retention for 20 min was investigated with or without ouabain perturbation in the presence of a high concentration of potassium chloride (KCl, 50 mM), sufficient to depolarize the neuronal cell membrane. DG retention under the high concentration of KCl (50 mM) was significantly higher than that in the control and that with ouabain treatment. To confirm that glucose metabolism is coupled with oxygen in the depolarization of neuron-type PC12 cells, [^{14}C]CO_2 production and the incorporation of carbons derived from [U-^{14}C]glucose into macromolecules were also investigated. The level of [^{14}C]CO_2 production in the treatment with 50 mM KCl was higher than that in the control. In contrast, there was no significant difference in the ^{14}C radioactivity retained in cells between cells treated with 50 mM KCl and the control.

4. Discussion

PC12 cells are thought to be a suitable model system for confirming the properties of glucose metabolism related to the expression of the neural phenotype, as the properties of tumor cells change markedly when converted to the neuronal phenotype in the presence of NGF, and the change in glucose metabolism is thought to be due to the dynamic changes in these cell properties. Morelli et al. [16] have reported an increase in glucose metabolism during the differentiation process in PC12, but their focus was primarily on energy production in the differentiation process. In previous reports, the activity of glucose metabolism has not been directly compared among neurons, other normal cells, and tumor cells, but based on the collected data from several reports, the glucose metabolic activity in neurons and glia seems to be higher than that in other normal and tumor cells [11,15,18]. As expected, DG retention of the neuron-type PC12 cells was found to be much higher than that of the tumor-type cells as well as other tumor cell lines and fibroblasts. The high glucose metabolic activity in neuron-type PC12 cells appears to be one of the most important factors in the expression of the neural phenotype, and this property is thought to be supported by high levels of both glucose transport and hexokinase activity.

Tumor cells are known to have a high level of lactate production even under normoxic conditions (so-called aerobic glycolysis). Conversely, oxygen consumption in the nervous system has been reported to be higher than that in other normal tissues [22]. In the present study, [^{14}C]CO_2 production from [U-^{14}C]glucose in neuron-type PC12 cells was also found to be higher than in tumor-type cells, but lactate production in the neuron-type cells was higher than that in tumor-type cells. An exact explanation of these results will require further investigation into the origins of lactate in neuron-type cells, but it can be said that neuron-type PC12 cells achieved high levels of glucose metabolic activity accompanied by high levels of oxidative phosphorylation concomitantly with acquiring the neural phenotype.

Glucose is used not only for energy production but also as a substrate for the production of cellular constituents such as lipids and amino acids. As described above, the retention of carbon atoms in glucose in the brain has also been demonstrated in experiments using [^{14}C]glucose [3,8,13,19,21,25]. Gaitonde et al. have shown that 65% of [^{14}C]carbon atoms in perchloric acid extracts of the brain are incorporated into amino acids 20 min after the injection of [^{14}C]glucose [9]. We therefore investigated the changes in the use of glucose carbon atoms, accompanying the change in cell type of PC12 cells. Although the difference in DG retention between neuron-type cells and tumor-type cells was 2.7-fold, 10-times the levels of [^{14}C]carbon retention in tumor-type cells was observed in neuron-type PC12 cells, accompanied by a reduction in the retention of both [^{3}H]uridine and [^{35}S]methionine–[^{35}S]cysteine. In addition, a high level of conversion of glucose to amino acids in neuron-type PC12 cells was confirmed, with this level being almost as high as that in brain, as reported by Gaitonde et al. [9]. This common property of brain tissue and neuron-type PC12 cells is therefore thought to be the most important character of glucose metabolism in cells of the nervous system. Considering these previous reports and the data from the present study, the utilization of glucose as a substrate for cellular constituents as seen in nervous systems increases very much concomitantly with the acquisition of neural phenotype in PC12 cells.

Under conditions of high neural activity in the nervous system, glucose metabolism is thought to increase to supply large amounts of energy in the form of ATP, energy that is necessary for the recovery of membrane potential after depolarization. However, the contribution of such recovery to glucose metabolism in neuron-type cells could not be estimated from the results described above. Therefore, we compared DG retention with or without depolarization in neuron-type PC12 cells. Based on the increased DG retention in response to depolarization and its suppression by ouabain, depolarization is thought to activate glucose metabolism to supply energy to Na^+–K^+-ATPase. These results are in consistent with the results using neurons ([12], review by Herts and Peng). In addition, as [^{14}C]CO_2 production was increased in depolarization, the oxidative phosphorylation of glucose appeared to become

more active, producing more energy during depolarization than under normal conditions. On the other hand, even in depolarization, the levels of ^{14}C in perchloric acid-treated cell residue were as high as those of ^{14}C in perchloric acid-treated cell residue under normal conditions. These results indicate that the property allowing the conversion of glucose to other substrates like amino acids may not be affected by depolarization.

Some of the properties in glucose metabolism reported previously in neuron and brain tissues were observed in this study in neuron-type PC12 cells such as high levels of $^{14}CO_2$ production. However, the biggest change in glucose metabolism before and after acquisition of the neural phenotype was the level of glucose use for the production of macromolecules. Our metabolite analysis also showed a high conversion of glucose to amino acids, accompanied by a decrease in amino acid uptake. From these results, it appears that the increase in glucose uptake after acquisition of the neural phenotype may be related to not only the efficient energy production necessary for Na^+–K^+-ATPase, but also the use of glucose for the production of macromolecules. The reason why such metabolic properties are necessary in neuron-type PC12 cells and how these properties are related to expression of the neural phenotype should be clarified in future studies.

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