Do crocodiles co-opt their sense of “touch” to “taste”? A possible new type of vertebrate sensory organ

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Abstract. We recount here two experiments carried out which suggest the existence of the first described integumentary osmoreceptor of its kind in a vertebrate. Domed pressure receptors, present on the cranial scales of alligators have previously been demonstrated to convey the sensation of “touch” when flattened by pressure. Here we find that morphologically similar domed sensory organs present on the post-cranial scales of crocodylid but not alligatorid crocodilians flatten when exposed to increased osmotic pressure, such as that experienced when swimming in sea water hyper-osmotic to the body fluids. When contact between the integument and the surrounding sea water solution is blocked, crocodiles are found to lose their ability to discriminate salinities. We propose that the flattening of the sensory organ in hyper-osmotic sea water is sensed by the animal as “touch”, but interpreted as chemical information about its surroundings.

Introduction

Sensory organs are essential for the survival of animals, providing information about the surrounding environment, both external and internal. The sensation of “touch” (mechanoreception), and “taste” (chemoreception) are two important senses served by two different major types of sensory organ: mechanoreceptors, in which the physical pressure of touch causes a change in the shape of the sensory organ, stimulating a nerve impulse, and chemoreceptors, such as taste buds, in which cell surface molecules recognise a particular chemical, inducing a chemical change in the chemosensory cell which is interpreted as information about that specific chemical (Liem et al., 2001).

But between mechanoreception and chemoreception there exists a grey area: osmoreception. First described by Verney (1946, 1947), osmoreceptors sense the osmolality of a surrounding solution, thus providing chemical information, but the mechanism by which the osmolality is sensed is mechanical: osmoreceptors are cells which expand or shrink in response to changes in the osmotic gradient between them and their surroundings. In vertebrates, osmoreceptors are groups of specialised nerve cells, located in various places in the circulatory system, where they sense changes in plasma osmolality, thus allowing hormonal regulation of NaCl in the blood (Sibbald et al., 1988; Oliet and Bourque, 1993; Bourque et al., 1994; Wells, 1998; Voisin and Bourque, 2002). Here we describe what we believe may be an entirely new type of vertebrate sensory organ: an osmoreceptor on the integument.

Animals inhabiting marine or estuarine environments must cope with the hyper-osmotic or fluctuating osmolality by either osmoconforming or osmoregulating. Like all tetrapod vertebrates, the Estuarine Crocodile, Crocodylus porosus (Meyer, 1795; Crocodylidae) osmoregulates (Taplin, 1988). It maintains a plasma osmolality of approximately 300 mOsm kg\(^{-1}\) (Mazzotti and Dunson, 1984; Taplin, 1984) while living in coastal waters in which the salinity may range from occasionally available fresh or hypo-osmotic sea water to full strength (35 ppt) sea water. C. porosus possesses a suite of adaptations which allow it to reduce osmotic water loss while immersed in hyper-osmotic sea water.

Interesting to the evolutionary biologist, these adaptations appear to be phylogenetically conservative traits, representing differences between crocodylid and alligatorid crocodilians,
as opposed to being specific attributes of \textit{C. porosus} (reviewed below). Although some caution must be exercised in making generalisations from studies of individual species, comparative physiological studies across crocodilian taxa have found that of the two main families of living crocodilians, the crocodylids (crocodiles) and the alligatorids (alligators and caimans), major physiological differences exist at the family level in ability to tolerate estuarine conditions. All crocodylids studied (including freshwater species) are morphologically adapted for hyper-osmotic conditions, while all alligatorids lack these adaptations. These adaptations and the geographical distribution of living crocodilians have been interpreted as good evidence in support of a marine phase in an ancestor of living crocodylids (e.g. Taplin and Grigg, 1989). Estuarine adaptations possessed by Crocodylids, and absent in alligatorids, include reduced integumental permeability (reviewed by Taplin, 1988), heavy keratinisation of the buccal epithelium (Taplin and Grigg, 1989), and lingual salt-secreting glands (Taplin and Grigg, 1981). An additional mechanism used is that of behavioural osmoregulation: the animal drinks opportunistically when the surrounding water is hypo-osmotic to its body fluids and avoids drinking when it is hyper-osmotic. Crocodylids, even freshwater species with no experience of sea water, will avoid drinking hyper-osmotic sea water, while freshwater alligatorids will freely drink full sea water, even to the point of death (Bentley and Schmidt-Nielsen, 1965; Mazzotti and Dunson, 1984; Lauren, 1985; Jackson et al., 1996a) (but see Discussion).

Here we explore a further morphological difference between crocodylid and alligatorid crocodilians: the distribution of sense organs on the integument. The study here presented investigates the possibility that there may a connection between sensory organs present on the integument of crocodylids but not alligatorids, and the capacity for sensation of sea water osmolality and thus behavioural osmoregulation.  

The study of sensory organs on the integument of crocodilians has been subject to great confusion as a result of inconsistent terminology. Two distinct types of integumentary sense organs exist: (1) facial mechanoreceptors, present in all crocodilians, and (2) sense organs present on the post-cranial integument in crocodylids and gavialids, but absent in alligatorids. The facial sense organs were determined on the basis of detailed morphological study (von During, 1973, 1974; von During and Miller, 1979) and physiological study (Soares, 2002) to be mechanosensory in function. These facial mechanoreceptors appear to the naked eye as small, black dots, present several per scale on all the cranial scales. Magnified they are seen to be dome-shaped protuberances, innervated beneath by branches of the trigeminal nerve. It is presumed that deformation of the dome by touch is perceived as mechanosensation.

The post-cranial sense organs were not always known to be sensory organs. These have received much attention because they are of phylogenetic interest, as they are absent in alligatorids. These were once thought to possibly be secretory pores (King and Brazaitis, 1971; Wermuth, 1978; Brazaitis, 1987; Grigg and Gans, 1993) or alternatively sensory structures. Brazaitis (1987) proposed the term “integumentary sense organs (ISOs)”, a term that stuck.

Jackson et al. (1996b) studied the morphology of the ISOs of \textit{C. porosus}, in detail, and found that the ISOs very much resemble von During’s facial mechanoreceptors (“touch papillae”) in structure, except that they are approximately 300% the size of the former, present only one per scale (rarely as many as three), and presumably innervated by spinal nerves rather than trigeminally (fig. 1). They concluded that the ISOs were definitely sense organs, but refrained, in the absence of physiological data, to commit themselves to mechanosensation as their function.

Soares (2002) generated terminological confusion by lumping together the two types of
sense organ under the single term “domed pressure receptors” (DPRs). Using “DPRs” as a blanket term for both crocodilian facial touch papillae and for post-cranial crocodylid/gavialid ISOs makes it impossible to discuss the two as possibly different types of sense organ. Since that is exactly what we propose to do here, we will modify Soares’ terminology further, dividing it into “facial DPRs” and “post-cranial DPRs” (“pcDPRs”).

The present study consisted of two experiments. The first tested the hypothesis that in *C. porosus*, sensory information from the post-cranial sense organs is necessary for behavourial osmoregulation to occur. The second experiment tested the hypothesis that an individual post-cranial sense organ could react mechanically to osmotic changes in its surroundings, providing a mechanism by which the pcDPR could function as an integumentary osmoreceptor (fig. 2).

Materials and methods

*Experiment #1*

This experiment was designed to test the hypothesis that crocodiles rely on sensory information from their skin to determine the salinity of the water in which they are swimming. The experiment was carried out at the Long Kuan Hung Crocodile Farm in Singapore, in an outdoor shed exposed to outdoor temperatures (approx. 30°C). Two groups of six one year-old *C. porosus* were kept out of the water until their body mass was reduced by 10% due to water loss (a quantifiable way of stimulating thirst). Mean mass of animals in the control group was 676 g ± 13.4 sem. Mean mass of the animals in the experimental group was 701 g ± 32.9 sem. (An unpaired, two-tailed t-test shows no significant difference in mass between the two groups, *p* = 0.5002). This drying out period took 2-3 days, and the animals were kept in the dark, in silence, in opaque plastic bins with lids in order to minimise stress. The animals in the experimental group were then coated with petroleum jelly, weighed, and allowed to swim in 35 ppt seawater for 15 min, after which they were weighed to determine whether any water had been ingested. They were then moved to fresh water and weighed again after 15 min exposure. The same protocol was followed with the control group except without the petroleum jelly. For the experimental group, the increase in mass following exposure to sea water was compared with the increase in mass following exposure to fresh water, using a paired t-test. The control group was tested in the same way.

*Experiment #2*

This experiment was designed to test the hypothesis that the height of the domed pcDPRs increases in fresh water, and decreases in seawater or other hyperosmotic solutions. The animal used was a one yr old *C. porosus* (total length 72 cm), housed under laboratory conditions at the University of Toronto, with water and air temperatures of 27°C and a 12:12 light:dark light cycle. The enclosure included swimming and basking areas, and the animal was maintained on a diet of neonate mice and live minnows.

Four tanks were prepared, deep enough for the crocodile to float in. Two were fresh water (dechlorinated reverse-osmosis tap water), while the other two held the experimental solutions. The first of these was 1000 mOsm (mOsm = mosmol·kg water⁻¹) (~35 ppt) seawater, mixed from Instant Ocean™ powder. The second was a solution of sucrose, iso-osmotic to the seawater solution (1000 mOsm).
Figure 2. A diagrammatic explanation of the hypothetical mechanism of osmosensation proposed here. In (a), as has been demonstrated experimentally, a cranial DPR responds to touch by flattening, thus transducing a nerve impulse. In (b) we propose that a pcDPR flattens similarly but in response to exposure to a hyper-osmotic sea water solution (full-strength sea water: 35 ppt). In this scenario the flattening of the dome generates a nerve impulse as for the mechanosensory cranial DPR, but this signal is interpreted by the animal as chemical information.

Osmolality of both solutions was verified by freezing point depression (Advanced InstrumentsTM micro-osmometer, model 3MO).

An area approximately 3 cm² of ventral scales on the crocodile was outlined in permanent marker. The crocodile was left in the fresh water tank for one hour. The animal was then removed from the water and held firmly ventral surface up, as quickly as possible. A layer of the light-bodied, low viscosity impression material, ReprosilTM hydrophilic vinyl polysiloxane impression material (light body) (Jackson, 1997) was immediately applied to the area of scales outlined, allowed to cure to a rubbery consistency for 7 min. The layer of PVS was then peeled off, having formed a high-resolution impression of each outlined scale and of each pcDPR. The crocodile was then left in the seawater tank for 10 minutes, and the procedure was repeated, covering exactly the same outlined area of scales. The animal was moved back to fresh water for a period of 10 min, and a cast was made of the outlined scales after their second time in fresh water. Finally the crocodile was made to swim in a tank of sucrose solution for ten minutes and a cast was made of the outlined scales.

The PVS impressions of the ventral skin were cut into individual scales. Each individual scale impression from the fresh water (1), sea water, fresh water (2), and sucrose solutions, was matched up so that the pcDPR of an identical individual scale was compared after exposure to all four solutions. The PVS impressions were used with light microscopy as a circular depression in the PVS cast. In order to make a “positive” copy from the “negative” created by the PVS
impression, each impression was filled using a hypodermic needle, with a droplet of Spurr’s low viscosity embedding medium (firm mixture) (Bozzola and Russel, 1992), which was allowed to cure for 24 hrs at a temperature of 70°C. This procedure produced a rigid, transparent, high-resolution cast of the scale and the pcDPR. Each cast was mounted with double-sided tape to a scanning electron microscope (SEM) stub, grounded with carbon glue, sputter-coated with gold, and examined using a Hitachi H-7000 SEM, at an acceleration voltage of 20 kV.

Examined with the SEM, each cast was tilted until the pcDPR cast appeared as a domed structure in lateral view. It was impossible to be certain that the cast in the image was perfectly in side-view, making height measurements of the dome unreliable. To overcome this problem, an initial side view was photographed, and then the stub was rotated and two more images captured at 120° angles from one another. The mean of these three measurements was used as the height of dome measurement. Measurements of dome height were made using Image JTM software.

Heights of ISO casts were compared using a series of paired t-tests, to determine whether the height of the domes changed following exposure to the three solutions: (1) The two fresh water groups, (2) the sea water and the sucrose groups, (3) the first fresh water group and the sea water group, and (4) the second fresh water group and the sucrose.

Results

Experiment #1

In the control group a paired t-test showed a significantly greater mass increase following exposure to fresh water as opposed to sea water (p = 0.0469, t = 2.266, 10 degrees freedom), indicating that the animals drank no sea water, but did drink when moved to fresh water, replicating the results of numerous other studies of crocodylid drinking behaviour (reviewed in Introduction). In the experimental group, by contrast, an increase in mass was observed following exposure to sea water as well as to fresh water, and a paired t-test revealed no significant difference between the mass increase in the two solutions (p = 0.3572, t = 1.014, 5 degrees freedom). This result indicated that the crocodiles drank fresh water and full sea water indiscriminately when the integument was coated with petroleum jelly (fig. 3). The (not significant) difference in the amount of fresh water ingested by animals in the two groups is thought to result from the experimental animals having partially filled their stomachs with sea water prior to exposure to fresh water.

Experiment #2

Measurements were made of casts from n = 10 individual pcDPRs, exposed to four experimental conditions: fresh water (first time), sea water, fresh water (second time), and sucrose solution. The resulting four columns of values were subjected to a series of paired t-tests. A comparison of the pcDPR dome height in following exposure to the two fresh water solutions found no significant difference (P = 0.5682, t = 0.59, df = 9). Similarly, a comparison of the sea water with the sucrose solution found no significant difference (P = 0.66, t = 0.46, df = 9). Comparisons of fresh water (first time) with sea water, and of fresh water (second time) with sucrose solution both revealed very significant differences (P = 0.0028, t = 4.07, df = 9 and P = 0.0021, t = 4.26, df = 9, respectively) (fig. 4). This result indicated that the domed pcDPR becomes flattened when exposed to hyper-osmotic solutions. There was no difference in the degree of flattening induced by exposure to hyper-osmotic solutions of two different solutes.

Discussion

Comparison of this osmoreceptor with others

Von Seckendorff Hoff and Hillyard (1993) and Nagai et al. (1999) in their studies of behavioural osmoregulation in the toad, Bufo punctatus (Baird and Girard, 1852; Bufonidae), concluded, based on trials of solutions of NaCl, urea, and amiloride in various combinations, that although the toads were detecting changes in NaCl osmolality, the mechanism by which this was accomplished was partially osmosensory, but also with a chemosensory component. This scenario is the only vertebrate example similar to the osmoreceptors described here that we are aware of. The ecological function (avoiding uptake of hyperosmotic NaCl solutions) is
Figure 3. Amount drunk, as inferred from mass increase, (mean ± sem) by crocodiles exposed to sea water (SW) and to fresh water (FW). Animals in the experimental group have the integument coated with petroleum jelly. Animals in the control group do not. (n = 6 in each group, mean mass = 688 g).

Figure 4. Height of casts of pcDPRs (mean ± sem) following exposure to fresh water, hyper-osmotic sea water, fresh water a second time, and hyper-osmotic sucrose solution. No significant difference between the two fresh water solutions (p > 0.5); No significant difference between sucrose and sea water solutions (p > 0.6); Significant difference between fresh water (first time) and sea water (p < 0.01); Significant difference between fresh water (second time) and sucrose (p < 0.01).

comparable, although the physiological mechanism is different.

The pcDPRs investigated in the present study differ greatly from previously described osmoreceptors. In location and function they are integumentary and sense the external environment. In structure they are enormous (approx 300 µm in diameter in a 1-m long crocodile).
They are multicellular structures as opposed to single, specialised neurons. In mechanism, they respond equally to solutions of sea water and sucrose. Surveying the literature, we have failed to find any sensory organ like them. We are not aware of other amniotes that have chemo- or osmo-sensory organs on the integument (Liem et al., 2001). We propose here to dispense with the cumbersome term “pcDPRs” in favour of the more descriptive “integumentary osmoreceptors” or “IOs”. Thus the cranial mechanoreceptors present in both crocodylids and alligatorids need a new name to replace “DPRs” that will distinguish them from the IOs. We propose reverting to Von During’s term, “touch papillae”.

Implications for crocodilian phylogeny

To date, studies of sensory organs in crocodilians have been restricted to crocodylids and alligatorids. The physiology of gavialids, sister-group to the latter two lineages is poorly studied. Several reasons account for this omission. The living Gavialidae are represented by only one (Brochu, 2003) or possibly two (Harshman et al., 2003) species worldwide: the gharial, *Gavialis gangeticus* (Gray, 1831; Gavialidae), and possibly also the “false” gharial, *Tomistoma schlegelii* (Müller, 1838; Crocodylidae), traditionally thought to be a crocodylid. Both species are restricted to South Asia in distribution and are endangered species. They are poorly represented in live collections, and are not captive-bred outside of India, all these factors making them relatively inaccessible to comparative physiologists. However, a further reason, which ought not to be overlooked, is the temptation by crocodilian physiologists to assume that what is true of the single species they have studied in detail, is applicable to all crocodilian species. Given the significant differences in many physiological adaptations to hyper-osmotic environments found between alligatorids and crocodylids (or at least those species from these two groups which have been studied!), it may be rash to make assumptions about the physiology of gavialids, which are the group the most distantly related to all other crocodilians.

With that caution in mind, structures appearing to the naked eye to be IOs like those of crocodylids, are present on the post-cranial scales of gavialids (K. Jackson, pers. obs.). Looking at the phylogenetic relationships among the living crocodilian families, two equally parsimonious, and equally puzzling hypotheses account for the phylogenetic distribution of IOs: Either IOs have been derived independently in crocodylid and gavialid lineages, or, the presence of IOs represents the plesiomorphic condition for modern crocodilians, and these structures have been secondarily lost in alligatorids. Detailed morphological and physiological study of the putative IOs of gavialids have the potential to resolve this question.

Sensing and interpreting

A further puzzle to be solved is the capacity for behavioural osmoregulation by estuarine populations of the alligatorid, *A. mississippiensis*, observed by Jackson et al. (1996a). Although all alligatorids of freshwater origin studied (including freshwater populations of *A. mississippiensis*) have been found to drink fresh water and hyper-osmotic sea water indiscriminately, as described in the Introduction, Jackson et al. (1996a) found hatchling alligators from a coastal population in Georgia, USA, to discriminate salinities and to avoid drinking 35 ppt sea water. To discriminate salinities, these individuals, which were morphologically indistinguishable from individuals from freshwater populations, must possess a mechanism to taste NaCl, and an instinct to avoid drinking hyperosmotic solutions of it.

Very similar to the above scenario for *A. mississippiensis*, differences in capacity for behavioural osmoregulation have been demonstrated between coastal and freshwater populations of snakes, *Nerodia* (Linnaeus, 1758; Colubridae) (Dunson, 1980) and turtles, *Chelydra* (Linnaeus, 1758; Chelydridae) (Dunson, 1986).
In both cases these are the same species with identical sensory organs morphology and physiology. The behavioural differences observed in drinking appear to be instincts evolved recently in populations exposed to estuarine conditions. Juveniles captive-bred in fresh water from parents of estuarine origin retain the capacity to avoid drinking hyperosmotic sea water.

But how are estuarine alligators able to determine the salinity of their surroundings, lacking IOs? An obvious possibility is that they taste NaCl with chemosensory organs on the tongue. Weldon and Ferguson (1993) demonstrated the capacity for lingual chemosensation in A. mississippiensis. However, no other crocodilian species were included in their study. If all crocodilians can sense NaCl lingually, what is the role of the IOs, and why do C. porosus lose their capacity to sense hyper-osmotic salinities when the IOs (but not the tongue) are covered?

Here we propose that a difference in capacity for lingual chemosensation may exist between crocodylids and alligatorids. In crocodylids the surface of the buccal epithelium is heavily coated with keratin (Taplin and Grigg, 1989). This is thought to represent an adaptation to reduce osmotic water loss across the large surface area of the buccal epithelium in a crocodile resting with its jaws open. One possible adaptive scenario is that the keratin coating, though effective at reducing osmotic water loss also had the effect of inhibiting lingual chemosensation. This may have set the stage for the adaptation of other sensory organs, the IOs, to take on the function of providing sensory information about sea water osmolality. Investigations of the capacity for lingual chemosensation in a broader range of crocodilian species may shed light on this question.

The result of our first experiment indicates that C. porosus is unable to behaviourally osmoregulate even with the lingual epithelium intact, when contact between the integument and the surrounding solution is blocked. From this result we can only conclude that either, (a) C. porosus cannot taste NaCl with its tongue, or (b) sensation of hyperosmotic NaCl from the tongue is not interpreted by the crocodile as a signal to avoid drinking. We believe the former scenario most likely to account for the results we observed in C. porosus. We propose a scenario like the latter to account for the drinking of hyper-osmotic sea water by freshwater alligatorids. That is, they sense but do not interpret.

Conclusions

From the results of the above experiments we conclude that C. porosus relies on sensory information from the integument to distinguish fresh water from hyper-osmotic sea water. Investigation of the IO, the only sensory organ present on the post-cranial integument, shows that this domed structure changes shape (flattens) on exposure to hyper-osmotic solutions. Much as flattening of the cranial touch papillae of A. mississippiensis has been shown by Soares (2002) to be sensed by the animal and to be interpreted by the animal as “touch”, we propose that similar shape change in similar structures on the post-cranial integument of C. porosus is similarly sensed, but is interpreted by the animal as chemical information about the osmolality of its surroundings. We predict that the same result will be found in other crocodylid (and possibly gavialid) species. We propose that the IO represents an addition to the arsenal of morphological adaptations to estuarine conditions that crocodylid crocodilians possess. To the best of our knowledge this is the first report of an integumentary osmoreceptor in any tetrapod vertebrate.

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New type of vertebrate sensory organ

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