The Role of Noradrenaline on the Lipid Metabolism of Water- and Air-Breathing Fish Species.

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β-Adrenergic Control of Plasma Glucose and Free Fatty Acid Levels in the Air-breathing African Catfish (*Clarias gariepinus*, Burchell 1822).

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Abstract

In several water-breathing fish species, β-adrenergic receptor stimulation by noradrenaline leads to a decrease in plasma free fatty acid (FFA) levels, as opposed to an increase in air-breathing mammals. We hypothesised that this change in adrenergic control is related to the mode of breathing. Therefore, cannulated air-breathing African catfish were infused for 90-min with noradrenaline or with the nonselective β-agonist, isoproterenol. To identify the receptor type involved, a bolus of either a selective β_1-antagonist (atenolol) or a selective β_2-antagonist (ICI 118.551) was injected 15 min prior to the isoproterenol infusion. Both noradrenaline and isoproterenol led to an expected rise in glucose concentration. Isoproterenol combined with both the β_1- and β_2-antagonist led to higher glucose concentrations than isoproterenol alone. This could indicate the presence of a stimulatory β-adrenoceptor different from β_1 and β_2-adrenoceptors; these two receptors thus seemed to mediate a reduction in plasma glucose concentration. Both noradrenaline and isoproterenol led to a significant decrease in FFA concentration. Whereas the β_1-antagonist had no effect, the β_2-antagonist reduced the decrease in FFA concentration, indicating the involvement of β_2-adrenoceptors. It is concluded that the air-breathing African catfish resembles water-breathing fish in the adrenergic control of plasma FFA and glucose levels.

Introduction

There is a fundamental difference between mammals and fish in how lipid metabolism is affected by hypoxic stress. In both animal groups, hypoxia results in strongly elevated catecholamine levels. Catecholamines strongly stimulate lipolysis in mammals (Fain and Garcia-Sainz, 1983; Smith, 1983) while the β-oxidation of fatty acids is impaired due to the oxygen shortage (Moore, 1985; Van der Vusse *et al*., 1992). These processes enhance each other and hypoxia thus results in elevated plasma free fatty acid (FFA) levels in mammals (Vogel and Hannon, 1966; Stock *et al*., 1978; Meerson *et al*., 1994; Roberts *et al*., 1996). High levels of FFA, however, can cause disruption of cell membranes resulting in cell leakage and tissue damage, as seen in the ischemic heart (Katz and Messineo, 1981; Hagve *et al*., 1990; Hütter and Soboll, 1992). In mammals, hypoxia normally does not occur at the organismal level. There appears to be no short-term adaptation to acute hypoxia, but only adaptation to chronic hypoxia (Roberts *et al*., 1996; De Glisezinski *et al*., 1999).

Some fish species on the other hand can frequently encounter hypoxia at the organismal level, as water is a relatively poor source of oxygen and, by nature, often has strongly fluctuating oxygen levels. As in mammals, catecholamine levels in fish are elevated during hypoxia, but FFA levels fall rapidly, particularly in hypoxia-tolerant fish species such as carp (*Cyprinus carpio*; Van Raaij *et al*., 1996a; Van Ginneken *et al*., 1998) and tilapia (*Oreochromis mossambicus*; Vianen *et al*., 2002). In tilapia, noradrenaline appeared to be solely responsible for mediating this decrease by a reduction of adipose lipolysis (Vianen *et al*., 2002). The suppression of plasma FFA levels by noradrenaline is possibly a protective mechanism against fatty acid poisoning in fish under hypoxia (Van den Thillart *et al*., 2002).

In mammals, α_2-adrenoceptors are known for their anti-lipolytic action (Fain and Garcia-Sainz, 1983; Smith, 1983) and, therefore, they were the most likely receptors to mediate a decrease in FFA levels in fish. However, both at the organismal level (Van den Thillart *et al*., 2001) and at the cellular level in adipose tissue (Vianen *et al*., 2002), α_2-adrenoceptors were not directly involved in mediating decreased FFA concentrations by noradrenaline. Activation of β-adrenoceptors, on the other hand, completely mimicked the effect of noradrenaline in lowering plasma FFA levels (Van den Thillart *et al*., 2001) and decreasing adipose tissue lipolysis (Vianen *et al*., 2002). This was a novel finding as β-adrenoceptor activation in mammals strongly enhances lipolysis.

Van den Thillart *et al*. (2001) hypothesised that this difference in the role of noradrenaline

between fish and mammals may be connected to the transition from water to air-breathing. Therefore, in the present study the effect of β-adrenoceptor stimulation on plasma FFA levels was investigated in the air-breathing African catfish (*Clarias gariepinus*). *Clarias* species are among the best known air-breathing fish species (Graham, 1997) and are mostly classified as facultative air-breathers (Magid, 1971; Jordan, 1976; Bevan and Kramer, 1987), meaning that they can live indefinitely on aquatic oxygen and breathe air only when necessary. When African catfish was subjected to low aquatic oxygen tensions, the air-breathing frequency increased resulting in a constant total oxygen consumption (Magid, 1971; Jordan, 1976). In this way, this species can sustain a complete aerobic metabolism at low aquatic oxygen tensions and it is thus unlikely that African catfish experiences functional hypoxia in its natural surroundings. Therefore, we hypothesised that the decreasing effect of β-adrenergic stimulation on plasma FFA levels would not be present in this species. Plasma glucose levels were measured because β-adrenergic stimulation has a known hyperglycemic effect.

Materials and Methods

Experimental animals

African catfish (*Clarias gariepinus*, Burchell 1822) of 1275 ± 37 gr were purchased from a commercial catfish farm (Fleuren Viskwekerij, Someren, The Netherlands). The fish were kept in groups (max. 20 per tank) in a well-aerated recirculation system (25ºC). They were fed once a day with Trouvit Biomeerval (Trouvit, Putten, The Netherlands) at maintenance level (~7 gr/kg BW). The light-dark cycle was 12:12 h. All fish were acclimatised to these conditions for at least 2 weeks.

Pre-experimental protocol

The experiments were conducted in flow chambers supplied with well-aerated water of 25ºC as part of a recirculation system of 3 m^3. The flow rate through the flow chambers was approximately 1 l/min. The fish could move back and forth freely without being able to turn. The flow chambers were closed with a darkened lid to prevent startling of the fish by outside movements. The flow chambers contained about 2 cm of air to allow air-breathing.

Before the start of an experiment, the fish were placed individually in the flow chambers and

were deprived of food from that moment on. After 3 days of acclimatisation, a fish was anaesthetised in a MS222 solution (300 mg/ml, tricaine methanesulphonate, Argent Chem. Lab., Redmond, U.S.A.). After cessation of gill movements, the fish was placed on an operation table with the ventral side up. Both gills were opened with operation clamps and continuously irrigated with well-aerated water, containing MS222 (150 mg/ml).

Fish were cannulated in the dorsal aorta after Soivio *et al*. (1975). After cannulation the fish were placed back into the flow chambers and allowed to recover for 2 days during which the cannulae were filled with a PVP (Poly-Vinyl-Pyrrolidon, Merck, Amsterdam, The Netherlands) solution with 4% sodium citrate as anticoagulant. During the experiment, the cannulae were filled with a 1% sodium citrate-saline solution. This 5 day pre-experimental protocol has been shown to minimise effects of handling, anaesthesia and surgery (Van Raaij *et al*., 1996a).

Experimental protocol

Five different infusion protocols were used. A control infusion was carried out with Ringer saline (Wolf, 1963). Two different agonists were infused: noradrenaline (α- and β-agonist, 154 μg/kg) and isoproterenol (nonselective β-agonist, 27 μg/kg). Based on a half-life of 10 and 100 min respectively (G.J. Vianen, unpublished results) and an extracellular volume of 8% of the body mass, these amounts would result in a 10^{-6} M concentration in the blood of the fish at the end of infusion. Similar concentrations of noradrenaline and isoproterenol evoked a significant effect in carp (Van den Thillart *et al*., 2001). In some experiments, isoproterenol infusion was preceded by a bolus injection of an antagonist: either atenolol (selective β_1-antagonist, 213 μg/kg resulting in 10^{-5} M) or ICI 118,551 (selective β_2-antagonist, 250 μg/kg resulting in 10^{-5} M). In carp, atenolol and ICI 118,551 have been applied at the same concentration and evoked clear and opposing effects on plasma FFA levels (Van den Thillart *et al*., 2001). These antagonists at this concentration were thus considered to be selective and appropriate.

The experiments started between 8.30 and 9.30 a.m. by taking 2 initial blood samples at t= -¾ and -¼ h before start of infusion. Together with the second blood sampling, the fish received a bolus of Ringer saline or a bolus of Ringer saline containing the antagonist. At t= 0 h, a 1½-h infusion period started using Ringer saline or Ringer saline containing the agonist plus 1 mg/ml ascorbic acid as antioxidant. To this purpose, a microinfusion pump (Fine Mechanical Dept., Leiden University, The Netherlands) was used at an infusion rate of 7.4 μl/min. During infusion, blood was sampled at t= ½ and 1 h. To allow sampling, the infusion pump was stopped for 300

sec, after which infusion was resumed using a 10x higher speed for 33 sec, followed by infusion at normal speed. Immediately after infusion, a blood sample was taken at t= 1½ h and subsequently at t= 2½, 3½, 5½, 9½ and 24 h.

Analytical procedures

Blood sampling (270 µl) was done using gastight microliter syringes (Hamilton) containing 30 µl of 4% sodium citrate as anticoagulant. On whole blood samples, the hematocrit (2x9 µl) and hemoglobin content (2x10 µl) were determined. The hematocrit was measured by filling heparinized capillaries followed by centrifugation in a mini-centrifuge (Compur M1100). The hemoglobin concentration was measured using a hemoglobin test kit (Roche, Almere, The Netherlands). The remaining blood was centrifuged for 5 min at 15,000 g and plasma was separated immediately. Aliquots of 50 µl of untreated plasma were stored at –80ºC for FFA determination. For glucose and lactate measurements, a plasma sample was added to 6% trichloric acetic acid in a 1:4 volume ratio, mixed and put on ice for at least 20 min to precipitate plasma proteins. After centrifugation, two aliquots of the supernatant were stored at –20ºC and analysed within a week.

After neutralisation with 1 M K_3PO_4, plasma lactate was measured according to the method of Hohorst (Bergmeyer, 1970) and plasma glucose was measured by an enzymatic test kit (Instruchemie, Delfzijl, The Netherlands). Plasma FFA was measured using an enzymatic test kit of Waco chemicals (Instruchemie, Delfzijl, The Netherlands).

Chemicals

Noradrenaline-bitartrate, isoproterenol-hydrochloride and ICI 118,551-hydrochloride were obtained form Sigma (Zwijndrecht, The Netherlands). Atenolol-hydrochloride was a kind gift from AstraZeneca (Macclesfield, Cheshire, UK). All other chemicals were of analytical grade.

Data analyses and statistics

Data are presented as means ± SEM. All values were normalised relative to the initial values to compensate for the effect of individual variation. The mean cellular hemoglobin content (MCHC) was calculated as hemoglobin concentration divided by the hematocrit. The area under

the curve (AUC) during infusion (0-1½ h) was calculated for the relative glucose data in %·h.

Statistical differences ($p<0.05$) were tested using Sigmastat 2.03. Differences between sampling points and initial values within each group were tested with a Repeated Measures Anova on Ranks according to Dunnett's method, while differences between groups were tested with a Mann-Whitney rank sum test or an Anova on Ranks.

Results

No significant differences between the five infusion groups were found in the initial values of the hematological parameters (Table 1). The initial hematocrit was 23.1 ± 1.0%, hemoglobin 4.26 ± 0.20 mM and MCHC 18.56 ± 0.41 mM. The saline infusion induced no significant changes in all three hematological parameters as compared to the initial values, as is shown in Fig. 1 for hematocrit. Also the isoproterenol infusion with and without antagonists induced no consistent changes in the three hematological parameters. The noradrenaline infusion, however, induced a rise in hematocrit to a relative maximum at the end of infusion of 145.2 ± 22.5% of the initial value. Due to the high variation, these values were only significantly different from the isoproterenol infusion values. The increase in hemoglobin concentration at the end of the noradrenaline infusion was smaller, being 116.9 ± 9.6%, but still significantly different from the isoproterenol infusion. As a result, the MCHC dropped to 13.9 ± 1.8, which was not significant, however.

The initial lactate concentration was 0.63 ± 0.06 mM. No significant changes occurred in all infusion groups except in the isoproterenol + atenolol group in which the lactate concentration came slightly above 1.0 mM from t= 1 to 5½ h. This increase was significantly different from the

Table 1. Initial values of parameters measured in the different experimental groups of cannulated African catfish. Values are expressed as mean ± SEM.

Infusion	Hematocrit [%]	Hemoglobin [mM]	MCHC	Lactate [mM]	Glucose [mM]	FFA [mM]	*n*
Saline	23.5±2.1	4.41±0.29	19.01±0.84	0.70±0.09	2.97±0.24	0.30±0.03	7
Noradrenaline	23.0±3.6	4.49±0.95	19.04±1.23	0.84±0.22	2.93±0.29	0.27±0.05	5-6
Isoproterenol (iso)	23.1±1.1	4.42±0.24	19.18±0.98	0.39±0.10	2.47±0.20	0.32±0.04	6
Iso + Atenolol	22.5±2.4	3.83±0.21	17.35±1.03	0.47±0.20	3.32±0.52	0.33±0.03	5
Iso + ICI 118,551	23.0±2.5	4.05±0.39	17.93±0.92	0.65±0.05	2.49±0.27	0.24±0.05	6
All groups	23.1±1.0	4.26±0.20	18.56±0.41	0.63±0.06	2.82±0.13	0.29±0.02	

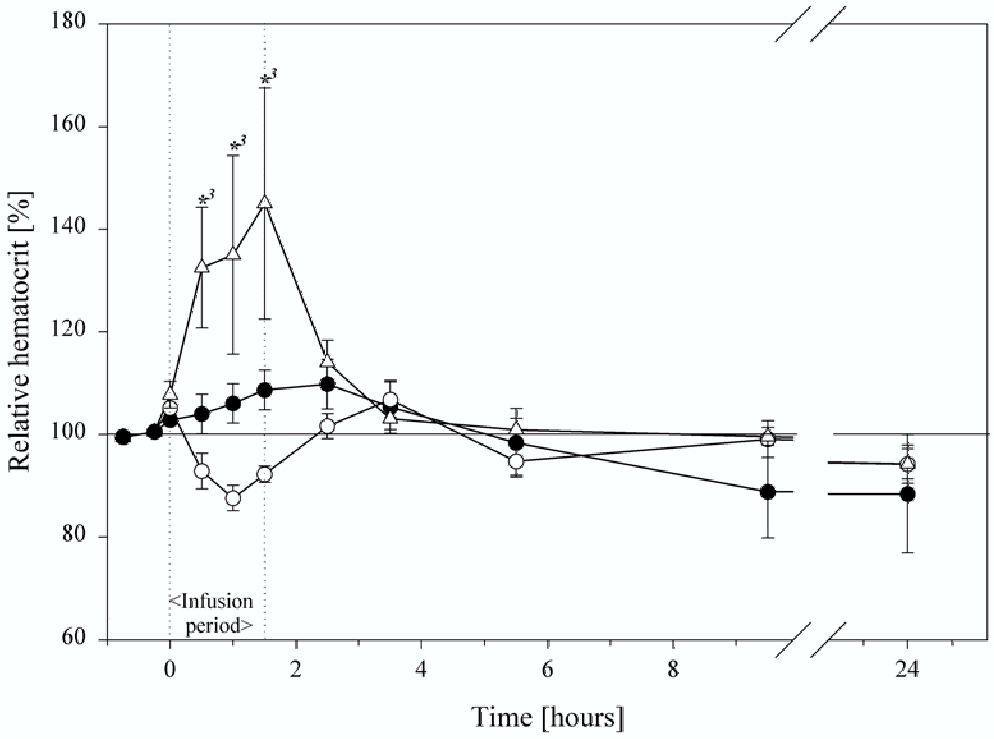

Figure 1. The relative hematocrit of African catfish infused with saline (●; *n*=7), isoproterenol (○; *n*=6; 27 µg/kg) or noradrenaline (△; *n*=5-6; 154 µg/kg) from t=0 to 1½ h. *[3]: p<0.05 vs. isoproterenol infusion.

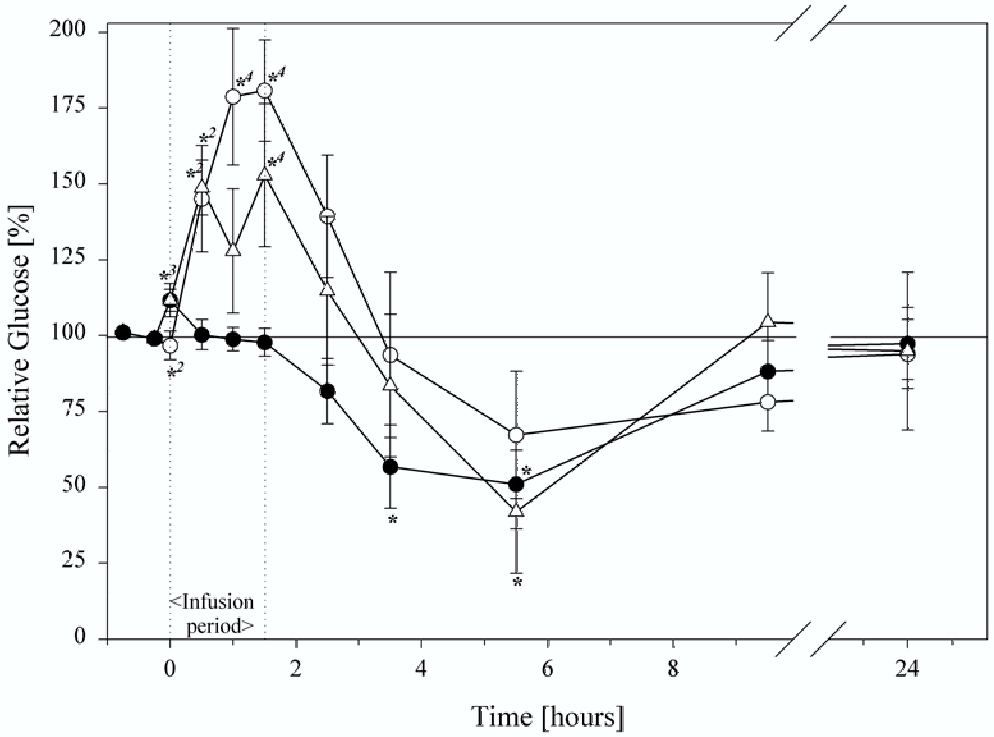

Figure 2. The relative plasma glucose concentration of African catfish infused with saline (●; *n*=7), isoproterenol (○; *n*=6; 27 µg/kg) or noradrenaline (△; *n*=5-6; 154 µg/kg) from t=0 to 1½ h. *: p<0.05 vs. initial values; *[2]: p<0.05 vs. saline infusion; *[3]: p<0.05 vs. isoproterenol infusion; *[4]: p<0.05 vs. initial values and saline infusion.

initial values, but was not different from the saline and isoproterenol infusion.

The initial plasma glucose concentration was 2.82 ± 0.13 mM. The glucose concentration in the saline infused group showed a marked decrease after the infusion, resulting in significantly different values at t= 3½ and 5½ h of 56.8 ± 13.7% and 51.0 ± 14.9% of the initial value, respectively. Subsequently, plasma glucose concentrations returned to the initial value (Fig. 2).

During infusion with noradrenaline, glucose concentration increased significantly at t= ½ and 1½ h compared to the saline group. The maximal effect of 152.9 ± 23.6% was reached after 1½ h. During isoproterenol infusion, the increase in glucose concentration during the first ½ h was the same as for noradrenaline, but the increase persisted resulting in a higher maximum level of 180.8 ± 16.7% at t=1½ h. The plasma glucose concentrations were significantly different from the saline infusion from t= ½ to 1½ h. After the infusion, the plasma glucose levels in both agonist groups decreased, while showing the same fluctuation as after the saline infusion.

When infusion of isoproterenol was accompanied by either of the two antagonists, plasma glucose levels significantly increased (Fig. 3). When the selective β_2-antagonist ICI 118,551 was used, the maximal glucose level at the end of infusion was 221.3 ± 32.5%, which was not significantly higher than in the absence of ICI 118,551. With the selective β_1-antagonist atenolol present, even higher maximum levels of 271.4 ± 33.4% were noticed, but again no significant difference from the infusion with isoproterenol alone was found. After infusion the glucose levels, as compared to the saline infusion, stayed significantly elevated with both antagonists up to t= 3½ h for ICI 118,551 and up to 5½ h for atenolol. No significant differences were found compared to the isoproterenol infusion, except for atenolol after 9½ h.

When the areas under the curve during infusion were calculated, both the isoproterenol and noradrenaline infusion had led to significant higher values as compared to the saline infusion being 79.4 ± 21.4 %·h and 48.4 ± 19.3 %·h respectively. Also in the presence of the antagonists, higher area values were found, and with atenolol the area of 145.3 ± 36.4 %·h was significantly higher than the isoproterenol infusion alone. For ICI 118,551, the area of 94.4 ± 27.4 %·h did not differ significantly from the isoproterenol infusion.

The mean initial FFA concentration amounted to 0.29 ± 0.02 mM. The FFA concentration in the saline group showed a marked decrease immediately after the beginning of the experiment from 106.8 ± 11.4% at t= -¾ h to 61.6 ± 5.1 % at t= ½ h. The FFA concentration was maintained at this low level up to t= 2½ h, after which a clear overshoot occurred to a significantly different value of 201.3 ± 23.1% at t= 9½ h. After 24 h, the FFA levels had returned close to the initial value (Fig. 4).

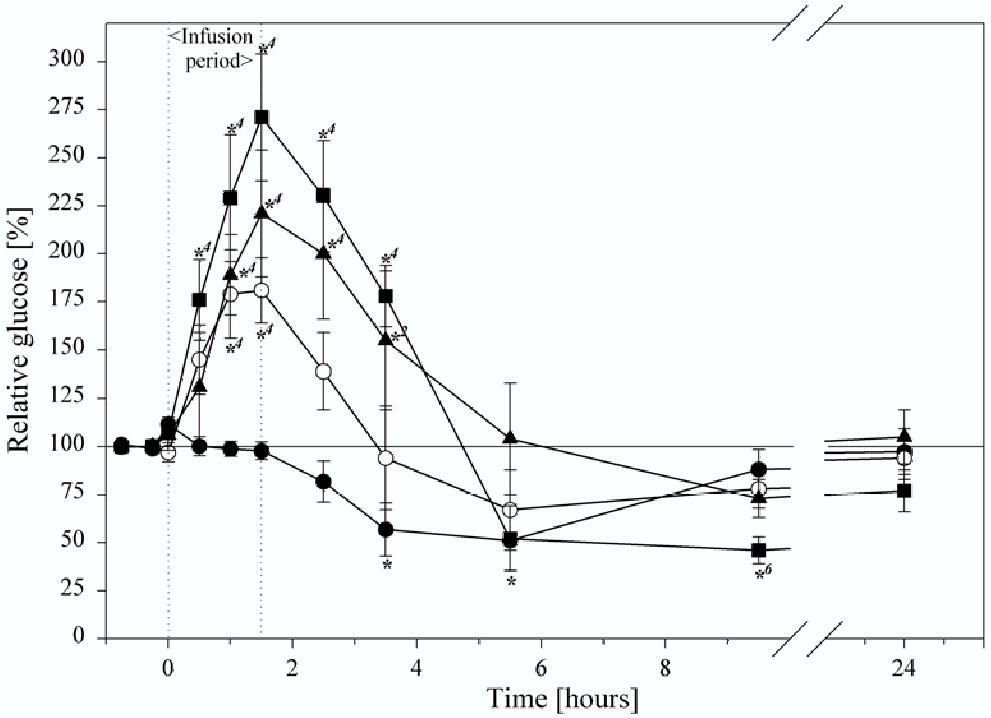

Figure 3. The relative plasma glucose concentration of African catfish infused with saline (●; *n*=7) or isoproterenol (○; *n*=6; 27 µg/kg) in combination with either atenolol (■; *n*=5; 213 µg/kg) or ICI 118,551 (▲; *n*=6; 250 µg/kg) from t=0 to 1½ h. *: $p<0.05$ vs. initial values; $*^2$: $p<0.05$ vs. saline infusion; $*^4$: $p<0.05$ vs. initial values and saline infusion; $*^6$: $p<0.05$ vs. initial values, saline and isoproterenol infusion.

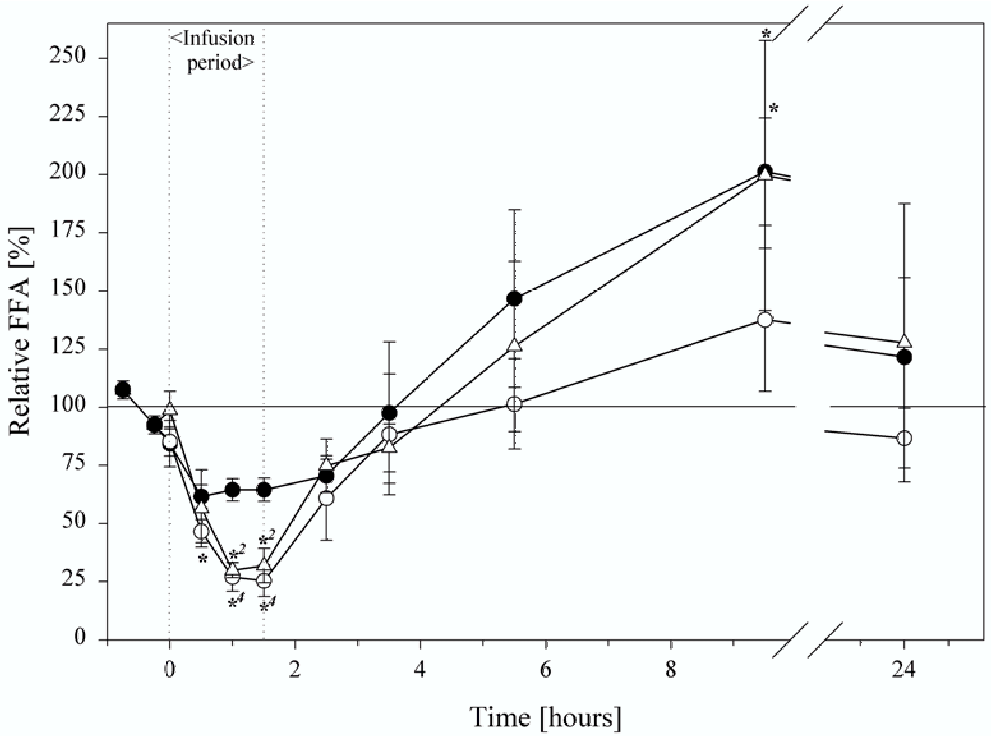

Figure 4. The relative plasma FFA concentration of African catfish infused with saline (●; *n*=7), isoproterenol (○; *n*=6; 27 µg/kg) or noradrenaline (△; *n*=5-6; 154 µg/kg) from t=0 to 1½ h. *: $p<0.05$ vs. initial values; $*^2$: $p<0.05$ vs. saline infusion; $*^4$: $p<0.05$ vs. initial values and saline infusion.

During infusion of noradrenaline, there was a significant decrease in FFA concentration as compared to the saline group at t= 1 and 1½ h. Minimal values of 30.0 ± 3.3% were reached after 1 h. Subsequently, a clear recovery to normal levels occurred, as within 1 h after the end of the infusion the significant difference from the saline infusion was no longer present, while the further courses of both graphs (saline and noradrenaline) were very similar. During the isoproterenol infusion, FFA levels decreased significantly both compared to the initial values and with respect to saline infusion. The lowest concentrations were reached after 1½ h with a mean of 25.4 ± 6.8% of the initial value; the reduction, as compared to the saline infusion, was –39.1%. The recovery from the isoproterenol infusion did not result in elevated FFA levels as it did during the recovery from the saline and noradrenaline infusion. However, no significant differences were found during the recovery period.

As FFA levels had already dropped during the infusion of saline, it was difficult to visually distinguish the effect of the antagonists. Therefore, the data of all three isoproterenol infusions were corrected for the saline infusion data (Fig. 5). With the selective β_1-antagonist atenolol

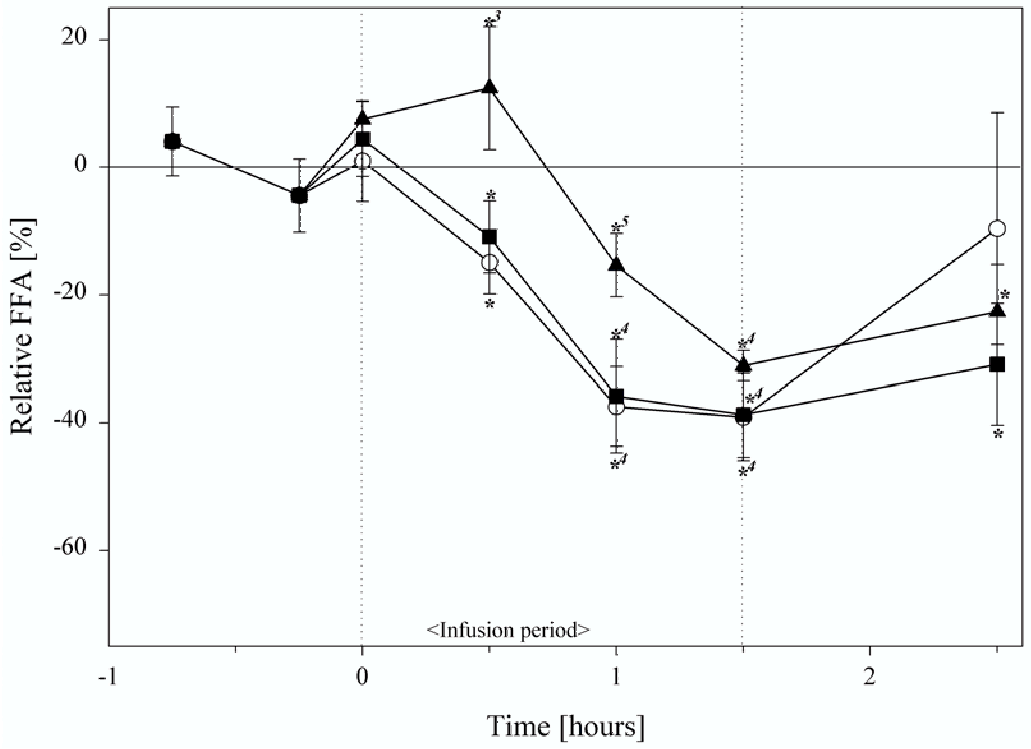

Figure 5. The relative plasma FFA concentration of African catfish infused with isoproterenol (○; *n*=6; 27 µg/kg) in combination with either atenolol (■; *n*=5; 213 µg/kg) or ICI 118,551 (▲; *n*=6; 250 µg/kg) from t=0 to 1½ h. Note that all values are corrected for the saline infusion. *: $p<0.05$ vs. initial values; $*^3$: $p<0.05$ vs. isoproterenol infusion; $*^4$: $p<0.05$ vs. initial values and saline infusion; $*^5$: $p<0.05$ vs. initial values and isoproterenol infusion.

present, the FFA concentration followed the same course as when only isoproterenol was administered; at t= ½ h, FFA levels were significantly different from the initial values only and at t= 1 and 1½ h they also differed significantly from the saline infusion. The FFA level at the end of infusion was decreased by 38.8 ± 6.8% as compared to saline infusion. With the selective β_2-antagonist ICI 118,551 present, the decrease of FFA levels was delayed by ½ h, resulting in a rightward shift of the time-response curve during the isoproterenol infusion. After ½ h, the FFA concentration was significantly different as compared to the infusion with only isoproterenol, but not significantly different as compared to the initial value and the saline infusion. At the end of the infusion, FFA levels were no longer significantly different from the isoproterenol infusion and the maximal reduction was similar, namely –31.1 ± 2.4%, being 33.5% of the initial value.

Discussion

General

In the experiments presented here, cannulated fish were used for infusion of catecholamines. The applied experimental protocol does not evoke a significant catecholamine release by the fishes during the complete experimental period (Van Raaij *et al*., 1995; Van den Thillart *et al*., 2001), in contrast to classical injection techniques (Woodward, 1982).

The infusion of saline induced a significant decrease in plasma glucose and FFA levels in African catfish. This reflects a diurnal fluctuation in both metabolites as was confirmed in a separate study with cannulated African catfish (Chapter 2). Comparable diurnal fluctuations in blood metabolites have been reported for numerous other fish species (see review by Boujard and Leatherland, 1992a). Such a decrease in metabolites is most likely linked to the moment of feeding and the concomitant release of hormones. Although the fish in our study were fasted for 3 days, a feeding-entrained hormonal release could still have been present (Gutierrez *et al*., 1984).

In contrast to isoproterenol, noradrenaline induced a rise in both hematocrit and, to a lesser extent, hemoglobin with a non-significant decline in MCHC. This means that the increase in hematocrit is mainly due to extra erythrocytes brought into circulation from the spleen, which releases erythrocytes upon stimulation of α-adrenoceptors (Nilsson and Grove, 1974). An increase in hematocrit due to erythrocyte swelling upon β-adrenergic stimulation was not evident (Soivio and Nikinmaa, 1981; Nikinmaa *et al*., 1987). A catecholamine-induced cell swelling is not present in all fish species (Perry and Reid, 1992). When present, as in carp, it is almost

absent in normoxic conditions as compared to hypoxic conditions (Salama and Nikinmaa, 1990). This explains why β-adrenergic stimulation by isoproterenol in normoxic carp (Van den Thillart *et al*., 2001), as in the African catfish in this study, did not have a significant effect on hematocrit and MCHC.

Glucose

Both catecholamines (noradrenaline and isoproterenol) induced in African catfish hyperglycemia as reported for numerous fish species (see review by Fabbri *et al*., 1998a). This is generally accepted to be mainly mediated by β-adrenoceptor stimulated glycogenolysis and β-adrenoceptor inhibited glycolysis in the liver (Birnbaum *et al*., 1976; Janssens and Lowrey, 1987; Mommsen *et al*., 1988; Wright *et al*., 1989; Reid *et al*., 1992). However, the presence of stimulatory α-adrenoceptors has been demonstrated *in vitro* (Brighenti *et al*., 1987a; Moon *et al*., 1993; Fabbri *et al*., 1995a, 1999). Although the fact that isoproterenol completely mimicked the effect of noradrenaline suggests that also in African catfish hyperglycemia was mainly mediated by β-adrenoceptors, our data do not allow differentiation between β and α-adrenoceptor effects.

Blockage of either of the β_1- or β_2-adrenoceptors did not inhibit the increase in plasma glucose levels to any extent, suggesting that both receptors are not the main receptors mediating this increase. Blockage of either or both β-adrenoceptors actually led to further enhanced glucose levels when compared to infusion of only isoproterenol, although the difference was only significant for the β_1-adrenoceptor. This finding could imply the presence of a stimulatory β-adrenoceptor type on the liver of African catfish other than β_1 and β_2. The only other β-adrenoceptor reported to be present in fish, is the β_3-adrenoceptor. The first report of a functional β_3-adrenoceptor was on adipose tissue of tilapia by Vianen *et al*. (2002). Nickerson *et al*. (2003) identified using molecular tools two types of the β_3-adrenoceptor in trout where it was expressed mainly in blood, gill and heart. In contrast to these two studies, the hepatic β-adrenoceptor from African catfish was not identified directly but indirectly using antagonists. Although the results from this study (see Fig.5) and from Van den Thillart *et al*. (2001) suggest functional selectivity of these antagonists, these data should be treated cautiously as several studies indicate a possible discrepancy in the characteristics of adrenergic ligands between mammals and teleost (Brighenti *et al*., 1987a; Moon and Mommsen, 1990; Fabbri *et al*., 1992). Additional experiments with African catfish hepatocytes using both pharmacological and molecular tools will identify the subtype of the hepatic β-adrenoceptor in this species.

Only one β-adrenoceptor (not β_1 and β_2) mediated the hepatic glucose release in African catfish. Also in eel (*Anguilla anguilla*; Fabbri *et al*., 2001) and trout, one β-adrenoceptor type was present on the liver (Fabbri *et al*., 1995a; Dugan and Moon, 1998); in trout, it was identified as a β_2-adrenoceptor (Reid *et al*., 1992; McKinley and Hazel, 1993). In rockfish hepatocytes (*Sebastes caurinus*), glucose release was mediated by the β_1-adrenoceptor although the presence of another adrenoceptor could not be excluded (Danulat and Mommsen, 1990). Both β_1- and β_2-adrenoceptors were responsible in carp for an increase in plasma glucose levels (Van den Thillart *et al*., 2001), although only one binding site appeared to be present in carp liver (Janssens and Lowrey, 1987). Also two different binding sites were present on hepatic membranes of the Australian lungfish (*Neoceratodus forsteri*; Janssens and Grigg, 1988) and of bullhead catfish (*Ictalurus melas*; Fabbri *et al*., 1992).

A straightforward explanation for the increasing effect of a β_1- and β_2-blockage on plasma glucose levels is that both adrenoceptor types had a suppressing effect on the glucose release. The presence of inhibitory β_1- and β_2-adrenoreceptors on the liver has not been reported in the literature as only stimulatory hepatic β-adrenoceptors have been found (see review by Fabbri *et al*., 1998a). However, an additional target organ involved in the potentiation by β_1- and β_2-adrenoceptor blockage is the pancreas. *In vivo* catecholamine administration both reduced and enhanced plasma insulin levels (Ince and Thorpe, 1977; Zelnik *et al*., 1977; Mommsen and Plisetskaya, 1991). *In vitro*, however, β-adrenergic stimulation of the pancreatic islet cells by isoproterenol consistently enhanced the basal release of insulin (Tilzey *et al*., 1985a,b; Milgram *et al*., 1991). The effects of adrenaline and noradrenaline *in vitro* were biphasic: inhibition at low adrenaline concentrations (10^{-10} M) and stimulation at high concentrations (10^{-6} M) in trout (*Oncorhynchus mykiss*; Tilzey *et al*., 1985a), while in anglerfish (*Lophius americanus*) increasing noradrenaline concentrations induced a switch from stimulation to inhibition (Milgram *et al*., 1991). These differential effects were most likely caused by differences in the ratio of inhibitory α- and stimulatory β-adrenoceptors (Milgram *et al*., 1991; Mommsen and Plisetskaya, 1991). Based on these published data, the injection of β-adrenoceptor antagonists in our study possibly blocked an isoproterenol-induced insulin release in African catfish. As insulin is a known hypoglycemic hormone in fish (Mommsen and Plisetskaya, 1991), β-adrenoceptor blockage could thus indirectly have resulted in a larger increase in plasma glucose than without this blockage. In future experiments, the infusion of cannulated African catfish with the same agonists/antagonist in combination with insulin measurements will demonstrate if this hypothesis is correct.

Free fatty acids

In carp, β-adrenergic stimulation was specifically responsible for reduced plasma FFA levels, because α-adrenergic stimulation appeared to have only indirect effects (Van den Thillart *et al*., 2001). Vianen *et al*. (2002) showed that the decrease in plasma FFA in tilapia was due to a β-adrenoceptor mediated decrease in adipocyte lipolysis; α-adrenoceptor blockage had no effect on a noradrenaline-mediated decrease in adipocyte lipolysis, suggesting no involvement of α-adrenoceptors. In African catfish, the β-agonist isoproterenol and the α- and β-agonist noradrenaline had comparable suppressive effects on the plasma FFA levels, suggesting that also in this species it is a mainly β-adrenoceptor mediated process. No specific β-adrenoceptor agonists have been studied in other fish species. Noradrenaline, however, has been used frequently and reduced plasma FFA levels in all studies (carp and bream (*Abramis brama*), Farkas, 1967a,b; goldfish (*Carassius auratus*), Minick and Chavin, 1973; pike (*Esox lucius*), Ince and Thorpe, 1975; carp, Van Raaij *et al*., 1995).

When the isoproterenol infusion was preceded by the β_1-antagonist, the decrease in plasma FFA levels was identical to when only isoproterenol was infused. Injection of the β_2-antagonist, on the other hand, delayed the decrease in plasma FFA levels significantly. This rightward shift in the time-response curve indicates that the decrease in plasma FFA levels in African catfish was mediated by β_2-adrenoceptors. The reduction in adipocyte lipolytic rate in tilapia was mediated by β_1 and/or β_2-adrenoceptors, in combination with the β_3-adrenoceptor (Vianen *et al*., 2002). In carp however, β_1-adrenoceptors mediate a decrease in FFA levels while β_2-adrenoceptors mediate an increase. These opposite effects were believed to result from a decreased adipose lipolysis and an increased hepatic lipolysis, respectively (Van den Thillart *et al*., 2001). The fact that no stimulatory effect was found in African catfish like in carp, implies that lipolysis in African catfish liver cannot or only barely be stimulated by β-adrenoceptors.

The data presented here indicate that β-adrenergic stimulation mediated the same physiological reaction in air-breathing African catfish as in other water-breathing fish species, namely suppression of plasma FFA levels. For *Clarias*, aquatic oxygen is still the primary source of oxygen as they are normally classified as a facultative air-breathers (Magid, 1971; Jordan, 1976; Bevan and Kramer, 1987). Apparently, air-breathing in this species did not lead to an evolutionary change in the control of lipolysis as we hypothesised. Recent experiments showed that environmental hypoxia is potentially a stress condition for African catfish, i.e. submersion without access to aerial oxygen (Chapter 3). African catfish is caught at a depth of over 50

meters in Lake Victoria (Goudswaard and Witte, 1997), which makes it highly unlikely that it will surface to breathe air. Hence, also in its natural habitat African catfish is likely to become hypoxic. Obligate air-breathers, such as adult lungfish (*Protopterus* sp.) on the other hand, are vitally dependent on aerial oxygen and can only live when allowed air-breathing (Graham, 1997). The respiratory behaviour of adult African lungfish (*Protopterus aethiopicus*) was indeed not affected by decreasing aquatic oxygen tensions when allowed air-breathing (Johansen and Lenfant, 1968) as opposed to African catfish (Johnston *et al*., 1983). Hence, environmental hypoxia, even when it only means submersion like in African catfish, is per definition not a physiological relevant situation for African lungfish, rendering this species as an interesting model fish for our hypothesis.

Air-breathing in fishes has evolved independently in several fish lineages. The evolution of air-breathing was originally thought of as a way to survive environmental hypoxia (Graham, 1997). Some authors have suggested, however, that air-breathing also functions to maintain activity levels when aquatic oxygen levels are low (Grigg, 1965; Burleson *et al*., 1998; Farmer and Jackson, 1998). Therefore, the main driving force for the evolution of air-breathing may not necessarily be survival of hypoxia but rather coping with hypoxia by sustaining high activity levels. As the suppressive role of noradrenaline on plasma FFA levels is hypothesised to be a survival mechanism during hypoxia (Van den Thillart *et al*., 2002), an alternative drive for the evolution of air-breathing is in corroboration with our finding that noradrenaline suppresses plasma FFA levels in air-breathing African catfish as in other water-breathing fishes.

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