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EXPERIMENTAL STUDY OF CARDIOVASCULAR RECEPTORS BEHAVIOR IN RAT HEART AND AORTA. THE IMPACT OF SERUM FACTORS FROM HYPERTENSIVE SUBJECTS

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Abstract

Hypertension or elevated arterial blood pressure is the most common risk factor for cardiovascular disease and death and by the year 2025 the number of people with arterial hypertension will reach 1.5 billion. Objectives are to get some insights concerning the mechanisms of induction of arterial hypertension (AHT). We used freshly collected fragments of rat heart and aorta incubated with serum from hypertensive (HT) subjects as well as with different Na_2HPO_4 concentrations in order to follow up the changes in $^{45}CaCl_2$ fluxes, in ^3H Serotonine, ^3H Noradrenaline (NA) as well as in ^3H Cortisol uptake in rat aorta and heart. Our data have pointed out significant changes in $^{45}CaCl_2$ uptake in rat aorta and heart incubated with serum from (HT) subjects which can be pathologically correlated with the mechanism of (AHT) induction. A significant decrease in ^3H Serotonine and ^3H NA has been recorded both in rat aorta and heart incubated with (HT) serum which can be accounted for a suppressing of normal inhibitory mechanism .As far as ^3H Cortisol uptake is concerned, no change was noticed in the case of incubation with sera from (HT) subjects both in heart and aorta. When different Na_2HPO_4 have been used, ^3H NA and ^3H Serotonine uptake in rat aorta have pointed out an increase in ^3H NA uptake at phosphate concentrations close to the physiology ones, while in the case of ^3H Serotonine, there is a proportional increase in its uptake at higher Na_2HPO_4 concentrations. It seems that $^{45}CaCl_2$ admission depends very much on organic Na_2HPO_4 concentration.

Key words*: rat heart muscle, rat aorta, cell receptors, ^3H serotonine,^3H noradrenaline, hypertension*

INTRODUCTION

Hypertension or elevated arterial blood pressure is the most common risk factor for cardiovascular disease and death and by the year 2025 the number of people with arterial hypertension will reach 1.5 billion (Wenzel et al., 2016).

This disorder is a major risk factor for many common causes of morbidity and mortality including stroke, myocardial infarction, congestive heart failure, and end-stage renal disease (Lifton, 2001).

Stiffening of large elastic arteries and aorta is a hallmark of vascular aging and one of the most important determinants of the age-related increase in blood pressure and cardiovascular disease events (Cecelja, 2016).

Increased intraarterial pressure causes more tension in the arterial wall, both in smooth muscle cells and endothelial cells and increased production of growth factors (Lagerlof, 1989). During ageing, the distension of the arterial wall decreases progressively (Lithel, 1988), stiffness is also due to glycation process of cellular matrix (Fleckenstein, 1987).

Abnormal vascular smooth muscle cell proliferation is thought to contribute to the pathogenesis of vascular occlusion including atherosclerosis.

Atherosclerosis is multifactorial process extremely complex, a disease with slow progression, its onset is related with childhood, but became obviously in the middle or advanced age, when clinical symptoms indicate organic lesions (Aviram, 1993). Atherosclerosis, or hardening of the arterial blood vessel wall, is a chronic progressive inflammatory disorder. The disorder presents with coronary artery disease, carotid artery disease, peripheral artery disease, or combined, cardiovascular disease (CVD) Aberrant endothelial cells (EC) - vascular muscle cells (VSMC) interaction could promote atherogenesis (Mana Li, 2018).

Atherogenesis is a process characterized by the formation of a neo intima lesion that progressively occludes arterial lumen and neo intima thickening is due to accumulation of cellular and extra cellular substances in the space between intima and the underlying medial vascular smooth muscle (Nilsson, 1986).
Endothelial dysfunction is characterized by lipid accumulation and increase adherence of monocyte/macrophage and T lymphocytes which then migrate through endothelium and localize sub endothelium (Gimbrone, 2016).
In patients with essential arterial hypertension (Linder (1987) pointed out the effects of a circulating factor on intracellular Ca in normal platelets.
Objective: In order to get some insights related to the induction mechanisms of hypertension, we studied the modifications in $^{45}CaCl_2$ transport as well as the uptake of ^{3}H Serotonine, ^{3}H Noradrenaline and ^{3}H Cortisol in rat aorta and heart incubated with serum from hypertensive subjects as well as in the presence of different phosphate concentrations

MATERIALS AND METHODS

The study was done on 10 white Wistar rats aged 8 months old, with a weight between 180-200 grams, fed on standard chew.
Following anesthesia, the animals were killed by cervical dislocation, and thoracic aorta and heart were quickly removed by surgical opening of thorax cadge and were placed on ice bath in physiological saline solution for being processed for radioisotope uptake experiments within one hour.
10 ml of peripherial blood were collected on heparin test tubes by vein punction from hypertensive subjects and then serum has been separated from plasma by 3000 rpm centrifugation.
The heart fragments and aorta rings (50 mg each) were placed in test tubes and incubated with:
a) 20 µl of serum from hypertensive patients and then with $^{45}CaCl_2$, ^{3}H Cortisol, ^{3}H Serotonin and ^{3}H NA;
b) The incubation medium of rat aorta/heart fragments included the following chemicals:
- Na_2HPO_4 0.6 mM, 1.2 mM, 2.5 mM
- Na_2HPO_4 1.2 Beta glycerol phosphate 2.5 mM
- Na_2HPO_4 1.2 mM beta glycerol phosphate 5 mM solutions and then radioactivated by adding the following radioisotopes in the following concentrations:
1) $^{45}CaCl_2$ 0.15 uCi/ml
2) ^{3}H Serotonine 0.05 uCi/ml
3) ^{3}H Cortisol 0.05 uCi/ml
4) ^{3}H Noradrenaline 0.025 uCi/ml
The tissue samples were incubated over the night at 37°C. The extraction was done on next day by introducing biological tissue fragments in HCl 1N after being previously rinsed 3 successive times in bidistilled water.
The next day, these have been processed for radioactive uptake: the tissue fragments have been removed from extraction medium and 0.2 ml of incubation and 0.2 ml of extraction media have been introduced in 5 ml vials containing scintillation liquid. Then, the samples from incubation and extraction media were subjected to estimation of radioactivity uptake assay has been done in fluid phase in Beta Berthold Scintillation Counter for beta radiations.

RESULTS AND DISCUSSIONS

The data have been statistically processed and their percentage values were referring to control values. The significance of the difference between the two groups of samples incubated with serum from hypertensive subjects, under study divided by standard deviation of the difference was then calculated:

$$t=d/Sd \qquad d=Xm-Xp$$

$$Sd=\frac{d2-(d2)/h}{n(n-1)}$$

P values (counter probability) was calculated in reference to the t value and the degree of experimental freedom. The difference between samples under study was considered more marked (Table 1).

Table 1. Representation of values statistically calculated (t and p) obtained after calculation of difference significance between the two groups of biological samples incubated with serum from HT subjects

Radioisotope	$^{45}CaCl_2$		^{3}H Serotonine		^{3}H Cortisol		^{3}H NA	
Biological material	t	p	t	p	t	p	t	p
Heart	2.3	5-2%	2.1	5%	0.11	60%	3.17	1%
Aorta	2.4	5-2%	2.8	2-1%	1.31	40-20%	1.94	5%

The normal behavior of the vascular and cardiac cell membrane permeability in terms of their ability to uptake $^{45}CaCl_2$ changes in contact with sera from hypertensive patients (an enhanced Ca uptake is to be noticed in the case of both aorta and heart tissue, p ranges from 5-2%) (Linder, 1987).

In the case of samples incubated with serum from hypertensive subjects, the uptake of ^{3}H Serotonine falls below the confidence levels. The drop is more marked in aorta p=2%-1% than in heart p=5%. The role of Serotonine in neurotransmission is hypothetical in some forms of experimental arterial hypertension.

Concerning ^{3}H Cortisol uptake, there are no changes in rat heart or aorta when these are incubated with serum from hypertensive subjects. When incubated with serum from HT subjects in the presence of ^{3}H NA the uptake is much lower in rat heart (p=1%), and close to the significance ranges in rat aortic tissue (p=5%).The depressed uptake of ^{3}H NA is relatively difficult to interpret; this phenomenon might mean a suppressing of a normal inhibitory mechanism (Table 2, fig.1).

Table 2. Mean values of $^{45}CaCl_2$, ^{3}H Serotonine, ^{3}H Cortisol and ^{3}H NA in rat aorta and myocardium incubated in Hanks physiological solution with serum from control patients (cpm/g tissue)

Biological material	$^{45}CaCl_2$	^{3}H Serotonine	^{3}H Cortisol	^{3}H NA
Heart	154.68	81.40	101.72	88.18
Aorta	151.17	74.10	121.49	86.83

There is almost constant values of radioactive uptake in rat aorta and heart in the presence of serum from control patients free of HTA pathology (fig. 2).

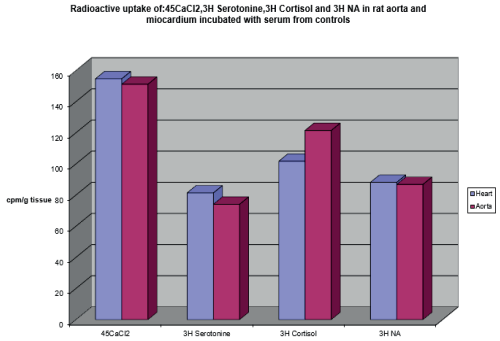

Figure 1. Histogram of of $^{45}CaCl_2$, ^{3}H Serotonin, ^{3}H Cortisol and ^{3}H NA in rat aorta and myocardium incubated in Hanks physiological solution with serum from Control patients (cpm/g tissue)

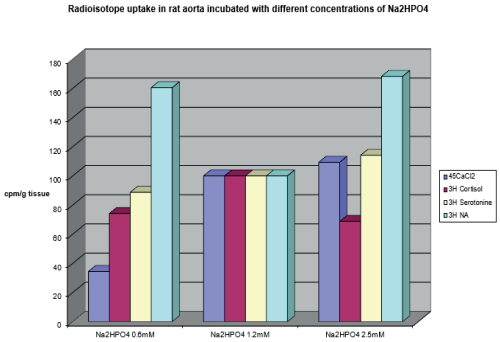

Figure 2. Radioisotope uptake in rat aorta in presence of different concentrations of Na_2HPO_4

Table 3. Radioisotope uptake in rat aorta incubated with different concentrations of organic and inorganic phosphate + mixture of 1.2 mM Na_2HPO_4 and 5 mM Beta Glycerol phosphate (B.G.P.)

Phosphate	Na_2HPO_4			Na_2HPO_4 1.2 mM	
Concentrations	0.6mM	1.2mM	2.5mM	B.G.P. 2.5mM	B.G.P. 5mM
Radioisotope $^{45}CaCl_2$	34.4	100	109.3	101.6	99.1
^{3}H Cortisol	74.2	100	68.8	69.1	93.4
^{3}H Serotonine	88.7	100	114.1	96.04	175.14
^{3}H N.A.	160.5	100	168.2	161.1	98.2

1.2 mM Na_2HPO_4 was considered physiological normal (fig. 3).

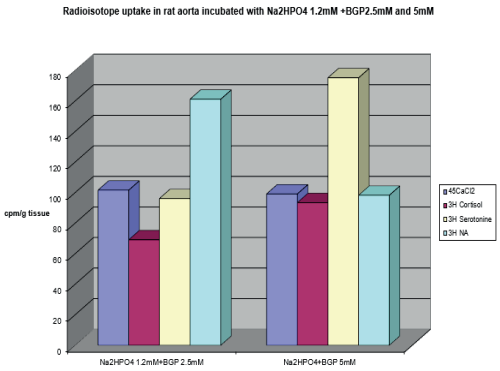

Figure 3. Radioisotope uptake in rat aorta incubated with Na_2HPO_4 1.2mM+B.G.P. 2.2mM and 5mM

From our data, it seems that $CaCl_2$ admission depends very much on organic phosphate concentration.

It is obvious that at lower concentrations to those physiological ones, Ca^{+2} transport is very much slowed down (only 34.4%) versus control (fig. 1) while at higher concentrations as well as different concentrations of organic phosphate, this process is not significantly modified.

Concerning ^3H Cortisol uptake, at both below and above normal phosphate concentrations there is a decrease in ^3H Cortisol uptake and the phosphate in higher concentration determines an uptake of ^3H Cortisol in rat aorta close to that found in controls (Tabel 3).

There are no significant differences between the radioisotope admission at 1.2 mM Na_2HPO_4 in comparison with the mixture of 1.2 mM Na_2HPO_4 and 5 mM Beta Glycerol phosphate (B.G.P.).

From our radioactive uptake of ^3H Serotonin in the presence of different phosphate concentrations, it seems that this process is not influenced by concentrations close to normal ones, but it is very much increased in presence of phosphate in excess.

The behaviour of ^3H NA uptake is very different. This uptake of ^3H NA is very much increased at different organic phosphate concentrations than physiological normal concentration and close to normal in the presence of phosphate in excess.

CONCLUSIONS

The data on $^{45}CaCl_2$, ^3H Serotonine, ^3H NA uptake by rat heart and aorta in the presence of serum from HT subjects pointed out a change in normal behavior of cell membrane receptors and in the ionic flow and neural mediators uptake.

In the presence of different Na_2HPO_4 concentrations, there are modifications of $^{45}CaCl_2$ incorporation in rat aorta, which can be pathologically correlated with the mechanism of arterial hypertension induction.

The decrease in ^3H Serotonine and ^3H NA uptake is relatively difficult to interpret, these phenomena can be accounted for a suppressing of a normal inhibitory mechanism.

Our studies seem to point out that in the case of arterial hypertension there are not any modifications in ^3H Cortisol uptake.

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