Regulation of glucose production in rainbow trout: role of epinephrine in vivo and in isolated hepatocytes

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WEBER, Jean-Michel, and Deena S. Shanghavi. Regulation of glucose production in rainbow trout: role of epinephrine in vivo and in isolated hepatocytes. Am J Physiol Regulatory Integrative Comp Physiol 278: R956–R963, 2000.—The rate of hepatic glucose production (Ra glucose) of rainbow trout (Oncorhynchus mykiss) was measured in vivo by continuous infusion of [6-3H]glucose and in vitro on isolated hepatocytes to examine the role of epinephrine (Epi) in its regulation. By elevating Epi concentration and/or blocking β-adrenoreceptors with propranolol (Prop), our goals were to investigate the mechanism for Epi-induced hyperglycemia to determine the possible role played by basal Epi concentration in maintaining resting Ra glucose and to assess indirect effects of Epi in the intact animal. In vivo infusion of Epi caused hyperglycemia (3.75 ± 0.16 to 8.75 ± 0.54 mM) and a twofold increase in Ra glucose (6.57 ± 0.79 to 13.30 ± 1.78 µmol·kg⁻¹·min⁻¹, n = 7), whereas Prop infusion decreased Ra from 7.65 ± 0.92 to 4.10 ± 0.56 µmol·kg⁻¹·min⁻¹ (n = 10). Isolated hepatocytes increased glucose production when treated with Epi, and this response was abolished in the presence of Prop. We conclude that Epi-induced trout hyperglycemia is entirely caused by an increase in Ra glucose, because the decrease in the rate of glucose disappearance normally seen in mammals does not occur in trout. Basal circulating levels of Epi are involved in maintaining resting Ra glucose. Epi stimulates in vitro glucose production in a dose-dependent manner, and its effects are mainly mediated by β-adrenoreceptors. Isolated trout hepatocytes produce glucose at one-half the basal rate measured in vivo, even when diet, temperature, and body size are standardized, and basal circulating Epi is responsible for part of this discrepancy. The relative increase in Ra glucose after Epi stimulation is similar in vivo and in vitro, suggesting that indirect in vivo effects of Epi, such as changes in hepatic blood flow or in other circulating hormones, do not play an important role in the regulation of glucose production in trout.

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examined whether trout hepatocytes can produce glucose at similar rates in vivo and in vitro when diet, temperature, and body size are standardized. This comparison was made to determine the integrated effect of the cell-isolation procedure (e.g., destruction of membrane integrity, abnormal stimulation of glycogen breakdown (17), and elimination of neural input), to evaluate how relevant in vitro measurements of glucose production are to the intact organism.

**METHODS**

**Animals**

Adult rainbow trout, Oncorhynchus mykiss (Walbaum), of both sexes were purchased in September from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) and held in a 1,300-liter flow-through tank at 13°C. They were kept in dechloraminated, well-oxygenated water under a 12:12-h light-dark photoperiod. The animals were fed low-fat trout chow (11% lipids, 42% protein, 20% carbohydrate) until satiation three times a week. They were acclimated to these conditions for 6 wk before the first experiment and were randomly divided into two groups for the in vivo and in vitro experiments, respectively.

**In Vivo Experiments**

Catheterizations. Double cannulation of the dorsal aorta was performed under anaesthesia (0.1 g/l ethyl-n-aminobenzozoate sulphonic acid, MS-222, buffered with 0.2 g/l sodium bicarbonate) (8). Cannulated animals were allowed to recover for at least 36 h in opaque acrylic boxes (60 × 16 × 18 cm) with a weak water current to ensure adequate oxygenation. Fish with hematocrits <20% after recovery from surgery were not used. During the experiments, the acrylic boxes were covered by an opaque plastic sheet to avoid light-induced effects of the cell-isolation procedure (e.g., destruction of membrane integrity, abnormal stimulation of glycogen breakdown (17), and elimination of neural input), to evaluate how relevant in vitro measurements of glucose production are to the intact organism.

Measurement of glucose kinetics. Ra glucose was measured by continuous infusion of 6-3H glucose as described and validated previously (7, 8). The infused isotope was prepared by drying 30 µl of plasma under N2, resuspending in 1 ml dechloraminated, well-oxygenated water under a 12:12-h light-dark photoperiod. The animals were fed low-fat trout chow (11% lipids, 42% protein, 20% carbohydrate) until satiation three times a week. They were acclimated to these conditions for 6 wk before the first experiment and were randomly divided into two groups for the in vivo and in vitro experiments, respectively.

**In Vitro Experiments**

Hepatocyte isolation. The fish were killed by a blow to the head (n = 22, mean body mass 715 ± 47 g), and a midventral incision was made to expose the liver. Hepatocytes were isolated according to Moon et al. (21). Media A, B, and C, described in detail by these authors, were used with the following modifications: the liver was digested with medium C, containing 30 mg/100 ml collagenase (type IV from Clostridium histolyticum, >125 collagen digestion units/mg solid), and cell incubations were carried out in medium B added with 2 mM alanine and 1.5 mM lactate. The final pellet was resuspended in medium B and left on ice for 1 h before the cells were counted and their viability assessed with the trypan blue exclusion method. Cell preparations with viability of <80% were not used in experiments.

Hepatocyte incubations. The rate of glucose production was measured by monitoring the accumulation of glucose in the incubation medium. For each treatment, 50 mg of cells were incubated for 15–60 min at 13°C with medium B alone (control) or added with Epi, Prop, or both. Incubations with Epi were performed at low (5 nM) or high (500 nM) concentrations to simulate normal levels at rest (low Epi) or after exhaustive exercise (high Epi). Incubations with Prop were performed at a concentration of 50 µM. In experiments in which both Prop and Epi were used, Prop was added 15 min before Epi to ensure that all β-adrenoreceptors were blocked. All incubations were carried out in the dark and stopped by adding perchloric acid. Two sets of experiments were performed. The first set consisted of control, Prop, and high-Epi treatments. The second set consisted of control, low-Epi, high-Epi, low-Epi + Prop, and high-Epi + Prop treatments.

Calculations and statistics. All in vivo Ra glucose values reported in this paper were calculated with the steady-state equation of Steel (30), because they were never significantly different from Ra glucose when both rates were calculated separately with the non-steady-state equation. To allow meaningful comparisons with in vivo Ra glucose, in vitro rates of glucose production measured in micromoles of glucose produced per gram of hepatocytes were converted to micromoles per kilogram per minute as follows: in vivo Ra glucose = [glucose produced (µmol/g hepatocytes) × 0.9 liver mass (g)/(body mass (g) × incubation time (min))]. Liver mass was multiplied by 0.9 because ~90% of all liver cells are hepatocytes (15). For all the comparisons between rates of glucose production in vivo and in vitro, measured in vivo Ra values were corrected for renal glucose production by subtracting 10.5% (7). For the in vivo experiments, changes in Mo2, glucose concentration, glucose specific activity, and Ra glucose were assessed by two-way ANOVA and Dunnett’s or Tukey’s test. Differences between in vitro treatments and compari-
sons between $R_a$ values measured in vivo and in vitro were tested with one-way ANOVA or Kruskal-Wallis one-way ANOVA on ranks when normality tests failed. Percentages were converted to the arcsine of their square root before analyses, and all the values presented are means ± SE.

RESULTS

In Vivo Experiments

Control saline infusions. Infusion of vehicle saline had no effect on $\dot{M}O_2$ (Fig. 1), plasma glucose concentration (Fig. 2A), glucose specific activity (Fig. 2B), or $R_a$ glucose (Fig. 2C; $P > 0.05$). Throughout the saline infusion experiments, $\dot{M}O_2$ averaged 38.69 ± 0.36 µmol O₂·kg⁻¹·min⁻¹ ($n = 6$), plasma glucose concentration 5.44 ± 0.06 mM ($n = 13$) and $R_a$ glucose 7.95 ± 0.08 µmol·kg⁻¹·min⁻¹ ($n = 13$). These values were not different from mean baseline levels measured before starting the administration of Epi or Prop (see Figs. 1, 4, and 5; $P > 0.05$). Mean hematocrit did not change significantly during the saline infusion experiments and averaged 24.9 ± 1.6% ($n = 3$).

Epi infusions. The infusion of Epi caused an increase in $\dot{M}O_2$ from a mean baseline value of 40.67 ± 0.39 to 61.36 ± 2.67 µmol O₂·kg⁻¹·min⁻¹ ($n = 7$, $P < 0.05$), and $\dot{M}O_2$ was still significantly higher than baseline 1 h after the end of Epi infusion ($P < 0.05$, Fig. 1). Figure 3 shows plasma catecholamine concentrations before, during, and after Epi infusion. Plasma Epi was 2.36 ± 0.72 nM ($n = 7$) before starting the exogenous administration of Epi and increased to 545 ± 23.91 nM ($n = 6$; $P < 0.001$) after 30 min of exogenous Epi infusion. Plasma Epi concentration returned to baseline 25 min after the end of infusion and remained at basal levels until the end of the measurements. Plasma norepinephrine concentration did not change significantly throughout the experiments ($P > 0.05$) and averaged 19.47 ± 5.34 nM ($n = 8$). The effects of Epi infusion on glucose concentration, glucose specific activity, and $R_a$ glucose are presented in Fig. 4. Before Epi infusion, plasma glucose concentration and $R_a$...
glucose averaged 3.75 ± 0.16 mM and 6.57 ± 0.79 µmol·kg\(^{-1}\)·min\(^{-1}\) (n = 7), respectively. Glucose concentration increased to a maximal value of 8.51 ± 0.54 mM (P < 0.001) after 20 min of Epi infusion but had returned to baseline within 1 h after the end of Epi infusion (Fig. 4A). The administration of Epi caused a twofold increase in \(R_g\) glucose to a maximal value of 13.30 ± 1.78 µmol·kg\(^{-1}\)·min\(^{-1}\) (P < 0.001, Fig. 4C). Glucose production declined progressively after the end of Epi infusion. It reached baseline values after 1 h of recovery and was 5.29 ± 0.43 µmol·kg\(^{-1}\)·min\(^{-1}\) after 2 h of recovery, a significantly lower rate than before Epi infusion (P < 0.05). Mean hematocrit did not change significantly during the Epi infusion experiments and averaged 24.5 ± 0.9% (n = 7).

Prop infusions. One-hour infusion of the \(\beta\)-adrenoceptor blocker Prop caused a decline in \(\dot{M}O_2\) from a baseline value of 41.06 ± 0.96 to 32.80 ± 1.37 µmol O\(_2\)·kg\(^{-1}\)·min\(^{-1}\) (n = 7, P < 0.05). A further decrease to 29.36 ± 1.47 µmol O\(_2\)·kg\(^{-1}\)·min\(^{-1}\) was observed after the end of the Prop infusion (Fig. 1). Changes in glucose concentration, glucose specific activity, and \(R_g\) glucose during and after Prop infusion are plotted in Fig. 5. Baseline glucose concentration and \(R_g\) glucose before Prop infusion were 4.98 ± 0.49 mM and 7.65 ± 0.92 µmol·kg\(^{-1}\)·min\(^{-1}\) (n = 10), respectively. Plasma glucose concentration decreased progressively to reach values significantly lower than baseline in the recovery period (Fig. 5A). \(R_g\) glucose declined progressively to 5.17 ± 0.54 µmol·kg\(^{-1}\)·min\(^{-1}\) (n = 10, P < 0.05) during Prop infusion and reached a minimal value of 4.10 ± 0.56 µmol·kg\(^{-1}\)·min\(^{-1}\) (P < 0.05) by the end of recovery (Fig. 5C). Hematocrit did not change significantly during the Prop-infusion experiments and averaged 23.9 ± 0.8% (n = 10). A comparison of the relative effects of vehicle saline, Epi, and Prop infusions is presented in Fig. 6, in which percent changes in \(R_g\) glucose are quantified.

In Vitro Experiments

Effects of Epi and Prop on glucose production by hepatocytes. Mean glucose production rates of isolated...
hepatocytes incubated for 60 min with or without Epi and/or Prop are summarized in Table 1. In each experiment, all treatment groups measured before starting the incubations (time 0) produced glucose at the same rate (experiment 1, \( P = 0.63 \); experiment 2, \( P = 0.95 \)). Over time, the control groups of experiments 1 and 2 were not different from each other (\( P > 0.05 \)). Low and high Epi concentrations stimulated glucose production in a dose-dependent manner, but these effects were abolished by Prop (Table 1). Figure 7 summarizes the relative changes in glucose production measured after 60 min of incubation. Low Epi caused a 23% increase in glucose release (\( P < 0.05 \); Fig. 7B), whereas high Epi stimulated glucose output by 110% and 133% in experiments 1 and 2, respectively (\( P < 0.001 \); Fig. 7, A and B). Prop had no effect in the absence of Epi (Fig. 7A), and it prevented both low and high Epi from stimulating glucose production (Fig. 7B).

Comparison of In Vivo and In Vitro Glucose Production Rates

Figure 8 shows a comparison of \( R_a \) glucose measured in the whole organism and in isolated hepatocytes. Rates of glucose production measured in vitro in micromoles per gram of hepatocytes (Table 1) were converted to micromoles per minute per kilogram body mass to compare them with baseline \( R_a \) glucose measured in vivo that averaged 7.31 ± 0.54 µmol·kg\(^{-1}\)·min\(^{-1} \) (\( n = 11 \)) and increased to 3.83 ± 0.16 µmol·kg\(^{-1}\)·min\(^{-1} \) (\( n = 22 \)) in the presence of low (5 nM) and high (500 nM) Epi concentration, respectively (\( P < 0.05 \)).

Table 1. Amount of glucose released by isolated hepatocytes incubated in different media

<table>
<thead>
<tr>
<th></th>
<th>15-min Control</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
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<tr>
<td>Control</td>
<td>6.5 ± 0.7</td>
<td>6.7 ± 0.8</td>
<td>12.6 ± 1.7</td>
<td>18.6 ± 2.2</td>
<td>20.6 ± 2.2</td>
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<tr>
<td>Prop</td>
<td>6.5 ± 0.7</td>
<td>6.2 ± 0.9</td>
<td>11.8 ± 1.4</td>
<td>16.9 ± 2.1</td>
<td>22.8 ± 2.6</td>
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<tr>
<td>High Epi</td>
<td>6.4 ± 0.6</td>
<td>12.2 ± 1.4</td>
<td>23.8 ± 2.9</td>
<td>34.4 ± 4.3</td>
<td>44.1 ± 5.6†</td>
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<td>Experiment 2</td>
<td></td>
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<tr>
<td>Control</td>
<td>4.4 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>8.8 ± 0.4</td>
<td>13.4 ± 0.6</td>
<td>17.5 ± 0.8</td>
</tr>
<tr>
<td>Low Epi</td>
<td>4.4 ± 0.2</td>
<td>5.5 ± 0.3</td>
<td>10.5 ± 0.6</td>
<td>15.8 ± 0.9</td>
<td>21.4 ± 1.1*</td>
</tr>
<tr>
<td>Low Epi + Prop</td>
<td>4.6 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>8.7 ± 0.6</td>
<td>13.7 ± 0.9</td>
<td>18.2 ± 1.1</td>
</tr>
<tr>
<td>High Epi</td>
<td>4.4 ± 0.2</td>
<td>9.9 ± 0.8</td>
<td>19.2 ± 1.1</td>
<td>27.2 ± 1.6</td>
<td>35.9 ± 2.1†</td>
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<tr>
<td>High Epi + Prop</td>
<td>4.4 ± 0.2</td>
<td>5.1 ± 0.5</td>
<td>10.5 ± 0.7</td>
<td>15.6 ± 0.9</td>
<td>20.7 ± 1.3§</td>
</tr>
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</table>

Values are means ± SE in µmol/g cells; \( n = 11 \) fish. First column (15-min control) shows glucose release for a subsample of each batch of cells used in both experiments, but without the addition of epinephrine (Epi) or propranolol (Prop). Low Epi (5 nM); high Epi (500 nM); Prop (50 µM). Symbols indicate significant differences between treatments after 60 min of incubation (\( P < 0.05 \)): *, different from control; †, different from low Epi; ‡, different from high Epi; §, different from low Epi; and ‡§, different from high Epi.
effects of Epi and Prop in vivo and in vitro are compared in Fig. 9. High Epi concentration had the same effect in vivo and in vitro, stimulating $R_a$ glucose by 102% and 89%, respectively. Prop alone caused a 46% decline in $R_a$ glucose in vivo but had no significant effect in vitro (+1.8%).

DISCUSSION

Earlier in vivo measurements of fish substrate kinetics had revealed high basal rates of hepatic glucose production and a limited ability to increase $R_a$ glucose (7–9, 29). Stresses such as moderate exercise (29) and acute changes in water temperature (9) were shown to depress glucose production, suggesting that resting unstressed fish already release glucose at close to maximal rates. Results from the present study demonstrate otherwise and provide a new minimal estimate of reserve capacity for in vivo glucose production. Except for a small and transient increase in $R_a$ observed during acute hypoxia (9), this is the first example in which fish strongly stimulate hepatic glucose production in vivo, in this case, responding to a hormonal signal. Elevated plasma Epi levels (Fig. 3) caused a twofold increase in $R_a$ glucose (Figs. 4 and 6), whereas previous experiments by others showed that cortisol (1, 34) and estrogen (35) had no significant effects.

Mechanisms of Epi Action on Hepatic Glucose Production

Arterial infusion of Epi caused hyperglycemia (Fig. 4A) as noted previously in the same species (22), in other teleosts (24, 33), and in humans (28). Although the observed increase in blood glucose had to be caused by a temporary mismatch between $R_a$ and $R_d$, the difference between these rates could not be detected statistically, because $R_d$ increased almost simultaneously with $R_a$. Therefore, hyperglycemia resulted from a rapid increase in $R_a$, whereas the Epi-induced reduction in $R_d$ known to occur in mammals (2) did not play a role in fish. The increase in trout $R_a$ glucose could be mediated through the direct activation of glycogenolysis and gluconeogenesis, and via indirect mechanisms involving changes in hepatic blood flow or in the levels of other circulating hormones such as glucagon, insulin, and cortisol. The respective contributions of these different factors cannot be determined from our results and further work will be needed to evaluate their potential impact separately.

In mammals, the initial increase in $R_a$ glucose after Epi administration is caused by the immediate stimulation of glycolysis, whereas gluconeogenesis is only activated later when glycogen stores are somewhat depleted (3, 28, 32). In vitro experiments on various species of fish show that Epi increases glycolysis by activating glycogen phosphorylase (10, 39). In isolated fish hepatocytes, glucose production is supported almost exclusively through the breakdown of glycogen, whereas gluconeogenesis only plays a minor role. However, this overwhelming dominance of glycolysis may be an artifact of the cell isolation procedure and gluconeogenesis may be more significant in vivo (17). High Epi levels are known to depress pyruvate kinase activity in fish liver, thereby causing a reciprocal increase in gluconeogenesis and decrease in glycolysis (17, 39). Furthermore, Epi may cause the peripheral mobilization of several gluconeogenic precursors and promote their uptake by the liver, as in mammals (28, 32).

Role of Epi in Maintaining Basal Glucose Production

Resting Epi levels are not responsible for sustaining basal glucose production in mammals (2), but our study shows that they can play a significant role in fish. When the $\beta$-adrenergic effects of resting Epi were blocked by the infusion of Prop in vivo, plasma glucose concentration and $R_a$ glucose of trout decreased by 14% and 46%, respectively (Figs. 5 and 6). Furthermore, glucose production by trout hepatocytes incubated at basal Epi concentrations showed a 20% decline when Prop was added to the medium (Fig. 7). Taken together, these results suggest that, in the resting state, Epi is present at higher concentrations in the portal circulation of fish than in mammals, and the observation that teleosts have much higher resting arterial Epi levels than mammals (26) supports this view. The absence of a decrease in the $R_a$ glucose of mammals after the administration of $\alpha$- or $\beta$-blockers has been attributed to the fact that their portal concentrations of catecholamines are negligible under basal conditions (2).

Glucose Production Rates: In Vivo vs. In Vitro

After diet, temperature, and body size were standardized, isolated trout hepatocytes incubated without hormones still produce glucose at less than one-half the basal rate observed in vivo (3.13 vs. 7.31 $\mu$mol·kg$^{-1}$·min$^{-1}$; Fig. 8). This difference is due to a combination of factors that may include hormonal and neural effects only acting in vivo as well as structural and/or functional damage incurred by the cells during isolation. Resting arterial Epi levels are responsible for a fraction of the difference, because the addition of 5 nM Epi to the incubation medium increases glucose production in vitro (Figs. 7B and 8), and Prop causes a decrease in $R_a$. 

Fig. 9. Relative effects of high epinephrine (HE) and propranolol (Prop) on hepatic glucose production of rainbow trout measured in vivo (solid bars) and in isolated hepatocytes (open bars).
glucose in vivo (Fig. 5) as well as in isolated cells incubated with basal Epi levels (Fig. 7). Isolated cells only approached in vivo rates when they were incubated with 500 nM Epi, a concentration simulating extreme arterial conditions after exhaustive exercise (Fig. 8). Therefore, other factors than basal Epi are also responsible for the twofold difference between basal glucose production rates observed in vivo and in vitro. Resting levels of glucagon, norepinephrine, and cortisol as well as direct sympathetic stimulation may be involved in maintaining basal Ra glucose in vivo. However, further experiments will be needed to clarify this issue. The mean basal rate of glucose production measured here in vitro (3.13 µmol·kg⁻¹·min⁻¹) falls within the range of values previously obtained by others for trout hepatocytes (1–5 µmol·kg⁻¹·min⁻¹, see METHODS for conversion to this unit) (4, 12, 14, 17). We could only find one in vitro study reporting glucose production rates as high as measured in vivo (22). Interestingly, these high in vitro values were obtained in experiments carried out on liver slices rather than isolated cells, suggesting that disrupting the integrity of the liver tissue impairs normal glucose production. In humans, Epi also stimulates Ra glucose indirectly by increasing hepatic blood flow and by activating glucagon secretion, which enhances liver gluconeogenesis (28). Here, high Epi concentration had similar relative effects in vivo and in vitro, causing baseline Ra glucose to double in both cases. These findings suggest that the indirect pathways of Epi stimulation acting in vivo in mammals do not play a significant role in trout.

β- Vs. α-Adrenergic Effects of Epi

Both in vivo and in vitro experiments show that the stimulating effect of Epi on the Ra glucose of rainbow trout is eliminated in the presence of Prop. The signal transduction pathway involving the binding of Epi to β-adrenoceptors, the activation of adenyl cyclase, and the upregulation of glycogen phosphorylase A have been well characterized in fish (5). The reverse phenomenon has been observed when treatment with Prop caused a three- to fivefold decrease in glycogen phosphorylase activity (39). Recently, the presence of α-adrenoceptors has been demonstrated in the membrane of rainbow trout hepatocytes, but no clear functional link with glucose metabolism has yet been established (6). Taken together, these results show that circulating Epi stimulates trout hepatic glucose production mainly via β-adrenoceptors and that α-receptors could only play a minor role if any.

Effects of Epi on Glucose Disappearance

Because the rates of glucose production (Ra) and glucose disposal (Rd) were never statistically different, all the figures showing changes in Ra also depict how Rd was affected. Arterial Epi infusion caused Ra glucose to double (Fig. 4), and two possible mechanisms for this response can be proposed: 1) a simple mass action effect mediated by hyperglycemia and/or 2) the activation of the glucose transporter (GLUT-4). The large augmentation in plasma glucose levels (Fig. 4A) expanded the diffusional concentration gradient driving glucose into cells, and this mechanism must be partly responsible for the observed increase in Ra glucose. However, an increase in the rate of glucose transport through the translocation of GLUT-4 to the cell membrane, as observed in mammals, is unlikely to be involved in the Epi stimulation of Rd in fish. To date, every attempt to demonstrate the presence of GLUT-4 in fish tissues has failed, with the possible exception of pancreatic cells in Tilapia (38).

Conclusions

By combining results from our in vivo and in vitro experiments, the following conclusions can be drawn from this study: 1) the Epi-induced hyperglycemic response of rainbow trout is caused by the stimulation of Ra glucose, but the concomitant reduction in Rd glucose observed in mammals does not occur in trout. 2) Basal levels of circulating Epi are partly responsible for maintaining resting Rd glucose in rainbow trout. 3) Epi stimulates hepatic glucose production in a dose-dependent manner, and its effects are mainly mediated by β-adrenoceptors. 4) Isolated trout hepatocytes produce glucose at about one-half the basal rates measured in vivo, even when diet, temperature, and body size are standardized, and basal arterial Epi is partly responsible for this discrepancy. 5) The relative increases in Ra glucose after Epi stimulation are similar in vivo and in vitro, suggesting that indirect in vivo effects of Epi, such as sympathetic stimulation, changes in hepatic blood flow, or changes in the levels of other circulating hormones, do not play an important role in trout.