

## Renal Responses of the Chilean Toad, *Calyptocephalella caudiverbera*, and the Mud Puppy, *Necturus maculosus*, to Mesotocin

Intravenous injections of mesotocin resulted in increased urine production in the Chilean toad, *Calyptocephalella caudiverbera*, and the mud puppy, *Necturus maculosus*. At low doses, the diuretic responses increase with the doses of hormone administered, but at high doses the diuretic response is considerably reduced. These findings confirm those reported previously for the bullfrog (P.K.T. Pang and W. H. Sawyer, 1978, *Amer. J. Physiol.* **235**(2), F151-F155). However, this is the first time that such data are reported for an aquatic toad and an aquatic urodele.

The neurohypophyseal hormones arginine vasopressin (AVP) and arginine vasotocin (AVT) have antidiuretic effects on many amphibians (Bentley, 1974). However, there is very little information on the functions of mesotocin (MT), the other neurohypophyseal hormone in lower tetrapods. Jard and Morel (1963) reported that pharmacological doses of MT can elicit a minimal diuresis in the European frog, *Rana esculenta*. These authors suggested that MT antagonized AVT. In some of our recent studies on the bullfrog (*Rana catesbeiana*), MT was found to be diuretic at doses much lower than those used by Jard and Morel. Moreover, this hormone was diuretic in a perfused kidney system in which AVT was absent (Pang and Sawyer, 1978). We feel that MT may be an amphibian diuretic hormone which can be of great physiological significance when the animals remain in the aquatic environment (Pang, 1977). Obviously, it is difficult to draw any conclusions based upon work performed on only one species. We have, therefore, tested the effects of MT on two other aquatic species of amphibians, the Chilean toad, *Calyptocephalella caudiverbera*, and the mud puppy, *Necturus*

*maculosus*. These preliminary data are reported here.

### MATERIALS AND METHODS

Experiments were performed on mixed sexes of Chilean toads (average body weight =  $420 \pm 60.2$  g) and mud puppies (average body weight =  $101.4 \pm 3.7$  g). The Chilean toads, collected by a local supplier near Santiago, Chile, were kept in outdoor tanks with running tap water for at least 3 weeks before use in experiments. The mud puppies, purchased from a commercial supply house in the United States, were kept in aquaria (Aquarium Systems, Inc.) in tap water at 18° for at least 2 weeks before use.

The toads were anesthetized with tricaine methanesulfonate (Finquel, Ayerst). Through a dorsal and a lateral incision, both ureters and the musculocutaneous vein were cannulated according to the method of Uranga and Sawyer (1960). Mud puppies were anesthetized similarly and, through a ventral incision, the ureter and the pulmonary vein were cannulated. The mud puppies were secured on a plastic board, but the toads remained free. The animals were housed in water in individual plastic boxes so that continuous ureteral urine collection and intravenous injections of hormones could be performed without disturbing them. After an overnight period of adaptation to the experimental boxes, control urine output was collected. The various doses of MT were injected into the animals in a random sequence. Subsequent injections of MT were administered only after the urine volume returned to the preinjection level. Following each injection of the hormone, the cannula was washed with 0.1 ml of heparin in 0.6% saline (5 U/ml).

The difference in urine volume between the periods immediately before and after the hormone injection was calculated. The hormone (kindly supplied by Dr. M. Manning), synthetic mesotocin, with 380 U/mg of oxytocic activity diluted in 0.05 M acetic acid and 0.5% chlorobutanol was used in the experiments.

## RESULTS AND DISCUSSION

Figure 1 presents the data from the Chilean toad experiment. Increasing the doses of MT from 50 to 250 ng/kg produced an increasing diuresis in most toads. However, at the highest dose, 500 ng/kg, the magnitude of the response is reduced in all experiments. Figure 2 summarizes the results obtained with the mud puppy. A dose of 1 ng/100 g had no diuretic effect while doses of 5–300 ng/100 g had distinct diuretic effects. Once again, higher doses (500 and 1000 ng/100 g) produced a much reduced effect. Injection of the solvent for the hormone had no effect on urine volume.

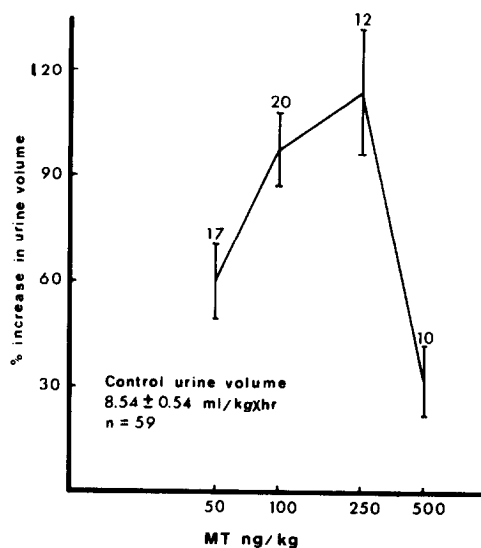


FIG. 1. Effects of increasing doses of MT on percentage changes of urine volume in Chilean toads.

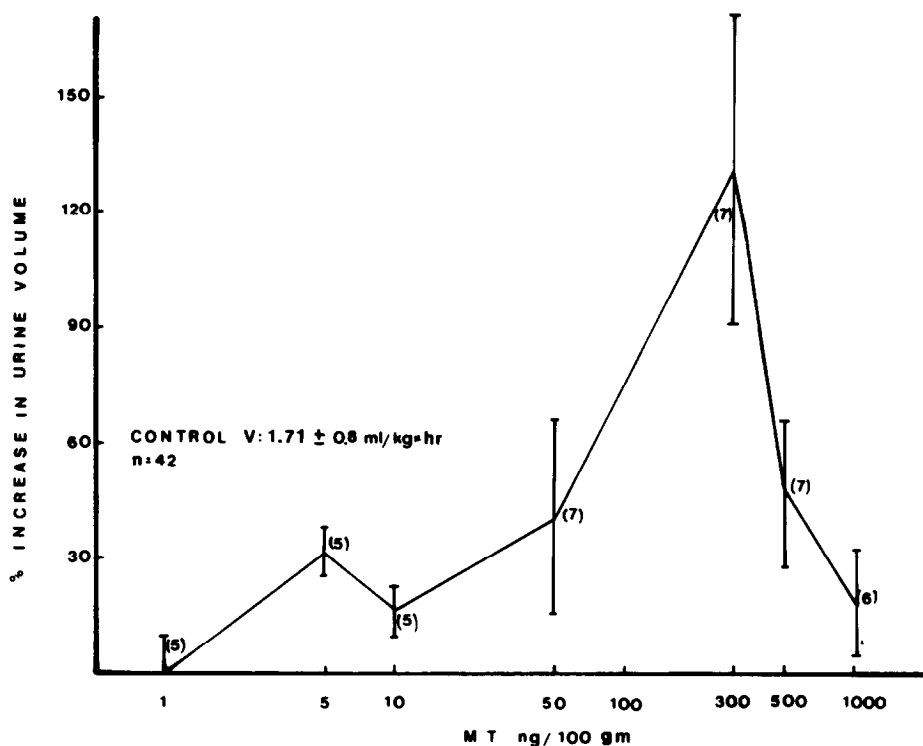


FIG. 2. Effects of increasing doses of MT on percentage changes of urine volume in mud puppies.

Our data clearly indicate that at low doses MT is diuretic. This confirms our previous findings with the bullfrog (Pang, 1977; Pang and Sawyer, 1978). A striking similarity between the present data and those obtained with the bullfrog is the decrease in response at high doses. At times these high doses, which are clearly pharmacological, produced antidiuresis in all three species. It is possible that since MT and AVT have similar molecular structures, MT may, at high doses, mimic the action of AVT. In some amphibian bioassays, MT has the same effect as AVT, although 100–200 times more MT is required to produce these similar effects (Bentley, 1969). On the other hand there may be other explanations for such reversal of effects. This requires further investigations.

In our studies with the perfused bullfrog kidney, we have demonstrated that MT can exert its effect in the absence of AVT and is, therefore, diuretic in itself. This effect is produced mainly by dilating the afferent arterioles of the glomeruli (Pang, 1977; Pang and Sawyer, 1978). Further studies are needed to confirm that MT acts by similar mechanism in Chilean toad and mud puppies.

This is the first time that MT has been shown to produce diuresis in a toad and a urodele. It is also important that all three species studied so far tend to spend their time in water. In the experiments the animals also were tested in water. This can be important physiologically. The three species studied thus far are aquatic and thus MT may function in enabling the animals to rid themselves of excess water.

The dose ranges for similar responses are similar for bullfrog and Chilean toads. However, 10 times higher doses of MT must be used with the mud puppies before the reduction in the diuretic response is ob-

served. This may represent a difference between anurans and urodeles in their response to MT.

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