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Renal responses to mesotocin in Western painted turtles compared with the antidiuretic response to arginine vasotocin

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Abstract

We have examined the renal responses to the two neurohypophysial peptides mesotocin (MT) and arginine vasotocin (AVT) in Western painted turtles (*Chrysemis picta*). Ureteral urine was collected, after removal of the urinary bladder, to exclude modification by trans-bladder fluxes of ions and or water. Test doses of MT ranged from 5 to 500 ng kg^{-1} i.v. but only the uppermost pharmacological doses of 300 and 500 ng kg^{-1} were followed by even a small reduction in GFR, a renal tubular antidiuresis, and a decrease in percent relative free-water one word clearance. A much lower, physiological, dose of 5 ng kg^{-1} of AVT attenuated significantly the GFR for 30 min and decreased the percent relative free-water clearance until the end of a 150 min observation period. This is the first report of the renal response to MT in a reptile. It was concluded that MT is not a diuretic hormone, as it is in amphibians and that AVT may be the naturally occurring antidiuretic hormone in the Chelonians.

Keywords: Turtle; Kidney; Bladder; Mesotocin; AVT

1. Introduction

Mesotocin (MT) and arginine vasotocin (AVT) are released from the neurohypophysis of lungfish, amphibians, reptiles, and birds. MT is accompanied by oxytocin (OXT) together with AVT in certain reptiles such as the cobra (*Naja naja*) (Pickering, 1967), marine turtles, and grass snakes (Follett, 1967; Perez-Figares et al., 1995). MT continues alone as the second neurohypophysial peptide in marsupials (Metatheria) such as the red kangaroo (*Macropus rufus*), and tammar (*Macropus eugenii*) (Chauvet et al., 1981) and is found together with oxytocin in species such as the Australian brush tailed opossum (*Trichosurus vulpecula*) (Hurpet et al., 1982) and the Australian northern bandicoot (*Isoodon macrourus*) (Acher et al., 1995).

Follett and Heller (1964) first provided pharmacological evidence for the occurrence of MT in lungfish and amphibian pituitary glands. Acher et al. (1964) subsequently extracted the peptide from frog pituitary glands, identified it chemically, and named it mesotocin. At relatively high, non-physiological doses, MT increased the water permeability of toad bladders and frog skins, but only if the quantity of peptide was at least 100 times the amount of AVT required to give the same response (Bentley, 1969). Relatively large doses of MT were required to produce even a weak diuresis in African lungfish (*Protopterus aethiopicus* Owen) (Sawyer, 1970). MT has induced a diuretic effect in nearly all amphibians studied (Pang and Sawyer, 1978; Stiffler et al., 1984). This increased urine flow rate was the direct consequence of an increased glomerular filtration rate (GFR). It has been suggested that in amphibians, water balance is maintained by an interplay between the glomerular diuretic action of MT

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and the antidiuretic action of AVT, which depresses GFR (glomerular antidiuresis) and increases the renal tubular reabsorption of free water (Hartenstein and Stiffler, 1990; Sawyer, 1957; Uranga and Sawyer, 1960). AVT also increased water uptake by the integument (hydroosmotic effect) and urinary bladder (Stiffler et al., 1984) in amphibians, so removal of the urinary bladder in *Chrysemis picta* excluded bladder transport as a complicating factor in assessing renal function in turtles.

AVT reduces the GFR in reptiles (Butler, 1972b; Dantzler, 1967; Dantzler and Braun, 1980) and decreases relative free-water clearance when given in physiological doses. AVT causes a glomerular and tubular antidiuresis in freshwater turtles (Butler, 1972b) and arid-zone agamid lizards(*Ctenophorus ornatus*) (Bradshaw and Bradshaw, 1996). AVT also increases the fractional reabsorption of solutes in reptiles (Butler, 1972b; Dantzler, 1989). Even though the powerful antidiuretic effect of AVT has been demonstrated in aquatic and terrestrial reptiles, virtually nothing is known about the physiology of MT in reptiles.

The objective of this study was to examine the renal responses to a range of doses of MT in an aquatic reptile, the Western painted turtle (*C. picta*) following recent observations that MT is a diuretic hormone in some amphibians. It was also important to measure the renal response to a lower, and possibly more physiological, dose of AVT than 10 ng kg^{-1} which has already been shown to produce a glomerular and tubular antidiuresis in *C. picta* (Butler, 1972b).

2. Materials and methods

2.1. Animals

Female Western painted turtles (*C. picta* belli) weighing 600–800 g were purchased from a commercial supplier in Oshkosh, Wisconsin, shipped to the Department of Zoology and held in plastic tanks supplied with flowing dechlorinated tap water maintained at a depth of 8 in. and a temperature of 15 °C. The light cycle was set at 12L:12D. Wooden platforms allowed the turtles to leave or enter the water. Turtles were starved during the experimental period but were normally fed chopped liver, worms, and lettuce ad libitum. All experimental protocols were approved by the Department of Zoology and University of Toronto Animal Care Committees under license, and performed within guidelines set by the Canadian Council of Animal Care.

2.2. Experimental groups

Turtles were selected randomly from the holding tanks. Six animals were placed in each of three experimental groups: (a) low doses of MT (5, 10, and 50 ng kg^{-1}, i.v.); (b) high doses of MT (100, 200, and 300 ng kg^{-1}, i.v.); and (c) AVT (one dose 5 ng kg^{-1}, i.v.).

2.3. Preparation of turtles for the collection of urine

2.3.1. Removal of the urinary bladder

The urinary bladder was removed using a surgical technique described by Butler (1972a). Urine then drains directly from the ureters with minimal contact with the cloacal epithelium. Otherwise trans-bladder fluxes of water and ions would modify bladder urine and prevent the accurate measurement of renal function. A 10-day post-operative recovery period allowed the turtles to stabilize before experimental observations were started.

2.3.2. Insertion of urine catheters

A plastic catheter (4 mm × 35 mm) was fashioned from a syringe needle guard by cutting off its tip and cutting perforations (5 mm in diameter) along its length. The catheter was inserted into the cloaca and tied in place with a purse-string ligature (size 3-0 surgical silk) after local anesthesia had been induced with 2% lidocaine i.m. Urine drained into a surgeon's finger cot which was tied over the open end of the cloacal catheter.

2.3.3. Insertion of blood catheters

The day before urine collection experiments were to start, (9 days post-operative recovery), a turtle was placed in the supine position and taped to a holding board. The left and right hind limbs were retracted, then PE 20 heparin-filled blood catheters were inserted into the left and right femoral veins following the induction of local anesthesia with 2% lidocaine. The catheters were then tied firmly in place and the incisions were closed with 9 mm stainless steel Michel clips (Clay Adams, Sparkville, NS, USA). Test solutions containing organic compounds or hormones were later injected via the right catheter and blood samples were collected from the left catheter.

2.4. Procedure for measuring creatinine clearance

Each turtle was restrained in the prone position by taping it to a piece of wood measuring 1.25 × 10 × 20 cm. It was given an i.v. injection of 30 ml kg^{-1} of a 2.8% aqueous solution of creatinine, followed by an i.p. injection of 30 ml kg^{-1} of distilled water to increase urine flow rates. A series of 8 or 9 consecutive, 30 min urine samples were started 2 h after the above injections. Each 30 min urine collection was preceded by the withdrawal of a 0.5 ml blood sample. Plasma was stored at −20 °C until plasma creatinine concentrations were measured.