Evidence for the presence of a glucosensor in hypothalamus, hindbrain, and Brockmann bodies of rainbow trout

Sergio Polakof, Jesús M. Míguez, Thomas W. Moon, and José L. Soengas

Laboratorio de Fisiología Animal, Departamento de Biología Funcional e Ciencias da Saúde, Facultade de Biología, Universidade de Vigo, Vigo, Spain; and Department of Biology and Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Ontario, Canada

Submitted 21 July 2006; accepted in final form 5 December 2006

Polakof S, Míguez JM, Moon TW, Soengas JL. Evidence for the presence of a glucosensor in hypothalamus, hindbrain, and Brockmann bodies of rainbow trout. Am J Physiol Regul Integr Comp Physiol 292: R1657–R1666, 2007. First published December 14, 2007; doi:10.1152/ajpregu.00525.2006.—The aim of this study was to evaluate the existence of a glucosensor in different regions of the brain and in the Brockmann bodies (BB) of the rainbow trout, Oncorhynchus mykiss. Five groups (n = 12) of trout were injected intraperitoneally with saline alone (control) or saline-containing bovine insulin (4 mg/kg), 2-deoxy-D-glucose (100 mg/kg), or D-glucose (500 mg/kg) to promote hyperglycemia or hypoglycemia (glucagon, D-glucose, 2-deoxy-D-glucose) or hypoglycemia (insulin). Six hours after injection, samples from four brain regions (hypothalamus, telencephalon, hindbrain, and midbrain) and the entire BB were taken. Our results demonstrate within the BB and both the hypothalamus and hindbrain a metabolic response different to that observed in other tissues (midbrain, telencephalon) but similar to that described in tissues known to be glucosensors in mammals. The metabolic responses of these areas to changes in plasma glycemia were characterized by parallel changes in GLUT-2 expression, hexokinase-IV, or glucokinase activity and expression, glycolytic potential, and levels of glycogen and glucose. These changes are similar to those reported in mammalian pancreatic β-cells and glucose-excited (GE) neurons, two cell types containing glucosensors. This study provides evidence for the presence of glucosensors responsive to hyper- and hypoglycemia in rainbow trout BB, hypothalamus, and hindbrain.

FEEDING BEHAVIOR AND GENERAL energy homeostasis are regulated by circulating levels of nutrients, including glucose. In mammals, sensors to detect levels of glucose are found in pancreatic β-cells (31), where phosphorylation of glucose by hexokinase-IV (HK-IV) or glucokinase (GK) plays a central role (27). HK-IV or GK has appropriate kinetics (low affinity for glucose) and lacks end-product inhibition and its selective inhibition by 2-deoxy-D-glucose treatment (28), and dietary carbohydrates (20, 21). GK transcripts were also isolated and characterized from the liver (4, 14, 20, 21) and brain (21, 36) of a few fish species but not from white muscle, heart, gills, or kidney (4, 21, 36). Additionally, experimentally induced hyperglycemia in some fish produces an increased glucose uptake in most tissues (3), including brain (2), and Panserat et al. (23) demonstrated that high levels of GLUT-2 transcript expression occurred in rainbow trout tissues, regardless of nutritional conditions.

Pancreatic cells in teleost fish are dispersed along the gastrointestinal tract, although a distinct grouping called the Brockmann bodies (BB) exists near to the gall bladder. The BB are formed by endocrine cells, mainly β-cells with fewer α- and δ-cells than found in the mammalian islets of Langerhans (44). Little information is reported in fish regarding the presence of glucosensors in pancreatic cells, and even these studies provide only indirect support for their existence. The data include 1) the presence of GK activity in the BB of Atlantic halibut (39), 2) the release of insulin from catfish BB induced by 2-deoxy-D-glucose treatment (28), 3) the necrosis of β-cells in tilapia treated with streptozotocin (41), and 4) the fact that insulin release is induced by increased levels of plasma glucose in rainbow trout (7).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Several recent studies in rainbow trout suggest the possible existence of brain glucosensors. These studies found 1) that experimentally induced hyperglycemia led to decreased food intake, whereas increased food intake occurred after intracerebroventricular treatment of rainbow trout with 2-deoxyglucose (35), 2) parallel changes between brain glycogen and GK activity and expression levels and changes in plasma glucose levels (25), and 3) that fish refed for 7 days after 14 days of food deprivation displayed in both the hypothalamus and hindbrain increased GK activity and expression and glycogen levels at the same time than plasma glucose levels increased (36). Each of these results is consistent with a typical mammalian glucosensor as found in β-cells and GE neurons (12, 43).

Thus, the purpose of this study was to obtain direct evidence for the existence of a glucosensor in BB and specific brain regions (hypothalamus, midbrain, hindbrain, and telencephalon) of the rainbow trout, Oncorhynchus mykiss. To do so, we induced experimental hyperglycemia with D-glucose, 2-deoxy-D-glucose, and glucagon injections or hypoglycemia with exogenous insulin treatments and assessed in these tissues the responses of several well-known metabolic indicators of the capacity for glucose sensing, including GK activity and expression, GLUT-2 expression, glycolytic potential, and glycogen levels.

**MATERIALS AND METHODS**

**Fish**

Rainbow trout (Oncorhynchus mykiss Walbaum) were obtained from a local fish hatchery (Soutorredondo, Spain). Fish were maintained for 1 mo in 100-liter tanks under laboratory conditions and a natural photoperiod in dechlorinated tap water at 14°C. Fish mass was 95 ± 3 g (n = 60). Fish were fed once daily (0900) to satiety with commercial dry fish pellets (Dihaq-Dipro; proximate food analysis was 48% crude protein, 6% carbohydrates, 25% crude fat, and 11.5% ash; 20.2 MJ/kg of feed). The experiments described complied with the Guidelines of the European Union Council (86/609/EU), and of the Spanish Government (RD 1201/2005) for the use of animals in research. Experiments were approved by the Ethics Committee of the University of Vigo, Vigo, Spain for the use of animals in research and were undertaken in February 2006.

**Experimental Protocol**

Following the 1-mo acclimation period, fish were randomly assigned to 100-liter experimental tanks, and each tank was randomly assigned to one of five experimental treatments. Fish were lightly anesthetized with MS-222 (50 mg/l) buffered to pH 7.4 with sodium bicarbonate, weighed, and intraperitoneally injected with 5 ml/kg body mass of Cortland saline alone (control, n = 12) or saline containing the different treatments: glucagon (100 μg bovine glucagon/kg body mass, n = 12; Sigma), insulin (4 mg bovine insulin/kg body mass, n = 12; Sigma), 2-deoxy-D-glucose (100 mg/kg body wt, n = 12), or D-glucose (500 mg/kg body mass, n = 12). Sampling was initiated 6 h after injection using fish fasted for 24 h before injection to ensure that basal hormone levels were achieved. These concentrations and times were used as they were shown in other studies to modify glycemia in rainbow trout (6, 7, 13, 19).

**Sampling**

Fish were removed from replicate holding tanks at 1500 (6 h after injection), anesthetized as described in Experimental Procedure and weighed. Four fish per group (two per tank) were used to assess mRNA expression in tissues, and the remaining 8 fish per group (four per tank) were used to assess enzyme activities and metabolite levels.

Blood was obtained by caudal puncture into ammonium-heparinized syringes. Plasma was obtained after centrifugation of the blood (1 min at 10,000 g) and divided into two aliquots. One aliquot was immediately frozen in liquid nitrogen for the assessment of plasma protein and cortisol, while the second aliquot (for the assessment of plasma metabolites) was deproteinized (6% perchloric acid, PCA) and neutralized (1 mol/l potassium bicarbonate) before freezing in liquid nitrogen; then it was stored at −80°C until assayed. The liver and BB were removed, freeze-clamped in liquid nitrogen, and stored at −80°C until assayed. The brain (without the pituitary) was removed, placed on a chilled Petri dish, and sectioned into four regions (hypothalamus, midbrain, hindbrain, and telencephalon), as described previously (36), and each section was freeze-clamped in liquid nitrogen and stored at −80°C until assayed.

**Enzyme and Metabolite Assays**

Plasma glucose and lactate levels were determined enzymatically using commercial kits (Spinreact, Spain) adapted to a microplate format. Plasma protein was measured using the bicinechonic acid method with bovine serum albumin as standard (Sigma). Plasma total α-amino acids were assessed colorimetrically using the ninhydrin method of Moore (17); alanine was used to develop a standard curve. Plasma acetoacetate levels were determined by decreases in absorbance of NADH at 340 nm after sample incubation with (in mmol/l) 0.1 NaHPO₄ and 0.1 NaH₂PO₄ (pH 7.0), 0.2 NADH, and excess β-hydroxybutyrate dehydrogenase. Cortisol levels were measured using an ELISA validated for rainbow trout (38).

Frozen tissues were quickly minced on a chilled Petri dish to very small pieces that (still frozen) were divided into two homogeneous portions to assess enzyme activities and metabolite levels. The tissue portions used to assess metabolite levels were homogenized immediately by ultrasonic disruption in 7.5 vol of ice-cooled 6% PCA, and neutralized (using 1 mol/l potassium bicarbonate). The homogenate was centrifuged, and the supernatant was used to assay tissue metabolites. Tissue glycogen levels were assessed using the method of Keppler and Decker (9). Glucose obtained after glycogen breakdown (after subtracting free glucose levels) was determined with a commercial kit (Biomérieux, Spain). Tissue glucose 6-phosphate levels were estimated by a decreases in absorbance of NADH at 340 nm after incubation of sample with (in mmol/l) 0.1 NaHPO₄, and 0.1 NaH₂PO₄ (pH 7.0), 0.2 NADH, and excess glucose 6-phosphate dehydrogenase. Tissue glyceraldehyde 3-phosphate levels were assessed by decreases in absorbance of NADH at 340 nm after incubation of sample with (in mmol/l) 50 imidazole (pH 7.6), 0.2 NADH, and excess α-glycerophosphate dehydrogenase and triosephosphate isomerase. Tissue lactate, total α-amino acids, and acetoacetate levels were assessed as described above for plasma samples.

Tissue portions used to estimate enzyme activities were homogenized by ultrasonic disruption with 9 vol of ice-cold buffer consisting of (in mmol/l) 50 Tris (pH 7.6), 5 EDTA, 2 1,4-dithiothreitol, and a protease inhibitor cocktail (P-2714; Sigma). The homogenate was centrifuged and the supernatant was used immediately for enzyme assays. Enzyme activities were assessed spectrophotometrically using a microplate reader (SPECTRAFluor; Tecan, Grödig, Austria). Reaction rates followed increases or decreases in absorbance of NAD(P)/H at 340 nm. The reactions were started by the addition of homogenates (15 μl), at a preestablished protein concentration, omitting the substrate in control wells (final volume 265–295 μl), and allowing the reactions to proceed at 20°C for preestablished times. Low-Kₘ HK (EC 2.7.1.1) and high Kₘ HK-IV (EC 2.7.1.2) or GK activities were estimated as described by Panserat et al. (20) by coupling ribulose-5-phosphate formation from glucose 6-phosphate to the reduction of NADP at 340 nm. GK activities were corrected for glucose dehydrogenase (GIDH; EC 1.1.1.47) activities. Thus, GK activity is calculated...
as total HK activity minus low $K_m$, HK activity minus 1/3 of GIDH activity (see also 36). The specific conditions for the remaining enzyme assays were described previously (25, 29, 30). Enzyme activities are presented as units (µmoles/min) per milligram tissue protein. Protein was assayed in homogenates using the bicinchoninic acid method as noted above.

RT-PCR Analysis of HK-I, HK-JV/GK, and GLUT-2 Gene Expression

Total RNA was extracted from frozen brains using TRI Reagent as recommended by the manufacturer (Sigma). The quality and quantity of the isolated RNA was assessed spectrophotometrically. Total RNA (2 µg) was reverse transcribed into first-strand cDNA when primed with 5′-pd(T)12–18-3′ (Amersham Biosciences) using Maloney murine leukemia virus reverse transcriptase for 1 h at 37°C by methods recommended by the manufacturer (Promega).

We have assessed the expression of single isoforms of three different genes: HK-I, HK-JV/GK, and GLUT-2. GK cDNA was PCR-amplified using specific primers developed for rainbow trout by Panserat et al. (20): 5′-TGATGTTGGTGAAGGTGGGG-3′ (forward) and 5′-TTCAGTGGATGCCCCGTGTC-3′ (reverse); amplification with these primers resulted in a 250-bp product. HK-I cDNA was PCR amplified using specific rainbow trout primers reported by Soengas et al. (36): 5′-GGCTTCTCAGAGAGGGATT-3′ (forward) and 5′-TTTCGAAATGGCCTCTGTC-3′ (reverse); amplification with these primers resulted in a 245-bp product. GLUT-2 cDNA was PCR-amplified using specific rainbow trout primers reported by Panserat et al. (23): 5′-CGTCTTACCATGGTGTCG-3′ (forward) and 5′-CCACAATGAACCCGAGGGATG-3′ (reverse); amplification with these primers resulted in a 222-bp product. The housekeeping gene used to assess the relative cDNA levels of HK-I, GK, and GLUT-2 was rainbow trout β-actin. Accordingly, β-actin cDNA was amplified by PCR using specific primers: 5′-ACCTCAGCTGTTGTAGA-3′ (forward) and 5′-ACCGATCCCGGTATGCGCAA-3′ (reverse) designed using the β-actin mRNA sequence for rainbow trout (GenBank accession no. AJ438158); amplification with these primers resulted in a 253-bp product.

The PCR reactions were carried out using a PTC-200 Peltier thermal cycler (MJ Research) in a final volume of 20 µl containing cDNA template (8 µl for HK-I, GK, and GLUT-2, and 2 µl for β-actin), 1 × buffer (50 mM KCl, 20 mM Tris-HCl and 0.1% Triton X-100), 0.2 mM dNTPs, 1.5 mM MgCl₂, 2 pmol of each primer (forward and reverse), and 1 U of Taq polymerase (EcoGen). The optimal number of cycles for amplification was established (reactions were terminated in the logarithmic phase of the PCR reaction). Amplification of cDNA was achieved with an initial denaturation at 94°C followed by 35 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s) and extension (72°C for 30 s); a final extension period of 10 min occurred before termination. Negative controls without reverse transcriptase or cDNAs were performed to ensure observed bands were not a result of contamination. The PCR products were sequenced using primers T7 and M13 (flanking the insert) and sequenced in both directions using the dRhodamine terminator cycle sequencing kit (Applied Biosystems). The reactions were run on an Applied Biosystems automated sequencer model ABI PRISM 310. The resulting sequences were compared with known sequences on GenBank using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/).

Statistics

Data are presented as means ± SE. Comparisons among groups were performed using one-way ANOVA (SigmaStat; SPSS). When necessary, data were log transformed to fulfill the conditions of the ANOVA. Post hoc comparisons were made using a Student-Newman-Keuls test, and differences were considered statistically significant at $P < 0.05$.

RESULTS

No differences existed between control and uninjected fish for any parameter assessed (data not shown). Changes in plasma metabolic parameters and cortisol levels are presented on Fig. 1. Plasma glucose levels significantly decreased in insulin-treated fish and increased in glucagon, 2-DG, and glucose-treated fish. There were no other significant differences with control fish for the other parameters assessed in plasma.

No significant differences were noted for HK-I expression in any tissue assessed (data not shown). GK and GLUT-2 expression were presented in Fig. 2. No significant differences existed between treatments in midbrain and telencephalon GK expression (data not shown). GK expression significantly decreased in hypothalamus, hindbrain, and BB in insulin-treated trout, and the expression in this group was always lower than in the other treatment groups. Relative GK expression was enhanced after glucagon (hypothalamus, hindbrain, and BB), 2-DG (hindbrain and BB), and glucose (hypothalamus and hindbrain) treatments. Relative GLUT-2 expression significantly decreased after insulin treatment (hindbrain and BB) and increased with glucagon (BB), 2-DG (hindbrain and BB) and glucose (hindbrain and BB). No changes were noted in GLUT-2 expression in hypothalamus (Fig. 2) or in the other brain regions assessed (data not shown).

GK activities decreased in the hypothalamus, hindbrain, and BB after insulin treatment, whereas an increase occurred in the same tissues after glucagon, 2-DG, or glucose treatments (Fig. 3). Low $K_m$ HK activities did not change in the brain regions studied, whereas in BB, insulin treatment resulted in a significant decrease in activity (Fig. 3).

Glycogen synthetase (GSase) activities in hypothalamus (Table 1) and hindbrain (Table 2) paralleled changes in glyco- gen levels in these tissues (Fig. 4); no significant differences were noted in the other brain regions (data not shown), and inadequate tissue mass was available to assess activities in BB. Pyruvate kinase (PK) activities in hypothalamus (Table 1) and hindbrain (Table 2) decreased with insulin treatment, whereas the activity ratio [activities at subsaturating phosphoenol pyruvate (PEP) concentration to activities at saturating PEP concentration] of this enzyme increased with glucose and 2-DG treatments. No significant differences were noted in PK activities in the other brain regions assessed (data not shown); again, inadequate tissue was available for the BB for these estimates.

Tissue glucose, glycogen, and glucose 6-phosphate levels are presented in Fig. 4. Glucose levels in hindbrain, midbrain, and BB significantly decreased after insulin treatment and increased in the hypothalamus, hindbrain, and midbrain after

Downloaded from http://ajpregu.physiology.org/ by 10.220.133.1 on October 6, 2016
glucose treatment. Glycogen levels decreased after glucagon treatment in the BB and insulin in hypothalamus, hindbrain, and midbrain, whereas levels increase with 2-DG in hypothalamus, hindbrain, and BB and with glucose in hypothalamus, hindbrain, and BB. Glucose-6-phosphate levels decreased with insulin treatment in hindbrain and increased with glucose in hypothalamus and BB.

No significant differences were noted after any treatment for acetoacetate and lactate levels in the brain regions (data not shown). α-Amino acid levels were increased only in the hypothalamus after treatment with 2-DG and glucose (Table 1). Glyceraldehyde 3-phosphate levels in hypothalamus (Table 1) and hindbrain (Table 2) increased after glucagon, glucose, and 2-DG treatments and decreased after insulin treatment, whereas no significant differences were noted in the other brain regions assessed (data not shown). Inadequate tissue was available to assess those metabolite contents in the BB.

**DISCUSSION**

Since gene expression assessed by conventional RT-PCR is not strictly quantitative, the mRNA expression data reported in Fig. 2 must be interpreted cautiously.

Plasma levels of cortisol displayed no significant differences among treatments, and in all cases, values were close to baseline values for this species (38), supporting that changes in metabolic parameters were not linked to any stress induced by our experimental conditions. Plasma glucose levels of treated fish displayed changes that clearly validated the experimental design, since both hypoglycemia (insulin) and hyperglycemia (glucose, 2-DG, and glucagon) were achieved. Changes observed in glycemia are consistent with those previously observed in fish after treatment with 2-deoxy-D-glucose (6), α-glucose (7), insulin (19), and glucagon (13). Further validation of this experimental design was achieved by assessing the effect of these treatments on several parameters in liver (glycogen levels, GSase, and PK activity, etc.) known to change under similar experimental designs. In general, the results obtained in liver (data not shown) agree with those previously observed in other studies, providing additional support for the validity of our experimental design (6, 7, 19).

**Brockmann Bodies**

Increased plasma glucose levels induce GK gene expression in β-cells of the mammalian pancreas (37). Therefore, we predicted changes in GK expression and activity in rainbow trout under altered glycemic conditions. In fact, GK activity and relative mRNA expression in the trout BB closely followed changes in plasma glucose levels, as observed in mammals (31). This is the first study, as far as we are aware, in which GK gene expression was assessed in piscine BB, although previous studies did evaluate GK activity (39). The fact that low $K_m$ HK activity and HK-I mRNA expression did not follow plasma glycemia reinforces the important role of GK in the trout BB. Thus, changes in the relative expression and activity of GK not
the low $K_m$ HK (HK-I) may provide the capacity for trout BB to detect changes in plasma glucose levels in a way similar to that observed in mammals (31).

This is also the first study in which GLUT-2 expression was assessed in fish pancreatic cells, as the only available fish study (23) did not examine pancreatic cells. Relative GLUT-2 mRNA expression decreased under hypoglycemic and increased under hyperglycemic conditions. Again, this response is similar to that reported in mammalian pancreatic β-cells, where GLUT-2 mRNA levels changed directly with blood glucose (18), and fasting reduced expression of GLUT-2 and GK, whereas refeeding enhanced expression of both transcripts (45). Interestingly, hyperglycemia induced by glucose did not induce GK mRNA expression in the trout BB, but it did induce GLUT-2 expression. This implies distinct regulatory signals governing GK and GLUT-2 expression and that glucose per se may not be the only inducer in this species. In fact, studies in fish (15) find that amino acids like arginine are better insulin secretagogues than glucose.

Another important component supporting the existence of a glucosensor in the mammalian β-cell model is that glycogen levels change in parallel with plasma glucose and GK activity, as glucose phosphorylation is a key step in the activation of glycogen synthesis through increases in intracellular glucose 6-phosphate leading to allosteric activation of glycogen synthase (GSase) or covalent activation or translocation of GSase (26, 33). In the present study, levels of glycogen generally paralleled plasma glucose and GK activities (except for glucagon treatment, where a decrease rather than an increase occurred). Changes observed in tissue levels of glucose and glucose 6-phosphate also agree in general with those observed for glycogen levels. Because of the small mass of the BB, there was inadequate mass for the assessment of other metabolic parameters as in the brain regions. Therefore, we cannot compare the available mammalian data with respect to changes in NADH production (42) or glycolytic potential (32) following changes in glycemia with those of fish in the present study.

These results support the existence of a metabolic response in BB comparable with that reported in mammalian β-cells, providing evidence that in rainbow trout, the BB act as a glucosensor and contribute to the increased release of insulin reported under hyperglycemic conditions in rainbow trout (7).

**Brain Regions**

Increased plasma glycemia induce GK gene expression in the mammalian hypothalamus (43). We therefore predicted changes in GK expression and activity in those brain regions that in trout could act as glucosensors when submitted to different glycemic conditions. Values of GK activity and relative mRNA expression in the brain regions assessed were similar to those previously reported in this species after food...
deprivation and refeeding (36). GK activity and expression in the hypothalamus and hindbrain closely followed changes in plasma glucose levels in a way similar to that observed in mammalian GE neurons (11); similar correlations were not found in the remaining brain regions assessed (midbrain and telencephalon). As noted for the BB, no differences were noted in the low $K_m$ HK activity or relative HK-I mRNA expression in any tissue assessed, in agreement with our previous study in

![Graphs showing GK activity and low $K_m$ hexokinase activity in different brain regions](image)

Fig. 3. GK and low $K_m$ HK activities in hypothalamus, hindbrain, midbrain, telencephalon, and Brockmann bodies of rainbow trout sampled 6 h after intraperitoneal injection of 5 ml/kg of Cortland saline alone (C) or saline containing GLN (100 μg/kg), INS (4 mg/kg), 2-DG (100 mg/kg), or GLU (500 mg/kg). Further details are given in Fig. 1.
fasted and refeed rainbow trout (36). These results demonstrate that GK in the hypothalamus and hindbrain respond to changes in plasma glucose levels, which is a key component of the glucose-sensing machinery.

Panserat et al. (23) was unable to detect GLUT-2 mRNA expression in the whole brain of the trout. However, it is clear from this study that GLUT-2 mRNA is expressed in specific brain regions. GLUT-2 relative expression increased only in the hindbrain, not the hypothalamus, unlike changes noted for GK activity and the expression that occurred in both regions. The absence of GLUT-2 expression changes by circulating glucose levels in the hypothalamus is similar to that observed in mammals where transport of glucose into the hypothalamus through GLUT-2 is considered not to be limiting (27). In contrast, transport of glucose into trout BB and hindbrain was induced by glycemia in the present experiment, suggesting a significant difference between tissues with respect to the control of glucose uptake by GLUT-2.

Levels of glycogen in the hypothalamus and hindbrain responded directly to changes in plasma glucose levels, whereas in the other brain regions only marginal changes in glycogen were observed. These changes occurred in the same direction as those observed for GK activity and expression in agreement with the known effect of increased GK activity in mammals stimulating GSase activity, leading ultimately to increased glycogen synthesis (33). Changes observed in glycogen levels in the present experiment can be also attributed to changes in GSase activity. The parallel change observed in GK activity/expression and glycogen levels was previously reported in fed, fasted, and refeed rainbow trout, for which we postulated that plasma glycemia was modulating the glucose-sensing mechanisms within the brain (36); our present data support this postulate. Moreover, changes noted in tissue levels of glucose and glucose 6-phosphate agree, in general, with those for glycogen levels. In addition to changes in glucose and glycogen levels, changes were also noted in levels of glyceraldehyde 3-phosphate, which increased in both the hypothalamus and hindbrain of fish under hyperglycemia and decreased under hypoglycemia. Therefore, levels of this glycolytic intermediate might be strongly associated with increased GK activity and with the finding that increased glycolytic potential also occurred in the hypothalamus and hindbrain under hyperglycemic conditions, whereas a decrease was noted under hypoglycemic conditions. This is in contrast to the mammalian situation in which hypoglycemia may lead to enhanced glycolysis in the hypothalamus (43) but is in agreement with a previous study in which GK activity in the same brain regions decreased in fasted fish and recovered to fed values in refeed fish (36). These results demonstrate that the hypothalamus and hindbrain of the trout brain increase both glycogen production and glycolysis possibly as a result of low activities of GSase and thus limited capacity for glycogen production.

In addition to glucose, glucose-sensing neurons in mammals respond to a variety of metabolites, including lactate, ketone bodies, and fatty acids (11). Moreover, the electrical activity of glucose-responsive hypothalamic neurons in mammals may be regulated by glucose through enhanced production of lactate by glial cells and its oxidation by neighboring neurons (1). In the present study, changes observed in plasma levels of lactate and amino acids generally paralleled those of plasma glucose.

Table 1. Glycogen synthase and pyruvate kinase activities and α-amino acid and glyceraldehyde-3-phosphate levels in hypothalamus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Glucagon</th>
<th>Insulin</th>
<th>2-Deoxyglucose</th>
<th>D-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSase activity, mU/mg protein</td>
<td>107.1 ± 9.2</td>
<td>145.3 ± 8.1</td>
<td>81.9 ± 8.27</td>
<td>148.9 ± 10.4</td>
<td>165.4 ± 19.6</td>
</tr>
<tr>
<td>PK activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimal activity, U/mg protein</td>
<td>8.90 ± 0.51</td>
<td>8.07 ± 0.39</td>
<td>4.53 ± 0.15</td>
<td>8.32 ± 0.27</td>
<td>7.32 ± 0.32</td>
</tr>
<tr>
<td>Activity ratio, %</td>
<td>24.9 ± 2.41</td>
<td>24.5 ± 2.64</td>
<td>25.5 ± 2.27</td>
<td>38.0 ± 2.17</td>
<td>38.8 ± 2.82</td>
</tr>
<tr>
<td>α-Amino acid levels, μmol/g wet wt</td>
<td>12.2 ± 1.43</td>
<td>16.1 ± 2.07</td>
<td>12.1 ± 1.25</td>
<td>24.1 ± 2.6</td>
<td>21.6 ± 1.04</td>
</tr>
<tr>
<td>GAP levels, mmol/g wet wt</td>
<td>23.5 ± 1.18</td>
<td>32.4 ± 1.54</td>
<td>11.5 ± 0.45</td>
<td>36.3 ± 1.23</td>
<td>33.5 ± 2.02</td>
</tr>
</tbody>
</table>

Glycogen synthase (GSase) and pyruvate kinase (PK) activities, α-amino acid and glyceraldehyde-3-phosphate (GAP) levels in hypothalamus of rainbow trout sampled 6 h after intraperitoneal injection of 5 ml/kg of Cortland saline alone (control) or saline containing bovine glucagon (100 μg/kg), bovine insulin (4 mg/kg), 2-deoxy-D-glucose (100 mg/kg) or D-glucose (500 mg/kg). Each value is the mean ± SE of n = 8 fish per group. *Significantly different from control group (P < 0.05). **Different letters indicate significant differences among treated groups (one-way ANOVA, P < 0.05).

Table 2. Glycogen synthase and pyruvate kinase activities and α-amino acid and glyceraldehyde-3-phosphate levels in hindbrain

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Glucagon</th>
<th>Insulin</th>
<th>2-Deoxyglucose</th>
<th>D-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSase activity, mU/mg protein</td>
<td>102.5 ± 16.1</td>
<td>182.5 ± 12.9</td>
<td>73.6 ± 8.40</td>
<td>192.7 ± 19.1</td>
<td>190.3 ± 16.3</td>
</tr>
<tr>
<td>PK activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimal activity, U/mg protein</td>
<td>11.86 ± 0.28</td>
<td>11.43 ± 0.45</td>
<td>8.48 ± 0.23</td>
<td>11.7 ± 0.49</td>
<td>11.3 ± 0.36</td>
</tr>
<tr>
<td>Activity ratio, %</td>
<td>17.1 ± 2.14</td>
<td>17.6 ± 1.32</td>
<td>11.6 ± 1.52</td>
<td>25.1 ± 14.1</td>
<td>27.7 ± 1.70</td>
</tr>
<tr>
<td>α-Amino acid levels, μmol/g wet wt</td>
<td>15.7 ± 1.30</td>
<td>16.2 ± 1.18</td>
<td>15.8 ± 1.51</td>
<td>17.1 ± 1.04</td>
<td>17.8 ± 0.96</td>
</tr>
<tr>
<td>GAP levels mmol/g wet wt</td>
<td>21.7 ± 3.45</td>
<td>47.4 ± 2.82</td>
<td>12.2 ± 1.12</td>
<td>46.2 ± 5.19</td>
<td>47.9 ± 4.17</td>
</tr>
</tbody>
</table>

GSase and PK activities, α-amino acid and GAP levels in hindbrain of rainbow trout sampled 6 h after intraperitoneal injection of 5 ml/kg of Cortland saline alone (control) or saline containing bovine glucagon (100 μg/kg), bovine insulin (4 mg/kg), 2-deoxy-D-glucose (100 mg/kg), or D-glucose (500 mg/kg). Each value is the mean ± SE of n = 8 fish per group. *Significantly different from control group (P < 0.05). **Different letters indicate significant differences among treated groups (one-way ANOVA, P < 0.05).
Interestingly, levels of amino acids increase in hypothalamus (but not in other brain regions) after some hyperglycemic treatments, suggesting that in this specific brain region a connection may exist between glucose sensitivity and the capacity for detecting and using other fuels by the fish brain (34).

The results as a group support our previous hypothesis (36) that proposed the existence of glucosensors in the rainbow trout hypothalamus and hindbrain. These glucosensors respond to changes in plasma glucose with similar changes in GK activity and mRNA expression and glycogen levels but with a different response regarding GLUT-2. This metabolic response may be involved in the orexigenic or anorexigenic responses observed in rainbow trout subjected to hypoglycemic or hyperglycemic conditions (35).

**Perspectives**

After experimentally induced hyperglycemia and hypoglycemia in rainbow trout, we demonstrated in both the BB and in the hypothalamus and hindbrain a metabolic response that was distinct from that noted in other brain areas (midbrain, telencephalon) and the liver (data not reported). The metabolic responses of the hypothalamus, hindbrain, and BB to changes in plasma glucose were characterized by parallel changes in relative GLUT-2 expression, GK activity and expression, glycolytic potential, and levels of glycogen. These changes are consistent with those reported in mammalian β-cells and GE neurons, providing support for the presence of glucosensors in rainbow trout responsive to hyperglycemia and hypoglycemia. Further studies are needed to elucidate whether the mechanisms involved in glucose sensing post-GK induction are also
similar to those previously reported in mammals (11, 31). Furthermore, considering that in fish amino acids (in particular, arginine and lysine) are considered to be stronger insulin secretagogues than glucose (15), whether expression profiling of β-cells exposed to altered glucose would reveal differential expression of genes involved in amino acid turnover and nitrogen disposal (T. P. Mommesen, personal communication) would be important to determine. Also, since glucose constitutes a small portion of the normal fish diet (40), it is possible that the fish pancreas developed the capacity to produce and secrete insulin independent of glucose, or at least in a nutrient milieu containing low glucose. Therefore, we can hypothesize additional roles for glucosensors being involved in insulin release produced not only by glucose but also by other secretagogues, including amino acids (BB), or in the detection of alternative fuels (BB, hypothalamus, and hindbrain), including amino acids and lactate.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Paloma Morán (Universidade de Vigo) for assisting in sequencing of genes.

GRANTS

This study was supported by research grants from Ministerio de Educación y Ciencia and European Fund for Regional Development (AGL2004–08137-C04–03(ACU), and Xunta de Galicia (PGIDT05PXIC31202PN). S. Polakof was recipient of a predoctoral fellowship from the Universidade de Vigo.

REFERENCES


...