J. exp. Biol. **142**, 177–189 (1989)
Printed in Great Britain © *The Company of Biologists Limited 1989*

RENAL RESPONSES TO CONTINUOUS AND SHORT-PULSE INFUSION OF ARGININE VASOTOCIN IN THE TOAD *BUFO MARINUS*

BY DANIEL P. TOEWS, MARYSE MALLET AND LAURA MACLATCHY
Acadia University, Wolfville, Nova Scotia, Canada, B0P 1X0

Accepted 12 August 1988

Summary

Chronically catheterized specimens of the toad *Bufo marinus* (L.) were given single bolus injections, periodic bolus injections, and continuous infusions (*via* peristaltic pump) of the neuropeptide arginine vasotocin (AVT). Urine flow and glomerular filtration rates (GFR) were monitored to quantify the antidiuretic response of the toad to AVT under these conditions. The response to single bolus injections was dissipated after 1 h, despite the continued presence of AVT, whereas continuous infusion of AVT at levels as high as 100 ng kg^{-1} was ineffective in lowering flow rates, suggesting that pulsatile increases in the hormone might be required to effect a more prolonged response. In toads which were 'primed' with AVT *via* continuous infusion with the pump, additional pulses of AVT were able to lower flow rates for 1 h, with sensitivity to the hormone decreasing with an increase in primed concentration of AVT. Pulses of AVT given to unprimed toads significantly lowered flow for over 2 h. This study is the first to show that phasic bursts of AVT are more effective in regulating the renal response of the toad than are continuous, nonpulsatile infusions.

Introduction

The following experiments were undertaken to establish the effects of dose-related single bolus injections, and long-term continuous infusion and periodic bolus injections, of the neuropeptide arginine vasotocin (AVT) on the glomerular filtration rates (GFR) and urine flow rates in the toad *Bufo marinus*. An attempt was also made to determine the *in vivo* pulse interval for the release of AVT.

Single bolus injections of AVT were given to estimate the extent and duration of the antidiuretic effect of the hormone in *Bufo marinus*. Long-term infusion experiments were then done in an attempt to mimic the conditions these anurans experience during lengthy periods of dehydration. In preliminary trials, we could not stop urine flow in the long term and we began to wonder whether the nature of hormone release *in vivo* might be more pulsatile, and continuous infusion was overloading receptor sites.

There is some evidence that the release of vasopressin in rats is in response to

Key words: arginine vasotocin, *Bufo marinus*, toads, episodic secretion.

the phasic firing of vasopressin-secreting neurones (Dyball & Leng, 1985; Dyball *et al.* 1985; Dyball & Paterson, 1983; Shaw *et al.* 1984). In animals such as dogs, sheep, horses and humans, where large continuous blood samples can be obtained for vasopressin analysis, there is evidence that the hormone is released in an episodic fashion (Katz *et al.* 1979; Redekopp *et al.* 1986; Weitzman *et al.* 1977).

There is also evidence that if isolated toad bladders are incubated with high concentrations of vasopressin, the bladders are somewhat refractory to further stimulation by lower concentrations of the hormone (Handler & Preston, 1981). Eggena & Ma (1986) suggest that the apparent insensitivity of bladders to intense stimulation by AVT might be caused by a 'downregulation' of the hormone receptors. More recently, Eggena (1987) has shown that the isolated toad bladder responds more readily to short bursts of exposure to AVT. The present experiments were thus designed to compare the effects of bolus injections and continuously injected amounts of AVT on some aspects of renal function in the freely moving but chronically catheterized toad.

Materials and methods

Bufo marinus were obtained from a commercial supplier (Charles D. Sullivan Ltd, Nashville, TN) and were maintained in sand-filled aquaria with access to water. Prior to the experiment, adult animals of either sex were chosen randomly and put in dechlorinated water for 2 days to decrease the blood level of AVT. They were then anaesthetized in a solution of tricaine methane sulphonate ($1{\cdot}5\,g\,l^{-1}$, buffered to pH 7 with $NaHCO_3$). Once anaesthetized, the animals were cannulated in the femoral artery (Boutilier *et al.* 1979*a*,*b*) for blood sampling. Ureters were cannulated for a continuous measure of urine flow using a technique modified from Tufts & Toews (1985): a dorsal incision was made in the skin between the posterior end of the sacrum and the iliac bone, and the underlying muscle was separated to expose the ureter. A cannula was inserted into the ureter and secured 0·5 cm upstream with surgical thread (Ethicon, CE-4). The cannula consisted of a 1 cm segment of polyethylene tubing (PE 90) inserted 0·5 cm into a length of silastic tubing (1 mm i.d.). The protruding tip of the polyethylene tubing was heat flared, this being the end that was inserted into and secured to the ureter. The trailing end of the cannula was then sutured to the skin of the animal posterior to the incision on the dorsal surface, and the incision was sewn shut and sealed with cyanoacrylate glue (no. 7432, Bostik Gmbtt, Oberursel, FRG). Bladders were cannulated with a length of Tygon tubing (0·13 cm i.d.) notched in several places in the terminal 2 cm to facilitate emptying of the bladder and infusion of 'isourine' (Na^+, $2\,mequiv\,l^{-1}$, K^+, $0{\cdot}3\,mequiv\,l^{-1}$, Cl^-, $1{\cdot}7\,mequiv\,l^{-1}$, Ca^{2+}, $0{\cdot}2\,mequiv\,l^{-1}$). A purse-string suture closed the cloaca around the exiting catheter and cyanoacrylate glue was applied to eliminate water entry or urine leakage.

Preparation of the 'minipump' and catheter for continuous infusion

The sciatic vein was catheterized with PE 50 polyethylene tubing in the same

manner as the femoral artery. The vein was used for the continuous infusion of synthetic AVT (Sigma Chemical Co.) with an Alzet mini-osmotic pump (Alza Co., Palo Alto, CA). In a preliminary study, a small mesenteric vein was chosen for infusion as we were concerned that hormone infused into the posterior circulation might enter the renal portal system and possibly be modified or eliminated by the kidney. In both cases, however, the effects were similar and the less invasive sciatic vein catheterization was used thereafter. After surgery the animals were allowed a 24-h recovery period.

For the delivery of AVT, the PE tubing exiting the animal was filled with isosmotic saline followed by hormone dissolved in saline. The tubing was then connected to the minipump which was filled with inert dye and placed in an isosmotic saline solution. The hormone solution was demarcated on either side by a 1 mm length of paraffin oil to prevent mixing and had inert dye added so that its movement could be observed. Using the pumping rate of the minipump ($0{\cdot}42\,\mu l\,h^{-1}$), and the exact length and internal volume of the catheters, it was possible to calculate (± 8 min) the time at which the hormone would start to enter the circulatory system. The catheter lengths were thus prepared so that the hormone entered the blood on the following day, 48 h post-surgery.

The bioactivity of the hormone was tested by diluting AVT to mimic the concentration which would be present in the delivering catheter. A single bolus of $100\,ng\,kg^{-1}$ was then injected into three animals. The same solution, left at room temperature, was injected once every day on days 1, 2 and 4 in the same three animals. Urine flow was monitored in all cases.

Experimental groups

There were four experimental groups, each involving a different set of animals.

1. *Single bolus injection of AVT*

After a control period, animals were injected with a single dose of synthetic AVT (Sigma Chemical Co.) dissolved in 0·2–0·5 ml of glucose-free MacKenzie's toad saline (de la Lande *et al.* 1962). Doses were of 0, 1, 10 and $100\,ng\,kg^{-1}$. Urine flow was monitored continuously and GFR measurements were made 10 min, and 1 and 2 h after injection of the hormone. The time between GFR determinations was dictated by the low urine flow after AVT injection.

2a. *Continuous infusion of AVT* via *minipump*

Following a control day of infusion with saline, the hormone reached the blood, and animals were continuously infused with the minipump to a final AVT dosage of 10 or $100\,ng\,kg^{-1}$ above the expected blood level. Urine flow was measured during both days.

The half-life for AVT in the blood of toads is about 33 min (Hasan & Heller, 1968). Thus the continuously infused hormone concentration followed an exponential increase such that 50 % of the expected final dosage was in the blood after 30 min, and 99 % was present after 220 min. After this, the exogenously intro-

duced AVT would be maintained at a constant level. The increase in AVT level can best be expressed by the formula:

$$T = c/k \times (1 - e^{-kt})/m,$$

where T is [AVT] in the blood at any time, t is the approximate time to reach steady state, c is hormone pumping rate ($ng\,kg^{-1}\,min^{-1}$), m is mass and k is the decay rate of AVT = ln2/33.

2b. *Periodic bolus injections*

Five hours after AVT had entered the circulatory system *via* the minipump (see 2a above), animals were given a series of bolus injections for 3 h. Based on a half-life of AVT in the toad of about 30 min, we calculated the required dosage which, if injected every 10 min, would maintain a theoretically constant AVT blood level of twice the primed concentration. These 'primed' toads were continuously infused with AVT *via* the minipump during the entire period. To obtain a $10\,ng\,kg^{-1}$ increase, the first injections were 7, 2 and 1 ng. Following that, a continuing series of injections every 10 min of 3·5, 1 and 5 ng would theoretically maintain a constant blood AVT concentration. To obtain a $100\,ng\,kg^{-1}$ increase, the preceding introductions were rapid and differed from the continuous slow infusion by the osmotic pumps. Urine flow was measured every 10 min and GFR was estimated less frequently (see Fig. 3).

3. *Continuous infusion of saline* via *minipump*

Animals underwent an experimental protocol similar to 2b, except that the pump was only delivering saline; they are referred to as 'unprimed'.

4. *Periodic bolus injections at various time intervals*

Following a 3-h control period, a group of unprimed animals was given bolus injections at either 15-, 20- or 30-min intervals over a 5-h period. The AVT dosage was maintained at $10\,ng\,kg^{-1}$. The injection sequences (in $ng\,kg^{-1}$) were as follows: 15-min interval (8, 2; 4, 1, 4, 1...), 20-min interval (5, 5, 2·5, 3·75, 2·5; 3·1, 2·9, 3·1, 2·9...) and 30-min interval (10; 5, 5...). Urine flow was measured every 15 min for the entire experimental period.

GRF measurement and fractional reabsorption of water

Following catheter preparation, the animals were put into a 3-l wide-mouthed glass experimental chamber. The bladder was emptied and isourine infused through the bladder catheter (a volume of 25 % of body mass). A volume of water 2 times body mass was added to the chamber and air flow in the chamber maintained at $600\,ml\,min^{-1}$. To measure the GFR, 1 μCi of [^{14}C]inulin was injected into the femoral artery. After a 5-h equilibration period, urine and plasma samples (100 μl) were taken every hour for at least 4 h and dissolved in 15 ml of scintillation fluid. Inulin concentrations in plasma and urine were determined radiometrically using a liquid scintillation counter (LS-230 Beckman). Urine flow

was measured continuously at 10- or 15-min intervals. The following equation was used to determine the GFR: GFR = ([U] × U)/[Pl]), where [U] and [Pl] denote urine and plasma inulin concentrations, respectively, and U denotes urine flow rate (ml $100\,g^{-1}\,h^{-1}$). Fractional reabsorption of water (%TH_2O) was estimated as:

$$\%TH_2O = [(GFR - U)/GFR] \times 100.$$

Statistics

Data are shown as means ± S.E. of the mean number of animals given in the figure legends. Differences between two protocol groups (e.g. Figs 3A and B and 4A and B) were assessed with paired *t*-tests ($P < 0{\cdot}05$). Post-infusion or -injection group differences were assessed by comparing the pre-infusion or -injection values shown with the subsequent values using paired *t*-tests.

Results

Single bolus injection of AVT

The urine flow rates and glomerular filtration rates were obtained from four groups of *Bufo marinus*: those given a sham injection of glucose-free MacKenzie's toad saline, and those given saline injections containing AVT at doses of 1, 10 or 100 ng kg^{-1}.

Urine flow rates and GFRs were not significantly altered by sham injections (Fig. 1). For the 1, 10 and 100 ng kg^{-1} doses, urine flow rates dropped significantly

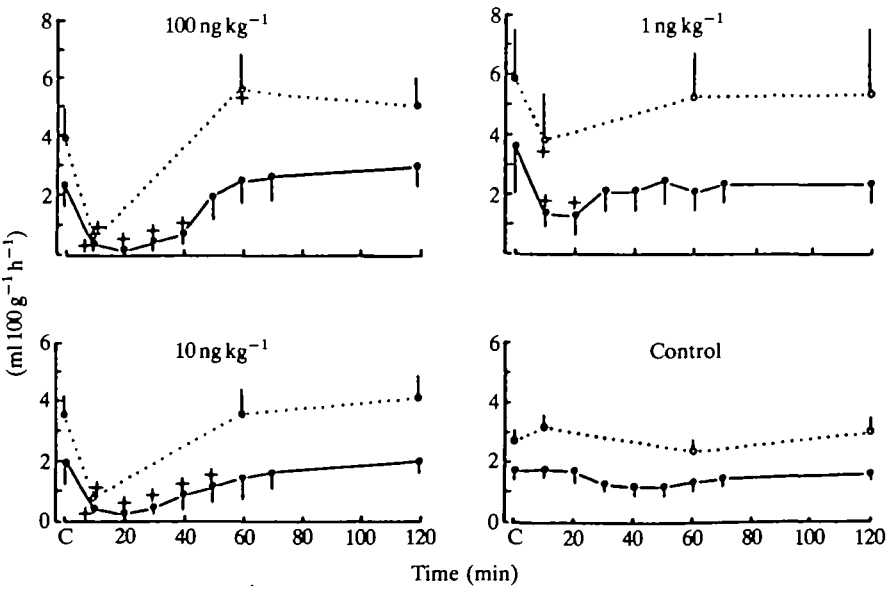

Fig. 1. Relationships among urine flow rates (●) and glomerular filtration rates (○) (GFR) for animals given sham injections of saline and animals given AVT injections of 100, 10 and 1 ng kg^{-1} ($N = 5$ for each dose). All values are given as means + S.E. A plus sign beside the mean indicates a significant difference from the control ($P \leq 0{\cdot}05$).

10 min after injection, and returned to pre-injection rates after 30 min for the 1 ng kg^{-1} dose and after about an hour for the 10 and 100 ng kg^{-1} doses. As with urine flow, GFRs were depressed 10 min after AVT injection. For the 10 and 1 ng kg^{-1} doses, the GFRs returned to normal after 1 h. For those toads given the supraphysiological 100 ng kg^{-1} bolus injections, GFRs were higher than pre-injection rates 1 h after injection and were normal 2 h after injection.

Toad urine flow and continuous AVT infusion via *osmotic pump*

Urine flow in toads is quite variable, with variations occurring naturally or stimulated by such uncontrolled events as lights being turned on or laboratory noises. In our experiments using minipump infusion (Fig. 2), urine flow (in ml 100 g^{-1} h^{-1}) varied from $1{\cdot}62 \pm 0{\cdot}25$ to $2{\cdot}69 \pm 0{\cdot}14$ for the 10 ng kg^{-1} dosage, whereas flows ranged from $2{\cdot}24 \pm 0{\cdot}36$ to $3{\cdot}07 \pm 0{\cdot}36$ for the 100 ng kg^{-1} dosage. In neither the 10 ng nor the 100 ng experiments did the gradual infusion of hormone by the osmotic pumps stimulate any statistically significant change in urine flow in the 17 animals studied.

Bolus injections of AVT into AVT-primed or unprimed toads

In all cases, the injection of a hormone bolus caused a significant depression in the toads' urine flow rates. The drop in urine flow was most pronounced in the unprimed animals in that a 7 ng injection produced an immediate 69 % drop in urine flow (Fig. 3A) and a 70 ng bolus produced an 81 % drop (Fig. 3B). If the AVT dosage was relatively low (i.e. 10 ng kg^{-1}), the AVT bolus injected into the 10 ng kg^{-1} primed animals lowered the flow by 69 % (Fig. 3A), but when the dose

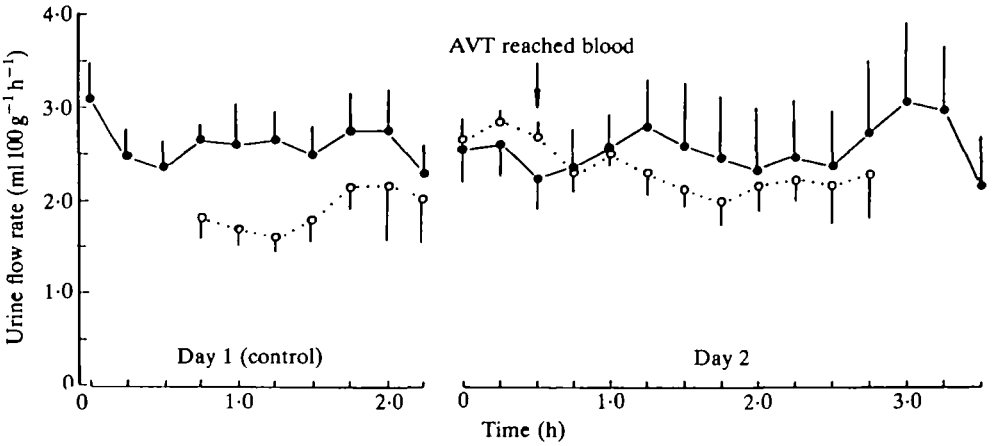

Fig. 2. Effects of osmotic pump infusion of 10 (○) and 100 (●) ng kg^{-1} AVT on urine flow in the toad. Although flow was quite variable, there were no significant changes in either the 10 ($N = 4$) or 100 ($N = 8$) ng kg^{-1} groups. There were no statistically significant changes in either group. All animals were chronically catheterized in the femoral artery, ureters and bladder and were moving freely.

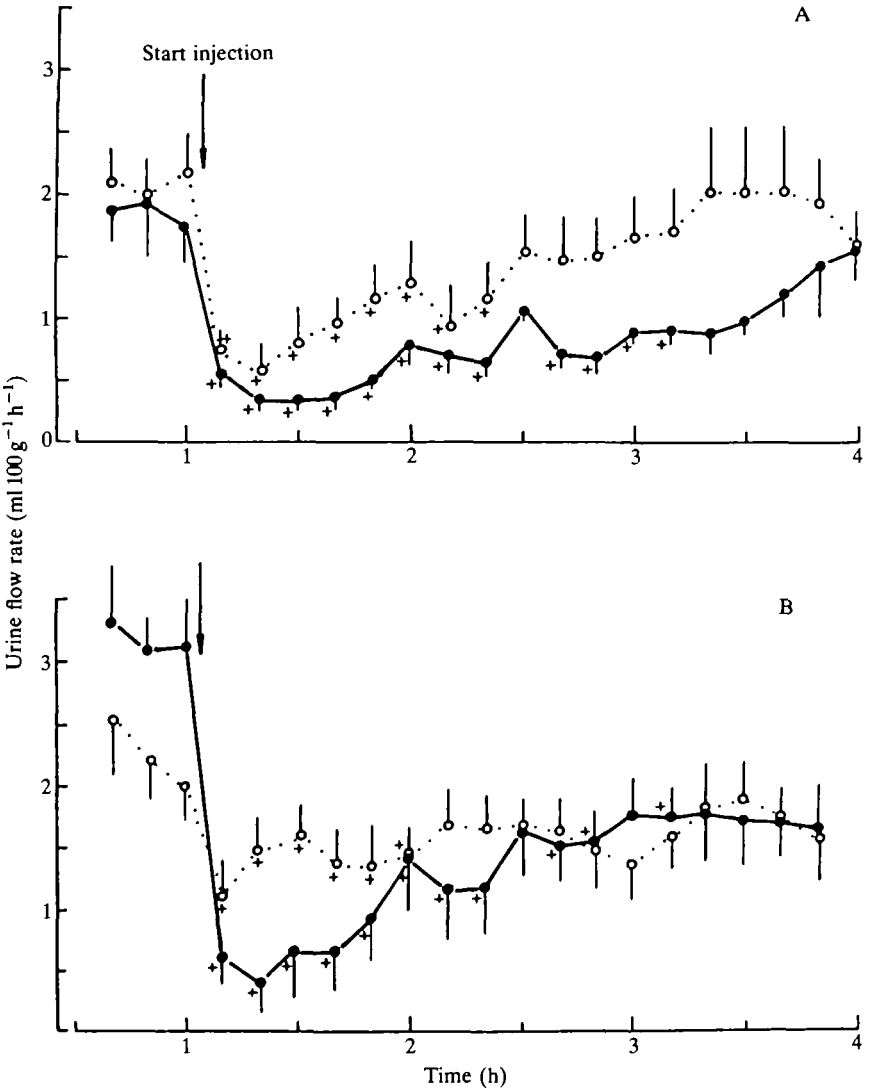

Fig. 3. Urine flow changes following periodic (every 10 min) bolus injections of AVT into primed (○) and unprimed (●) animals. (A) 10 ng kg^{-1} bolus injections; (B) 100 ng kg^{-1} bolus injections. A plus sign beside the mean indicates a significant difference from the control ($P \leq 0{\cdot}05$). For the 100 ng kg^{-1} animals, there was also a significant difference between primed and unprimed groups between 20 and 50 min after the injection of AVT.

was kept at high levels (100 ng kg^{-1} bolus into the 100 ng kg^{-1} primed animals), the AVT injected dose decreased urine flow by only 42% (Fig. 3B).

Another important difference between the AVT-primed and unprimed animals is that the effects of periodic bolus injections persisted for a longer period in unprimed animals. Nonetheless, the continuous injection of AVT did not maintain

urine flow at low levels, since in all cases urine flow was starting to return to normal within 1 h after the manual infusion of the hormone.

The GFR results (Fig. 4A,B) show the same pattern as the urine flow measurements, with a smaller initial decrease in the primed (41 % and 9 % for the 10 and 100 ng kg^{-1} doses, respectively) than in the unprimed animals (69 % and 53 % for the 10 and 100 ng kg^{-1} doses, respectively). In both the 10 and 100 ng kg^{-1} unprimed animals, the GFR remained depressed for at least 1 h. In the primed animals, the GFR depression was less pronounced but still lasted for 1 h in the 10 ng kg^{-1} animals, although it was absent in the 100 ng animals.

Although changes in urine flow can be mediated by a number of factors, it appears that the initial and subsequent decreases in flow were mostly glomerular in origin and not tubular. This is demonstrated by the close relationship between the

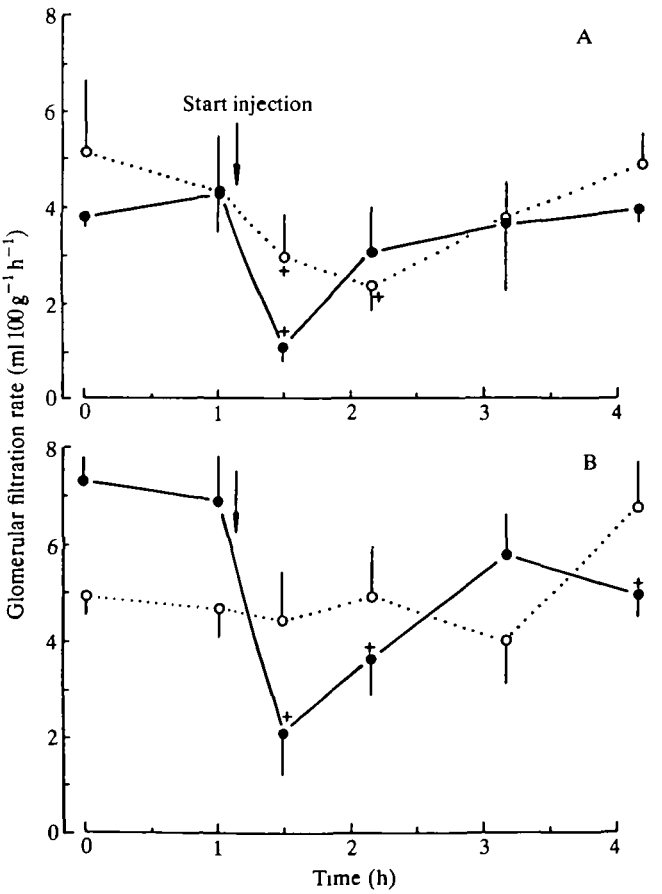

Fig. 4. Effect of periodic bolus injections of AVT on GFR in *Bufo marinus*. (A) 10 ng kg^{-1} injections in primed (○) ($N = 9$) and unprimed (●) ($N = 7$) animals. (B) 100 ng kg^{-1} injections in primed (○) ($N = 10$) and unprimed (●) ($N = 5$) animals. All values given as means + S.E. A plus sign beside the mean indicates a significant difference from the control ($P \leq 0{\cdot}05$).

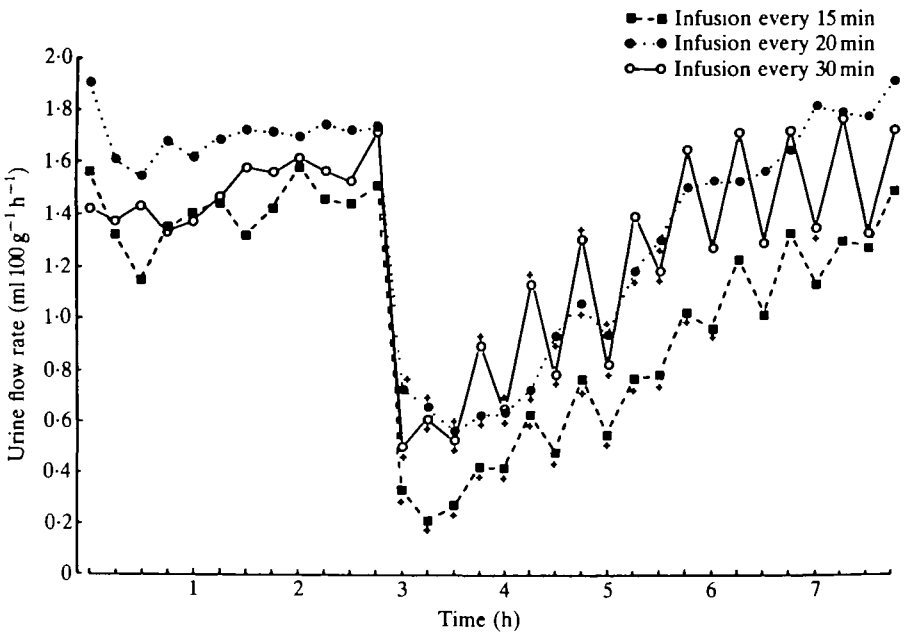

Fig. 5. Urine flow changes following infusion of AVT at intervals of 15 ($N = 13$), 20 ($N = 15$) and 30 ($N = 14$) min. AVT dose was 10 ng kg^{-1}. A plus sign beside the mean indicates a significant difference from the control value ($P \leq 0{\cdot}05$).

change in GFR and urine flow following single and periodic bolus infusions of AVT (see Figs 3 and 4). In general, the effects of either single bolus or continuous injections of AVT on tubular water reabsorption were minimal. Although there was an increase in the fractional reabsorption of water for up to 1 h after injections began in the primed and unprimed groups, the changes, with one exception (at 1 h for the 10 ng kg^{-1} unprimed group), were not significant.

Periodic injections at various intervals in unprimed toads

The urine flow values remained significantly lower than controls for 2 (30-min interval) to 3 (15- and 20-min intervals) hours (see Fig. 5). However, in all cases, the urine flow started to return to normal 45 min to 1 h after the first AVT injection, as was observed for the 10-min injection interval in the unprimed animals (Fig. 3A,B).

Bioactivity

The results of the test for the bioactivity of AVT over the experimental period showed that the hormone was just as effective after 48 h as it was after 24 h. After 96 h, the initial effect of AVT injection was the same as it was after 24 or 48 h. The time it took for the urine flow to recover to pre-injection values varied from 80 (after 24 h) to 50 min (after 96 h) as shown by paired *t*-tests.

Discussion

The present study is the first to demonstrate that AVT-mediation of urine flow in the freely moving toad *Bufo marinus* is most effectively regulated by pulses of AVT and not by a protocol whereby AVT levels are increased gradually and maintained at one level.

In the first experiment, it was found that the antidiuretic response to a single bolus injection of up to $100\,ng\,kg^{-1}$ of AVT was greatly dissipated after 1 h (Fig. 1). The half-life of AVT in toads is approximately 33 min (Hasan & Heller, 1968), so there should still have been sufficient AVT in the plasma to effect an antidiuretic response for at least 3 h. Gradual introduction of the hormone and the carrier saline *via* the osmotic pump did not increase the length of the response: in fact, even when the given AVT blood dosage had reached levels as high as $100\,ng\,kg^{-1}$ animal, there was no change in urine flow (Fig. 2). We have shown, however, that the toad kidney is sensitive enough to modulate flow when AVT doses as small as $1\,ng\,kg^{-1}$ are injected into the blood in the form of a bolus (Fig. 1). Both experiments suggest that the AVT receptors are unresponsive to the continuous presence of AVT and require pulsatile increase in the hormone to cause a change.

That AVT is most probably released in a pulsatile fashion was demonstrated in a third experiment in which AVT was injected periodically over a 3-h period (Fig. 3A,B). In these experiments, urine flow was maintained for over 2 h at a level significantly lower than the control in both the 10 and $100\,ng\,kg^{-1}$ unprimed groups. In the primed groups, the urine flow returned to normal within an hour, in a manner similar to that observed for the single bolus injection.

This episodic secretion pattern of hormone release (with the hormone arginine vasopressin or AVP) has been shown to occur during lengthy periods of dehydration in the dog and is not simply a random occurrence (Weitzman *et al.* 1977). There is also evidence that AVP secretion is pulsatile or episodic in normal active humans (Katz *et al.* 1979) and the horse (Redekopp *et al.* 1986).

The AVT-stimulated movement of water across the toad bladder (hydro-osmotic response) occurs most readily when the isolated bladders are exposed to short pulses of the hormone (Eggena, 1987). Eggena (1987) has shown that continuous exposure of the bladders to AVT abolishes the hydro-osmotic response and that bladders with a previous history of AVT exposure show a diminished response. Our results for the antidiuretic response *in vivo* almost mirror these results for the isolated toad bladder.

The frequency at which normal pulses of AVP or AVT occur during dehydration, or the frequency at which pulses of hormone should be introduced to give a maximum response, is difficult to establish in a small animal such as a toad, where serial blood sampling is limited. In dogs and sheep that were dehydrated, the time between AVP spikes in the plasma averaged 11·6 min (Weitzman *et al.* 1977). If the release of AVT in the dehydrated toad is episodic, and it appears that this is the case, present methods (radioimmunoassays) based on serial and large sampling sizes make it difficult to determine when these pulses occur. In our

experiments, none of the three interpulse times was effective in completely stopping urine flow.

The primed animals were exposed to the hormone for a long time, hence the receptor reserve available must have been very low. Nonetheless, the receptors available did effect a reduction in urine flow when pulsed with AVT. What is surprising is that a significant reduction in flow occurred with bolus injections even when the animals were exposed to continuously infused doses of AVT of $100\,ng\,kg^{-1}$ for several hours. The decrease in sensitivity to the hormone with an increase in the primed concentration suggests a degree of receptor saturation. There is no question, however, that the phasic burst of hormone introduction is more effective in stimulating a drop in toad urine flow than is a continuous and nonpulsatile AVT infusion.

The initial decrease in urine flow following AVT bolus injections in *Bufo marinus* can be attributed, in all but one case, to a reduction in GFR. In the animals primed with $100\,ng\,kg^{-1}$ AVT, the decrease in urine flow is not accompanied by a corresponding change in GFR (Figs 3B and 4B). It is possible that the drop in GFR could have taken place between our sampling periods and an increase in tubular reabsorption could have occurred since the GFR values were unchanged from control for the entire experimental period, and the urine flow values remained depressed for over an hour. In addition, it has been shown that, in response to bouts of dehydration in *Bufo marinus*, precipitous and rapid decreases in urine flow are of both glomerular and tubular origin (Tufts & Toews, 1985). The presence of tubular reabsorption is evident in both the single bolus and periodic injections of AVT. The increase, however, is small compared with the changes observed when the animals are subjected to dehydration (Tufts & Toews, 1986), where a constant and significant increase in the fractional reabsorption component is observed even though the blood level of AVT is possibly no higher than $10\,ng\,kg^{-1}$.

It seems likely that the rapid physiological response of *Bufo marinus* to dehydrating conditions involves a rapid release of AVT which suppresses glomerular filtration and another (neuronal?) reflex which enhances tubular reabsorption. Since the renal portal system supplies a significant portion of the peritubular blood and tubular perfusate, whereas the glomerular filtrate results almost exclusively from the arterial circulation (Deeds *et al.* 1977), it seems reasonable to hypothesize different control mechanisms for the two very different blood delivery systems.

What was perhaps most perplexing was our inability to keep the urine flow rates reduced even with periodic but regular (10-min interval) AVT injections. Subsequent attempts were made to determine the appropriate pulse rate interval. Changing the injection frequency to 15, 20 and 30 min did not alter the response. In all cases, the urine flow started to return to normal 45 min to 1 h following the first AVT injection (see Fig. 5). It may be that the true *in vivo* AVT pulse frequency is close to one of these four intervals.

Eggena (1986) found that during dehydration *Bufo marinus* had a circulating

level of AVT in the plasma of $4{\cdot}86\times10^{-10}$ mol l^{-1} (4 ng kg^{-1}) compared with $1{\cdot}53\times10^{-10}$ mol l^{-1} for a hydrated toad (1 ng kg^{-1}). Osmotically stimulated *Bufo marinus* had plasma AVT levels of $1{\cdot}4\times10^{-10}$ mol l^{-1} (10 ng kg^{-1}). The importance of hydration of the animals prior to experimentation is thus crucial to ensure a minimum circulating level of the hormone in the blood. To conduct our experiments, low initial circulating levels of AVT are required, hence animals are kept in water. Under these conditions, AVT injections will stimulate an increased movement of water into the animal. To mitigate against a potential oedematous condition, the responses could well be a decrease in the tubular reabsorption of water and possibly the release of the diuretic hormone mesotocin.

Financial support was provided by NSERC (Canada) Operating and Equipment Grants (DPT) and by an NSERC Undergraduate Summer Research Scholarship (LM).

References

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. & TOEWS, D. P. (1979*a*). Acid–base relationships in the blood of the toad, *Bufo marinus*. I. The effects of environmental CO_2. *J. exp. Biol.* **82**, 331–344.

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. & TOEWS, D. P. (1979*b*). Acid–base relationships in the blood of the toad, *Bufo marinus*. II. The effect of dehydration. *J. exp. Biol.* **82**, 345–355.

DEEDS, D. G., SULLIVAN, L. P., FENTON, R. A., TUCKER, J. M. & CUPPAGE, F. E. (1977). Function and structure of perfused bullfrog kidney. *Am. J. Physiol.* **233**, F481–F490.

DE LE LANDE, I. S., TYLER, M. J. & PRIDMORE, B. J. (1962). Pharmacology of the heart of *Tiliqua* (Trachysaurus) *rugosa* (the sleepy lizard). *Aust. J. exp. Biol. med. Sci.* **40**, 129–137.

DYBALL, R. E. J., BARNES, P. R. J. & SHAW, F. D. (1985). Significance of phasic firing in enhancing vasopressin release from the neurohypophysis. In *Neurosecretion and the Biology of Neuropeptides* (ed. H. Kobayashi, H. A. Bern & A. Urano), pp. 239–246. Japan Sci. Soc. Press. Town: Springer-Verlag.

DYBALL, R. E. J. & LENG, G. (1985). Supraoptic neurones in Brattleboro rats respond normally to changes in plasma osmotic pressure. *J. Endocr.* **105**, 87–90.

DYBALL, R. E. J. & PATERSON, A. T. (1983). Neurohypophysial hormones and brain function: The neurophysiological effects of oxytocin and vasopressin. *Pharmac. Ther.* **20**, 419–463.

EGGENA, P. (1986). Disk method for measuring effects of neurohypophysial hormones on urea permeability of toad bladder. *Am. J. Physiol.* **250**, E31–E34.

EGGENA, P. (1987). Hydroosmotic responses to short pulses of vasotocin by toad bladder. *Am. J. Physiol.* **252**, E705–E711.

EGGENA, P. & MA, C. L. (1986). Downregulation of vasopressin receptors in toad bladder. *Am. J. Physiol.* **250**, C453–C459.

HANDLER, J. S. & PRESTON, A. S. (1981). Vasopressin-elicited refractoriness of the response to vasopressin in toad urinary bladder. *Am. J. Physiol.* **240**, F551–557.

HASAN, S. H. & HELLER, H. (1968). The clearance of neurohypophysial hormones from the circulation of non-mammalian vertebrates. *Br. J. Pharmac. Chemother.* **33**, 523–530.

KATZ, F. H., SMITH, J. P. & LOEFFEL, D. E. (1979). Plasma vasopressin variation and renin activity in normal active humans. *Hormone Res.* **10**, 289–302.

REDEKOPP, C., ERVINE, C. H. G., DONALD, R. A., LEVESEY, J. H., SADLER, W., NICHOLLS, M. G., ALEXANDER, S. L. & EVANS, M. J. (1986). Spontaneous and stimulated adrenocorticotropin and vasopressin pulsatile secretion in the pituitary venous effluent of the horse. *Endocrinology* **118**, 1410–1416.

SHAW, F. D., BICKNELL, R. J. & DYBALL, R. E. J. (1984). Facilitation of vasopressin release from the neurohypophysis by application of electrical stimuli in bursts. *Neuroendocrinology* **39**, 371–376.

TUFTS, B. L. & TOEWS, D. P. (1985). Partitioning of regulatory sites in *Bufo marinus* during hypercapnia. *J. exp. Biol.* **119**, 199–205.

TUFTS, B. L. & TOEWS, D. P. (1986). Renal function and acid–base balance in the toad *Bufo marinus* during short-term dehydration. *Can. J. Zool.* **64**, 1054–1057.

WEITZMAN, R. E., FISHER, D. A., DISTEFANO, II, J. J. & BENNETT, C. M. (1977). Episodic secretion of arginine vasopressin. *Am. J. Physiol.* **233**, E32–E36.