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EFFECTS OF CONFINEMENT/CROWDING ON UREOGENESIS IN THE GULF TOADFISH *OPSANUS BETA*

PATRICK J. WALSH, BONNIE C. TUCKER AND TODD E. HOPKINS

Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149, USA

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Summary

In order to elucidate the cues for, and the biochemical mechanisms of, the transition to ureogenesis in the gulf toadfish *Opsanus beta*, experiments on the effects of confinement/crowding were carried out. Confinement of toadfish to small volumes of water initiated a switch to nearly complete reliance on ureogenesis for nitrogen excretion within 24–48 h. Further experiments suggested that this switch was probably due to the physical confinement *per se*, rather than to a measurable build-up of ammonia in the water. However, the possibility of the response being triggered by a very low concentration of a pheromone-like substance was not excluded by our experimental design. The activities of several enzymes of ureogenesis, ornithine–citrulline transcarbamoylase, aspartate amino transferase and glutamine synthetase, increased in the liver of toadfish during confinement. Notably, glutamine synthetase activity increased almost fourfold within 24 h, and this increase preceded increases in urea excretion. A number of plasma and liver amino acid concentrations changed during confinement: there were declines in plasma asparagine, glutamate and glycine levels and an increase in plasma valine, as well as a decline in liver alanine and an increase in liver arginine concentrations. Liver glutamine was not detectable. When the amino acid data are taken together with the enzyme activity changes, it appears that the switch to ureogenesis occurs primarily upstream of the ornithine–urea cycle, at the level of supply of nitrogen to the pathway. The results are discussed in the context of the habitat of toadfish.

Introduction

Although the vast majority of teleost fish excrete their waste nitrogen principally as ammonia (ammoniotely), recent studies have expanded the list of teleosts which excrete the bulk of their waste nitrogen as urea (ureotely) (Wood, 1993). Ureotely in teleosts can be obligate or facultative. For example, the Lake Magadi tilapia, *Oreochromis alcalicus grahami*, is exclusively ureotelic, an adaptation which contributes to an overall strategy enabling it to survive in the highly buffered, alkaline (pH 10) water of Lake Magadi, Kenya (Randall *et al.* 1989; Wood *et al.* 1989). Other teleosts, such as the gulf toadfish *Opsanus beta*, are able to switch between nearly complete ammoniotely and nearly

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complete ureotely (Walsh *et al.* 1990) over short periods. Investigation of the switching to ureogenesis in facultatively ureotelic fish is likely to lead to interesting mechanistic insights for two reasons. First, unlike the situation in obligate ureoteles (e.g. mammals, the Lake Magadi tilapia, elasmobranch fishes, etc.), where control is focused on fine-tuning rates of an already functioning pathway, changes in pathway flux in facultatively ureotelic fish are likely to be larger and more abrupt. Second, although models for the study of such on/off regulation of ureogenesis are already available in the lungfish (e.g. Funkhouser *et al.* 1972) and amphibians (e.g. Helbing *et al.* 1992), the biochemical architecture of ureogenesis in teleosts is sufficiently different to provide interesting comparisons. In particular, (1) ureogenic teleosts possess a different isoenzyme of carbamoylphosphate synthetase (CPS III which utilizes glutamine as a nitrogen donor, instead of the CPS I found in non-piscine vertebrates which utilizes ammonia), and (2) the enzyme glutamine synthetase is an important first step in the teleost pathway, whereas it plays only an ancillary role (downstream scavenging of excess ammonia) in other non-piscine systems (Mommsen and Walsh, 1991).

Among facultatively ureotelic teleosts, the toadfish have received recent attention. In toadfish, ureogenesis appears to function primarily in times when normal pathways of ammonia excretion are blocked (Walsh *et al.* 1990; Barber and Walsh, 1993), and ureogenesis does not appear to be an important part of strategies for osmoregulation (Walsh *et al.* 1990) or acid–base balance (Walsh *et al.* 1989; Barber and Walsh, 1993). Ureogenesis can be activated in toadfish by air exposure and NH_4Cl exposure (up to 150 μmol l^{-1}) in the laboratory (Walsh *et al.* 1990), although these conditions may not be experienced in the wild. The biochemical mechanisms responsible for this activation are not known, but several observations have led Mommsen and Walsh (1991) to propose a pivotal role for glutamine synthetase in the activation of ureogenesis in this species. The present study of the gulf toadfish was undertaken to examine some of the potential biochemical mechanisms responsible for activation of ureogenesis as well as to examine more subtle (and perhaps more ecologically important) factors which might cue the transition to ureogenesis. Our results demonstrate that, in the toadfish, ureogenesis is activated by conditions of confinement/crowding and appear to support the concept of a pivotal role for glutamine synthetase in the activation of ureogenesis.

Materials and methods

Experimental animals

Individuals of the gulf toadfish, *Opsanus beta* (Goode and Bean), were captured with a roller trawl by commercial shrimpers in Biscayne Bay, Florida, USA, between September 1992 and July 1993. Toadish (30–210 g) were held in an outdoor tank at the shrimpers' holding facility with running sea water (with ambient seasonal conditions) for up to 4 days following capture, then transferred to the laboratory where they were kept in 80 l glass aquaria with flowing, aerated sea water (sand-filtered and ultraviolet-sterilised) for up to 1 week prior to the start of an experiment. Temperatures in these holding tanks and during the experiments described below were 22±2 °C. Fish typically

had food in their digestive tracts after transfer from the outdoor tanks, but were not fed once they were brought into the laboratory. Although gender was recorded when fish were killed, fish were randomly assigned to control or test groups without regard for gender.

Experimental design

Three experimental series were designed to examine conditions causing toadfish to become more ureotelic and eliciting changes in the underlying biochemical aspects of ureogenesis. In the first experimental series (series I), toadfish were either left in the 80 l glass aquaria with flowing sea water as described above with no disturbances (referred to as *control* conditions) or transferred to small plastic tubs (30 cm long × 25 cm wide × 10 cm high) with 6 l of aerated filtered sea water, without flow, which was changed every 24 h (referred to as *confined-static water* conditions). In series I (and series II below), all transfers of fish were made by gently netting the fish and exposing them to air for less than 5 s. Fish were similarly transferred at water changeover every 24 h. Typically, three fish (with a total mass of approximately 250 g) were placed in each tub, while the 80 l aquarium had 6–10 fish of similar size. At various time intervals, 10 ml of sea water was withdrawn from the tubs and frozen (−20 °C) for later analysis of ammonia and urea. At the end of an experiment, fish were anesthetised in 0.5 g l^{-1} tricaine methanesulfonate (buffered with sodium bicarbonate) and kidney, liver and brain were dissected, quick-frozen in liquid nitrogen and stored at −80 °C for later analysis of enzyme activities. Additionally, in parallel groups of fish, following anesthesia, blood samples were taken by caudal puncture and livers were freeze-clamped in tongs precooled with liquid nitrogen. Plasma was separated by centrifugation at 13 000 ***g*** for 1 min and plasma and livers were stored at −80 °C for later analysis of metabolites.

In the second experimental series (series II), in addition to the *control* and *confined-static water* treatments described above, toadfish were also exposed to a third condition, flowing aerated sea water in the same plastic tubs as the *confined-static water* treatment, with the same total volume of 6 l; flow rates were such that the water was turned over once every 2–3 min (referred to as *confined-flowing water* treatment). [These flow rates, relative to biomass, gave approximately the same wash-out rates as for the control fish and, thus, probably the same approximate ammonia concentrations. Ammonia concentration in both control and confined-flow treatments were below the limits of detection (see below).] Additionally, some of the confined-flow-treated fish were subsequently shifted to confined-static conditions (by simply turning off the water flow with no initial transfer, but with subsequent net transfers at each 24 h water changeover), and water samples were taken as above and stored for ammonia and urea analyses. Some fish were killed at various points after these exposures, and their livers were frozen and stored as above for later enzyme analysis.

In a final experimental series (series III), fish were subjected to treatments as above, but with more varied and controlled handling of fish. For example, some fish were prodded with a net but not netted or exposed to air (disturbed), some fish were netted and exposed to air, some were transferred without netting, etc. Samples of liver were taken and handled as above and analyzed for glutamine synthetase activity.

Analytical methods

Water samples were analyzed for ammonia by the method of Ivancic and Deggobis (1984), which has an approximate detection limit of 1–2 μmol l^{-1}, and for urea by the method of Price and Harrison (1987) as previously applied to toadfish (Barber and Walsh, 1993). Plasma samples were analyzed directly for amino acids and related compounds as previously described (Barber and Walsh, 1993). For the analysis of amino acids, frozen livers were pulverized in a mortar and pestle precooled with liquid nitrogen and then homogenized on ice with 19 vols of chilled 3 % 5′-sulfosalicylic acid using a Brinkman polytron. Homogenates were centrifuged at 4000 ***g*** for 20 min to remove debris, and the supernatant was analyzed for amino acids and related compounds as described by Barber and Walsh (1993). Liver glutamine was not detectable by this method (detection limit was 5 μmol g^{-1}) (see below), so a second group of livers was homogenized as above but in 9 vols of 10 % trichloroacetic acid, centrifuged as above, and rapidly neutralized with 2 mol l^{-1} $KHCO_3$. The neutralized samples were then assayed enzymatically for glutamine, according to the method of Lund (1983), which has an approximate detection limit of 2.5 μmol g^{-1}.

For analysis of enzyme activity, tissues were homogenized on ice in 4 vols of homogenization buffer (20 mmol l^{-1} K_2HPO_4, 10 mmol l^{-1} Hepes, 0.5 mmol l^{-1} EDTA, 1 mmol l^{-1} dithiothreitol, 50 % glycerol, adjusted with NaOH to pH 7.5 at 24 °C) using a Brinkman polytron. Homogenates were spun at 8000 ***g*** for 20 min at 4 °C in a Jouan CR412 centrifuge. The supernatant or a 1:10 dilution was used directly for the assay at 24 °C of alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT), glutamate dehydrogenase (GDH), glutamine synthetase (GNS), carbamoylphosphate synthetase (CPS), ornithine–citrulline transcarbamoylase (OCT), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL) and arginase (ARG) by previously described methods (Mommsen and Walsh, 1989; Barber and Walsh, 1993).

All biochemicals were purchased from Sigma Chemical Co (St Louis, MO) and all other chemicals were reagent grade. Values were tested for significance at the 0.05 level using an unpaired Students' *t*-test or analysis of variance (ANOVA) (Zar, 1974). For excretion measurements, *N* is the number of tubs, but for amino acid and enzyme activity measurements *N* is the number of fish.

Results

Confinement of toadfish in small water volumes (series I) decreased total nitrogen excretion during the first 24 h (Fig. 1A). This decrease was preceded by an initial pulse of nitrogen release in the first 3 h, probably representing an initial voiding of the bladder (Fig. 1A). However, beginning in the 24–48 h flux period (Fig. 1A), and continuing to 168 h (Fig. 1A,B), nitrogen excretion increased markedly, with the majority as urea. During these measurements, water ammonia concentration reached a maximum of only approximately 15 μmol l^{-1} at the end of each 24 h period (Fig. 1C).

The confined-static water treatment gave rise to clear metabolic effects. First, the activity of several enzymes involved in urea synthesis increased in the liver (Table 1). One enzyme within the ornithine–urea cycle showed a modest increase in activity (OCT)

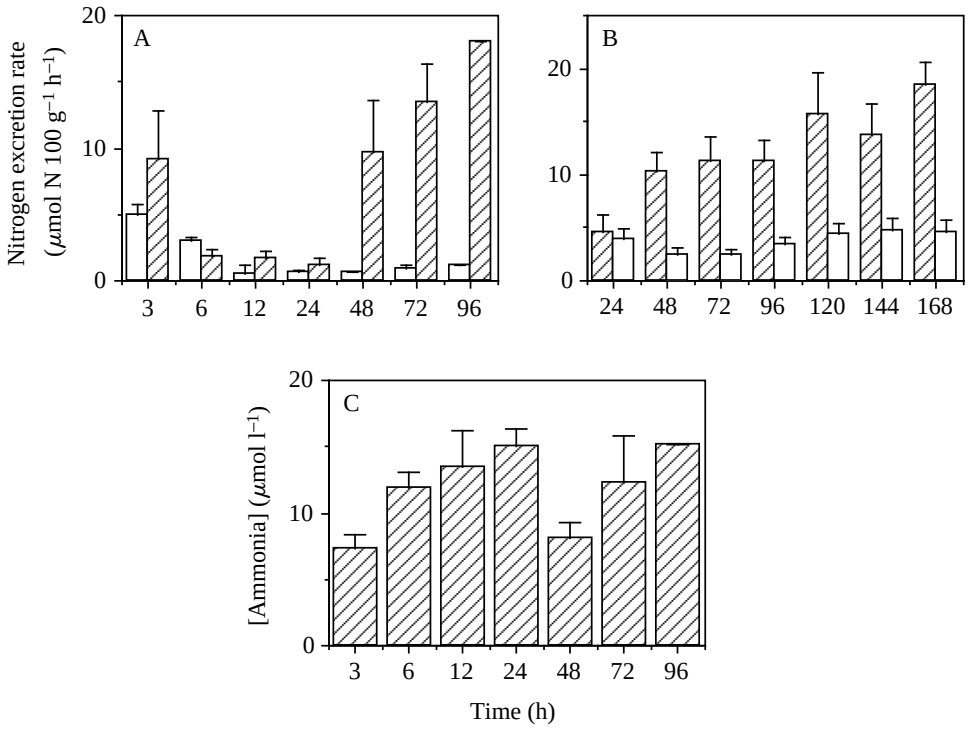

Fig. 1. Series I. (A) Plot of nitrogen excretion rate (ammonia-N, open bars; urea-N, hatched bars) in toadfish *versus* time after transfer to confined-static water conditions. Times note the end of a flux period (e.g. 3 h means the 0–3 h time period). (B) Plot of nitrogen excretion (ammonia-N, open bars; urea-N, hatched bars) in toadfish *versus* time after transfer to confined-static water conditions for longer periods. (C) Actual [ammonia] in the tubs at the end of the flux period. Note that the water was changed every 24 h. Values are means + 1 S.E.M. (N=3–6 replicate tubs with three fish per tub, except 96 h point in A, which is one tub). Error bars are not visible in the 24 and 48 h ammonia excretion values.

and the two enzymes which feed nitrogen into the pathway (AspAT and GNS) showed increases. The effect of the treatment on GNS activity is particularly pronounced (a 3.6-fold increase), and the activation is completed by 24 h and persists for the remainder of the 96 h treatment (Fig. 2). The activation by 24 h precedes the onset of enhanced urea excretion in the 24–48 h flux period (Fig. 1A), indicating a possible delay for synthesis, transport to the bladder and excretion from the bladder (as suggested by Walsh *et al.* 1990). Also noteworthy is the non-significant trend towards increased activity in AlaAT (P=0.07, Table 1).

The confined-static treatment led to modest declines in plasma concentrations of three amino acids (asparagine, glutamate and glycine) and a modest increase in one amino acid (valine) (Table 2). The treatment also led to a significant decline in liver [alanine] and a significant increase in liver [arginine] (Table 3); however, the significant increase in [arginine] results from only three out of six individuals having arginine levels above the detection limits. Interestingly, glutamine was not detectable in liver homogenates by the

Table 1. *Effects of confined-static water treatment (series I) on activities of ornithine–urea cycle and related enzymes for selected tissues of the toadfish*

Tissue Enzyme	Experimental treatment Control	 96 h confined-static
Kidney		
AlaAT	6.18±1.59	4.23±1.17
AspAT	28.57±7.58	29.73±5.74
GDH	14.58±3.88	16.67±2.83
Brain		
AlaAT	0.94±0.26	1.45±0.34
AspAT	31.74±3.80	31.74±4.03
GDH	1.76±0.51	1.60±0.34
GNS	22.55±3.14	19.67±3.46
Liver		
AlaAt	32.86±4.99	45.61±3.80
AspAT	42.79±4.84	66.76±6.61*
GDH	42.55±11.21	45.45±3.18
GNS	3.21±0.63	11.45±2.29*
CPS	0.16±0.02	0.17±0.03
ASS	0.22±0.13	0.15±0.10
ASL	0.51±0.05	0.61±0.07
OCT	44.34±3.23	61.28±6.75*
ARG	36.92±8.10	51.34±12.1

Units are μmol substrate converted to product min^{-1} g^{-1} wet tissue mass and values are means ± 1 S.E.M. (N = 6 per treatment).

* indicates a value significantly different from control, at $P < 0.05$.

Abbreviations are explained in Materials and methods.

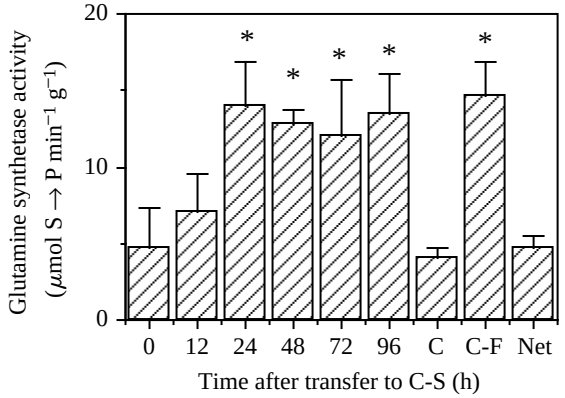

Fig. 2. Plot of liver glutamine synthetase activity for toadfish *versus* time after transfer from control to confined-static water conditions. C is a control group at 96 h, C-F is a group 96 h after transfer from control to confined-flowing water, and Net is a group briefly netted and air-exposed from control conditions and returned to control conditions for 96 h. Values are means + 1 S.E.M. (N=3–6 per group). * indicates a significant difference from C, P<0.05.

Table 2. *Effect of confined-static water treatment (series I) on plasma amino acid concentrations in the toadfish*

	Experimental treatment	
Amino acid	Control	96 h confined-static
Taurine	147.8±35.5	155.6±35.5
Aspartate	48.9±4.7	45.9±8.6
Threonine	142.7±11.8	122.1±31.3
Serine	102.9±7.1	100.2±8.9
Asparagine	59.0±4.6	43.4±3.4*
Glutamate	95.0±2.1	76.8±6.8*
Glutamine	85.9±8.6	71.5±13.4
Proline	34.7±3.3	21.2±7.1
Glycine	170.7±11.3	129.7±11.0*
Alanine	122.3±7.3	109.9±4.4
Citrulline	31.2±11.6	26.8±5.6
Valine	104.1±1.5	120.8±4.3*
Methionine	44.2±2.6	45.3±2.5
Isoleucine	50.8±3.0	58.1±5.1
Leucine	136.7±9.4	158.6±10.5
Tyrosine	54.9±3.2	44.9±3.4
Phenylalanine	51.2±5.6	50.9±3.3
Tryptophan	10.1±0.9	9.7±1.1
Ornithine	310.1±78.6	334.5±32.2
Lysine	325.0±20.9	356.1±27.0
Histidine	43.3±1.2	46.3±3.2
Arginine	ND	ND

Amino acid concentrations are given as μmol l^{-1}.
Values are means ± 1 S.E.M. (N = 4 per treatment).
* indicates a value significantly different from control, $P < 0.05$; ND, not detectable.

two methods applied to both control and confined-static water groups (Table 3). In the enzymatically based method where samples were rapidly neutralised, our recovery of glutamine from spiked liver samples was greater than 85 %. Also noteworthy among the liver amino acid values are the non-significant trends towards decreasing [aspartate] and increasing [asparagine] (P=0.07 and 0.20, respectively, Table 3).

Since the differences between the control and experimental treatments in series I were multifaceted, the changes in urea excretion rates and the underlying metabolic aspects we observed could be due to netting, air-exposure, build-up of waste products (e.g. ammonia) in the water, the neurochemical aspects of physical confinement/crowding, or any combination of these factors. In the second series of experiments (series II), our goal was to pre-condition the toadfish to confinement for 96 h, but with flowing water so as to avoid the complication of elevated waste product build-up. If ureogenesis were already fully activated when the water flow was finally turned off (to make excretion measurements), this result would indicate that confinement, and not highly elevated amounts of waste products in the environment, was the key stimulus. We netted and transferred toadfish to the tubs used for confined-static water conditions, but supplied

Table 3. *Effect of confined-static water treatment (series I) on liver amino acid concentrations in the toadfish*

	Experimental treatment	
Amino acid	Control	96 h confined-static
Taurine	16877.7±1912.1	13217.4±1337.0
Aspartate	1097.9±245.5	575.0±174.5
Threonine	771.0±92.4	415.4±114.0
Serine	136.2±31.7	114.9±15.9
Asparagine	495.4±52.2	2259.4±1474.7
Glutamate	28326.2±3663.4	21623.9±4497.0
Glutamine	ND	ND
Proline	ND	ND
Glycine	2886.1±289.9	2201.6±171.9
Alanine	2009.8±360.4	798.4±194.6*
Citrulline	23.8±13.2	59.1±20.8
Valine	26.4±17.5	30.4±18.3
Isoleucine	81.7±7.0	62.2±11.1
Leucine	147.5±14.7	130.0±18.4
Tyrosine	46.4±15.9	23.1±8.6
Phenylalanine	1057.2±255.3	1035.3±120.3
Ornithine	1199.2±495.4	1144.3±195.7
Lysine	795.1±91.0	777.6±97.6
Histidine	584.9±37.9	568.2±26.2
Arginine	ND	79.2±40.2*

Amino acid concentrations are given as μmol g^{-1} fresh mass.
Values are means ± 1 S.E.M. (N = 6 per treatment).
* indicates a value significantly different from control, $P < 0.05$; ND, not detectable.

them with flowing water for 96 h; in other words, they were pre-conditioned. The fish were then subjected to confined-static conditions by turning off the water flow, without physical transfer. The control group for this series first stayed in control conditions for 96 h and was then transferred by netting and air-exposure to confined-static conditions at the same time that water flow to the experimental group was turned off. Urea and ammonia excretion and liver glutamine synthetase activity (as an index of ureogenesis) were measured in both groups. Toadfish pre-exposed to confined-flowing water conditions showed enhanced urea excretion rates in the first 24 h measurement period (Fig. 3A), which were comparable to the rates achieved by confined-static fish only after 48 h following transfer from control conditions in both this series (Fig. 3A) and series I (Fig. 1B). The rate of urea excretion in the fish pre-conditioned to confined-flow conditions continued to increase (Fig. 3A), and ammonia excretion rates remained elevated while controls showed a decrease with time (Fig. 3B). Liver glutamine synthetase rates were elevated in fish after 96 h of exposure to confined-flow conditions (Fig. 2, C-F) and exhibited a time course of activation similar to that of the confined-static group (results not shown). Thus, the enhanced ability to excrete urea is again correlated with elevated liver GNS activity.

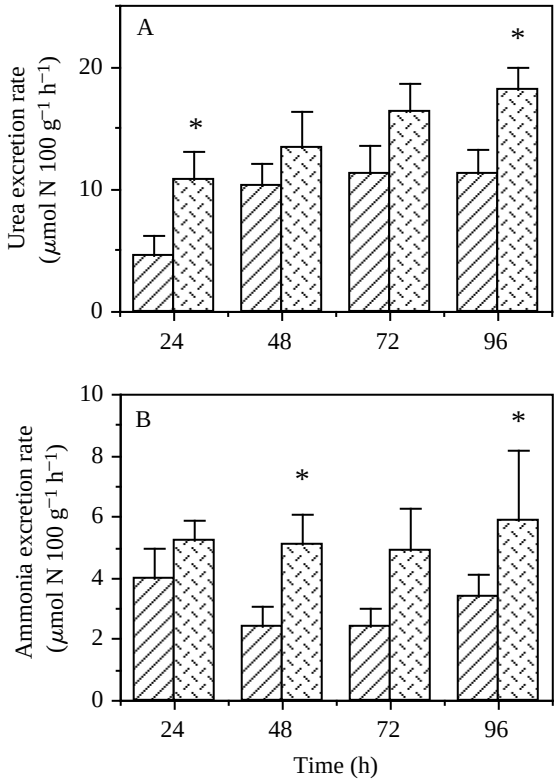

Fig. 3. Plot of urea excretion rate (A) and ammonia excretion rate (B) in toadfish *versus* time after transfer to confined-static water conditions from control conditions (hatched) or from confined-flowing water conditions (stippled). Values are means + 1 S.E.M. (N=6 replicate tubs per treatment). * indicates a significant difference between the groups, P<0.05.

The experiments in series I and II did not rule out the possibility that activation of liver GNS activity and enhanced urea excretion could be the result of netting and/or air-exposure. In series III, control toadfish were netted and air-exposed as during a typical transfer, but returned to control conditions. This treatment did not cause an elevation of GNS activity after 96 h (Fig. 2, Net). Also in this series, toadfish were transferred to confined-static conditions without air-exposure, and water changes were accomplished by flushing, rather than by transfer; under these conditions, liver GNS values were 13.29±2.07 μmol g^{-1} (N=5), equivalent to those observed in the other treatments that enhanced GNS activity (Fig. 2) and urea excretion rates. These results taken together allow us to conclude that the conditions of physical confinement and crowding enhance rates of ureogenesis.

Discussion

Prior studies of ureogenesis in the gulf toadfish have demonstrated that exposure to air or to elevated NH_4Cl levels in the laboratory can enhance rates of urea excretion (Walsh

et al. 1990). Although these treatments might be useful tools for activating ureogenesis, it is not clear how often toadfish experience these conditions in nature and whether these treatments would elicit underlying biochemical changes in a biologically relevant way. Gulf toadfish spend up to several weeks in confined water volumes while breeding and caring for young (Breeder, 1941), and they have been observed to occur in small volumes of water (similar to the confinement conditions in the present study) and, more rarely, to undergo air-exposure during low tides (personal observations of T. E. Hopkins for the West Coast of Florida; personal observations of D. Gleeson, Gulf Specimen Marine Laboratories, Panacea, FL, for the Florida Panhandle), although we know of no useful water ammonia data in this regard. The present study suggests that the cues of increased water ammonia level or air-exposure may be secondary to the cue of confinement in activating ureogenesis in the toadfish in nature in that (1) confinement to small volumes of water would presumably happen in advance of ammonia build-up or air-exposure, and (2) substantial ammonia build-up was not necessary to activate ureogenesis (Figs 1C, 2A). Our results suggest that a classic stress response may be important in activating ureogenesis, but this interpretation should be considered as speculative for three reasons. First, although confinement and crowding conditions normally associated with eliciting the stress response in fish (Barton and Iwama, 1991) clearly activated ureogenesis, we have yet to examine in detail the hormonal (e.g. cortisol) basis of this response. Second, although some of our treatments involved minimal intervention, very different patterns of nitrogen metabolism and excretion are possible in toadfish which *choose* to enter (and possibly defend) confined spaces rather than those that have the condition imposed externally, as in the present study (and in most studies of stress in fish). Also important in this context is the possibility that there may have been chemical communication between the crowded fish in our experiments. Although we are confident that ammonia accumulations in control and confined-flow treatments were roughly equivalent and below detectable limits, other water-borne substances may have been differentially exuded or retained in the two treatments. Lastly, this study focused on basal nitrogen excretion in unfed fish and it cannot be assumed that the patterns observed apply to fed fish, where total nitrogen excretion will be much higher. The observed transition to ureogenesis and the possibility of a stress response fit well with our prior observation of two patterns of response of nitrogen excretion to air-exposure in toadfish (Walsh *et al.* 1990). Clearly, detailed studies on the precise behavioral cues that trigger ureogenesis, and any allied stress response, during voluntary and solitary confinement in the toadfish will be a fruitful line of further investigation.

The activation of ureogenesis by crowding/confinement in the present study was accompanied by several biochemical changes which allow us to draw preliminary conclusions regarding sites of regulation. Generally the activation of urea production under these circumstances appears primarily to be through changes in nitrogen supply upstream of the actual ornithine–urea cycle. Only a modest increase in one cycle enzyme (OCT) was seen in our treatments, but the activities of the enzymes of the two reactions which feed nitrogen to the pathway (AspAT and GNS) increased, that of GNS markedly so. Plasma and liver amino acids levels appear to reflect this upstream strategy as well. In terms of aspartate supply to the pathway, there is an increase in the ratio of liver to

plasma asparagine, which may indicate its use for the production of aspartate *via* asparaginase. However, the most noticeable increase in aspartate production appears to be *via* AspAT. The nitrogen donor for this reaction, glutamate, is present in very high titers in the liver. Glutamate, which is also a substrate for the GNS reaction, is probably supplied mostly by glutamate dehydrogenase, which uses free ammonia to aminate α-ketoglutarate from the tricarboxylic acid cycle (TCA) cycle. However, it may be partly supplied by plasma glutamate (which showed a significant decline) and by AlaAT, which uses oxaloacetate from the TCA cycle and alanine (which also showed a significant decline in liver levels) as a nitrogen donor. The changes in glycine and valine concentrations may be related to roles in priming the TCA cycle at the level of pyruvate and succinylCoA, respectively, bearing in mind that free ammonia generated by these substrates would also be a source of nitrogen for the GDH reaction. The increased liver arginine concentration may reflect incomplete adaptation of the pathway at the level of arginase or at the level of a transporter that may enable arginine to enter the mitochondria for use by mitochondrial arginase. This conclusion is further supported by the observation that only half the confined-static individuals showed measurable quantities of hepatic arginine. However, recent studies suggest that these treatments increase arginase activity in both cytoplasmic and mitochondrial compartments (P. M. Anderson and P. J. Walsh, unpublished data), so it is possible that both compartments play a role in the generation of urea.

Finally, the observation of very low liver glutamine levels is rather interesting. Taken together with the large increase in GNS activity, the exceptionally large pool of the substrate for GNS, glutamate, and the lack of change in CPS activity, these observations make it tempting to conclude that GNS rapidly channels glutamine to CPS; verification of this suggestion must await verification of non-detectable glutamine by additional methods and, specifically, measurements of *intramitochondrial* levels of glutamine and *N*-acetylglutamate (a potent activator of CPS). However, these observations point towards GNS as a key regulatory or rate-limiting site for ureogenesis in the toadfish. In addition to the observations of the present study (1) glutamine synthetase is inducible by dexamethasone (Mommsen *et al.* 1992), (2) there is a lack of metabolic zonation of glutamine synthetase (and urea synthesis) within the liver of toadfish (Mommsen and Walsh, 1991), consistent with a flux-generating role rather than as a downstream scavenging role as is seen in mammals (Jungermann and Katz, 1989), and (3) glutamine is the likely *in vivo* substrate for CPS III in the toadfish (Mommsen and Walsh, 1991; P. M. Anderson and P. J. Walsh, unpublished data). The mechanisms of the rapid activation of GNS are an interesting avenue for further research. Purified enzyme studies should be performed to determine whether the K_m for ammonia is low, as in elasmobranch GNS (Shankar and Anderson, 1985). If this were the case for the toadfish, the enzyme would typically be saturated with both glutamate and ammonia, and its main mode of activation would probably be through an increase in the number of GNS molecules, a facet which would make the ureogenic toadfish an excellent model system for future studies of the molecular mechanisms of activation of ureogenesis.

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