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Response of the hammerhead shark olfactory epithelium to amino acid stimuli

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Abstract Sharks and rays are highly sensitive to chemical stimuli in their natural environment but several hypotheses predict that hammerhead sharks, with their expanded head and enlarged olfactory epithelium, have particularly acute olfactory systems. We used the electro-olfactogram (EOG) technique to compare the relative response of the scalloped hammerhead shark (*Sphyrna lewini*) olfactory epithelium to 20 proteinogenic amino acids and determine the sensitivity for 6 amino acids. At micromolar concentrations, cysteine evoked the greatest EOG response which was approximately twice as large as that of alanine. The weakest response was obtained for proline followed by aspartic acid and isoleucine. The olfactory epithelium showed adaptation to sequential stimulation, and recovery was related to the inter-stimulus time period. Estimated EOG response thresholds were in the sub-nanomolar range for both alanine (9.2×10^{-11} M) and cysteine (8.4×10^{-10} M) and in the micromolar range for proline and serine. These thresholds from 10^{-10} to 10^{-6} M for the scalloped hammerhead shark are comparable or lower than those reported for other teleost and elasmobranch species. Future work should focus on binary and more complex compounds to test for competition and cross-adaptation for different classes of peripheral receptors, and their responses to molecules found in biologically relevant stimuli.

Keywords Amino acid · Chemoreception · EOG · Hammerhead shark · Olfactory epithelium

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Introduction

Soluble chemical stimuli provide environmental cues or signals for the detection and location of prey, predators, and mates in aquatic environments. In jawed fishes, olfaction is mediated by a sensory epithelium that responds to dissolved substances that flow through the nares and across chemosensory receptors that respond to several forms of organic compounds, especially amino acids and nucleotides that are common constituents of natural prey. Additional compounds such as steroids and prostaglandins are also involved in chemical mediation of social and reproductive behaviors (Hara 1992; Sorenson 1992).

Olfaction in sharks is an important sense used in the detection and localization of prey. The sensory epithelium consists of microvillous sensory and ciliated support cells that form the superficial layer of the olfactory rosettes contained within large bilateral olfactory cavities on the ventral surface of the snout (Kleerekoper 1978; Zeiske et al. 1987). In carcharhinid sharks, primary water flow over the rosette is produced passively by the pressure difference between the incurrent and excurrent nares during forward motion of swimming. Behavioral studies show that

occlusion of the olfactory nares eliminates the ability to locate an odor source (Parker 1914). Olfactory stimuli evoke a klinotaxis behavior in the nurse shark where the animal searches across the stimulus gradient to locate the source, whereas lemon sharks presented with a point-source chemical stimulant respond with accelerated swimming and a strong rheotaxis behavior to the strongest water current (Mathewson and Hodgson 1972; Hodgson and Mathewson 1978; Kleerekoper 1978). Differential injection of chemical stimuli into the nares can mediate orientation behaviors that vary in stagnant or flowing water (Johnsen and Teeter 1985). These behavioral experiments indicate the importance of olfaction for source localization, the detection of differential concentrations by the nares, and also the existence of environment-specific behavioral responses to chemical stimuli. However, the behavioral response thresholds to dissolved concentrations of specific amino acids in the wild are not known.

Knowledge of the physiological response properties of the elasmobranch olfactory epithelium is also incomplete. Previous studies on neural responses to chemical substances focused on the evoked potential electro-olfactogram (EOG) from the receptors or evoked neuron potentials in the olfactory bulb and forebrain (Gilbert et al. 1964; Mathewson and Hodgson 1972; Hodgson and Mathewson 1978; Silver 1979). These studies characterized the features of neural excitation to olfactory stimulation but the relative response to different classes of amino acids is reported only for two elasmobranch species. In addition, EOG thresholds in elasmobranchs are known only for two proteinogenic amino acids. Previously reported response thresholds to methionine were between 10^{-7} and 10^{-8} M for a single lemon shark, *N. brevirostris*, and $10^{-7.4}$ M in the Atlantic stingray, *Dasyatis sabina*; and for alanine at $10^{-7.8}$ M in *D. sabina* (Silver et al. 1976; Silver 1979; Zeiske et al. 1986). However, lower threshold responses to amino acid stimuli were reported for single cells in the olfactory tract of the black skate (Nikonov et al. 1990).

Hammerhead sharks (Carcharhiniformes, Sphyrnidae) have a dorso-ventrally compressed head morphology in which the nasal capsules are laterally expanded within a wing-like 'cephalofoil' that may confer several advantages for resolving odor gradients and chemical sources (Kajiura et al. 2005). First, it is generally accepted that the widely spaced incurrent nares of hammerhead sharks may enhance their ability to directly localize odor gradients (Hasler 1957; Tester 1963; Nelson 1969) by simultaneous sampling of odorant concentrations that may mediate tropotactic orientation. A second potential olfactory advantage is that the broad incurrent nares and long prenarial grooves may augment the volume of water sampled and presumably increase the probability of odorant detection (Tester 1963, Kajiura et al. 2005). Finally, the expansion of the cephalofoil has resulted in large nasal capsules that contain elongated nasal organs (Gilbert 1967; Compagno 1984, 1988) and provide a correspondingly large population of chemosensory receptor cells. Thus, knowledge about physiological responses of the olfactory epithelium to a wide range of amino acid stimuli are needed to assess the absolute and relative sensitivities of the olfactory organ in the hammerhead shark relative to other elasmobranch species.

We performed initial tests on the hypothesis that the hammerhead olfactory epithelium is more sensitive to amino acid odorants than that of other elasmobranch taxa. The standard EOG technique was used to quantify the physiological response of juvenile scalloped hammerhead sharks, *Sphyrna lewini*, for comparison with published studies on two other species. Our primary goals were to characterize olfactory responses to the full suite of proteinogenic amino acid stimulants and to determine the detection threshold concentration for a subset of relevant amino acids. This study confirms the variation in olfactory response to different amino acids among elasmobranch species and also a stimulus response threshold for the hammerhead shark in the 10^{-11} M range, the lowest yet reported for an elasmobranch fish.

Methods

Shark capture and handling

Female adult scalloped hammerhead sharks, *Sphyrna lewini*, give birth to young in protected inshore waters of Kaneohe Bay, Hawaii and juveniles remain in this nursery area for 1–2 years before migrating to open coastal waters (Duncan and Holland 2006). For this study, we collected 22 juvenile sharks with a total body length between 53 and 67 cm by hand line fishing with barbless hook in Kaneohe Bay. Fish were transferred to a hemispherical tank and transported to the Hawaii Institute of Marine Biology where they were either used immediately or maintained for up to 4 days in a 2.5 m diameter tank supplied with flow through seawater. Captured sharks were fed a maintenance diet of fresh thawed squid on alternate days.

Experimental apparatus

Electro-olfactogram responses to amino acid stimuli were recorded on sharks in a $16'' \times 30'' \times 8''$ deep acrylic tank supplied with flow through seawater. Anesthetized sharks (see below for protocol) were inverted and mounted on a soft neoprene-covered rack with a center groove for the dorsal fin. The shark cephalofoil, trunk and caudal peduncle were secured to the rack with straps. Inflow water

was both mechanically (25 μm polyscreen) and chemically (activated charcoal) filtered prior to entering the tank, and distributed through a three channel manifold with each channel controlled by a separate ball valve. One channel provided respiratory ventilation by water perfused into the mouth (sealed with a neoprene bite grip) that expanded the branchial chamber and maintained a constant flow of water through all gill slits. A second channel provided the main water flow through the tank which was continuously drained to reduce ambient metabolites and olfactory stimulants in the surrounding water. The third channel provided the fresh seawater source that flowed into the left olfactory capsule. Amino acid stimulants were delivered to the naris by injection into a port on the incurrent surgical tubing that was encased within a stiff vinyl tube to minimize mechanical vibration. The delivery of a constant stream of fresh seawater to and past the olfactory epithelium was confirmed by injection of dye into the injection port and observation of a stream from the excurrent medial opening of the naris. Fresh seawater used for naris flushes and pre-stimulus trials was extracted from the incurrent port with a large syringe.

Experimental protocol

Test subjects were placed in a 0.8 m diameter acrylic hemisphere containing an anesthetic seawater solution of MS222 (1:25,000–1:10,000 wt:vol) until sedate and then irrigated with anesthetic for an additional 2 min. The shark was then immobilized by injection of pancuronium bromide (0.1–0.4 mg) into the dorsal musculature, removed from the anesthetic bath, secured in the experimental tank and perfused through the mouth with single-pass fresh seawater. The odor delivery pipette was mounted in a micromanipulator and positioned into the lateral margin of the left incurrent naris at a standard position relative to the olfactory epithelium. Water flow into the incurrent naris was regulated at 2 cc s^{-1} based on natural flow estimates from the product of cross-sectional area of the incurrent naris (0.2 cm^2) and a swimming velocity of 10 cm s^{-1}. The recording electrode consisted of an Ag–AgCl wire held inside a glass pipette with bent tip and was inserted into the naris medial to the stimulus delivery pipette. A second similar reference electrode was positioned on the skin or in the right naris. Source signals were differentially amplified (Warner DP301) at 10,000×, band passed at DC-1000 Hz and notch filtered through a 60 Hz noise eliminator (Humbug). Conditioned signals were digitized on a CED 1401 running under Spike2 software and stored on a computer. Heart rate was recorded by EKG throughout the experiments to monitor for stress and condition. Animals were ventilated with fresh flowing seawater on the bench for 1 h before initiation of an experiment.

The dilution factor was determined for injected stimulant solutions following transport to the olfactory epithelium. The tubing between the injection port and pipette tip in the incurrent naris had a seawater volume of 15 cc. A 0.5 cc volume of fast green dye was injected at the same rate as test substances (0.25 cc s^{-1}), a series of 0.5 cc samples of the diluted dye collected at the pipette tip, and their diluted concentrations determined on a spectrophotometer. Replicate tests showed that the stimulus at the olfactory epithelium was diluted to 4.1% of that injected. All stimulus concentrations are thus reported as that delivered at the opening of the stimulus delivery pipette (e.g. a peak concentration of 40 μM at the incurrent naris from injection of a 10^{-3} M test solution).

Stock solutions in filtered seawater (10^{-3} M) were prepared weekly and stored at 4°C. We measured pH for 1 mM concentrations of all test amino acids and confirmed that pH of stock solutions (7.1–8.4) were similar to that of seawater (7.1–7.9). Test concentration dilutions were made daily and kept in a water bath at the same temperature as the inflowing water. Preliminary trials were run to determine a standard stimulus injection volume for our experiments. EOG responses to 0.1, 0.25, 0.5 and 1.0 cc volumes of 10^{-3} M alanine showed a strong linear relationship between injection volume (amount of stimulant) and EOG response (EOG (peak mV) = 4.40 × [10^{-3} M alanine (cc)] + 0.69, $R^2 = 0.98$, $p = 0.011$). This indicates that more receptors are likely recruited by the addition of more stimulant and are not saturated within this linear range. From these tests, we chose a standard injection volume of 0.5 ml to be delivered over a period of 2 s (0.25 cc s^{-1}) to control for this volume-dependent effect.

Our experimental procedures were very similar to previous EOG studies on other elasmobranchs (Silver et al. 1976; Silver 1979; Zeiske et al. 1986) and we attempted to minimize adaptation and cross-adaptation of the sensory epithelium due to the serial application of amino acid stimulants. We empirically determined a standardized stimulant delivery time interval by presentation of seven consecutive applications of 4 × 10^{-4} M alanine at intervals of 1, 5 and 10 min. These trials revealed a strong adaptation effect that followed the first presentation and persisted for periods >10 min. Recovery was approximately 50% of the first stimulus response for 1 min application intervals but improved to about 70–80% of the initial response magnitude for the 5- and 10-min inter-stimulus periods. Therefore, in order to reduce the effects of stimulus adaptation in our sequential applications but maximize the number of tests performed on each fish, we chose a standard inter-stimulus interval of 5 min.

The relative dose responses to 20 proteinogenic L-isomer amino acid solutions were determined. After acclimatization of the subject in the test tank, 0.5 cc of fast

green dye was injected into the flow stream to confirm water flow through the olfactory capsule and out the excurrent flap of the olfactory capsule, followed by a rapid clearing flush of 5 cc seawater. A control stimulus of 0.5 cc of fresh seawater was then delivered. This was then followed by the first injection of the 10^{-3} M alanine (diluted to 40 μM at the naris) to provide a standard response for comparison with other stimulants. After recovery of the EOG waveform, the olfactory capsule was again flushed by a vigorous injection of 5 cc of seawater at the injection port. The sequence of application of the 20 amino acids and their concentrations were randomized. Each test series was ended with a second injection of the alanine standard to confirm that the responsiveness of the sensory system had been maintained. We also determined concentration responses for the most and least stimulating amino acids by recording EOG response to concentrations ranging from 4×10^{-1} to 4×10^{-11} M delivered at the olfactory epithelium.

Analysis

The relative sensitivity of the olfactory epithelium to each amino acid was estimated by its proportionate response to that of the alanine standard. The reference response to alanine was calculated as the average of the first and final tests for each animal. The relative response to other amino acids was calculated as the ratio of their response to alanine. The average response to each amino acid was determined for each animal, the median calculated among individuals and the amino acid responses ranked. Response data were compared by the Kruskal–Wallace non-parametric test on ranks followed by a post hoc Dunn's test for paired comparisons. This latter test used a critical p value of 0.008 to discriminate differences among the 20 amino acids tested.

EOG stimulus threshold levels were estimated for both high- and low-potency amino acid stimulants. The responses to each test concentration were averaged for each shark to avoid pseudoreplication, and then the mean response for each concentration determined among individuals. The dose–response parameters for each amino acid were estimated by fit of the data to a power function and log–log plotted. At stimulus concentrations greater than 4×10^{-4} M, the response curves sometimes flattened (or accelerated possibly due to pH affects for some amino acids). For example, the EOG responses to alanine at 4×10^{-3} and 4×10^{-2} M did not differ indicating probable saturation of chemoreceptors at the higher concentration. At low stimulus concentrations, the minimum threshold detectable in our apparatus was limited by background noise and brief, weak transient potentials associated with injections at the port. Therefore, data from test concentrations delivered at the naris of $>4 \times 10^{-4}$ M and those that evoked responses below the average noise floor recorded for the amino acid were not used for calculation of the dose–response regressions. Threshold concentrations were estimated for the intersection of the dose–response curve and mean response to injection of pre-stimulus seawater (noise floor) as done in previous studies (Silver et al. 1976; Silver 1979). We also confirmed EOG responses as they neared these estimated threshold levels.

Results

The hammerhead EOG is similar to that reported for other elasmobranchs and characterized by a negative potential that reached peak magnitude within a few seconds of excitation (Fig. 1 inset) followed by a slower recovery that could last for several minutes at high stimulus concentrations. The peak response varied with position of the recording electrode near the incurrent naris and distance from the sensory epithelium. However, this technique showed reasonable repeatability among individuals (e.g. $\bar{x} = 149.9 \pm 69.5$ SE μV for a 40 μM alanine stimulus, $n = 10$ sharks). A slow recovery to pre-stimulus levels is typical in this flow through technique (Silver et al. 1976; Silver 1979; Zeiske et al. 1986) that may be due to retention of the stimulus within the capsule due to slow water flow and perfusion across the deep sensory lamellae.

The EOG technique revealed distinct differences in the response of the olfactory epithelium to amino acid stimulants. Figure 1 shows representative traces of the EOG response to 40 μM alanine, cysteine, proline and the seawater control. The magnitude of the response varied dramatically among amino acids with the greatest response magnitudes observed for cysteine and alanine. A large magnitude response required a proportionally longer time for the trace to return to baseline levels. The seawater control treatment elicited either no measurable response or several μV above background.

The median responses of the olfactory epithelium to the 20 amino acid stimulants show great variation in relative sensitivity at a 40 μM stimulus concentration (Fig. 1). The greatest response was evoked by cysteine which was approximately twice that of second ranked alanine (alanine: $\bar{x} = 199.9$ μV ± 123.2; cysteine: $\bar{x} = 432.1 \pm 287.4$ μV, paired t test, two tail $p = 0.024$, $df = 8$). All other amino acids showed response values with glycine, arginine, tryptophan, leucine, valine, isoleucine, aspartic acid and proline at less than or equal to half that of alanine. The post hoc analysis also shows this latter group elicited a response lower than that of the higher ranked stimulants (Kruskal–Wallis, $H = 99.120$, $p < 0.0001$, 19df; Dunn's test).

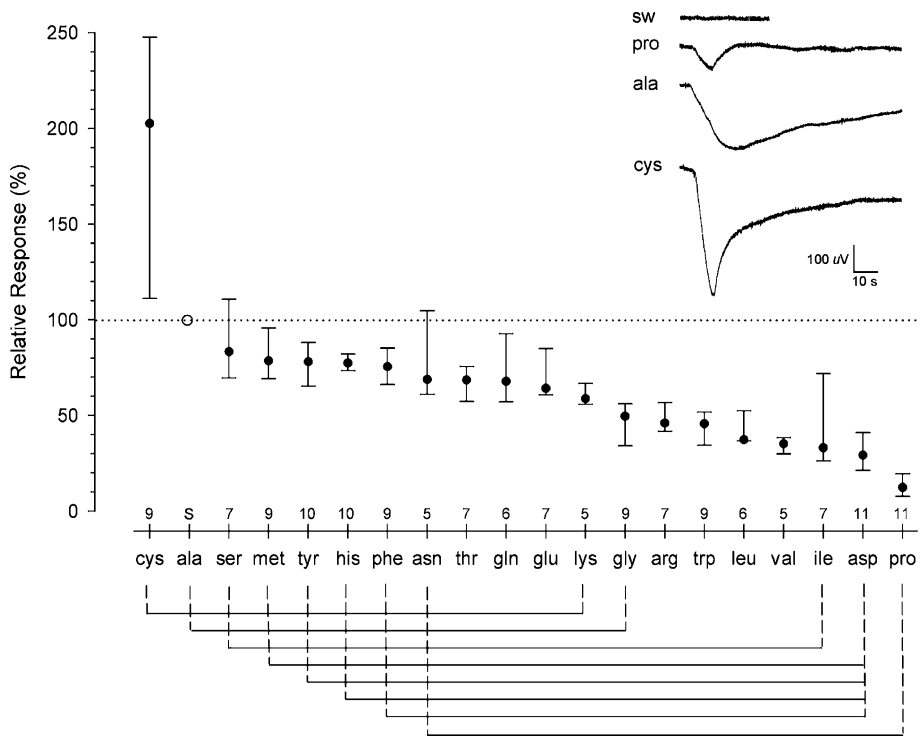

Fig. 1 Electro-olfactogram responses to 20 amino acids by the olfactory epithelium of the scalloped hammerhead shark, *Sphyrna lewini*. Responses are shown as medians and quartiles and are ranked with respect to the alanine standard. *Horizontal bars* show groups of stimulants that did not differ in rank (Kruskal–Wallace test on ranks followed by the post hoc Dunn's method for paired comparisons). Cysteine elicited the strongest response. Amino acids with responses ≤50% of alanine fall within a group (glycine through proline) that showed a lower response than the group of higher ranked stimulants (cysteine through lysine). All stimuli were delivered at 40 μM concentrations at the entrance to the naris. *Numbers* indicate number of sharks tested for each amino acid. Traces (*inset*) show representative responses to applications of a seawater control and representative amino acids. *cys* cysteine, *ala* alanine, *ser* serine, *met* methionine, *tyr* tyrosine, *his* histidine, *phe* phenylalanine, *asn* asparagine, *thr* threonine, *gln* glutamine, *glu* glutamic acid, *lys* lysine, *gly* glycine, *arg* arginine, *trp* tryptophan, *leu* leucine, *val* valine, *ile* isoleucine, *asp* aspartic acid, *pro* proline, *sw* fresh seawater

Dose–response functions were determined for alanine, the high-ranked (cysteine, serine, methionine and histidine) and the low-ranked (proline and aspartic acid) amino acids. Response thresholds were estimated for the intercept of each regression with the average response to the respective seawater control. Peak response levels to seawater controls ranged from 15.1 to 22.8 μV with a mean of 17.6 μV (Table 1) and are averaged to show the mean noise floor in Fig. 2. Based on the regression intercepts with their respective noise floor value, alanine showed the lowest response threshold at 9.2×10^{-11} M, which is very near the 4×10^{-11} M response confirmed for some individuals (Table 1). Similar matches or slight differences between estimated and observed thresholds were found for cysteine (8.35×10^{-10} M), methionine (1.36×10^{-8} M), proline and serine. However, the estimated threshold for aspartic acid was approximately two orders of magnitude below the lowest observed at 4×10^{-6} M, thus we defer to the observed response threshold for this compound. The regression slopes shown in Fig. 2 are not parallel (ANCOVA, $df = 6$, $F = 2.40$, $p < 0.05$) thus the relative responses are concentration dependent. For example, at high test concentrations, cysteine evoked a greater response than alanine, but the responses converged at lower concentrations. In addition, the responses of alanine and methionine were equivalent at high test concentrations of 4×10^{-4} M but diverged at lower stimulus concentrations (Fig. 2).

Discussion

Soluble amino acids are widely distributed in marine environments and involved in numerous metabolic processes of aquatic organisms. In shark olfaction these compounds are best associated with the detection and localization of prey, but may also function as social or reproductive signals as demonstrated for other fishes (see Hara 1994 for review). Thus, information on the relative excitability of amino acids to the shark olfactory system provides important insight into their relative efficacy as putative environmental signals and cues.

Table 1 EOG dose–response functions and estimated olfactory epithelium thresholds for amino acid stimulants in the scalloped hammerhead shark, *Sphyrna lewini*

Amino acid	EOG regression	R^2	N	Noise floor average (μV)	Estimated threshold (M)	Min observed response (M)
Alanine	$y = 676.3x^{0.157}$	0.91	10	18.1	9.20×10^{-11}	4×10^{-11}
Aspartic acid	$y = 236.4x^{0.152}$	0.99	5	19.2	6.37×10^{-8}	4×10^{-6}
Cysteine	$y = 3{,}748.0x^{0.266}$	0.95	6	14.5	8.35×10^{-10}	4×10^{-11}
Histidine	$y = 545.1x^{0.158}$	0.93	3	15.5	NC	4×10^{-8}
Methionine	$y = 2{,}221.2x^{0.276}$	0.95	5	15.1	1.36×10^{-8}	4×10^{-8}
Proline	$y = 178.9x^{0.163}$	0.97	6	22.8	3.28×10^{-6}	4×10^{-5}
Serine	$y = 17{,}695x^{0.448}$	0.98	5	18.4	2.16×10^{-7}	4×10^{-8}

Power function regressions were calculated from peak response of the sensory epithelium to different concentrations of the amino acid stimulant. Response thresholds were estimated at the intersection of the EOG regression and the corresponding average response to seawater controls (noise floor). Note that lowest thresholds were found for alanine and cysteine. Thresholds for histidine were not calculated (*NC*) because we lacked sufficient data at low concentrations to extrapolate to seawater control noise levels. N = number of sharks tested for each peptide. Dose–response regression equations show EOG response (y = μV) and dose (x = molar concentration). Noise floor average was determined from the small peak response to injections of seawater controls and is indicated for each amino acid. The minimum test concentration that evoked a response above noise for at least one individual is also shown

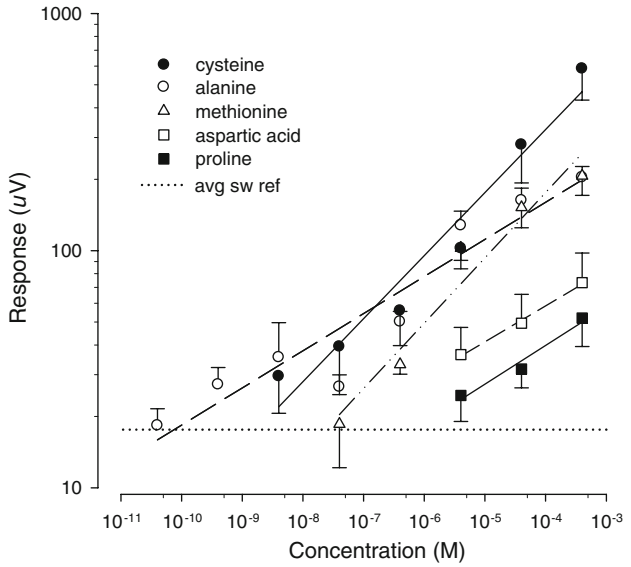

Fig. 2 Electro-olfactogram dose–response curves for a sample population of hammerhead sharks for five high- and low-ranking amino acids. The dose concentrations reflect the maximum delivered at the entrance to the naris. Note that at higher concentrations cysteine evoked the strongest EOG response but that several amino acids converge in excitability at lower concentrations with alanine showing the lowest threshold at 9.2×10^{-11} M

The EOG response

Like other fishes, the hammerhead EOG is characterized by a rapid negative peak potential followed by a slower recovery phase. The slow EOG recovery observed in our study (and others) may be due partially to retention of the stimulant in the capsule due to low rate of turnover and correspondingly low rate of perfusion across the deeper epithelium. We made efforts to enhance recovery from sensory adaptation to residual amino acids by use of the peak response, randomized order of amino acid delivery and concentrations followed by continuous sea water flow, long inter-stimulus intervals and vigorous seawater washes. In addition, it is assumed that the concentration-dependent EOG as measured near the lateral margin of the olfactory epithelium (our recording site) is a good indicator of the relative response of the receptor population across the sensory rosette. Further work is needed to determine the distribution of different receptor types and their response and time course of stimulation across the olfactory epithelium.

Relative amino acid sensitivity

As reported for other cartilaginous fishes, the peak EOG responses in the hammerhead shark varied among amino acid stimulants but showed a different rank response pattern. For example, at 40 μM concentrations cysteine elicited the greatest response that was approximately twice that for alanine, and showed greater differences at higher concentrations (Fig. 1). In contrast, the response to cysteine in the Atlantic stingray was only 61% of that for alanine (Silver 1979) and of "intermediate" effectiveness relative to alanine in the lemon shark (Zeiske et al. 1986). Furthermore, the observed high variability in response to cysteine compared to other amino acids may be due to fixed or transient factors, such as sex, age or hormonal condition among individuals, but these remain to be tested. The proximate mechanism for the strong excitation of the hammerhead shark epithelium by cysteine is unknown, but may be related to its relatively free and reactive sulfur group. The only other tested amino acid that contains a sulfur group is methionine, in which the sulfur is more closely bound. In addition, methionine and alanine were more effective stimulants than cysteine in the lemon shark

(Zeiske et al. 1986), but both evoked lower responses than cysteine in the hammerhead shark. Serine, another potent amino acid for the hammerhead shark, is also a neutral, short-chain amino acid like cysteine and alanine. The response to serine in the Atlantic stingray was 112% that of alanine (Silver 1979) but less (83%) in our hammerhead study.

The least stimulatory imino/amino acids in the hammerhead shark were proline, aspartic acid, isoleucine, valine, and leucine all of which elicited a median response of less than 50% of alanine. Of note, proline was the lowest ranked L-isomer amino acid for the Atlantic stingray (Silver 1979), a "relatively ineffective" amino acid for the lemon shark, *Negaprion brevirostris* (Zeiske et al. 1986), and the least stimulatory for the hammerhead shark in this study. Proline, isoleucine, valine and leucine vary in side chain structure but are hydrophobic, non-polar, non-charged molecules. These latter characteristics are shared by alanine, which has a methyl side group, and was much more excitatory in the hammerhead shark. In contrast to the other low-ranking amino acids, aspartic acid is polar and negatively charged; characteristics it shares with the intermediately ranked glutamic acid. Some of the least stimulatory (e.g. leucine, valine and isoleucine) are neutral long-chain amino acids. The most excitatory amino acids for the lemon shark olfactory epithelium were categorized as L-alpha amino acids with long, neutral side chains (Zeiske et al. 1986). This does not appear to be the case for the hammerhead shark, as the three most stimulatory amino acids (cysteine, alanine, and serine) are all neutral short chain.

The results from the present and previous two studies above indicate that there is no single or combination of molecule characteristics that can be used to predict the relative stimulation of the elasmobranch sensory epithelium by different amino acids. The robust response of the hammerhead olfactory epithelium to cysteine may be explained by cysteine-specific or non-specific receptors in the recording region of the olfactory epithelium. Amino acid olfactory receptors are not yet characterized for elasmobranch fishes, but are described for several teleosts (reviewed in Michel 2006). Recent EOG experiments using cross-adaptation and other protocols indicate the existence of at least three classes of amino acid receptors (cysteine, arginine and glutamate) in the trout (Hara 2005). These techniques could be applied to elasmobranch fishes to compare epithelial response properties among amino acid stimulants and elasmobranch species. More work is necessary to determine the proximate mechanisms that generate these differences in field potentials produced by the sensory epithelium, such as receptor type and density, receptor cross-reactivity and adaptation as observed in teleosts (Hara 2005; Michel 2006).

Amino acid EOG thresholds

The lowest estimated thresholds for the hammerhead shark sample population were 9.2×10^{-11} M for alanine and 8.4×10^{-10} M for cysteine, with confirmed excitatory responses to 4×10^{-11} M solutions for both amino acids in some individuals. The hammerhead threshold for alanine is well below the $10^{-7.8}$ M threshold reported for the Atlantic stingray (Silver et al. 1976; Silver 1979). No thresholds are published for cysteine in other elasmobranch fishes but the response was much less than that of alanine at a 10^{-4} M test concentration in the Atlantic stingray (Silver 1979). The 1.4×10^{-8} M response threshold for methionine in the hammerhead was similar to the 10^{-8}–10^{-7} M range reported for a single lemon shark (Zeiske et al. 1986) and $10^{-7.4}$ ($=3.9 \times 10^{-8}$) M reported for the Atlantic stingray (Silver 1979). This hammerhead threshold range of about 10^{-10}–10^{-6} (Table 1) is also below the 10^{-9}–10^{-7} M lower limit reported for the catfish olfactory epithelium (Caprio 1978). Thus, overall the hammerhead shows different response ranks and similar or lower threshold ranges to amino acid stimuli compared to several teleosts and the few studied elasmobranch fish (Hara 1992). However, although the greater observed sensitivity of the scalloped hammerhead shark may result from different properties of the sensory epithelium, it is also important to consider the different experimental techniques and factors such as sample size, individual variation and the background level of amino acids in the ambient seawater that can also affect apparent threshold (Caprio 1982). More comparative data are needed using the same experimental methods to confirm that the scalloped hammerhead olfactory epithelium has a sensitivity to amino acids that is approximately one order of magnitude lower than other elasmobranch taxa. In addition, future work should test for competition and cross-adaptation of binary and more complex compounds in peripheral receptors, and their response to molecules found in biologically relevant stimuli.

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