THE EFFECTS OF ANTIDIURETIC HORMONE (VASOPRESSIN) ADMINISTRATION ON THE CONTRACTILITY OF THE ARTERIAL PREPARATIONS IN RATS COMPARED WITH PETS

SORIN IOAN BEȘCHEA CHIRIAC, LUMINITA HRITCU, VASILE BOGHIAN, GHEORGHE SOLCAN

University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Iași. Faculty of Veterinary Medicine. Departament of Internal Medicine, Iași, Romania

sbeschea@yahoo.com

Abstract: *Vasopressin known as antidiuretic hormone is responsible for regulating plasma osmolality and volume. It acts as a neurotransmitter in the brain to control circadian rhythm, thermoregulation, and adrenocorticotrophic hormone release. The therapeutic use of vasopressin has become increasingly important in the critical care environment in the management of cranial diabetes insipidus, bleeding abnormalities, oesophageal variceal haemorrhage, asystolic cardiac arrest, and septic shock.*

The present study aims toward identifying similarities and differences between the resistance arteries belonging from various mammal species that are most involved in veterinary practice: rats, cats and dogs. Smooth muscle has been studied as circular preparations from rat aorta, cat and dog coronary gastric aorta. Force generation has been studied using isometric transducers while stimulation of preparations was made pharmacologically at various doses. Results were expressed as percentage of inhibition or stimulation of the control contraction.

Force generation, frequency and amplitude of spontaneous contraction have been recorded. Administration in isolated preparations where made using desmopressin acetate (Ferring) as vials of 4 mg/ml. The preparation is a synthetic analogue of the natural hormone 8-arginine-vasopressin, as arginine-vasopressine-monoacetatetrihydrate. Dosages varied from 10^{-12}M to10^{-8}M. Several methods have been tried for normalizing maximal isometric force developed by smooth muscle from various locations, vessel dimensions and animal species. We have measured in vitro the amount of force generated by arterial rings harvested from the same areas, very rigorously cleaned of adventice and surrounding tissues and the force, expressed as mN was ratioed to the wet weight of the preparations.

The results were statistically investigated using the t-test and ANOVA testing. In preparations of rat aorta and splachnic arteries from cat and dog, the vasopressin induced a tonic contraction, with an aspect of descending plateau.

In conclusion, vasopressin contraction has several special characteristics concerning its dynamics. The contractile plateau is kept only for about 10-15 minutes, after which it fades, and new administration in a space of approximately 16 minutes does not induce the initially effect (probable tachyphylaxis).

Key words:*vascular, reactivity, arterial, vasoconstrictor agent*

INTRODUCTION

Vasopressin, also known as arginine vasopressin (AVP), antidiuretic hormone (ADH) or argipressin is a neurohypophysial hormone found in most mammals (dog, cat, horse, etc). Its two primary functions are to retain water in the body and to constrict blood vessels. Vasopressin regulates the body's retention of water by acting to increase water reabsorption in the kidney's collecting ducts, the tubules which receive the very dilute urine produced by the functional unit of the kidney, the nephrons (Caldwell et al., 2006; Babar, 2013). It also, increases peripheral vascular resistance, which in turn increases arterial blood pressure. ADH plays a important role in homeostasis, by the regulation of water, glucose and salts in the blood. It is derived from a preprohormone precursor that is synthesized in the hypothalamus

and stored in vesicles at the posterior pituitary. Most of it is stored in the posterior pituitary to be released into the bloodstream. However, some AVP may also be released directly into the brain, and accumulating evidence suggests it plays an important role in social behavior, sexual motivation and pair bonding, and maternal responses to stress (Moncada et al., 1991; Rozenfeld et al., 2000).

Vascular reactivity is one of the three pillars on which lies the regulation of arterial pressure in living organisms. Arterial pressure is one of the main determinants of the activity state of various organs and systems both in healthy and in pathologically-altered states.

The aim of this study is to investigate the most common modifications encountered in the veterinary practice in the vascular arterial reactivity that could be involved in the pathogenesis of various animal species. We also wish to make a comparative investigation of the vascular reactivity at the arterial segments collected from different mammal species the veterinary pathology has most frequently to deal with, segments which are histologically and functionally similar.

The investigation of the methods that are at the basis of the arterial tonus adjustment and of the metabolism of the arterial smooth muscle fiber relied in the last two decades on the very well known isometric transducers pattern and on that of the annular preparation of different arteries. The arterial duct of election used for these types of investigations is the rat aorta, as it combines most of the stability, accessibility, disposability and controllability conditions required for a trustworthy investigation. The price is also an important criterion in this matter.

MATERIAL AND METHODS

The organ parts investigated were taken from the Medical and the Surgery Clinique's from the Faculty of Veterinary Medicine, from dead animals that were not subject of legal euthanasia nor had affections with vascular implications (Legea nr 471 din 9 iulie 2002).

The reactivity of the arterial rings was measured in terms of both absolute force, measured as force index and relative reaction towards a standardized witness. Also, where possible (considering the preparations availability) curves dose/effect were made, involving the majority of the known vasorelaxing and vasoconstrictor substances that are pharmacologically well characterized.

The comparative study was made on similar arteries in what their dimensions are concerned, being part of the resistance segment, in this situation branches from the gastric coronary artery or the superior mesentery which had similar dimensions: maximum length: 2 mm, Φ = 1 mm and weight: 10-15 mg. The force of contraction was quantified in N/mg wet weight (Rozenfeld et al., 2000).

After dissection the vessels were exsanguinated, washed in a solution of physiological salt, sectioned in fragments of 5-10 cm and then put into Krebs-Henseleit serum and transported in maximum 30 minutes to the place of the experiment. The aorta fragments were fixated through a metallic serfina on the bottom of the isolated organ baths, and the ring tensioned through the verniers of the tensiometrical stamps to an initial tension of 100 mN.

The vascular endothelium was removed by gentle rubbing with damp filter paper whenever the experimental characteristics required it. The presence of the functioning

vascular endothelium was pharmacologically verified using carbachol and through direct microscopy.

The aorta rings were set in organ baths containing 4 ml of Krebs-Henseleit (NaCl - 118; KCl - 4.7; $MgSO_4$ -1.64; $NaHCO_3$ - 24.88; KH_2PO_4 - 1.18; glucose - 5.55), thermostated at 37°C and bubbled with carbogen (a mixture of 95% oxygen and 5% carbon dioxide).

Isometric force transducers connected to a computerized system for data acquisition were used for detecting the contractions of the vascular smooth muscles. The preparations were allowed to equilibrate for 60-90 minutes under a rest tension of 100 mN.

The aorta rings were afterwards precontractated with phenylephrine (10^{-7} – 10^{-6}M) and K+ (40-70 mM) and treated with carbachol (10^{-6}M) for releasing endothelial NO (Jiang et al., 1991) The absolute magnitude of the contractions was of 175±25 mN for the phenylephrine (10^{-6} M) and K+ (40-70 mM).

RESULTS AND DISCUSSIONS

In the rat aorta preparations and in splanchnic arteries from cat and dog, vasopressin produced a tonic contraction, with the appearance of a descending plateau over a period of 10-15 minutes (Fig. 1). The force indices obtained at the vasopressin contraction are presented in table 1.

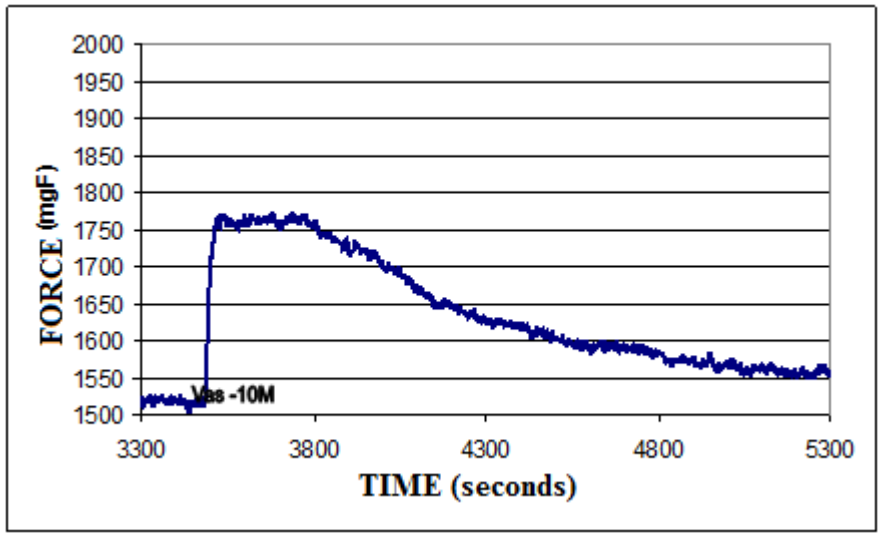

Figure 1. Characteristic aspect of vasopressin contraction on the rat aorta

Table 1.

Force index after administration of ADH 10^{-8} M

ANIMAL	FORCE INDEX (FI)
Rat	3,9 ± 1,25
Cat	4,1 ± 0,75
Dog	6,5 ± 1,5

Regarding the force produced, this is the most powerful of all preparations administered, except for the α-adrenergic agonist phenylephrine. From the dose-effect curve configuration (Fig. 2) it can be seen that very low doses (10^{-12} M – physiological) produce

little contractile effects, while with higher (pharmacological) doses, from 10-10 M, the contractile effects are close to maximum, having a curve with biphasic aspect. The curve aspect suggests the presence of quantum effects at the V1-type receptors.

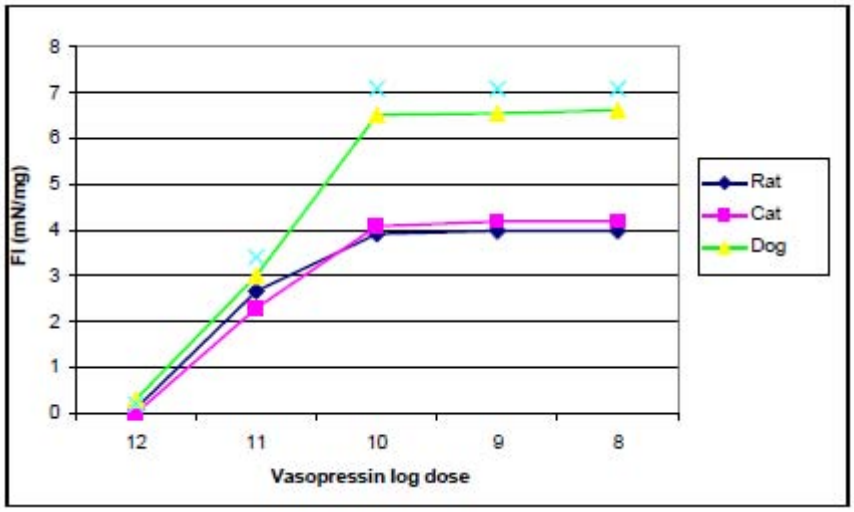

Figure 2. Vasopressin dose-effect curve in endothelized preparations from various species

Vasopressin contraction has a number of particular features concerning the dynamic. The contractile plateau lasts for only 10-15 minutes and after that it ceases and a new administration no longer produces the original effect (possibly tachiphylactic phenomena).

It also should be said that in dog's case, the effect was significantly stronger, considering the fact that the thickness of the muscular layer was smaller than that of the cat's, which leads to the assumption that dogs have a particular reactivity in what the vasopressin mediation is concerned (Fig. no 3).

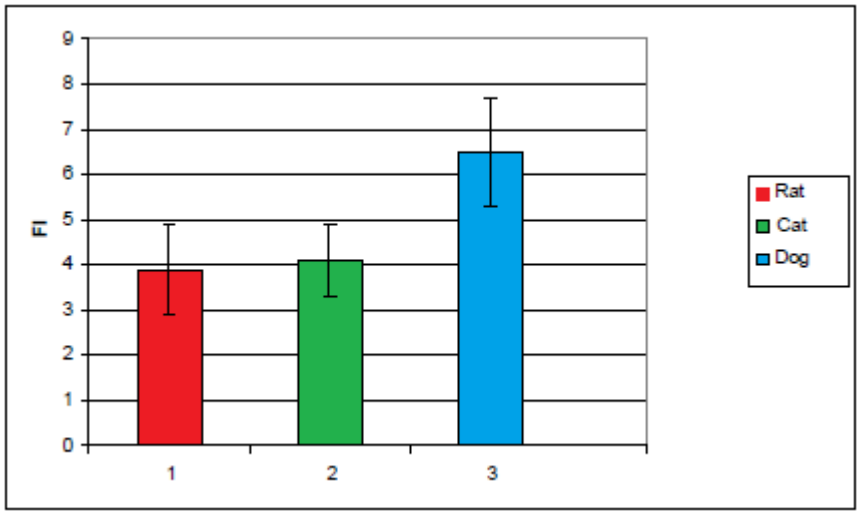

Figure 3. Vasopressin contraction force after administration of 10^{-10} M desmopressin. The differences between endothelized and de-endothelized preparations do not exhibit statistical significance

The ADH hormone is an essential neuropeptide for the cardiovascular homeostasis. Vasopressin was among the first peptide hormones ever described and it is clinically used for more than six decades, especially in treatment of diabetes insipidus and of upper gastrointestinal bleeding due to oesophageal varices (Caldwell et al, 2006). Vasopressin is also more and more often used in therapeutic management of shock, whether it is septic or vasodilator due to different reasons (Moncada et al, 1991).

The ADH is a nonapeptide having a disulphide bridge between two cysteine amino acids. It is synthesized in the paraventricular and supraoptic nucleuses of the hypothalamus, transported coupled to the neurophysins along the hypothalamohypophyseal tract to the neurohypophysis and stored in granules (Guimareas et al., 2001; Babar, 2013).

The effect of this hormone is achieved through two types of receptors, V1, found in the blood vessels (on the vascular smooth muscle) and which mediates the vasoconstriction through a cascade of mediators involving phospholipase C and release of calcium ions from intracellular stores through the inositol-phosphate system (Haulica et al, 2003).

The V2 receptors are located in the distal renal tubule and kidney collecting tube. Their effect is to stimulate the expression of aquaporin's (water channel proteins) in the tubule, thus allowing re-uptake of water and antidiuretic effect (Nielsen et al., 1995).

In normal conditions, ADH has little effect on arterial pressure and the doses at which its vasoconstrictor effect becomes noticeable are at least 10 times higher than normal plasmatic concentrations. However, in conditions of hypotension, its plasmatic concentration increases greatly and its vasoconstrictor effect allows keeping a high arterial pressure in the initial period. But as the ADH neurohypophysis reserves lessen, its plasmatic concentration decreases and its benefic effects – those of keeping the arterial pressure at quasi-physiological levels are fading.

The administrations in isolated preparations were made using the Desmopressin Acetate (Ferring) preparation in form of ampoules of 4 μg/mL. The preparation is a synthetic analogue of the natural 8-arginine-vasopressin hormone, under the form of arginine-vasopressin-monoacetate-trihydrate. The doses were administered starting from 10^{-12} M to 10^{-8} M.

CONCLUSIONS

Vasopressin reactivity produces the highest force, except for the α-adrenergic agonist phenylephrine. From the dose-effect curve configuration it can be seen that very low doses (10^{-12} M – physiological) produce little contractile effects, while with higher (pharmacological) doses, from 10^{-10} M, the contractile effects are close to maximum, having a curve with biphasic aspect. The curve aspect suggests the presence of quantum effects at the V1-type receptors.

In dog's case the effect was significantly stronger, considering the fact that the thickness of the muscular layer was smaller than that of the cat's, which leads to the assumption that dogs have a particular reactivity in what the vasopressin mediation is concerned.

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