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VASCULAR ARTERIAL REACTIVITY AT SEVERAL MAMMAL SPECIES TO THE INHIBITORY EFFECTS OF D_{600} (GALLOPAMIL)

REACTIVITATEA VASCULARĂ ARTERIALĂ LA MAI MULTE SPECII DE MAMIFERE LA EFECTELE INHIBITORII ALE D_{600} (GALOPAMIL)

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ABSTRACT | REZUMAT

The aim of the present study is to identify the similarities and differences between the resistence of arteries belonging from various mammal species that are involved in veterinary practice: cats, dogs and horses.

The arterial fragments were sampled from animals dead due to various clinical and traumatic conditions, unrelated to vascular pathology and 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, kept in the thermostat 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 vasoconstrictor agents used were: phenylephrine (synthetic derivative of epinephrine), potassium chloride (KCl 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 vascular-induced pathological states.

Keywords: vascular reactivity, arterial contractility, relaxing agent

Scopul studiului de faţă este de a identifica similitudini şi diferenţe între rezistenţele arterelor diferitelor specii de mamifere implicate în practica medicală veterinară: pisici, câini şi cai.

Fragmentele arteriale prelevate de la animale decedate în urma a diferite stări clinice şi traumatice neaparţinând patologiei vasculare au fost normalizate în urma sistemului nou introdus de cuantificare, sistemul indicelui de forţă. Acesta a fost calculat folosind parametrul greutăţii umede şi forţa generată după administrarea a diferiţi agenţi farmacologici vasoconstrictori. Fragmentele arterelor au fost plasate în băi de organ pe bază de soluţie salină Krebs-Henseleit, termostatate la 37° C şi îmbogăţite cu un amestec de 95% O_2 şi 5% CO_2. Endoteliul vascular a fost păstrat sau îndepărtat prin frecare uşoară cu hârtie de filtru umezită. Controlul îndepărtării endoteliului a fost făcut atât funcţional, cu carbacol (derivat sintetic al acetilcolinei) cât şi microscopic, după testare.

Forţa generată a fost măsurată prin transductori de forţă izometrică, cuplaţi la un sistem electronic de obţinere a datelor. Agenţii farmacologici vasoconstrictori au fost reprezentaţi de fenilefrină (derivat sintetic al epinefrinei), clorură de potasiu (KCl 40-80 mM, agent depolarizant) angiotensină II şi vasopresină.

Prelucrarea statistică a rezultatelor s-a făcut prin testul t şi testul ANOVA. Rezultatele preliminare indică o dependenţă a forţei generate de proporţia de muşchi prezentă la diferitele specii de la care s-au prelevat arterele, un răspuns crescut la angiotensină specific felinelor şi un răspuns crescut la vasopresină specific canidelor. Aceste rezultate vor fi folosite drept control în alte teste efectuate în diferite stări patologice şi pentru alţi agenţi farmacologici utilizaţi în terapia stărilor patologice induse pe cale vasculară.

Cuvinte cheie: reactivitate vasculară, contractilitate arterială, agent de relaxare

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 goal was to conduct a comparative investigation of

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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 (1). 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 (2, 3).

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 METHODS

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-effect 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 present study was made on arteries that were similar in terms of size and 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.

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 aorta rings were mounted in organ baths containing 4 ml of Krebs-Henseleit physiological saline solution, (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), kept in the thermostat 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 pre-contractated with phenylephrine (10^{-7} – 10^{-6})M and K^+ (40-70 mM) and treated with carbachol (10^{-6}M) for releasing endothelial NO [6]. The absolute magnitude of the contractions was of 175 25 mN for the phenylephrine (10^{-6}M) and K^+ (40-70 mM).

RESULTS AND DISCUSSION

The relaxation or inhibitory effect of the contraction was assessed as follows: phenylephrine was administered as a contracting agent, very well characterized in what the dose, the aspect of the contraction curve and the duration of effect were concerned. Then, the relaxation effect was tested by administering doses during the contractile plateau phase and quantifying the doses percentage wise compared to the contraction of 10^{-6}M phenylephrine witness.

The administration of D_{600}(gallopamil) has a relaxation effect by blocking the slow Ca^{2+} channels. D_{600} is a phenyl-alkyl-amine derivative from the verapamil family, whose effect relies on blocking the voltage-dependant Ca^{2+} channels in L-group, with minimum or no effect on the receptor-dependant channels (4) or on releasing Ca^{2+} from the endoplasmic reticulum. This mode of action ensures its vascular selectivity without significantly affecting the vein bed or the non-vascular smooth muscle (such as the bronchial smooth muscle)

The administration of gallopamil in vascular smooth muscle preparations, regardless of whether it is administered during the pretreating or during the plateau phase of the contraction, produces a 40% decrease in the contraction force caused by 10^{-5}M Phe.

The administration during the plateau phase of the contraction has a different appearance from that of the carbachol, which produces a rapid decrease in vascular tone with a collapsing aspect of the contractile plateau. The administration of D_{600} produces a slow de-

crease in the tone that is induced by α-adrenergic stimulation, which reduces the contractile state by an average of 40±5% (Fig. 1).

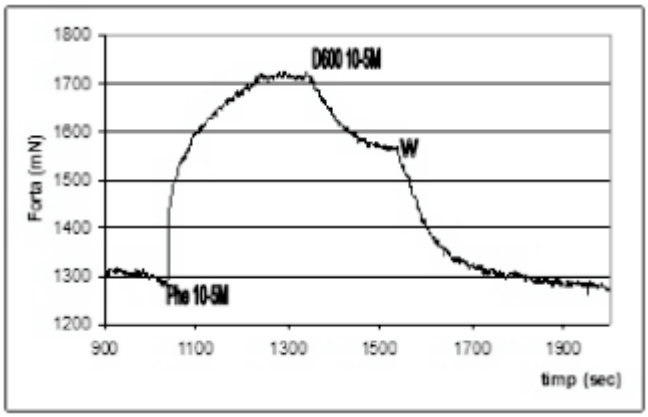

Fig. 1. Characteristic aspect of D_{600}- induced relaxation on the phenylephrine contraction

On the other hand, pretreating the arterial smooth muscle preparations with 10^{-5}M D_{600} produces an inhibition of the phenylephrine contraction that can be calculated in a similar way, in relation to the contraction of 10^{-5}M phenylephrine witness.

The administration of D_{600} produced the expected relaxation effects, according to Table 1.

Gallopamil has inhibitory effects on both the contractility of the mesenteric muscle of the dog, cat and horse and on the rat aorta, as it can be seen in the presented data.

Table 1

Contraction inhibition as percentage of 10^{-5}M Phe contraction witness

Species	Inhibitory effect after administration of 10^{-5}M D600 during pretreatment	Inhibitory effect upon administration of 10^{-5}M Phe during the plateau phase
Rat	38 ± 6 %	41 ± 7 %
Cat	55 ± 11 %	58 ± 12 %
Dog	27 ± 10 %	34 ± 10 %
Horse	24 ± 10 %	35 ± 8 %

The contractility was reduced when the preparation was incubated with D_{600}, which, statistically, significantly decreased the contractile response to α-adrenergic stimulation in all preparations ($p< 0.05$ according to ANOVA test).

The administration of 10^{-5}M D_{600} in the contraction plateau phase induced by 10^{-5}M Phe produced also a reduction of the tonic plateau, which was greater than when it was administered during pretreatment. The relation between the percentages of inhibition was 36±7,3 compared to 42±5,55, which probably means that the slow Ca^{2+} channels play a more important role in maintaining the contraction induced by α-adrenergic agonists. Acknowledging the fact that D_{600} has a low affinity for the low channels in the resting phase and that they have to pass to the open state before the substance affects the calcium flux (5, 6), it becomes clear why the inhibition of the contraction is greater when administered during the contraction plateau (when the Ca^{2+} channels are open) and reduced before the administration of α-adrenergic agonist, when most of the channels remain closed (7).

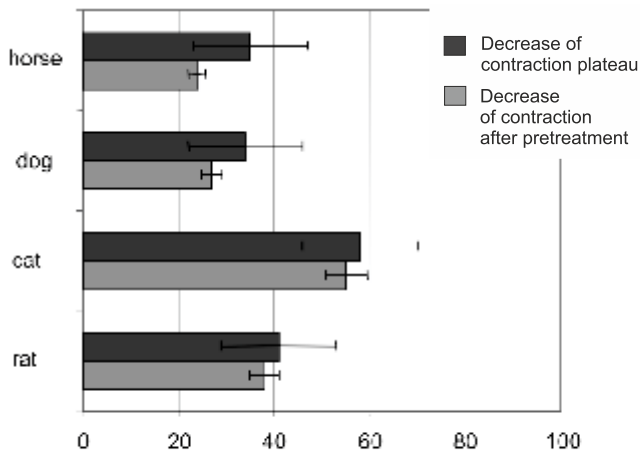

Fig. 2. Percentage of decreasing of phenylephrine contraction after pretreating with 10^{-5}M D600

The statistically significant difference is equally interesting (55,5%±11,5 compared to 29,6±4,25% during pretreatment and 36,6 ± 2,1% when administered during the plateau) between the inhibitory effects of D_{600} when administered in cat preparations and its effects in other preparations.

So, as Figure 3 shows, the inhibition induced by D_{600} in both pretreatment and plateau phases was a lot stronger than in the other 3 species in the study. The comparison was made between the average results of the mesenteric cat preparations and the other species' average results pooled into a single dataset.

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This behavior can be explained in two ways: firstly, the density of slow Ca channels, which are under the influence of gallopamil (D_{600}), is possibly higher in cat mesenteric artery than in other animal species. On the other hand, previous results (1) showed that in feline mesenteric vascular bed there is a higher density of both α- and β-adrenergic receptors, and blocking the latter leads to a more powerful vasoconstrictor response. Therefore, considering the fact that the response is more powerful in felines, it is also possible that the number of channels is larger than in other species.

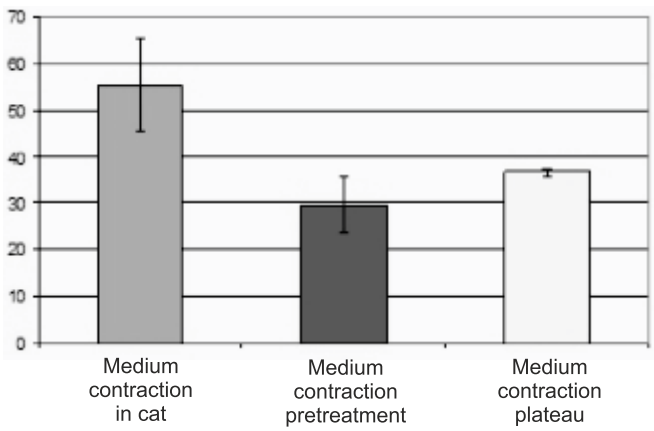

Fig. 3. Differences between the results of phenilephrine contraction inhibition by 10^{-5} M D600 in cats and the results in the other species in the study

Another explanation could be given by the interspecific variability of calcium sources necessary for the vascular contraction. If the calcium sources used by the cat vascular smooth muscle have an important extracellular component, then the contractile dependence on Ca^{2+} channels is greater, which could explain the powerful effects of D_{600} on adrenergic contraction in feline mesenteric arteries.

CONCLUSIONS

1. Contraction inhibition induced by phenylephrine is the same in cholinergic mediators.

2. In feline arteries the inhibitive effect is significant, which could mean that there is a great variability in the distribution of slow calcium channels at this level as well as an increased responsivity to pharmacologically active constrictor agents that rely on the opening of L-type calcium channels.

3. It is possible that the density of slow calcium channels which are under the influence of gallopamil (D_{600}) is higher in cat mesenteric artery than in other animal species.

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