Comparative study of vascular arterial reactivity in several mammal species: 1. Reactivity of the arterial smooth muscle to vasoconstrictor agents

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Abstract

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 present study aims at identifying similarities and differences between the resistance arteries belonging from various mammal species that are most involved in veterinary practice: rats, cats, dogs and horses. The arterial fragments harvested from animals dead due to various clinical and traumatic conditions unrelated to vascular pathology were normalized using a newly-introduced system of quantification, the force index system. This has been calculated using the wet-weight parameter and the force generated after administration of various pharmacological agents that cause vasoconstriction. The artery fragments were fitted in organ baths using the Krebs-Henseleit saline, thermostated at 37° C and bubbled with a mixture of 95% O_2 and 5%CO_2. Vascular endothelium was either kept or removed using gentle rubbing with moist filter paper. Control of endothelial removal was made both functionally, using carbachol (synthetic derivative of acetylcholine) and microscopically, after testing. The force generated was measured using isometric force transducers coupled to a computerized acquisition system. The pharmacological vasoconstricting agents used were phenylephrine (synthetic derivative of epinephrine), KCl (potassium chloride 40-80 mM, as depolarizing agent) angiotensin II, and vasopressin. The results were statistically investigated using the t-test and ANOVA testing. The preliminary results show a dependence of the force generated an the amount of muscle present in the various species from which the arteries were taken, a specifically increased response of feline-derived arteries to angiotensin and a specifically increased response of canine-derived arteries to vasopressin. These results will be used as controls for further testing in various pathological conditions and for various other pharmacological agents used in the therapy of vascularly-induced pathological states.

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

Introduction

The present study aims to investigate the modifications commonly encountered in the veterinary practice in vascular reactivity of arteries that may be involved in the pathogenesis of different animal species. Our scope is to conduct a comparative investigation of the vascular reactivity in histologically and functionally similar arterial segments that have been collected from various mammal species that the veterinary pathology frequently deals with.

In the past few decades the investigation of the mechanisms underlying the adjustment of the arterial tonus and of the arterial smooth muscle fiber has relied on the well-known isometric transducers pattern and on that of the annular preparation of different arteries. The arterial duct typically used for these types of investigations is the rat aorta because it meets most of the conditions of stability, accessibility, disposability and controllability that a trustworthy investigation calls for. The price is also an important factor to be taken into consideration in this matter.

Although the aforementioned pattern is widely known, the rat still isn't a perfect model in what the cardiovascular modeling is concerned; it is not similar to humans and even less so to other mammals. This experimental model has been used as from half a century ago [3].

Hence an experimental comparative investigation was conducted using fragments of arteries collected from dogs, cats and horses as well as thoracic aorta rings taken from Wistar rats.

Material and method

The reactivity of the arterial rings was measured in terms of both absolute force, measured as force index (the force in mN of the preparation reported at its weight in mg) and relative reaction towards a standardized witness. Dose-response curves were also produced where possible (considering the availability of preparations) involving the majority of the known vasorelaxing and vasoconstrictor substances that are pharmacologically well characterized.

The comparative study was made on arteries that were similar in terms of size, and that were assigned to the resistance segment, namely branches from the gastric coronary artery or the superior mesentery which had similar dimensions: maximum length: 2 mm, Φ = 1 mm, weight 10-15 mg. The quantification of the contraction force was expressed as N/mg wet weight. The organ parts were taken from the Medical Clinique and the Surgery Clinique at the Veterinary Medicine Faculty and were collected from dead animals that had not been subjected to legal euthanasia nor had they affections with vascular implications.

After the dissection the vessels were exsanguinated, washed in physiological salt solution, sectioned in 5-10 cm length fragments and then put into Krebs-Henseleit serum (prepared according to the formula), and transported to the place of the experiment in 30 minutes maximum.

The aorta fragments were fixated using a metallic serfina on the bottom of the isolated organ baths where the ring was tensed through the verniers of the tensiometric stamps to an initial tension of 100 mN.

The vascular endothelium was removed by gently rubbing with a damp filter paper where the characteristics of the experiment called for it. The presence of the vascular endothelium was verified both pharmacologically (using carbachol) and by direct microscopy.

The aorta rings were mounted in organ baths containing 4 ml of Krebs-Henseleit physiological salt, (composition (mN): NaCl 118; KCl 4.7; 2.52; $MgSO_4$ 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 to record the contractions of the vascular smooth muscles.

The preparations were allowed to equilibrate for 60-90 minutes under a resting 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 [**Eroare! Fără sursă de referință.**]. 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

Phenylephrine is a synthetic α-adrenergic used in the medical practice as mydriatic agent and nasal decongestant, and in the veterinary practice as cardiotonic agent. Its receptor is the adrenoceptor α 2 A.

It is a ubiquitous G protein-coupled receptor receptor localized in the sarcolemma of the smooth muscle. It produces effects by the medium of the inhibition of adenylate cyclase through the action of q-type G protein. Once activated, these proteins stimulate the activity of *Phospholipase C, stimulating the release of IP3 and DAG which act as secondary messengers that mediate the release of intracellular* Ca^{2+} *with immediate effect on the muscular contraction and, in subsidiary, activate PKC [4].*

It acts primarily on α2-adrenergic receptors in the arterial smooth muscle, regardless of their localization, producing vasoconstriction.
Administration of phenylephrine produces a strong and stable effect which can be replicated in all arterial preparations. This substance was elected also because it is completely hydrosoluble and stable in solution for several weeks, given that it is a synthetic substance (*fig. 1*).

Due to its availability, the substance was used as contraction witness in all subsequent experimentations.

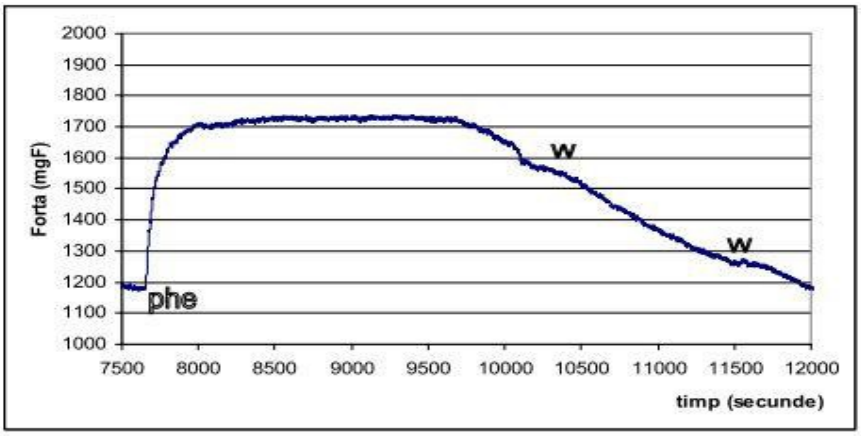

Figure 1 - Typical aspect of phenylephrine-induced contraction of an artery in the resistance segment

A dose-response curve to phenylephrine was done for preparations both in the presence as well as in the absence of the vascular endothelium (*fig. 2*).

The investigation of the effects of the vasoconstrictor substances was done as an evaluation of both the force index (F/gu) and the contraction relative to the 10^{-6} M phenylephrine-induced contraction, which was regarded as reference contraction (100%).

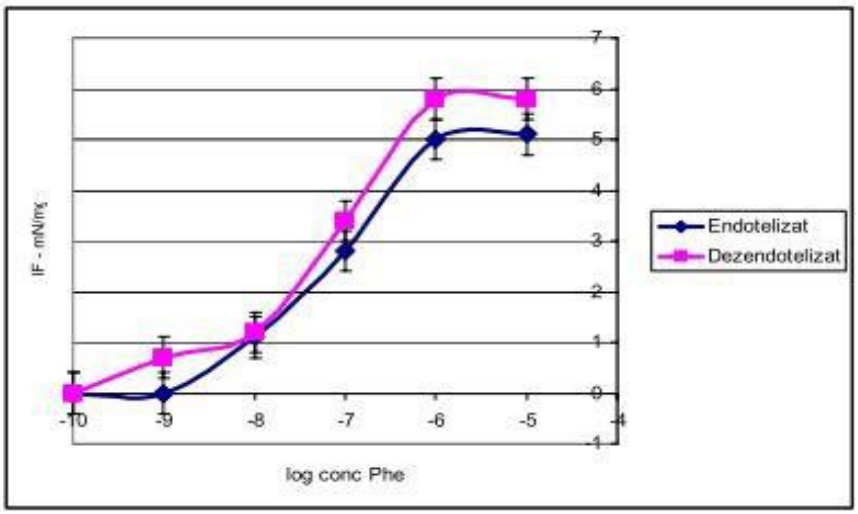

Figure 2 - Dose-response curve to phenylephrine - control group

Force indices of endothelialized vessels harvested from animals that had no vascular affections.

Rat – FI = F/gu = 2,5 gf/5mg – 25mN/5 = 5

Cat – FI = F/gu = 3,1 gf/5mg = 6,2

Dog – FI = F/gu = 3,4 gf/5mg = 6,8

Horse – IF = F/gu = 4,8gf/5mg = 8, 16

Where: *F* represents the force developed by the preparation, expressed as mN (N x 10^{-3}), and *gu* is the wet weight expressed as milligram tissue.

As it can be seen in *fig. 3*, the force of the arterial ring preparations harvested from big animals was also bigger. Therefore, the adrenergic responsiveness proved to be directly proportional to the quantity of the vascular smooth muscle in preparations.

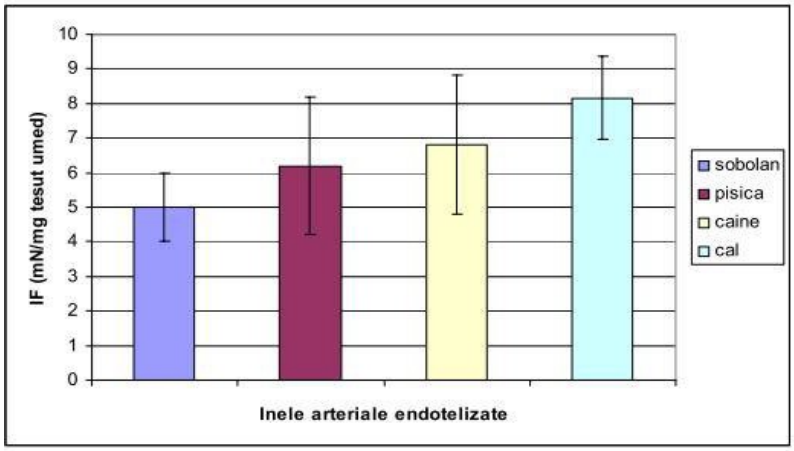

Figure 3 - Force index variation of arterial ring after administration of 10-6 M Phe

The response of *de-endothelialized* preparations to phenylephrine: as it can be seen in *fig. 4*, de-endothelialization led to an increased reactivity to phenylephrine by values ranging from 16% (rat), 9,25% (cat), 8,39 (dog) and 9,27% (horse). This phenomenon is in accordance with the results recorded by literature and with our expectations based on previous results.

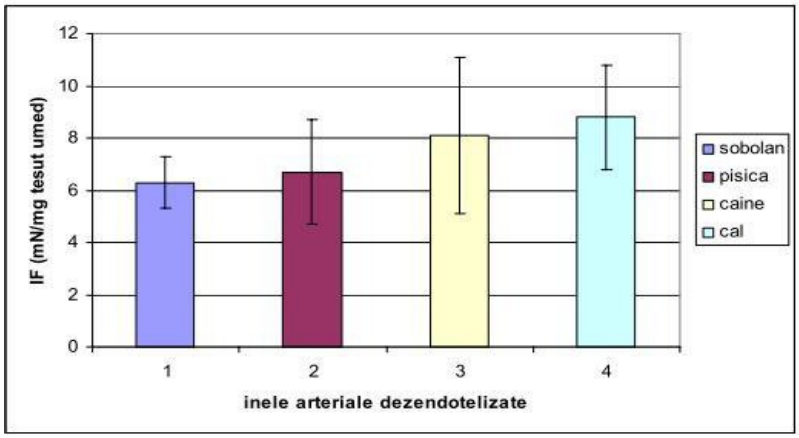

Figure 4 - Force index variation of de-endothelialized arterial rings after administration of 10^{-6} M Phe

Fig. 5 shows the cumulative graph of the two data sets. It can be seen that the stimulation of the contractile response is a more diminished effect in arteries harvested from big animals (horse, dog), which can imply that *catecholamines* involve the *basal vascular tone* regulation less *when resistance arteries show very large muscle masses.*

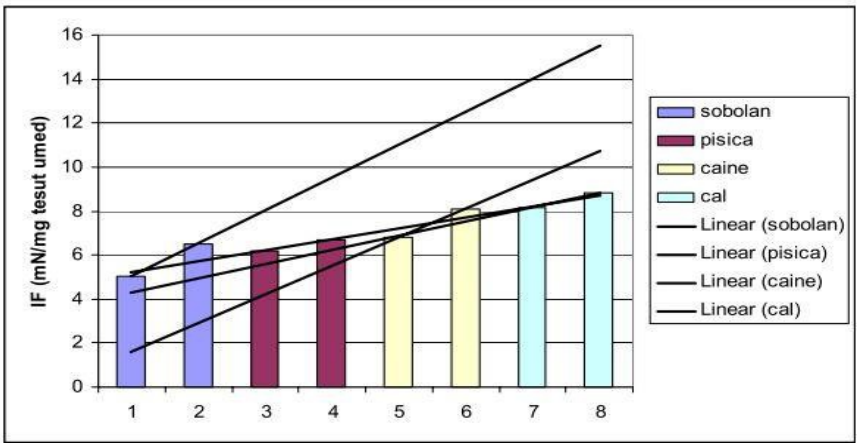

Figure 5 - Cumulative results (endothelialized and de-endothelialized) to which regression lines of the variations between the contraction of the endothelialized preparations and that of the de-endothelialized preparations were added

Conclusions

From the results shown above, the following can be inferred:

The α-adrenergic reactivity is similar in all species in the study, dose-dependent.

The only significant differences recorded are quantitative, possibly caused by the quantity of the vascular smooth muscle present in the different types of arteries used in the study.

Referring to histological data, it is safe to say that, given the percentage of smooth muscle, the results are within normal limits.

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