Effects of acetylsalicylic acid treatment on thyroid hormones, prolactins, and the stress response of tilapia

*Oreochromis mossambicus*

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Van Anholt, Rogier D., Tom Spanings, William Koven, and Sjoerd E. Wendelaar Bonga. Effects of acetylsalicylic acid treatment on thyroid hormones, prolactins, and the stress response of tilapia (*Oreochromis mossambicus*). *Am J Physiol Regul Integr Comp Physiol* 285: R1098–R1106, 2003. First published July 3, 2003; 10.1152/ajpregu.00731.2002.—The cyclooxygenase (COX) pathway converts arachidonic acid (ArA) into prostaglandins (PGs), which interact with the stress response in mammals and possibly in fish as well. Acetylsalicylic acid (ASA) is a COX inhibitor and was used to characterize the effects of PGs on the release of several hormones and the stress response of tilapia (*Oreochromis mossambicus*). Plasma PGE2 was significantly reduced at 100 mg ASA/kg body wt, and both basal PGE2 and cortisol levels correlated negatively with plasma salicylate. Basal plasma 3,5,3′-triiodothyronine (T3) was reduced by ASA treatment, whereas prolactin (PRL)18S increased at 100 mg ASA/kg body wt. ASA depressed the cortisol response to the mild stress of 5 min of net confinement. As expected, glucose and lactate were elevated in the stressed control fish, but the responses were blunted by ASA treatment. Gill Na+/K+-ATPase activity was not affected by ASA. Plasma osmolarity increased after confinement in all treatments, whereas sodium only increased at the high ASA dose. This is the first time ASA has been administered to fish in vivo, and the altered hormone release and the inhibition of the acute stress response indicated the involvement of PGs in these processes.

Acetylsalicylic acid (ASA; aspirin) is a potent and irreversible inhibitor of the COX pathway (36) and provides an excellent tool to determine the functions of PGs in fish. In the present study, we therefore inves...
tigated the effects of ASA treatment on the release of several hormones, the osmoregulation, and the response to an acute stressor of a well-studied model species, tilapia (*Oreochromis mossambicus*; Peters, 1952).

**MATERIAL AND METHODS**

The experiments described in this study were conducted in accordance with the current law on animal experimentation in the Netherlands.

*Fish.* Male and female freshwater tilapia (*O. mossambicus*) were obtained from laboratory stock (Univ. of Nijmegen, Nijmegen, The Netherlands). Fish were maintained in aerated, partially recirculated and filtered tap water at 25°C with a 12-h photoperiod. Tilapia were acclimated over a 3- to 4-wk period, during which they were fed standard pellets at 2% of their body weight per day (crude protein 38%, total lipid 10%, ash 10%, moisture 8%; TL4.5 Tilapia, Trouw Nutrition).

**Uptake of ASA.** Transparent gelatin capsules (size 4; Lamempro) were used to administer ASA. Capsules were filled with 100 mg of crushed pellets and the required amount of ASA (Sigma-Aldrich). The filled capsules resembled the standard pellets in size and appearance, and when fed together with pellets they were eaten indiscriminately within 5–10 min, enabling a stress-free administration of ASA. Prior to experimentation, the time course of the uptake of ASA into the bloodstream after oral administration was determined in a separate test. To this end, 16 adult tilapia (174 ± 7 g) from the same cohort, separated in two consecutive trials. Both trials consisted of 60 tilapia divided over 6 aquaria (80 liters, 10 fish/aquarium), and 2 aquaria were used per treatment. During the acclimation period of 25 days, they were fed 2% of body weight twice a day. Two doses of ASA were used, 10 mg or 100 mg ASA/kg body wt, while the control treatment was fed capsules without ASA. In contrast to the pilot study, the ASA dose was not determined for each individual fish but on the basis of the mean weight of 10 fish per aquarium. ASA was fed twice a day and on the third day, 4 h after their last meal or dose of ASA, several fish were sampled for baseline values. The remaining fish were immediately subjected to 5 min of submerged net confinement, after which they were released back into the aquarium and sampled after either 5 min or 30 min, depending on the parameter. Fish were anesthetized in a 1.5% 2-phenoxyethanol solution, and blood samples were collected using heparinized needles. Plasma samples were separated after centrifugation and stored at −20°C until analysis. Gill arches were dissected and stored frozen in SEI buffer (300 mM sucrose, 10 mM Na<sub>2</sub>EDTA, 100 mM imidazole, pH 7.4 with HEPES-Tris) until analysis of Na<sup>+</sup>–K<sup>+</sup>–ATPase activity.

**Salicylate.** Levels of plasma salicylate were determined with a commercial kit for human plasma (Sigma-Aldrich). To determine the recovery in tilapia plasma, an additional standard curve was created on the basis of addition of ASA in final concentrations of 0.36–1.81 nmol/l to plasma of a control fish. Up to 0.72 nmol/l salicylate, the regression was linear with a 95% recovery, and all tested samples were within this range. Undiluted samples were measured in triplicates, and assay blanks were used to correct for turbidity. Absorbance was read at 550 nm in a Victor<sup>®</sup> Wallac multilabel counter.

**PGE<sub>2</sub>.** Plasma levels of PGE<sub>2</sub> were assayed with a high-sensitivity chemiluminescence enzyme immunoassay (CILIA 91001; Assay Designs). Plasma samples were diluted five times and assayed according to the manufacturer’s protocol. Cross-reactivity of the antibody with PGE<sub>1</sub> is 33.2%, PGB<sub>1</sub> 7.0%, 13,14-dihydro-15-keto-PGF<sub>2α</sub> 2.8%, PGE<sub>1</sub> <0.01%, and ArA <0.01%.

**COX activity.** A commercial kit (907-003; Assay Designs) was used to determine whether ASA inhibited the COX activity of tilapia tissue homogenates in vitro. To this end, gill and kidney tissue samples of five control tilapia homogenized in SEI buffer were used. Fifty microliters of homogenate supernatant (~2.5 mg protein/ml) were added to the microplate in duplicates. A concentrated stock solution of 2.8 mM ASA (Sigma-Aldrich) in ethanol was diluted 100× in distilled water, of which 25 µl were added to a second duplicate set of wells to a final concentration of 400 µM. This concentration approximates the average value measured in plasma of the tilapia 4 h after administration of ASA. All samples were incubated for 20 min at room temperature, and chemiluminescence was determined according to protocol using a Victor<sup>®</sup> Wallac multilabel counter with an automated dispenser unit. The percentage of inhibition by ASA was calculated as the differences between the COX activity in the control samples and those with ASA, corrected for the non-specific increase in signal strength caused by ASA alone.

**RIAs.** Plasma total thyroxine (T<sub>4</sub>) was determined by RIA using a modification of a procedure developed for human serum and rat plasma (13). Because of the very low levels of T<sub>4</sub> in fish plasma, 100–µl samples of plasma (instead of 5 µl) were used for analysis. The standards used for measurement in human serum consisted of 0–360 nmol/l T<sub>4</sub> added to 5 µl of T<sub>4</sub>-free human serum that had been prepared by repeated treatment with charcoal (15 g/100 ml) and centrifugation. For this assay, these standards were supplemented with 100 µl of T<sub>4</sub>-free fish plasma prepared in the same way. Conversely, 100 µl of unknown fish plasma samples were supplemented with 5 µl of human T<sub>4</sub>-free serum to provide full matrix compatibility between samples and standards. By using 100-µl samples, the concentration range was 0–18 nmol/l, with a detection range of 0.5 nmol/l. Accuracy was tested by spiking a pooled fish sample with T<sub>4</sub>. Recoveries were 104 and 97%. At a level of 2.95 nmol/l, the intra-assay variation was 5.6%, and the interassay variation was 10.6%.

Plasma total 3,5,3’-triiodothyronine (T<sub>3</sub>) was assessed on the Centaur automated immunoassay system (ACS; Bayer) using an acridinium-labeled anti-T<sub>3</sub> antibody. Fish plasma was diluted twice, and the interassay variation was <3% at the levels assayed. Plasma samples of the two forms of PRL, PRL<sub>188</sub> and PRL<sub>177</sub>, were measured by homologous RIAs according to previous studies (1, 48).

**PRL cortisol levels were determined with a commercially available antibody, with some minor modifications of the manufacturer’s protocol (Campro Scientific).** All constit-
Antipyretic and antiinflammatory effects of aspirin in tilapia (Oreochromis niloticus) were evaluated by measuring plasma salicylate, corticosterone, and PGE2 levels, and by assessing body temperature and behavior. Plasma salicylate levels were elevated 1.5 h after the tilapia had received a single dose of 100 mg ASA/kg body wt, although this was not significant until 3.5 h after administration. Maximum plasma levels were reached after 5.5 h and remained significantly elevated until 15 h later. Repetitive sampling did not alter the salicylate levels of the two control fish, which were 66.4 ± 6.8 μmol/l salicylate for the sampling period.

In the follow-up experiment, two doses of ASA were tested. The administration of 10 mg/kg did not significantly elevate plasma salicylate levels after 4 h compared with the control group (stressed and nonstressed fish combined). However, at 100 mg ASA/kg body wt, plasma salicylate levels were significantly elevated (P = 0.000) compared with the controls (Table 1).

Plasma PGE2 was not significantly affected by the low dose of ASA, and the high ASA dose reduced the average plasma PGE2 by ~34%, which was significantly different from the low dose (P = 0.025; Table 1). Plasma PGE2 levels were significantly negatively correlated (R2 = 0.889, P = 0.001) with the plasma salicylate levels in individual fish (Fig. 2).

At a concentration of 400 μM in the medium, ASA inhibited the COX activity of kidney homogenates with
Table 1. Plasma levels of salicylate and PGE₂

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salicylate, μmol/l</th>
<th>PGE₂, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.0 ± 5.7†</td>
<td>1.95 ± 0.25‡†</td>
</tr>
<tr>
<td>10 mg/kg ASA</td>
<td>88.8 ± 21.3‡†</td>
<td>1.89 ± 0.12‡</td>
</tr>
<tr>
<td>100 mg/kg ASA</td>
<td>402.0 ± 47.3§</td>
<td>1.29 ± 0.25§</td>
</tr>
</tbody>
</table>

Values are means ± SE. Plasma levels of salicylate and prostaglandin (PG) E₂ in tilapia fed 2 different doses of acetylsalicylic acid (ASA): 10 and 100 mg/kg body wt. Samples were taken 4 h after the last dose of ASA. Different symbols indicate significant differences.

43.9 ± 1.9%, but the COX activity in gill homogenates was too low to be detected with this assay.

At 100 mg/kg ASA, the mean plasma T₃ level was reduced significantly (P = 0.014) compared with the control, and the low ASA dose had no effect on plasma T₃ (P = 0.109; Fig. 3). ASA administration did not significantly affect plasma T₄ levels at 10 mg/kg (P = 0.992) or at 100 mg/kg body wt (P = 0.083) compared with the control.

Although plasma PRL₁₇₇ levels increased slightly with increasing ASA dose, this effect was not significant (P = 0.672; Fig. 4). Basal PRL₁₈₈ levels were significantly enhanced at the high ASA dose compared with the low dose (P = 0.044; Fig. 4).

Administration of ASA did not result in a significant change of mean basal levels of plasma cortisol (Fig. 5). However, when comparing the values of individual fish, there was a significant negative exponential correlation (R² = 0.598, P = 0.005) between individual plasma salicylate levels and plasma basal cortisol of the nonstressed fish (Fig. 2). Plasma cortisol levels were significantly influenced by both the level of ASA (P = 0.010) and the time of sampling, i.e., confinement (P = 0.000; Fig. 5). At 100 mg/kg ASA, the postconfinement levels were significantly (P = 0.020) lower than the postconfinement levels in the control. Both 5 and 30 min after confinement, cortisol levels were significantly (P = 0.000) increased compared with the unstressed levels in all treatments, but there was no significant difference between 5 and 30 min after confinement (P = 0.051).

Plasma glucose levels were significantly influenced by both the ASA administration (P = 0.006) and the confinement (P = 0.000). At the low dose of ASA, the response to confinement was significantly (P = 0.007) reduced compared with the controls (Fig. 6). In all treatments, at both 5 and 30 min after confinement, levels were significantly elevated compared with the controls (P = 0.000), and the difference between 5 and 30 min was no longer significant.

ASA treatment and time of sampling both significantly (P = 0.000) influenced plasma lactate levels (Fig. 7). There was also a significant interaction effect between time and treatment (P = 0.000). Both the low...
and high dose of ASA significantly reduced the lactate response compared with the controls (P = 0.010 and 0.024, respectively). Lactate levels were significantly elevated at both 5 and 30 min after confinement compared with the unstressed fish (P = 0.000), and at 30 min the lactate levels were also significantly higher than after 5 min (P = 0.000; Fig. 7).

The lowest basal gill Na\(^+\)-K\(^+\)-ATPase activity was observed at the high dose of ASA. However, variation was high and the difference with the control group was not significant (Table 2).

Plasma osmolarity levels exhibited similar transient increases after confinement in all treatments (P = 0.000), but the dose of ASA had no significant influence on this response (Table 2). At 5 and 30 min after confinement, plasma osmolarity was significantly elevated compared with controls (P = 0.000), whereas the increase after 30 min was not significantly different with the levels after 5 min.

Plasma sodium levels were significantly affected by both the dose of ASA as well as the confinement (P = 0.021 and 0.000, respectively; Table 2). Although neither the low nor the high dose of ASA differed significantly from the controls, sodium levels were significantly elevated at 100 mg/ASA/kg body wt compared with 10 mg ASA/kg body wt (P = 0.039). Overall, after 5 and 30 min, sodium levels were significantly elevated compared with the unstressed fish (P = 0.010 and 0.000, respectively). Plasma chloride levels were significantly affected by confinement (P = 0.000) but not by the dose of ASA. Within 5 min after confinement, plasma chloride levels were significantly higher compared with unstressed fish (P = 0.000). Thirty minutes after confinement, plasma chloride levels had dropped significantly below the levels at 5 min (P = 0.000) and were no longer significantly elevated compared with the unstressed fish. Plasma potassium levels were not significantly affected by either administration of ASA or confinement.

**DISCUSSION**

This is the first time, to our knowledge, that ASA was used to study the effects of fatty acid-derived PGs in fish. The administration of 100 mg ASA/kg body wt via gelatin capsules resulted in elevated plasma levels of salicylate within 3.5 h, reaching a maximum within 5.5 h (~650 \(\mu\)mol/l), which was well within the range considered therapeutic for humans (<1,440 \(\mu\)mol/l) and considerably lower than toxic (>2,170 \(\mu\)mol/l) and lethal (>4,340 \(\mu\)mol/l) limits (41). The tilapia did not discriminate between capsules containing ASA and normal pellets, and therefore we consider this method superior to injections or forced feeding, which are always stressful. This was confirmed by the low basal cortisol, glucose, and lactate levels observed both in the
controls and after the administration of ASA in our tests. Cortisol is the major glucocorticoid in tilapia and is released from the head kidney after the stress-induced activation of the HPI axis (2, 46). These low basal values also argue against a nonspecific toxic effect of ASA in our experiments, which is supported by the practically identical basal plasma osmolarity and ion levels, the lack of behavioral changes or mortality, and the absence of hemorrhages.

One of three major pathways of ArA metabolism is the COX pathway converting ArA mainly into PGs (PGE₂, PGD₂, PGF₂α), and thromboxanes (TXa₂) (18). ASA has no direct effect on the activity of PLA₂ that releases ArA from the membranes (14), but it is a highly specific and irreversible inhibitor of the COX pathway by acetylating the COX enzymes. Hence, new enzyme synthesis is required before new PGs can be produced (36). Four hours after administration of ASA, the levels of circulating PGE₂ were significantly lower in the fish that had received the high dose compared with those that received the low dose. The strong negative correlation between individual PGE₂ and salicylate levels also suggested a reduction of COX activity in these tilapia, which was supported by the inhibitory effect of ASA on the COX activity of kidney homogenates in vitro.

PGs are known to influence a wide range of physiological processes by enhancing the release of hormones and/or altering the sensitivity of target organs (18, 23). Langer et al. (19) found that ASA (analog) treatment reduced plasma levels of TSH, T₃, and T₄ in humans, which indicated the involvement of PGs in the release of thyroid hormones. Studies on mammals further showed that PGs enhanced the response of thyroid tissue to TSH, resulting in higher T₃ and T₄ levels (18). In our study, basal levels of T₃ were significantly reduced after ASA treatment, suggesting that PGs have a similar regulatory function in tilapia. Thyroid hormones play an important role in growth and development of fish larvae and particularly flatfish metamorphosis (29, 30). Furthermore, thyroid hormones enhance the osmoregulatory ability, principally in saltwater fish (33) but also in freshwater fish, as they have been reported to promote gill Na⁺⁻K⁺-ATPase activity, an important ion-transporting enzyme, in tilapia (28).

PRL is also a hormone with osmoregulatory functions in fish (1, 16, 34). In mammals eicosanoids are thought to promote PRL release by decreasing the PRL release-inhibiting factor (PIF) and increasing the PRL-releasing factor (PRF) from the hypothalamus, without having a direct effect on the pituitary (26). ASA treatment has also been shown to attenuate the PRL response to exercise in humans, thereby providing indirect evidence for the role of COX metabolites in the control of PRL release (10). Surprisingly, in our experiments PRL levels were not reduced when the COX pathway was blocked, but instead plasma PRL levels were elevated after administration of 100 mg ASA/kg body wt. It might be that PRL release is under inhibitory control by PGs in tilapia, or ArA itself has a stimulatory effect on the release of PRL in fish, several polyunsaturated fatty acids were capable of inducing PRL secretion from rat pituitary cells in vitro, independently of the conversion to PGs (17).

The stress response in fish, similar to that in mammals, is generally characterized by activation of the brain center that control the release of corticotropin-releasing hormone (CRH) by the hypothalamus, which in turn induces the release of ACTH from the pituitary. ACTH is an important corticotropic hormone inducing the release of cortisol from interrenal cells in fish, although α-melanocyte-stimulating hormone and β-endorphin appear to be involved as well (3). In our tilapia, 100 mg ASA/kg body wt clearly reduced the cortisol response to net confinement, demonstrating the involvement of PGs in vivo. This supports the observations by Gupta et al. (12), who found that PGs were able to induce the release of cortisol from interrenal tissues of trout in vitro, and Wales (44), who demonstrated that injections of PGE₂ stimulated the cortisol release in hagfish in vivo. Several studies on humans have shown that ASA administration resulted in a blunted cortisol response, suggesting that PGs have a stimulatory effect on adrenal steroidogenesis (8). According to these studies, endogenous PGs can generate their effect on the release of cortisol at several levels: PGs can enhance hypothalamic CRH release but also

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, min</th>
<th>Na⁺⁻K⁺-ATPase, μmol P₁·h⁻¹·mg prot⁻¹</th>
<th>Osmolarity, mosM</th>
<th>[Na⁺], mmol/l</th>
<th>[K⁺], mmol/l</th>
<th>[Cl⁻], mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>1.90 ± 0.47</td>
<td>320.0 ± 4.7</td>
<td>168.2 ± 2.9</td>
<td>5.1 ± 0.3</td>
<td>169.4 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>339.4 ± 3.7</td>
<td>173.3 ± 3.7</td>
<td>5.1 ± 0.3</td>
<td>176.3 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>388.0 ± 7.7</td>
<td>180.5 ± 2.2</td>
<td>5.2 ± 0.2</td>
<td>168.2 ± 2.3</td>
</tr>
<tr>
<td>10 mg/kg ASA</td>
<td>0</td>
<td>1.89 ± 0.22</td>
<td>310.5 ± 3.6</td>
<td>167.4 ± 2.2</td>
<td>4.7 ± 0.2</td>
<td>166.9 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>327.8 ± 3.9</td>
<td>174.0 ± 3.5</td>
<td>4.5 ± 0.1</td>
<td>178.1 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>337.2 ± 8.4</td>
<td>185.4 ± 2.1</td>
<td>4.9 ± 0.2</td>
<td>165.8 ± 3.7</td>
</tr>
<tr>
<td>100 mg/kg ASA</td>
<td>0</td>
<td>1.63 ± 0.22</td>
<td>311.5 ± 4.8</td>
<td>169.1 ± 3.2</td>
<td>5.2 ± 0.2</td>
<td>167.5 ± 1.9</td>
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<tr>
<td></td>
<td>5</td>
<td></td>
<td>337.8 ± 5.4</td>
<td>180.7 ± 2.1</td>
<td>4.9 ± 0.3</td>
<td>181.5 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>330.0 ± 8.8</td>
<td>186.1 ± 2.6</td>
<td>4.0 ± 0.3</td>
<td>169.1 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Samples were collected 4 h after the last dose of ASA for nonstressed fish or 5 min and 30 min after net confinement. For significant differences, see RESULTS. [Na⁺], [K⁺], and [Cl⁻], concentrations of Na⁺, K⁺, and Cl⁻, respectively; prot, protein.
restrain CRH-induced ACTH secretion at the pituitary level as well as enhance the response of the adrenal cortex to ACTH stimulation, resulting in an increase in steroidogenesis (10, 25). In our study, the basal plasma levels of cortisol were slightly reduced by ASA administration, and a strong correlation was found between individual plasma salicylate levels and plasma cortisol levels in the nonstressed fish. This is in contrast to the studies on humans (8), which suggests that basal and induced releases of cortisol are under different controlling mechanisms.

Basal lactate and glucose levels in our tilapia were not affected by the ASA administration, and the high levels of glucose and lactate after confinement in the control group closely resembled those found previously in tilapia in response to stress (43). At a dose of 10 mg/kg body wt, the ASA administration resulted in a blunted glucose response within 5 min. Although cortisol regulates gluconeogenesis in fish, this requires modification of hepatic enzyme activity, which has been shown to take at least 12 h in tilapia. Instead, rapid hyperglycemia is most likely the result of enhanced hepatic glycogenolysis induced by catecholamine release (43). The blunted glucose response by ASA therefore altered either the catecholamine release or, alternatively, the adrenergic response of liver cells, although there is no previous evidence that the latter can occur in fish. Nevertheless, it has been shown in rats that brain PGE₂ can mediate the central sympathethic outflow of catecholamines and that other COX products, thromboxanes, are involved in the central adrenomedullary outflow (27, 51).

Although the initial stress-induced rise in plasma lactate remained unaffected, ASA at both doses strongly augmented the lactacidemia observed after 30 min. Lactacidemia points to increased muscle glycolysis or incomplete oxidation as a result of hypoxemia in muscle cells. There is only very limited knowledge on the effects of fatty acids or eicosanoids on lactate metabolism, and no studies have been performed on fish. In humans, ASA treatment had no effect on lactate levels after exercise (10). On the other hand, in young rats, it was shown that feeding n-3 fatty acids attenuated stress-induced lactacidemia, in contrast to n-6 fatty acids that enhanced the lactate response. In that study, increased gluconeogenesis was thought to be responsible for the attenuated lactacidemia, although this was not due to inducible PGs but most likely related to an altered response of liver cells to catecholamines (35).

Several studies have demonstrated both inhibitory or stimulatory effects of PGs and fatty acids on Na⁺-K⁺-ATPase (21, 38, 39, 42). Nevertheless, the effects of ASA administration on gill Na⁺-K⁺-ATPase, plasma ions, and plasma osmolarity were very limited. While ASA treatment had no effect on plasma chloride and potassium levels, only net confinement in combination with the high dose of ASA resulted in a slight increase in plasma sodium. Generally, plasma ion levels tend to drop in freshwater fish after stress because of an increased permeability of (branchial) membranes. However, during acute stress, plasma water can also move out of the circulation into the tissues, reducing the blood plasma volume (46), explaining the transient increase in plasma ions in our experiments.

Although it appears that the observed effects of ASA administration are the result of reduced PG production, two alternative hypotheses can be proposed. First, ArA itself could be a controlling factor. Once released from cell membranes, free ArA not only functions as a precursor to eicosanoids but also acts directly as a ligand, affecting either negatively or positively the binding of steroid hormones to their specific intracellular receptors. In addition, fatty acids can also coregulate glucocorticoid-dependent gene expression by modulating the activity of protein kinases involved in the phosphorylating transcription factors (37). An intracellular increase in free ArA, as a result of ASA treatment, might therefore be responsible for the augmented cortisol response to the stressor in this study. Second, we should be aware that blocking the COX pathway might have redirected free ArA to other enzymatic pathways, the two main alternative pathways being the oxygenation into leukotrienes and hydroxyeicosatetraenoic acids (HETEs) by lipoxygenase and the conversion into epoxyeicosatrienoic acids (EETs) by epoxygenase (36). These alternative eicosanoids are potential modulators of the HPI axis in fish, as epoxygenase and lipoxygenase metabolites have been shown to stimulate ACTH and β-endorphin secretion from rat pituitary cells (9, 24, 47), and lipoxygenase products stimulated steroidogenesis in bovine adrenal cells in response to ACTH (45, 49). However, in our experiments, these pathways might have been of subdominant importance, as all these studies demonstrated a stimulatory effect of the alternative metabolites on ACTH, β-endorphin, and cortisol secretion, which does not correspond to the attenuated cortisol response after ASA administration in our tilapia. Unfortunately, information on the specific accumulation and conversion of ArA in pituitary and interrenal cells of teleost fish is still lacking.

The results of this study clearly indicated that inhibiting the COX pathway by ASA modified the release of several important hormones and inhibited the response of tilapia to an acute stressor. Administration of ASA in vivo is an excellent tool to investigate the involvement of ArA-derived metabolites in the hormone release and stress response of fish. It enables the differentiation between the PG-controlled and ArA-mediated effects, which will help to further understand the underlying mechanism of dietary control of the stress response and hormone release in fish (20).

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