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Beta-adrenergic control of plasma glucose and free fatty acid levels in the air-breathing African catfish *Clarias gariepinus* Burchell 1822

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Summary

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, isoprenaline. 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 isoprenaline infusion. Both noradrenaline and isoprenaline led to an expected rise in glucose concentration. Isoprenaline combined with both the β_1- and β_2-antagonist led to higher glucose concentrations than isoprenaline 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 isoprenaline 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 reflects water-breathing fish in the adrenergic control of plasma FFA and glucose levels.

Key words: β-adrenergic stimulation, FFA, noradrenaline, isoprenaline, air-breathing, African catfish, *Clarias gariepinus*.

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 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 relative 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 like carp *Cyprinus carpio* (Van Raaij et al., 1996; 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 change in the role of noradrenaline from fish to 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 are 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, African catfish can sustain a complete aerobic metabolism at low aquatic oxygen tensions and it is thus unlikely that it 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 g 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 (25°C). They were fed once a day with Trouvit Biomeerval (Trouvit, Putten, The Netherlands) at maintenance level (~7 g kg^{-1} body mass). The light:dark cycle was 12 h:12 h. All fish were acclimatised to these conditions for at least 2 weeks. The experiments were approved by the board on Experimentation on Animals of the Leiden University.

Pre-experimental protocol

The experiments were conducted in flow chambers supplied with well-aerated water of 25°C as part of a 3 m^3 recirculation system. The flow rate through the flow chambers was approximately 1 l min^{-1}. 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 deprived of food from that moment on. After 3 days of acclimatisation, a fish was anaesthetised in a MS222 solution (300 mg ml l^{-1}, tricaine methanesulphonate, Argent Chem. Lab., Redmond, WA, USA). 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 l^{-1}).

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 the effects of handling, anaesthesia and surgery (Van Raaij et al., 1996).

Experimental protocol

Five different infusion protocols were used. A control infusion was carried out with Ringer's saline (Wolf, 1963). Two different agonists were infused: noradrenaline (α- and β-agonist, 154 μg kg^{-1}) and isoprenaline (nonselective β-agonist, 27 μg kg^{-1}). 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} mol l^{-1} concentration in the blood of the fish at the end of infusion. Similar concentrations of noradrenaline and isoprenaline evoked a significant effect in carp (Van den Thillart et al., 2001). In some experiments isoprenaline infusion was preceded by a bolus injection of an antagonist: either atenolol (selective β_1-antagonist, 213 μg kg^{-1} resulting in 10^{-5} mol l^{-1}) or ICI 118,551 (selective β_2-antagonist, 250 μg kg^{-1} resulting in 10^{-5} mol l^{-1}). In carp, atenolol and ICI 118,551 were 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 therefore considered to be selective and appropriate.

The experiments started between 08.30 h and 09.30 h by taking two initial blood samples at time t=–0.75 and –0.25 h before start of infusion. Together with the second blood sampling, the fish received a bolus of Ringer's saline or a bolus of Ringer's saline containing the antagonist. At t=0 h, a 1.5 h infusion period started using Ringer's saline or Ringer's saline containing the agonist plus 1 mg ml l^{-1} 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^{-1}. During infusion, blood was sampled at t=0.5 and 1 h. To allow sampling the infusion pump was stopped for 300 s, after which infusion was resumed using a 10× higher speed for 33 s, followed by infusion at normal speed. Immediately after infusion a blood sample was taken at t=1.5 h and subsequently at t=2.5, 3.5, 5.5, 9.5 and 24 h.

Analytical procedures

Blood sampling (270 μl) was done using gas-tight microliter syringes containing 30 μl of 4% sodium citrate as

anticoagulant. On whole blood samples the hematocrit (2×9 μl) and hemoglobin content (2×10 μl) were determined. The hematocrit was measured by filling heparinized capillaries followed by centrifugation in a mini-centrifuge (Compur M1100, Bayer, München, Germany). The hemoglobin concentration was measured using a hemoglobin test kit from Roche (Almere, The Netherlands). The remaining blood was centrifuged for 5 min at 15 000 ***g*** and plasma was separated immediately. 50 μl samples of untreated plasma were stored at –80°C 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 samples of the supernatant were stored at –20°C and analysed within a week.

After neutralisation with 1 mol l^{-1} K_3PO_4, plasma concentrations of lactate were measured according to the method of Hohorst (Bergmeyer, 1970) and glucose by an enzymatic test kit (Instruchemie, Delfzijl, The Netherlands). Plasma FFA concentrations were measured using an enzymatic test kit (Waco Chemicals, Instruchemie).

Chemicals

Noradrenaline-bitartrate, isoprenaline-hydrochloride and ICI 118,551-hydrochloride were obtained from 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 ± S.E.M. 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 (0–1.5 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 analysis of variance (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 mmol l^{-1} and MCHC 18.56± 0.41 mmol l^{-1}. No significant changes were induced by the saline infusion in any of the three hematological parameters as compared to the initial values, as is shown in Fig. 1 for hematocrit. Also the isoprenaline 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, the values for the noradrenaline infusion were only significantly different from the isoprenaline 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 isoprenaline 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 mmol l^{-1}. No significant changes occurred in any infusion groups except for the isoprenaline + atenolol group, in which the lactate concentration came slightly above 1.0 mmol l^{-1} from t=1 to 5.5 h. This increase was significantly different from the initial values, but was not different from the saline and isoprenaline infusion.

The initial plasma glucose concentration was 2.82±0.13 mmol l^{-1}. The glucose concentration in the saline infused group showed a marked decrease after the infusion, resulting in significantly different values at t=3.5 and 5.5 h of 56.8±13.7% and 51.0±14.9% of the initial value, respectively. Subsequently, the plasma glucose concentration returned to the initial value (Fig. 2).

During infusion with noradrenaline, glucose concentration increased significantly at t=0.5 and 1.5 h compared to the saline group. The maximal effect of 152.9±23.6% was reached after 1.5 h. During isoprenaline infusion, the increase in

Table 1. *Initial values of parameters measured in the different experimental groups of cannulated African catfish*

Infusion	Hematocrit (%)	[Hemoglobin] (mmol l^{-1})	MCHC	*N*	[Lactate] (mmol l^{-1})	[Glucose] (mmol l^{-1})	[FFA] (mmol l^{-1})
Saline	23.5±2.1	4.41±0.29	19.01±0.84	7	0.70±0.09	2.97±0.24	0.30±0.03
Noradrenaline	23.0±3.6	4.49±0.95	19.04±1.23	5–6	0.84±0.22	2.93±0.29	0.27±0.05
Isoprenaline	23.1±1.1	4.42±0.24	19.18±0.98	6	0.39±0.10	2.47±0.20	0.32±0.04
Iso + Atenolol	22.5±2.4	3.83±0.21	17.35±1.03	5	0.47±0.20	3.32±0.52	0.33±0.03
Iso + ICI 118,551	23.0±2.5	4.05±0.39	17.93±0.92	6	0.65±0.05	2.49±0.27	0.24±0.05
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

Values are expressed as mean ± S.E.M.
MCHC, mean cellular haemoglobin content; Iso, isoprenaline.

glucose concentration during the first 0.5 h was the same as for noradrenaline but the increase persisted, resulting in a higher maximum level of 180.8±16.7% at t=1.5 h. The plasma glucose concentrations were significantly different from the saline infusion from t=0.5 to 1.5 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 isoprenaline was preceded 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 isoprenaline alone was found. After infusion the glucose levels, as compared to the saline infusion, stayed significantly elevated with both antagonists up to t=3.5 h for ICI 118,551 and up to 5.5 h for atenolol. No significant differences were found compared to the isoprenaline infusion, except for atenolol after 9.5 h.

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

The mean initial FFA concentration amounted to 0.29±0.02 mmol l^{-1}. 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=–0.75 h to 61.6±5.1% at t=0.5 h. The FFA concentration was maintained at this low level up to t=2.5 h, after which a clear overshoot occurred to a significantly different value of 201.3±23.1% at t=9.5 h. After 24 h, the FFA levels had returned close to the initial value (Fig. 4).

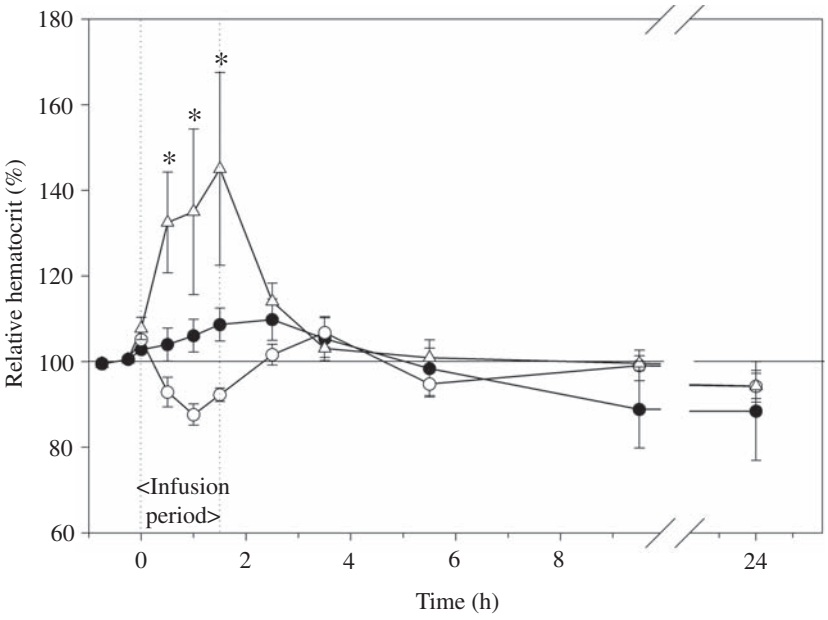

Fig. 1. The relative hematocrit of African catfish infused with saline (filled circles; N=7), isoprenaline (open circles; N=6; 27 μg kg^{-1}) or noradrenaline (triangles; N=5–6; 154 μg kg^{-1}) from t=0 to 1.5 h. *P<0.05 *vs* isoprenaline infusion.

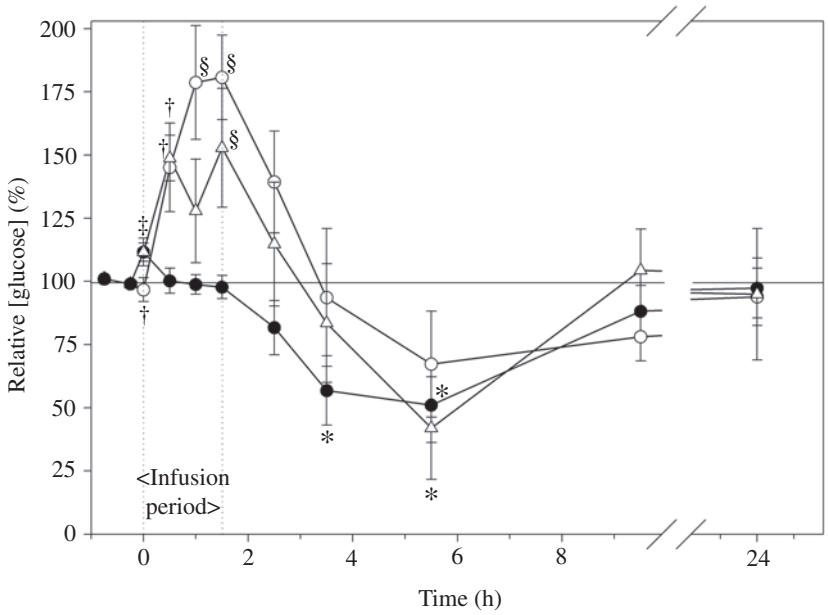

Fig. 2. The relative plasma glucose concentration of African catfish infused with saline (filled circles; N=7), isoprenaline (open circles; N=6; 27 μg kg^{-1}) or noradrenaline (triangles; N=5–6; 154 μg kg^{-1}) from t=0 to 1.5 h. *P<0.05 *vs* initial values; †P<0.05 *vs* saline infusion; ‡P<0.05 *vs* isoprenaline infusion; §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 1.5 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 isoprenaline infusion FFA levels decreased significantly, both compared to the initial values and with respect to saline infusion. The lowest concentrations were reached after 1.5 h with a mean of 25.4±6.8% of the initial value; the reduction, as compared to

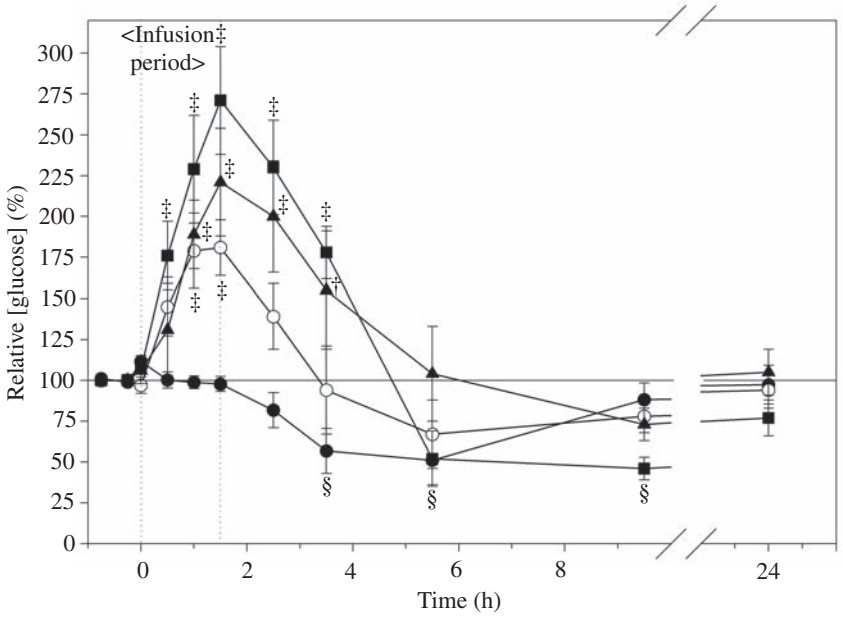

Fig. 3. The relative plasma glucose concentration of African catfish infused with saline (filled circles; N=7) or isoprenaline (open circles; N=6; 27 μg kg^{-1}) in combination with either atenolol (filled squares; N=5; 213 μg kg^{-1}) or ICI 118,551 (filled triangles; N=6; 250 μg kg^{-1}) from t=0 to 1.5 h. *P<0.05 *vs* initial values; $^{\dagger}P$<0.05 *vs* saline infusion; $^{\ddagger}P$<0.05 *vs* initial values and saline infusion; $^{\S}P$<0.05 *vs* initial values, saline and isoprenaline infusion.

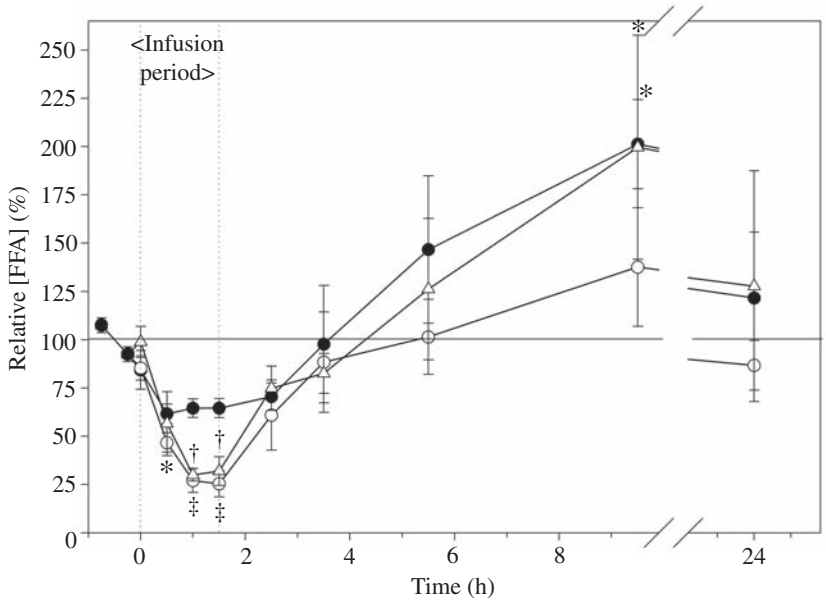

Fig. 4. The relative plasma FFA concentration of African catfish infused with saline (filled circles; N=7), isoprenaline (open circles; N=6; 27 μg kg^{-1}) or noradrenaline (triangles; N=5–6; 154 μg kg^{-1}) from t=0 to 1.5 h. *P<0.05 *vs* initial values; $^{\dagger}P$<0.05 *vs* saline infusion; $^{\ddagger}P$<0.05 *vs* initial values and saline infusion.

the saline infusion, was –39.1%. The recovery from the isoprenaline 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 the 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 isoprenaline infusions were corrected for the saline infusion data (Fig. 5). With the selective β_1-antagonist atenolol present, the FFA concentration followed the same course as when only isoprenaline was administered; at t=0.5 h the FFA levels were significantly different from the initial values only and at t=1 and 1.5 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 0.5 h, resulting in a rightward shift of the time–response curve during the isoprenaline infusion. After 0.5 h, the FFA concentration was significantly different as compared to the infusion with only isoprenaline, but not significantly different as compared to the initial value and the saline infusion. At the end of the infusion the FFA levels were no longer significantly different from the isoprenaline 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. An equal experimental protocol did not evoke a significant catecholamine release throughout 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, which reflects a circadian fluctuation in both metabolites (Van Heeswijk et al., 2005). Comparable circadian fluctuations in blood metabolites have been reported for numerous other fish species (see review by Boujard and Leatherland, 1992). 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 isoprenaline, noradrenaline induced a rise in

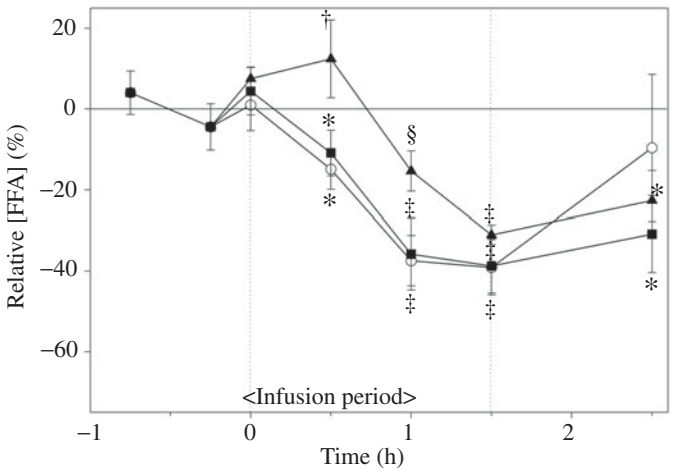

Fig. 5. The relative plasma FFA concentration of African catfish infused with isoprenaline (open circles; N=6; 27 μg kg^{-1}) in combination with either atenolol (squares; N=5; 213 μg kg^{-1}) or ICI 118,551 (triangles; N=6; 250 μg kg^{-1}) from t=0 to 1.5 h. Note that all values are corrected for the saline infusion. *P<0.05 *vs* initial values; $^{\dagger}P$<0.05 *vs* isoprenaline infusion; $^{\ddagger}P$<0.05 *vs* initial values and saline infusion; $^{\S}P$<0.05 *vs* initial values and isoprenaline infusion.

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), which explains why β-adrenergic stimulation by isoprenaline in normoxic carp (Van den Thillart et al., 2001), as in African catfish in this study, did not have a significant effect on hematocrit and MCHC.

Glucose

Both catecholamines (noradrenaline and isoprenaline) induced hyperglycemia in African catfish, as reported for numerous fish species (see review by Fabbri et al., 1998). 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., 1987; Moon et al., 1993; Fabbri et al., 1995, 1999). Although the fact that isoprenaline completely mimicked the effect of noradrenaline also suggests that 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 these 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 with isoprenaline only, 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) using molecular tools identified 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. 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, however, as several studies indicate a possible discrepancy in the characteristics of adrenergic ligands between mammals and teleost (Brighenti et al., 1987; 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 there was only one β-adrenoceptor type present in the liver (Fabbri et al., 1995; Dugan and Moon, 1998); in trout it was identified as a β_2-adrenoceptor (Reid et al., 1992; McKinley and Hazel, 1993). In rockfish *Sebastes caurinus* hepatocytes, 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). 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 literature as only stimulatory hepatic β-adrenoceptors have been found (see review by Fabbri et al., 1998). 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 isoprenaline 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} mol l^{-1}) and stimulation at high concentrations (10^{-6} mol l^{-1}) 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 literature data, the injection of β-adrenoceptor antagonists in our study possibly blocked an isoprenaline-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; 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 isoprenaline and the α- and β-agonist noradrenaline had comparable suppressive effects on the plasma FFA levels, suggesting that in this species it is also 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 isoprenaline infusion was preceded by the β_1-antagonist, the decrease in plasma FFA levels was identical to that measured when only isoprenaline 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 β_2-adrenoceptors mediated the decrease in plasma FFA levels in African catfish. 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 mediated a decrease in FFA levels, while β_2-adrenoceptors mediated 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 waterbreathing 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 airbreathers (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 (J.C.F.v.H., J. van Pelt and G.E.E.J.M.v.d.T., unpublished observations). African catfish is caught at a depth of over 50 m in Lake Victoria (Goudswaard and Witte, 1997), which makes it highly unlikely that it will surface to breathe air. Hence, in its natural habitat African catfish is also likely to become hypoxic. Obligate air-breathers like adult lungfish (*Protopterus* sp.), on the other hand, are vitally dependent on aerial oxygen and can only live when allowed to air-breathe (Graham, 1997). The respiratory behaviour of adult African lungfish *Protopterus aethiopicus* was indeed not affected by decreasing aquatic oxygen tensions when allowed to air-breathe (Johansen and Lenfant, 1968) as opposed to African catfish (Johnston et al., 1983). Hence environmental hypoxia, even when it only means submersion as in African catfish, is by definition not a physiologically relevant situation for African lungfish, rendering this species 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 the function of air-breathing is 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. The suppressive role of noradrenaline on plasma FFA levels is hypothesised to be a survival mechanism during hypoxia (Van den Thillart et al., 2002). Therefore, an alternative drive for the evolution of air-breathing is supported by our finding, that noradrenaline has a similar suppressive effect on plasma FFA levels in air-breathing African catfish, as in other waterbreathing fishes. Hence, air-breathing in African catfish most likely did not evolve as a survival mechanism for hypoxia.

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