Differential role of adrenoceptors in control of plasma glucose and fatty acids in carp, *Cyprinus carpio* (L.)

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During recent years, the hormonal regulation of carbohydrate metabolism in fish, especially in liver, has received increasing attention. It has been demonstrated that the mobilization of glucose from the liver is stimulated by hormones such as catecholamines, cortisol, and glucagon (4, 5, 10, 25), in particular during stress conditions such as hypoxia (43). In contrast, little information is available on the regulation of lipid metabolism during hypoxia in teleost fish. Whereas in mammals, hypoxia results consistently in increases of free fatty acid (FFA) levels (23, 24), the effect of hypoxia on FFA levels in fish is not clear, and the available results are conflicting. In the hypoxia-tolerant goldfish, carp, and bream, plasma FFA levels were found to decrease, whereas FFA concentrations increased in trout, lamprey, and plaece (7, 13, 22). Recently, we demonstrated that a maximal exposure to severe hypoxia, under well-controlled conditions, resulted in a considerable decrease of plasma FFA levels in carp, while a small decline was found in rainbow trout (38).

It has been proposed that a decline of FFA levels would be a secondary effect of the increased availability of carbohydrates during hypoxia or catecholamine administration by enhancing the rate of fatty acid reesterification (7, 22, 32). Moreover, the increased availability of carbohydrates was assumed to result in a stimulation of insulin secretion, leading to inhibition of lipolysis and stimulation of lipogenesis (13, 17). However, several arguments may be proposed against this hypothesis. First, a decrease of plasma FFA levels is not consistently accompanied by hyperglycemia in several fish species (13). Second, elevated plasma glucose levels do not necessarily result in an increase of insulin levels in fish (18). Third, the uptake of blood glucose in fish is normally quite low because of the low activity of hexokinase (11, 21). Finally, during stress conditions in mammals, when fuel mobilization is favored (e.g., exercise or hypoxia), insulin levels are suppressed as a result of a catecholamine-mediated inhibition of the insulin secretion (20, 30). Thus a decrease of plasma FFA levels during hypoxia must be caused by a reduction of lipolysis. Species-specific differences in plasma FFA responses were observed when fish were injected with catecholamines: a decrease of plasma FFA in goldfish, carp, and bream (7, 13, 16, 17), and an increase of plasma FFA in rainbow trout, perch, and scorpion fish (13, 22). Because hypoxia is a potent stimulator of the release of catecholamines in fish, these hormones may play a pivotal role in the mobilization of FFA.

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In a study (37) we showed that, although both catecholamines induce a significant hyperglycemia, stress-free infusion of epinephrine induces an increase of plasma FFA levels in carp whereas infusion of nor-epinephrine induces a significant decrease of plasma FFA concentrations. Mammalian adipose tissue and adipocytes always respond to catecholamines with lipolysis. The response of carp to norepinephrine, i.e., a decrease of blood FFA levels, was a new phenomenon. Considering the strong lipolytic character of these hormones in mammals, a reduction of lipolysis during hypoxia in fish is puzzling and strongly suggests a different mechanism of regulation. From a biological point of view, it is evident that suppression of lipolysis can have a protective effect on the animal under hypoxic conditions. Since the flux through the β-oxidation is high during normoxic conditions, fatty acids and their metabolites accumulate rapidly during anaerobiosis in mammalian tissues (19) and will quickly start to disrupt biomembranes. Anoxia-induced membrane damage, however, does not occur in fish (37), which may be related to a better control of lipid mobilization.

On the basis of the mammalian literature, it is expected that β-adrenergic stimulation results in a stimulation of lipolysis. However, suppression of FFA levels may be possible via the stimulation of α2-adrenoceptors, which are mostly coupled to adenyl cyclase via the inhibitory G Protein, resulting in a decrease of cAMP levels, thus decreasing triacylglycerol lipase activity (12, 34). However, at present it is not known whether α2-adrenoceptors are expressed in fish lipid depot tissues. In this study we first tested the hypothesis that the decrease of plasma FFA in carp is mediated by the α2-adrenoceptor. The results, however, indicate that, in addition to the α2-adrenoceptor, the β1-adrenoceptor is importantly involved. The first hypothesis was tested by selectively inhibiting and stimulating α2-adrenoceptors using yohimbine and clonidine, respectively. Since the outcome did confirm the hypothesis only in part, while, in contrast, isoproterenol infusion strongly reduced FFA levels, it was as-

Materials and Methods

Chemicals. Isoproterenol HCl, yohimbine HCl, and clonidine HCl were obtained from Sigma (St. Louis, MO). Norepinephrine HCl was obtained from BDH (Poole, UK), and atenolol HCl and ICI-118,551 were kind gifts from Zeneca (Wilmslow, Cheshire, UK).

Animals. Common carp (1.0–2.5 kg) were purchased from the Dutch Fisheries Organisation (OVB, The Netherlands). They were fed ad libitum with carp feed from Trouw (Putten, The Netherlands) and were kept in local running tap water in the laboratory for at least 2 mo. The fish were acclimated to a temperature of 20°C and a 14:10-h light-dark cycle. In total 51 experimental animals were used.

Preexperimental protocol. Experiments were performed in a recirculation system as described by Van Dijk et al. (35). Fish handling and surgery was performed as described by van Raaij et al. (38). The cannula was connected to a Y-piece just outside the fish and was split into two PE-50 tubes. One tube (45 cm) was used for blood sampling while the other tube (90 cm) was used for infusion. After cannulation, the fish were placed in the flow boxes and left for 2 days to recover from surgery before starting the experiments. The PE cannula was filled with a viscous solution of polyvinylpyrrolidone (PVP; Merck, Darmstadt, Germany; 1 g PVP/ml saline) containing 500 IU/ml heparin (Sigma). The heparin in the PVP solution prevents clot formation in the cannula, whereas the diffusion of heparin into the blood is negligible, thus preventing possible activation of endothelium-bound lipoprotein lipase.

Experiments. Experiments were started between 8:30 and 9:30 A.M. by taking two blood samples from each fish at t = 0.5 and 0 h (before infusion) to determine initial values. After the second blood sample, the 90-cm PE tube was connected to a 0.5-ml Hamilton syringe containing one of the five solutions: 1) carp Ringer, or carp Ringer containing 2) norepinephrine, 3) yohimbine (α2-antagonist) + norepinephrine, 4) clonidine (α2-antagonist), 5) isoproterenol (β-agonist). Because the volume of PE tubes was known, we were able to fill the cannula with the test solution just before entering the circulation (negligible dead space). The syringe was then placed in a microinjection pump (Fine Mechanic Service Dept., Leiden, The Netherlands), which was set at a rate of 3.6 μl/min. The solutions were infused in the dorsal aorta at t = 0 h. Norepinephrine was infused over a period of 90 min at a rate of 1 n mole/min × kg−1× h−1 (n = 5). Yohimbine was infused as a bolus of 5 mg/kg, followed by the same norepinephrine infusion protocol. Isoproterenol was infused over 90 min at 1 n mole/min × kg−1× h−1 (n = 6). Clonidine was infused at three, stepwise-increasing levels, i.e., 0.01, 0.1, and 1 n mole/min × kg−1× h−1 (n = 6) each for 1 h. Control animals (n = 6) received an infusion of carp Ringer saline only.

With the above infusion protocols (1, 2, 3, 5), the blood samples were drawn via the second PE-50 tube at t = 0.25, 0.5, 1.5, 2.5, 3.5, 5.5, 9.5, 24, and 48 h. The sampling times for the clonidine infusion protocol (protocol 4) were t = 0.75, 1.75, 2.75, 3.25, 4, 5, 9, 24, and 48 h. After each blood sample an equal volume of carp saline was injected and the cannula was subsequently refilled with PVP (1 g/ml). For blood samples taken during the infusion period, a different procedure was followed. The microinjection pump was stopped briefly, and blood was drawn into the cannula until it passed the tripod connector. A fresh blood sample was then taken via the other PE-50 tube, thus excluding interference of the test solution. After blood sampling, an equal volume of saline was injected and the test solution was reintroduced into the cannula. Then, the microinjection pump was switched on again, completing the procedure within 1 min.

For the characterization of the β-adrenoceptor subtype, infusions were made as a bolus in 4 different bolus protocols: 1) bolus saline (n = 6) at t = 0 (control); 2) bolus isoproterenol (19.8 μg/kg; n = 7) at t = 0; 3) bolus atenolol at t = −0.25 h, followed by a bolus isoproterenol at t = 0; 4) bolus ICI-118,551 at t = −0.25 h, followed by a bolus isoproterenol at t = 0. Before infusion of the bolus, three blood samples were taken from each fish (at t = −1.25, −0.75, and −0.25 h) to determine the initial values. In bolus protocols 3 and 4 the isoproterenol infusion (19.8 μg/kg) was preceded by a bolus infusion with either 213 μg/kg atenolol (β1-antagonist, n = 6) or 250 μg/kg ICI-118,551 (β2-antagonist, n = 6). The antagonists were infused over 2 min, 15 min before the isoproterenol bolus.

Infusion quantities. The chosen infusion rates and concentrations of the different drugs were mainly based on the
predicted end concentrations and on previously published data. The effective dose for norepinephrine in carp is taken from recent hypoxia studies: during normoxia norepinephrine levels were found to be 0.25 ± 0.07 ng/ml; this level rose to 15 ng/ml during moderate hypoxia and during anoxia to 49 ng/ml (38). This level corresponds to 0.5 × 10⁻⁶ M. The applied infusion protocol for norepinephrine was similar to the one we used previously (37), i.e., 2 µg·min⁻¹·kg⁻¹. Since the clearance of isoproterenol is ~10-fold lower than that of norepinephrine (preliminary observations), we infused isoproterenol at 1 µg·min⁻¹·kg⁻¹, which at an extracellular volume of 8% would end up, after 90 min infusion, at ~10⁻⁵ M. The yohimbine dose was based on mammalian literature: 0.2–3.0 mg/kg (2, 12, 40). We applied 5 mg/kg, assuming that this high concentration would block α₂-adrenoceptors virtually completely. To choose the appropriate concentration for clonidine was more difficult, because it is a partial α₂-agonist, and such agonists may turn into antagonists at higher concentrations. Clonidine is thus far only applied in mammals and such agonists may turn into antagonists at higher concentrations: 0.01, 0.1, and 1.0 µg·min⁻¹·kg⁻¹. The bolus infusion of isoproterenol was calculated to obtain an initial level of ~10⁻⁶ M, which is enough to activate the β-adrenoceptors. The doses of ICI-118,551 and atenolol chosen were reported previously to induce an effective blockade of mammalian vascular β₁- and β₂-adrenoceptors (28).

Analytical procedures. Blood samples (450 µl) were taken with ice-cooled, heparin-flushed, gastight precision syringes (Hamilton) and were directly placed on ice. Of the whole blood, 50 µl were used for hemoglobin (Hb) and hematocrit (Hct) measurements; the remainder was centrifuged for 5 min at 10,000 g (Eppendorf 5415), and plasma was separated directly. Aliquots of untreated plasma were stored at ~80°C until used for analysis of FFA (50 µl). For the measurement of lactate and glucose, 100 µl of plasma were diluted to 20% with trichloro-acetic acid solution (6% vol/vol) to precipitate plasma proteins. The supernatant was neutralized with 1 M K₂HPO₄ and stored at 20°C for up to 3 days until analysis. For the assay of catecholamines, 100 µl of plasma were mixed immediately with 15 µl of a preservative solution (22 mg EDTA/ml) and stored at ~80°C until analysis.

The FFA concentration was measured with the standard colorimetric assay of WAKO [nonesterified fatty acid (NEFA) C method, Instruchemie, Hilversum, The Netherlands]. Glucose was measured with an enzymatic method based on glucose-6-phosphate dehydrogenase, a test kit from Instruchemie (Hilversum). Lactate was measured according to the method of Hohorst (1). The catecholamines were analyzed by the HPLC method of Remie et al. (28).

Presentation and statistics. The results of the 1.5-h infusion experiments were normalized to the mean of the first two sample points at t = −0.5 and 0 h (control=100%) and are presented as means ± SE. The results of the bolus infusion experiments are presented as means ± SE. Statistical significance between experimental data points and initial values and/or control (saline-infused group) values was defined at P < 0.05. Statistical analysis was performed with Sigmastat using the Student’s two-tailed t-test. Since the data in Table 2 are not normally distributed, statistical differences were analyzed with the nonparametric Friedman test (Friedman repeated-measures analysis of variance on ranks), followed by the multiple-comparisons Dunnett’s test; comparisons were made with respect to the initial value (t = −0.5 h). Comparisons between the different series and the saline controls were based on the Mann-Whitney rank sum test.

RESULTS

Data on Hb, Hct, and mean cellular hemoglobin concentration (MCHC) are not presented because no changes were observed. Hct values were ~30 and declined by maximally 10% during the experiments because of blood sampling. The MCHC values stayed constant at 18 throughout the experiments.

The plasma levels of lactate and epinephrine hardly changed during the different protocols. Therefore a limited number of data are presented in Table 1: 4 of 10 sampling points (t = −0.5, 1.5, 5.5, and 48 h). Under all conditions the lactate levels remained below 0.6 mmol/l, indicating the absence of struggling and anaerobic metabolism. The epinephrine levels at the reference points (first 2 samples in each series) and in the control series were extremely low, quite often below the detection level (10 pg/ml), also indicating low-stress conditions.

All norepinephrine levels measured in the nine series are presented in Table 2. The norepinephrine concentrations remained rather low during and after the different infusions except for the infusion experiments (norepinephrine and yohimbine + norepinephrine). Over the period t = 0.5 to 3.5 h all levels were significantly increased. With the norepinephrine infusion the highest value occurred at t = 3.5 h: 297 ng/ml, which is about a 100-fold increase compared with the saline controls. The combination yohimbine + norepinephrine resulted in even much higher values: the highest level of 3,545 ng/ml was found at t = 1.5 h, which is roughly a 1,000-fold increase compared with the saline controls.

The initial values of the FFA and glucose levels during the different protocols are presented in Table 3. The levels are close to previously described levels for carp (37).

The relative changes in plasma FFA and glucose content due to infusion of the different compounds are shown in Figs. 1–5. Figure 1 shows the FFA and glucose values of the control (saline) series; the data stay constant throughout 48 h and can thus be used for reference. Infusion of norepinephrine (Fig. 2A) results in a transient increase of glucose and was accompanied by a transient decrease of FFA. The glucose increase was significant at the end of the 1.5-h infusion period and stayed elevated until t = 9.5 h. The glucose recovery was most prominent between t = 3.5 and 5.5 h. In contrast to glucose the FFA decline was already significantly lower at 15 min after the start of the norepinephrine infusion. A 50% decline was reached after 1.5 h of infusion, after which a very slow recovery occurred; at t = 9.5 h the plasma FFA level was still 25% below the control level. At 24 and 48 h the data were not different from the controls.

The same experiment was repeated for the combination of norepinephrine with the α₂-antagonist yohimbine (Fig. 2B). The glucose data were very similar to norepinephrine alone (Fig. 2A), with the same first significant point after 1.5 h infusion, and with the rapid recovery between 3.5 and 5.5 h. Remarkably
different, however, was the FFA pattern: the FFA decline in the presence of yohimbine was clearly retarded, reaching the first significant point at 2.5 h; thereafter the data were similar to those without yohimbine.

The effect of infusion of the β2-agonist clonidine is shown in Fig. 3. The concentration was increased stepwise (0.01, 0.1, and 1.0 μg·min⁻¹·kg⁻¹), with each concentration being infused during 1 h. The glucose level did not significantly change except at t = 9.0 h, where the concentration was reduced by 50%. The plasma FFA levels were decreased to some extent at 0.75 and 1.75 h, the other points particularly after 3.25 h, and later showed a large variance with values around 30–40% above control.

Infusion with the nonselective β-adrenoceptor agonist isoproterenol (Fig. 4) showed opposite effects for plasma glucose and FFA levels. A strong and highly significant increase during the infusion was observed for glucose. After the infusion the levels stayed high at ~300% of the control level and declined to control values between 3.5 and 9.5 h. The FFA response was also very clear but inverse. A decline of 40% below control occurred during the infusion; the levels stayed low until 3.5 h and slowly recovered to control values at 9.5 h.

To distinguish between β₁- and β₂-adrenoceptor effects, combinations of isoproterenol with selective β₁- and β₂-antagonists were tested, which were injected as bolus infusions. At several time points before and after the bolus injection(s) (t = −1.25, 0.25, 1.5, and 48 h), the isoproterenol concentration in the plasma was measured. Injection of isoproterenol caused a strong but transient increase in concentration. After 15 min the values were 1,000–6,000 ng/ml (5–25 × 10⁻⁶ M) followed by a decrease to values of 100–600 ng/ml 1.5 h postinjection. After 48 h no differences with the controls were observed anymore. In Fig. 5 the effects on glucose and FFA levels are shown. The plasma glucose levels (Fig. 5A) increased rapidly after the infusion with isoproterenol. The highest level was reached at t = 1.5 h; the plasma concentrations stayed elevated until t = 5.5 h. After infusion with isoproterenol + atenolol and isoproterenol + ICI-118,551, the glucose levels increased significantly above the control values in a pattern similar to isoproterenol alone. The infusion with each of the antagonists, however, resulted in significantly lower glucose levels at t = 2.5, 3.5, and 5.5 h. This indicates that both β₁- and β₂-adrenoceptors are involved in glucose mobilization.

In Fig. 5B the effects of the different infusion protocols on the plasma FFA levels are shown. Infusion with a bolus of isoproterenol showed a small but nonsignificant decline of the FFA levels. The combination of isoproterenol + ICI-118,551 showed a fast decline of the plasma FFA level with a significant point at t = 0.5 h, recovering to control values at t = 3.5 h. Infusion with isoproterenol + atenolol, however, showed a completely opposite effect: at three time points the values were significantly higher than the initial levels. These observations indicate that the plasma FFA levels are governed by inhibitory β₁- and stimulatory β₂-adrenoceptors.

### Table 1. Concentrations of lactate and epinephrine in plasma of common carp before, during, and after infusion of specific adrenoceptor (ant)agonists (4 of 10 sample points)

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Time, h</th>
<th>Lactate, mmol/l</th>
<th>Epinephrine, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>−0.5</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Lactate, mmol/l</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.26 ± 0.06</td>
<td>0.30 ± 0.07</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>NE</td>
<td>0.35 ± 0.11</td>
<td>0.37 ± 0.13</td>
<td>0.36 ± 0.10</td>
</tr>
<tr>
<td>Yohimbine + NE</td>
<td>0.42 ± 0.10</td>
<td>0.43 ± 0.05</td>
<td>0.59 ± 0.08†</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.22 ± 0.06</td>
<td>0.53 ± 0.11*</td>
<td>0.51 ± 0.14*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>0.22 ± 0.03</td>
<td>0.34 ± 0.04</td>
<td>0.34 ± 0.04*</td>
</tr>
<tr>
<td><strong>Epinephrine, ng/ml</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.054 ± 0.019</td>
<td>0.018 ± 0.008</td>
<td>0.014 ± 0.004</td>
</tr>
<tr>
<td>NE</td>
<td>0.090 ± 0.037</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Yohimbine + NE</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.071 ± 0.028</td>
<td>0.057 ± 0.030</td>
<td>0.043 ± 0.017</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>&lt;0.010</td>
<td>0.038 ± 0.023</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td><strong>Bolus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Atenolol + isoproterenol</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>ICI-118,551 + isoproterenol</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 5–8 independent experiments. NE, norepinephrine. Significant differences: *P < 0.05 vs. initial value; †P < 0.05 vs. saline; ‡P < 0.05 vs. initial value and saline.
DISCUSSION

**α-Adrenoceptors.** The results shown in Fig. 2 confirm our earlier findings that norepinephrine increases plasma glucose and decreases plasma FFA in carp (37), the latter being in sharp contrast to the mammalian response. The FFA decline observed after norepinephrine infusion (Fig. 2A) is similar to the one described in our former study: a significant fall already at 15 min, declining further to 50% at the end of the infusion period, followed by a very slow recovery. Since we found in carp that epinephrine increased the plasma FFA level (37), it was hypothesized that the antagonistic effect of both catecholamines may be mediated by their differential effects on α2- and β-adrenoceptors. α2-Adrenoceptors act through Gi proteins, which inhibit adenyl cyclase. On the other hand, β-adrenoceptors stimulate adenyl cyclase via Gs proteins. Recently it has been suggested that the ratio of α2- to β-adrenoceptors determines the lipolytic capacity in adipose tissues (34). Thus the ratio of activated α2- to β-adrenoceptors could determine the lipolytic rate in the adipose tissue, which could be controlled by the ratio of norepinephrine to epinephrine.

To test the involvement of α2-adrenoceptors we used the α2-antagonist yohimbine in combination with norepinephrine. In Fig. 2B we see that yohimbine suppresses the FFA decrease induced by norepinephrine during the first few hours. The effect of yohimbine wears off; after ~3.5 h the pattern is not different from the protocol with norepinephrine alone. This finding indicates that α2-adrenoceptors are involved in the decline of plasma FFA levels. To demonstrate more explicitly a regulatory role of α2-adrenoceptors on lipol-

### Table 2. Concentrations of NE in plasma of common carp before, during, and after infusion of specific adrenoceptor (ant)agonists (all samples)

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Sample Time, h</th>
<th>NE, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-1  -0.5  0   0.25 0.5 1.5 2.5 3.5 5.5 9.5 24 48</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.47 ± 0.76 2.47 ± 0.97 1.53 ± 0.49 3.47 ± 2.71 1.98 ± 2.09 3.35 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>4.51 ± 3.06 3.30 ± 50.9 177 ± 183 297 ± 28.6 719 ± 15.4</td>
<td></td>
</tr>
<tr>
<td>Yohimbine + NE</td>
<td>5.27 ± 8.92 9.09 ± 13.0 3.31 ± 257 3.545 ± 700 2.97 ± 55.9 536 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>Yohimbine</td>
<td>0.14 ± 0.19 0.20 ± 0.21 0.18 ± 0.16 0.18 ± 0.19 0.23 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 5–8. Iso, isoproterenol. Significant differences: †P < 0.05 vs. saline group; ‡P < 0.05 vs. initial value (t = -0.5 h) and saline group.

### Table 3. Initial values of glucose and FFA plasma concentration in all series

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Glucose, mM</th>
<th>FFA, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.18 ± 0.91</td>
<td>0.43 ± 0.19</td>
</tr>
<tr>
<td>NE</td>
<td>3.71 ± 0.68</td>
<td>0.33 ± 0.11</td>
</tr>
<tr>
<td>Yohimbine + NE</td>
<td>4.09 ± 0.74</td>
<td>0.52 ± 0.21</td>
</tr>
<tr>
<td>Clonidine</td>
<td>4.02 ± 0.52</td>
<td>0.46 ± 0.26</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>2.68 ± 0.38</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>3.47 ± 0.39</td>
<td>0.43 ± 0.13</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>3.70 ± 0.67</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td>Isoproterenol + atenol</td>
<td>3.17 ± 0.39</td>
<td>0.45 ± 0.08</td>
</tr>
<tr>
<td>Isoproterenol + ICI-118,551</td>
<td>3.09 ± 0.38</td>
<td>0.30 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 independent experiments. FFA, free fatty acids.
ysis, the inhibitory effect of the α2-antagonist yohimbine has to be confirmed by a stimulating effect of a selective α2-agonist, for which we applied clonidine. As far as we know the α2-agonist clonidine has been used only in mammalian studies and appears to be a partially selective α2-agonist (3, 8, 9). Because the effective dose of clonidine in fish is not known, we decided to increase the dose exponentially in three steps to the dose known to be effective in mammals, i.e., 60 μg/kg.

It is evident that already at very low levels clonidine exerts an inhibitory effect on lipolysis. The effect is, however, not maintained at the highest dose, and after the infusion a very fast rebound occurs. Thus it is evident from Fig. 3 that clonidine does mimic the lipolysis-inhibiting effect of norepinephrine only partially. It decreases the FFA level in plasma during infusion over the first 2 h, and higher concentrations have no further effect.

Clonidine is known as a partial α2-agonist, which fits well with our results because we observed a decrease of 20% in FFA levels, whereas with norepinephrine a decline of 40–50% was found. The strong rebound that occurs immediately after the infusion is puzzling and does not correspond to a direct effect of α2-adenoreceptors on lipolysis. A possible explanation is that α2-adenoreceptors may have an indirect effect via the circulation. When stimulation of α2-adenoreceptors leads to vasoconstriction, then yohimbine will enhance and clonidine will diminish the blood flow through adipose tissue. Vasoconstriction by clonidine may disappear rapidly after the perfusion by additional relaxation factors, in which case the rebound can be understood by the drainage of accumulated FFAs.

During norepinephrine infusion the norepinephrine levels rose to ~300 ng/ml at 3.5 h; thereafter they declined rapidly (Table 2; between t = 3.5 and 5.5 h). When the animals were given yohimbine before the norepinephrine infusion, the norepinephrine levels rose faster and reached a much higher level (3,545 ng/ml at t = 1.5 h). This observation shows the role of presynaptic α2-adenoreceptors on the sympathetic nerve terminals, a well-known system in mammals (29). The presynaptic α2-adenoreceptors are responsible for a negative feedback on the norepinephrine release. Inhibition of these adrenoreceptors by yohimbine prevents an attenuation of the norepinephrine release and results therefore in transmitter overshoot.

Yohimbine is an often-used α2-agonist in mammalian studies. Effective in vivo doses are between 0.2 mg/kg (in dog; Ref. 12) and 3.0 mg/kg (in rat; Ref. 40). Blanchard et al. (2) showed that yohimbine was an effective α2-blocker over the range of 0.5 to 2.0 mg/kg in mice. Yohimbine has been used before in fish to investigate whether glycogenolysis is activated by β- and α-adenoreceptors. The fact that there was no effect (10), however, does not demonstrate that yohimbine does not bind to fish α2-adenoreceptors. In our study it appeared that the applied dose of yohimbine (5 mg/kg) is effective in carp and results in a temporary blockade of the α2-receptors, which is the first positive observation in fish.

β-Adrenoceptors. The involvement of β-adrenoceptors in lipolysis was tested by the infusion of isoproterenol. This general β-adrenoceptor agonist has been applied in many studies including fish. The effect of isoproterenol infusion is presented in Fig. 4 and shows an increase of blood glucose and a decrease of plasma FFA. The first effect, i.e., glucose release from the liver by β-adrenoceptor stimulation, was expected. The metabolic effect of stimulation of β-adrenoceptors on fish hepatocytes has been studied extensively, although these studies were almost exclusively aimed at carbohydrate metabolism (6, 27). Both epinephrine and norepinephrine stimulate predominantly glycogenolysis, and at a lower rate also gluconeogenesis (10, 41). The adrenoceptors responsible for glucose release in trout liver are, according to McKinley and Hazel (14), of the β2-type, not the α- or β1-type. Also Reid et al. (27) showed that β-adrenoceptors in trout hepatocytes are of the β2-type. However, the results shown in Fig. 5A suggest the presence of two different β-adrenoceptors on the liver cells. Atenolol and ICI-118,551 are selective β1- and β2-adrenoceptor antagonists, respectively.
Fig. 2. Effect of arterially infused norepinephrine (A) and norepinephrine in combination with yohimbine (B) on the plasma concentrations of glucose (○) and FFA (■) in common carp. Yohimbine was infused as a bolus (5 mg/kg) followed by the same norepinephrine infusion protocol as presented in A. Values are normalized with respect to the mean initial concentration (t = −0.5 and 0 h) and are expressed as means ± SE. Significant differences: *P < 0.05 vs. initial value, †P < 0.05 vs. control group, ‡P < 0.05 vs. initial value and control group.

Fig. 3. Effect of arterially infused clonidine on the plasma concentrations of glucose (○) and FFA (■) in common carp. Values are normalized with respect to the mean initial concentration (t = −0.5 and 0 h) and are expressed as means ± SE. Significant differences: *P < 0.05 vs. initial value, ‡P < 0.05 vs. initial value and control group.
Fig. 4. Effect of arterially infused isoproterenol on the plasma concentrations of glucose (○) and FFA (■) in common carp. Values are normalized with respect to the mean initial concentration (t = -0.5 and 0 h) and are expressed as means ± SE. Significant differences: *P < 0.05 vs. initial value, ‡P < 0.05 vs. initial value and control group.

Fig. 5. Effect of an arterially injected bolus infusion of saline (○), isoproterenol alone (19.8 µg/kg; ♦), and isoproterenol preceded by a bolus infusion of atenolol (213 µg/kg; ▼) or ICI-118,551 (250 µg/kg; ●) on the plasma concentrations of glucose (A) and FFA (B). Values are expressed as means ± SE. Significant differences: *P < 0.05 vs. initial value; ‡P < 0.05 vs. initial value and control group; ‡‡P < 0.05, all 3 isoproterenol treatments vs. initial value and control group; #P < 0.05, atenolol or ICI-118,551 treatment vs. isoproterenol alone.
whereas isoproterenol is a nonselective β-adrenoceptor agonist. In Fig. 5A the conditions isoproterenol + atenolol and isoproterenol + ICI-118,551 both reveal a significantly lower glucose response than the infusion with isoproterenol alone, which shows that β₁- as well as β₂-adrenoceptors are involved in glucose release. The experiments of McKinley and Hazel (14) and Reid et al. (27) were carried out with trout, whereas our results were with carp. Thus there may possibly be species-related differences. Also our experiments are whole animal responses; therefore other tissues may be involved as well. To prove that the glucose response of carp is mediated by both β₁- and β₂-adrenoceptors on the liver, appropriate in vitro experiments should be carried out with hepatocytes.

Isoproterenol infusion (Fig. 4) also shows, apart from an increase of plasma glucose, a decrease of plasma FFA. Since β-adrenoceptor stimulation is generally known both for mammals and for lower vertebrates (15, 34) to be connected with stimulation and not with inhibition of lipolysis, this observation points to a novel mechanism. The stimulation of β-adrenoceptors usually results in an increase of cAMP. Accumulation of cAMP in adipocytes stimulates hormone-sensitive lipase (HSL), thus leading to FFA release (15, 42). Migliorini et al. (15) showed that FFA release by adipose tissues of fish is stimulated by cAMP, forskolin, and phosphodiesterase inhibitors, suggesting the presence of HSL in fish adipose tissues. In Fig. 5B the effect of preincubation with atenolol and ICI-118,551 in combination with isoproterenol on FFA levels is shown. The bolus isoproterenol has a lower impact than the 1.5-h infusion with isoproterenol, which is evident from both the FFA and the glucose response (compare Fig. 4 with Fig. 5, A and B). Remarkably, the effect of the selective β₁-antagonist atenolol shows an increase of the FFA release, indicating a stimulation of the lipolysis. On the other hand ICI-118,551, a selective β₂-antagonist, shows the opposite, i.e., a decrease of the FFA level. Thus we must conclude that β₁- and β₂-adrenoceptors mediate opposite effects: β₁-adrenoceptors appear to inhibit and β₂-adrenoceptors appear to stimulate lipolysis. Both receptors may be located on the fat cell membranes, in which case there must be a difference in sensitivity for catecholamines to maintain metabolic control. On the other hand it is also likely that there is an organ separation: the β₁-adrenoceptors could be located on the fat cells, while the β₂-adrenoceptors are located on the liver cells. From the glucose experiments we find evidence of the location of both adrenoceptors on liver cells (see above). β₂-Adrenoceptors on the liver cells are known to stimulate lipolysis (14). The β₁-adrenoceptors on the fat cells inhibit lipolysis, as can be concluded from this study.

Perspectives

Our in vivo experiments suggest that the glucose response of carp is mediated by both β₁- and β₂-adrenoceptors on the liver cells. To obtain further proof, appropriate in vitro experiments will be carried out with hepatocytes. Because this may be related to the overall food requirements of the species, comparisons with other species are planned.

The regulation of lipolysis in fishes by catecholamines is an intriguing problem. The involvement of β-adrenoceptors in inhibition of lipolysis as described in this study is a novel phenomenon and suggests a mechanism different from the well-described mammalian system. For analysis of this mechanism it is necessary to carry out in vitro experiments with adipose tissues or adipocytes. Ongoing experiments with isolated adipocytes from different fish species aim to characterize the receptor(s) and transduction mechanisms involved. The inhibition of lipolysis by noradrenaline in some fish species is likely a protection mechanism against accumulation of amphi- philes. This accumulation is responsible for tissue damage in mammals after ischemic and hypoxic insults. The transition from water to air breathing during evolution may be connected to a changed function of noradrenaline. Comparative studies are planned to test this hypothesis.

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REFERENCES


