Control of salt gland activity in the hatchling green sea turtle, *Chelonia mydas*

Abstract We studied the control of salt gland secretion in hatchling *Chelonia mydas*. The threshold salt load to activate salt secretion was between 400 μmol NaCl 100 g bodymass (BM)$^{-1}$ and 600 μmol NaCl 100 g BM$^{-1}$, which caused an increase in plasma sodium concentration of 13% to 19%. Following a salt load of 2700 μmol NaCl 100 g BM$^{-1}$, salt gland secretion commenced in 12 ± 1.3 min and reached maximal secretory concentration within 2–7 min. Maximal secretory rate of a single gland averaged 415 μmol Na 100 g BM$^{-1}$ h$^{-1}$. Plasma sodium concentration and total osmotic concentration after salt loading were significantly higher than pretreatment values within 2 min. Adrenalin (25 μg kg BM$^{-1}$) and the cholinergic agonist methacholine (1 mg kg BM$^{-1}$) inhibited salt gland activity. Atropine (10 mg kg BM$^{-1}$) reversed methacholine inhibition and stimulated salt gland secretion when administered with a subthreshold salt load. Arginine vasotocin produced a transient reduction in sodium secretion by the active gland, while atrial natriuretic factor, vasoactive intestinal peptide and neuropeptide Y had no measurable effect on any aspect of salt gland secretion. Our results demonstrated that secretion of the salt gland in *C. mydas* can be modified by neural and hormonal chemicals in vivo and that the cholinergic and adrenergic stimulation of an exocrine gland do not appear to have the typical, antagonist actions on the chelonian salt gland.

Key words Osmoregulation · Salt glands · Adrenergic · Cholinergic · Chelonian

Abbreviations *Adr* Adrenalin · *ANF* atrial natriuretic factor · *AVT* arginine vasotocin · *BM* bodymass · *MeCh* methacholine · *NPY* neuropeptide Y · *PBS* phosphate buffered saline · *VIP* vasoactive intestinal peptide

Introduction

The salt secreting lacrymal gland of marine turtles provides an efficient route of extrarenal salt removal. In all species of marine turtles examined the gland is capable of elaborating a solution in which the solute fraction is almost entirely sodium chloride at a concentration well above that found in seawater (Schmidt-Nielsen and Fänge 1958; Holmes and McBean 1964; Hudson and Lutz 1985, 1986; Marshall and Cooper 1988). Excess salt ingested with food or through intake of seawater can therefore be excreted with a much smaller water volume than could be achieved via the kidney, which cannot produce hyperosmotic urine. The effectiveness of the salt gland as an avenue for salt removal is demonstrated by unfed hatchling loggerhead turtles, *Caretta caretta*, which can gain mass by actively drinking seawater without increasing plasma sodium concentration over a period of 10–15 days (Bennett et al. 1986).

Activation of the salt gland in green sea turtle hatchlings, *Chelonia mydas*, occurs very rapidly following a salt load, with the sole stimulus for activation appearing to be an elevation of plasma sodium concentration (Marshall and Cooper 1988), unlike the avian salt gland which responds to elevated plasma sodium concentration or osmolarity (Gerstberger and Gray 1993). The first account of a salt gland in a marine turtle by Schmidt-Nielsen and Fänge (1958) reported that salt gland secretion could be initiated by methacholine (dose not reported), a cholinergic, muscarinic agonist. Methacholine causes the salt gland of the euryhaline diamondback terrapin, *Malaclemys terrapin*, to secrete in vivo at a dose of
10 mg kg$^{-1}$ (Dunson 1970) and increases oxygen consumption of dissociated salt gland cells in vitro (Shuttleworth and Thompson 1987). The lingual salt glands of the estuarine crocodile, *Crocodylus porosus*, will secrete following systemic injection of methacholine (Taplin et al. 1982). In birds, salt gland secretion was shown to be stimulated by injection of acetylcholine or methacholine and inhibited by injection of adrenalin (Fänge et al. 1958), suggesting that the autonomic nervous system (ANS) regulates salt gland activity. Histochemical data indicated the presence of both adrenergic and cholinergic innervation (Ellis et al. 1963; Haase and Fourman 1970) of the secretory tubules and vascular elements of salt glands in birds which had been acclimatized to salt water (Ash et al. 1969; Peaker and Linzell 1975). It seemed likely then, that the neural control of secretion in marine turtles was similar to that of marine birds, with cholinergic innervation initiating and maintaining gland secretion. However, the report by Schmidt-Nielsen and Fänge (1958) remains the only instance where cholinergic stimulation was investigated in a strictly marine turtle. The role of adrenergic stimulation on salt gland activity has never been investigated in marine turtles.

In addition to the neural control, a variety of hormones or neuropeptides may also be involved in controlling turtle salt gland function, as occurs in elasmobranch and avian salt glands (reviewed by Greger et al. 1988; Gerstberger and Gray 1993). Holmes and McBean (1964) investigated the action of corticosterone on salt gland activity in hatchling *C. mydas* by administering amphenone B to chemically simulate adrenectomy. Following amphenone B treatment, the salt gland response to a salt load was diminished and replacement therapy with corticosterone restored the response to control levels. However, there was no enhancement of the secretory response by corticosterone in the absence of amphenone B treatment, and secretion following a salt load still occurred at about 50% of control rates, despite injection with amphenone B. No direct effect on salt gland activity was apparent and it is more likely that some long-term, permissive role on gland maintenance and circulation was played by steroid hormones, as was the demonstrated for the duck *Anas platyrhynchos* (Butler 1987).

Peptide hormones or neuropeptides that are implicated in control of the avian and elasmobranch salt glands include arginine vasotocin (AVT, Peaker 1971), atrial natriuretic factor (ANF, Schütz and Gerstberger 1990; Solomon et al. 1985), vasoactive intestinal peptide (VIP, Torchia et al. 1992; Hammel et al. 1980; Gerstberger et al. 1988) and neuropeptide Y (NPY, Silva et al. 1993). The role that these peptides play in the regulation of the sea turtle salt gland has never been investigated.

The purpose of this study was to determine the threshold for activation of the salt gland in hatchling *C. mydas* and the effect of salt loading on salt gland secretion. The response of the salt gland to an experimental salt load was quantified so that the ability of methacholine, adrenalin or various peptides to modify secretion could be investigated. The ability of chemicals to influence the salt gland secretion was examined in two ways: (1) initiation of secretion, and (2) modification of secretion from a gland already secreting. Information regarding the influence of potential modifiers of gland activity is necessary to describe control of the chelonian salt gland.

### Materials and methods

**Animals**

Animals were collected under permits G93/085, G94/080 and G94/561 issued by the Great Barrier Reef Marine Park Authority, Australia. Experiment protocols were examined and approved by The Australian National University Animal Experimentation Ethics Committee and issued permits F.BTZ.18.93 and F.BTZ.47.94.

We collected hatching green sea turtles *C. mydas* (approximately 25–30 g body mass) from the Great Barrier Reef, Australia, as they emerged from the nest and made their way to the sea. We maintained animals and conducted experiments at the Heron Island Research Station, University of Queensland. Hatchlings lived in fibreglass aquaria 1.7 m × 0.7 m, filled to a depth of approximately 15 cm with continuously flowing seawater (approximately 28 °C). Spontaneous feeding typically commenced after about 5 days and thereafter hatchlings were fed shelled raw shrimp daily. We returned hatchlings to the aquarium following experiments and observed them for several days before releasing them to the ocean.

**Collection of samples**

We used 5-μl micropipettes to collect tears directly from the single salt gland excretory duct in the corner of the eye; this technique had been previously employed by Marshall and Cooper (1988). When one end of the micropipette touched the corner of the eye where the secretory duct emerged, tears were drawn up into the pipette as they were secreted and before any evaporation. We calculated the rate of secretion (ml min$^{-1}$) by measuring the time taken to fill the pipette. Collected tears (5 μl) were absorbed onto filter-paper discs and total osmolality (mosmol kg$^{-1}$) measured immediately (Wescor Vapour Pressure Osmometer 5500). The osmometer measures osmotic pressure per kilogram of solution rather than per litre, thus the term ‘osmolality’ (mosmol kg$^{-1}$) is used, not ‘osmolarity’ (mosmol l$^{-1}$). Discs were removed following osmolality measurement, and sealed in Eppendorf tubes for subsequent analysis for sodium and potassium concentration (mmol l$^{-1}$) by atomic absorption spectrophotometry (Varian Techtron) and chloride concentration by ion chromatography ( Dionex QIC analyser). This procedure permitted ion concentration and total osmolality to be measured on the same samples.

We collected blood samples of approximately 0.1 ml from the cervical sinus of hatchlings in heparinised 1.0-ml insulin syringes (Terumo, 27G × 1/2). The cervical sinus is a paired, blood filled cavity lying on either side of the vertebral column and is a suitable location for sampling venous blood (Owens and Ruiz 1980; Bennett 1986). Plasma was separated by centrifugation and frozen immediately at −70 °C for subsequent analysis of sodium concentration (technique described above).

**Experiments**

Generally it is preferable to administer chemicals through a cannulated blood vessel supplying the salt gland; this technique has