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β - OXIMA PROMOTES RELAXANT EFFECT IN RAT AORTA THROUGH KATP AND KIR POTASSIUM CHANNELS

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Abstract: Oximes are called organic compounds whose general formula is RR'C=-NOH, considering an organic substituent on R' and a hydrogen or any other organic group on R. They can be pharmacologically active when they release NO through oxidative cleavage of the C=NOH functional group and thus play an important role in the vascular system in relation to the vasorelaxation process. This study used male *Rattus norvegicus* which were sacrificed in accordance with the procedures approved by the UNIVASF Animal Experimentation Committee (Protocol No. 0003/111213). The organs were removed, dissected and sectioned into 3-5 mm rings, which were mounted in glass vats (10 ml) containing a medium conducive to keeping the organ alive. Contractions and relaxations were monitored by force transducers coupled to a digital acquisition system. The work presents pharmacological evidence to support the hypothesis that β-oxime has a relaxing effect reaching its maximum effect of 100% in a concentration-dependent and endothelium-independent manner in isolated rat aorta through the following mechanism of action, the relaxation of aortic smooth muscle promoted by β-oxime depends on potassium channels of the type K_{ATP} and K_{IR} ; there is no dependence on the direct nitric oxide pathway, nor on the synthesis or participation of$_{s}$ GC, with the relaxing effect promoted by this oxime. However, other mechanisms need to be investigated in order to better elucidate cell signaling.
Keywords: aorta, β-oxime, smooth muscle, nitric oxide.

INTRODUCTION

Oximes are organic molecules with a general formula RR'C=NOH, where an organic substituent is present on R' and any organic group, or even a hydrogen, is present on R. These molecules are obtained from condensations between aldehydes or ketones with hydroxylamines (PERES, 2009; VERAS, 2009).

These compounds are capable of promoting the donation of nitric oxide (NO). This donation has motivated studies using oximes as intermediate precursors in the synthesis and development of drugs, natural and industrial products (ARAUJO; GONSALVES, 2015; COMPENDIUM, 2012). The production and consequent release of NO reported in vascular endothelium, cerebellum, macrophages and other cells mediates various physiological phenomena, such as platelet adhesion and aggregation, relaxation of the human corpus cavernosum and endothelium-dependent vasorelaxation (CERQUEIRA, 2002; DIAS, 2011).

Derived from L-arginine and synthesized in the vascular endothelium by nitric oxide synthase (NOS), NO is a fat-soluble gas that is directly related to the process of relaxing the smooth muscles of blood vessels, as it is one of the factors responsible for maintaining and modulating vascular diameter and resistance (CERQUEIRA, 2002; DIAS, 2011). In vascular smooth muscle, it promotes vasorelaxation via NO-GC-PKG and direct activation of some proteins and ion channels (MENDES-JÚNIOR et al., 2015).

Under physiological conditions, vasorelaxation occurs when endothelial cell membrane receptors are activated by soluble stimuli (for example: acetylcholine, adenosine diphosphate and bradykinin) or when there is an increase in the friction exerted by circulating cells on the endothelial layer (shear stress), resulting in the activation of e-NOS present in these cells and the consequent production of NO (FURCHGOTT, ZAWADZKI, 1980).

Alterations in the function and structure of vessel smooth muscle and consequently an increase in peripheral vascular resistance are characteristic of hypertension (TAHVANAIMEN et al., 2006). Vascular smooth muscle is the contractile component of arteries, arterioles and veins and its contraction and relaxation derives directly from intracellular and extracellular signals, which when altered can cause this muscle to contract, thus contributing to increased resistance to the passage of blood and thus raising blood pressure, resulting in pathologies such as ischemia and myocardial infarction, angina pectoris and Systemic Arterial Hypertension (SAH) (RANG, 2007; GUYTON; HALL, 2011).

SAH, which is defined as a multifactorial clinical condition characterized by high and sustained levels of blood pressure (BP), has been shown to be an endemic problem with low control rates and high prevalence (BRASIL, 2010; BOMBIG, 2014; GUIDELINES, 2020). Hypertension is one of the main modifiable risk factors and one of the most important public health problems, despite the fact that several drugs have already been developed and used to treat this disease. Scientific evidence reports that small reductions in blood pressure (BP) have a major impact on reducing cardiovascular morbidity and mortality (BRASIL, 2010; BOMBIG, 2014; GUIDELINES, 2020).

Mortality associated with hypertension has remained high, making research into new therapeutic alternatives for treating this condition one of the main objectives of various research groups around the world (LESSA, 2010). In Brazil, data from the Ministry of Health has shown that hypertension currently affects between 20% and 40% of the young population. This data, as well as the resulting clinical complications, means that hypertension plays a prominent role in public health programs (BRASIL, 2010; LESSA, 2001; DIRETRIZES, 2020).

The main function of hypertension treatment is to reduce cardiovascular morbidity and mortality. Thus, antihypertensive drugs should not only reduce blood pressure, but also fatal and non-fatal cardiovascular events, and consequently the mortality rate (KANNEL, 1996; GUIDELINES, 2020). The drugs used in the direct treatment of AH are known to target the heart, kidneys and blood vessels, especially small-caliber arteries, inducing relaxation of the vascular smooth muscle and thus reducing resistance to the passage of blood through it (CLARK; PYNE-GEITHMAN, 2005).

A wide range of biological systems can be used as experimental prototypes for preclinical pharmacological trials, with the aim of developing new drugs for various pathologies, so with the treatment of AH as a focus, the ideal model for study would be the cardiovascular system (SILVA, 2014).

With this knowledge, pharmacological, epidemiological, clinical and genetic studies have been carried out to better understand the mechanisms involved in the development of hypertension, as well as to improve methods of diagnosis, treatment and prevention (BOMBIG, 2014; SILVA, 2014).

Knowing that the release of NO produced by the endothelium plays a crucial role in regulating and maintaining the functioning of most biological systems, especially the cardiovascular system, and seeking to overcome the limitations of classic NO donors, *in vitro* studies involving the measurement of smooth muscle contraction and relaxation, as well as the elucidation of the mechanism of action of molecules capable of promoting this activity are relevant in the attempt to discover new therapeutically useful, safer, more potent and effective drugs (CAMORETTI-MERCADO, 2009; WATTERSON, RATZ, SPIEGEL, 2005).

METHODOLOGY

SUBSTANCES AND SALTS

Oxime test (NE)-N-[1-naphthalen-1-yloxy-3-(propan-2-ylamino)-propan-2-ylidene] hydroxylamine (Figure 1B). All the substances were solubilized in pure water or, when this was not possible, in suitable solvents indicated by the manufacturers, as long as these were not overly toxic to the isolated organs used in this study.

NUTRITIONAL SOLUTIONS

Krebs nutrient solution was used, with its pH adjusted to 7.4 with HCl (1N) and aerated with a carbogenic mixture (95% O_2 and 5% CO_2), the composition of which is as follows: NaCl (118 mM), KCl (4.6 mM), $MgSO_4$ .7H_2 O (5.7 mM), KH_2 PO_4 .H_2 O (1.1 mM), $CaCl_2$ .2H_2 O (2.5 mM), $NaHCO_3$ (25 mM) and glucose (11 mM). The salts were solubilized in distilled water.

PREPARATION OF THE OXIMA STOCK SOLUTION

The stock solution was prepared by diluting the solid, crystalline oxime in dimethyl sulphoxide (DMSO), thus obtaining a solution of 10^{-2} M (stock solution), which was stored at -20°C and then diluted successively to obtain concentrations of 10^{-3} M, 10^{-4} M and 10^{-5} M.

EQUIPMENT

To record isometric and isotonic contractions, rat aorta segments were suspended in vats (10 mL) of a bath system for isolated organs model EFF-321 (Insightâ Instruments, Brazil). To monitor isometric and isotonic contractions, force transducers model TRO015 (Panlabâ, S.L., Spain) were used, coupled to a bridge system force amplifier (Insightâ Instruments, Brazil), connected to a computer, where contraction and relaxation records were observed and stored using DATAQ® software. To measure the pH of the nutrient solutions, a benchtop digital pH meter model pH250 (Policontrolâ, Brazil) was used. All the substances were weighed on an analytical balance model FA2104N (Celtacâ, Brazil) or semi-analytical balance model MARK300 (Belâ, Brazil) and the animals on a common balance model 9094C/4 (Toledoâ, Brazil).

ANIMALS

Wistar rats (*Rattus norvegicus*) weighing between 250 and 350g were used, all from the UNIVASF Central Animal Facility. Before the experiments, the animals were kept under strict dietary control with a balanced pellet diet and free access to water, with constant ventilation and temperature (22 ± 1°C), subjected to a 12-hour light-dark cycle every day, with the light period lasting from 06:00 to 18:00. All the experiments were carried out between 07:00 and 20:00.

ETHICAL ASPECTS

All the planned experiments were carried out in accordance with the ethical principles of animal experimentation of the UNIVASF Animal Experimentation Ethics Committee (Protocol No. 0003/111213) (Annex I) and the Brazilian College of Animal Experimentation (COBEA). For the experiments, all the animals were euthanized quickly and painlessly, always by a professional trained in animal husbandry or by the veterinarian responsible for the vivarium, in compliance with Decree No.º 24.645 of July 10, 1934 and Law No.º 6.638 of May 8, 1979, which establishes rules for the didactic-scientific practice of animal vivisection. After being euthanized, the animals were buried in a suitable place, in accordance with good animal handling practices, or placed in a suitable cold room designed for this purpose, and then buried or incinerated. The drugs and substances used in

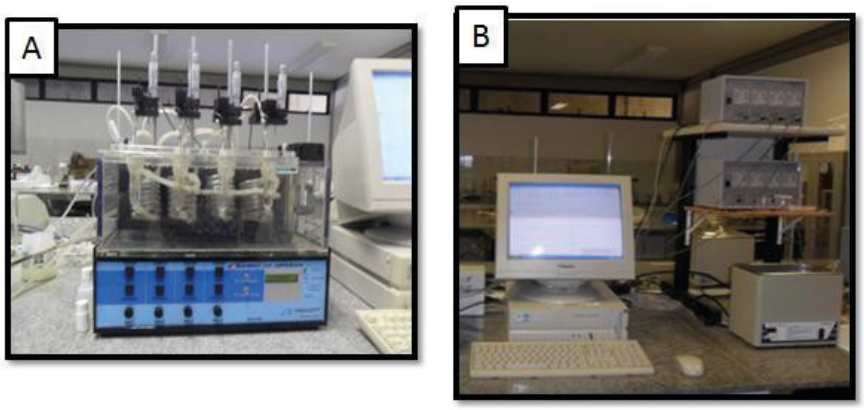

Figure 2 - photos of the equipment used to carry out the experiments (A) bath for isolated organs model EFF-321 (Insight[å] Instruments, Brazil. (B) force transducers model TRO015 (*Panlab*[å] , *S.L.,* Spain) coupled to a bridge system force amplifier (Insight[å] Instruments, Brazil), connected to a computer.

Source: author's own

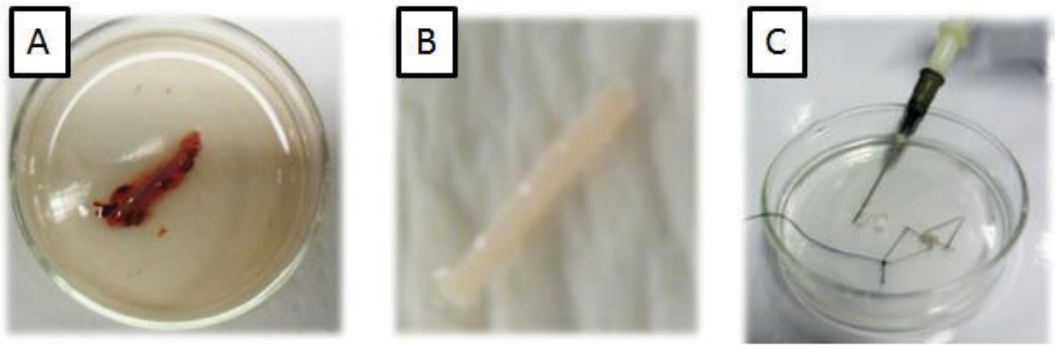

Figure 4 - (A) Photos of rat aorta before cleaning (B) Cleaned aorta ready to be sectioned into 3-5mm rings (C) Petri dish with aortic ring on metal rod attached to cotton thread immersed in Krebs solution and aerated with carbogen.

Source: Author's own.

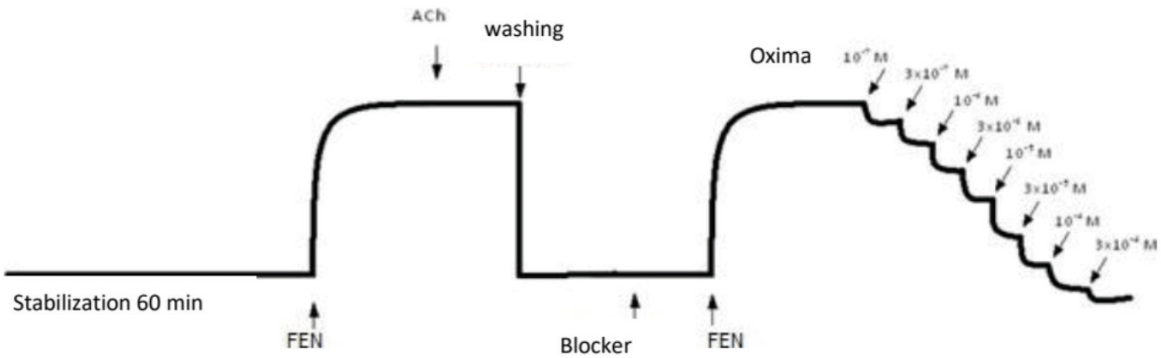

Figure 5 - Diagram showing the addition of substances throughout the experiment.

the experimental procedures, as well as some consumables and other materials (syringes, needles, disposable latex gloves, remains of animal viscera, scalpel blades, etc.) were treated in the same way as hospital waste in the municipality of Petrolina-PE, according to ANVISA's RDC 306 of December 7, 2004, which sets out the technical regulations for the management of health service waste.

PREPARATION OF ISOLATED RAT AORTIC RINGS

The rats were sacrificed by cervical dislocation followed by sectioning of the cervical vessels. The animal's thorax was opened and dissected, the thoracic aorta was carefully removed (Figure 4A) and 3-5 mm aortic rings were obtained free of connective and adipose tissue (Figure 4B). To obtain the isometric responses, the rings were suspended individually by stainless steel rods attached by cotton threads (Figure 4C) to force transducers, in glass vats (10 mL) containing Krebs solution at 37 °C and aerated with a carbogenic mixture (95% O_2 and 5% CO_2). The aortic rings were left to rest for a period of 60 min, during which time they were kept under an initial tension of 1 g. During this period, the nutrient solution was renewed every 15 min to prevent interference due to the accumulation of metabolites (ALTURA; ALTURA, 1970).

After the stabilization period, a contraction was induced with FEN at a concentration of 10^{-6} M. The integrity of the vascular endothelium was checked by adding ACh (10^{-6} M) to the vat during the tonic phase of the first FEN-induced contraction (FURCHGOTT, ZAWADZKI, 1980). Rings without functional endothelium were obtained by mechanically removing the endothelial layer through friction caused by the metal rod in contact with the inner wall of the vessel. Removal of the endothelium was confirmed by the absence of ACh-induced relaxation, which was considered intact when the aortic rings showed ACh--induced relaxation equal to or greater than 50% (in relation to the maximum force of the initial contraction) or when it was less than 10%. After checking whether or not the endothelium was present, the bath solutions were changed and the preparations washed every 15 min with Krebs solution for a total period of 30 min. After this time, a potassium channel blocker or another blocker of the pathway to be analyzed was added and soon after a second contraction was induced by adding 10^{-6} M of FEN (Figure 5) and during the tonic and sustained phase of this second contraction, the drugs to be tested were added to the vat in a cumulative manner, and their mechanism of action of the relaxing effect, efficacy and potency determined in rat aortic rings in the absence of functional endothelium.

After the maximum relaxing effect (E_{max}) induced by the tested products was reached, the bath solution was changed every 15 min, for a total period of 30 min, and after this time, a third contraction was induced by FEN, where it was observed whether the maximum tension reached was of a similar magnitude to the first one contraction, which was indicative of the reversibility of the relaxing effect of the products tested.

All relaxations were expressed as the reverse percentage of the maximum tension obtained by adding FEN to the tank, where maximum relaxation was obtained when the recorded tension was reduced to the initial baseline levels.

STATISTICAL ANALYSIS

All data obtained is expressed as mean ± standard error of the mean. The value of "n" refers to the number of animals used in each experiment in a given protocol, which can be up to a maximum of 7 animals, so that the result obtained could be representative and at the same time not too many animals were used.

Differences between the means were compared statistically using the unpaired Student's t-test, where these differences were considered significant when the calculated p-value was less than 0.05. Statistical analyses were carried out using the Graph-Pad Prism program© 5.0 (GraphPad Software Inc., San Diego, CA, USA).

DEVELOPMENT

After obtaining the results, it is possible to observe that this study has productive relevance for understanding the possible pathways through which oximes are able to promote a relaxing effect in rat aortic smooth muscle. During the 1980s, Furchogott and Zawadzki were pioneering researchers in studying and demonstrating the importance of the vascular endothelium in controlling tone and the relationship between the endothelium and a relaxation factor dependent on it in vasodilation, a factor that was later named EDRF (CERQUEIRA; YOSHIDA, 2002; ZAGO; ZANESCO, 2006; PERES, 2009).

With this knowledge and knowing the possible involvement of endothelial components, it was necessary to first check for the presence of a functional endothelium in order to later assess the participation, and relationship, of EDRF in the response promoted by oxime.

The presence or absence of functional endothelium was assessed by adding ACh (10^{-6} M). The original representative recordings showed that the rings with functional endothelium were those with a relaxation of more than 80% (in relation to the maximum force of the initial contraction) and rings without endothelium, with a relaxation of less than 10%.

In the field of pharmacology, the search for different types of NO donors through synthetic chemistry has been a focus for the development of new drugs for some time (LOHSE; FORTERMANN; SCHMITT, 1998). In order to carry out studies with these molecules, it is important to take into account the type of NO donor, as several studies have suggested that the mechanism of vascular relaxation may vary between different NO donors (WANSTALL, et al., 2001), thus leading to different responses to the possible mechanisms involved in the effect.

It should also be remembered that NO donors are pharmacologically active substances that release NO spontaneously or through other pathways (BOUCHER, et al., 1992). The oxidative cleavage of the C=NOH functional group present in oximes can be catalyzed not only by NO synthase, but also by the microsomal cytochrome P450 complex or by peroxidases present in the environment (RENAUD, et al., 1993; JOUSSERANDOT, et al., 1998).

β-oxime (10^{-7} to $3x10^{-4}$ M) promoted relaxation in a concentration-dependent and endothelium-independent manner in isolated rat aortic rings pre-contracted with FEN (10^{-6} M), with a maximum efficacy ($E_{máx}$) of 100% in the absence and presence of functional endothelium.

The results obtained are compatible with data in the literature showing that some oximes function as a type of NO donor leading to the consequent vasorelaxation of rat aortic rings, demonstrating involvement with the NO-cGMP pathway (JAROS, et al., 2007; CHALUPSKY, et al., 2004). The existence of a NO synthase-independent pathway capable of oxidizing some compounds with the C=NOH bond, present in oximes, has also been demonstrated in previous studies, and may be a pathway involved in the effect of β-oxime (VETROVSKY et al., 2002). It has therefore been proven that some oximes can be used as NO donors in arteries where NO synthase activity is compromised (JAROS, et al., 2007).

To prove that the solvent used did not interfere with the relaxing effect promoted by oxime, a control was carried out by adding 200μL of absolute ethanol to the vat, where it was possible to see that there was no relaxation in a time interval of seventy minutes.

Some studies show that non-selective β-blockers, such as propranolol, can act on up to three subtypes of these receptors. The β_1 receptors are responsible for the increase in cardiac output, heart rate and ejection fraction volume, the release of renin from juxtaglomerular cells and the lipolysis of adipose tissue (BORTOLOTTO; CONSOLIM- COLOMBO, 2009). The β_2 type are polymorphic adrenergics that predominate in the smooth muscles of the bronchi. They are also responsible for the lipolysis of adipose tissue, glycogenolysis and gluconeogenesis, inhibition of the release of histamine by mast cells, among other things. And those of the β_3 type whose functions include stimulating the lipolysis of adipose tissue (BORTOLOTTO; CONSOLIM-COLOMBO, 2009).

Some β-blockers have a vasodilator action, such as carvedilol and labetalol, which are also able to antagonize peripheral α_1 receptors, and nebivolol, which stimulates the production of nitric oxide (HELFAND; PETERSON; DANA, 2007). However, considering that the oxime studied comes from changes in the structure of the propranolol molecule, and that propranolol is a β-blocker that has no relaxing effect on blood vessels, it is suggested that the relaxing effect obtained comes from the molecular responses promoted by the modified portion of the molecule.

It is important to note that previous studies on the ketoxime of propranolol have shown that despite the molecular alterations made to obtain the β-oxime in one of its pharmacophoric groups responsible for interaction through hydrogen bonds with the active site, the β-blocking activity of the molecule is not significantly altered (BODOR, et al., 1988; BARREIRO; FRAGA, 2008).

Thus, the endothelium-independent relaxing effect promoted by β-oxime is consistent with the results demonstrated in the literature and studies carried out with other oximes.

In addition to evaluating the involvement of potassium channels in the relaxing effect promoted by oxime, it was also necessary to evaluate the involvement of the nitric oxide pathway, since the test substance is an oxime and it was expected that there would be spontaneous donation of NO by the molecule. Other important elements for this pharmacological study of endothelial function are the blockers of the effects and synthesis of EDRF/NO. Among these, the most relevant are: methylene blue (blocking cyclic GMP); hemoglobin ("sequester" or scavenger of EDRF/NO); and the nitric (NG-nitro-L-arginine, L-NOARG) and methylated (NG-monomethyl-L-arginine, L-NAME) forms of L-arginine, which are nitric oxide synthase blockers (EVORA, 1993).

Among the EDRFs, nitric oxide (NO) is the main factor produced and released by the endothelium, which through a paracrine action on the vascular smooth muscle induces its relaxation (IGNARRO et al., 1987). This EDRF is produced by the endothelial nitric oxide synthase enzyme, eNOS, which converts L- Study of the mechanism of action of the monoterpene borneol in the cardiovascular system 2013 95 arginine and molecular oxygen into L-citrulline and NO, and which can be competitively inhibited by false substrates such as L-NAME (FELETOU; VANHOUTTE, 2006).

In mammalian vascular endothelium, various extracellular signals can stimulate NOS and consequently increase NO production (FURCHGOTT AND ZAWADZKI, 1980). Nitric oxide produced by the endothelium diffuses rapidly into the smooth muscle cell where it activates soluble guanylyl cyclase (sGC), which in turn generates cyclic guanosine monophosphate (cGMP) that mediates vasodilation (MONDACA et al., 1991). A traditional approach to assessing NO production is through the use of L-NAME, since the expected effect of blocking NO production would be an increase in basal tension.

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The data obtained in this study showed that even after inhibiting nitric oxide synthesis, oxime was able to promote its relaxing effect independently of this pathway.

In the control condition and in the presence of L-NAME at a concentration of 10^{-4} M, the value of $E_{máx}$ was 94.4%, which was reached at a concentration of $3x10^{-4}$ M of β-oxime. This suggests that there is no involvement of the nitric oxide pathway through the production of NO by eNOS in the relaxing effect promoted by β-oxime, as there was no significant difference between the EC values$_{50}$, as can be seen in the concentration x response curve.

The soluble guanylyl cyclase pathway is associated with endothelial dysfunction, microcirculatory derangement, severe fluid loss, and critical hypotension. Inhibition of the 3',5'-cyclic guanosine monophosphate/soluble guanylate cyclase (cGMP-CGs) pathway by methylene blue (MA), a matrix thiazide, has been widely used in pharmacological studies associated with the down-regulated release of nitric oxide by the action of inducible nitric oxide synthase, indicate that the nitric oxide/cyclic guanosine 3',5'-monophosphate (NO/CGMP) pathway may be a potential target for numerous therapeutic interventions (BALDO, 2013).

The results obtained in this study show that this oxime promotes its relaxing effect independently of the nitric oxide pathway and in a manner dependent on potassium channels. The data suggest that perhaps this oxime is not releasing NO from its molecule and that the complete molecule is promoting its effect independently of sGC.

Literature data shows that activation of potassium channels in vascular smooth muscle cells can cause vasodilation and increase blood flow, as well as lowering blood pressure. With this knowledge, it is possible to conclude that the consequent inhibition of these channels causes vasoconstriction. To date, it has been known that there are four types of potassium channels, K_v , K_{Ca} , K_{ir} and K_{ATP} , which regulate cell membrane potential (NELSON, QUAYLE, 1995).

Studies show that Ca sparks^{2+} are capable of activating the synthesis of NO, endothelins, prostanoids, among other endothelium-derived factors (BOLTON, 2006), as well as Ca activated potassium channels^{2+} (K_{Ca}^{2+}), which modulate membrane potential and cell excitability itself (JACKSON- WEAVER et al., 2013) and are identified as one of the components of the endothelium-derived hyperpolarizing factor (EDHF) (SCHMIDT et al., 2010). In addition, there is evidence in the literature that TEA, which is a relatively effective blocker of K_{Ca} (LANGTON et al., 1991), can reduce the action of EDHF on vascular smooth muscle (CHUNG et al., 2012).

Among the currently known K^+ channel blockers of pharmacological importance is TEA, which can block a diverse range of potassium channels at concentrations close to 10 mM, but it is at concentrations of up to 1 mM that TEA is selective in blocking only BK_{Ca} (NELSON, QUAYLE, 1995; JACKSON, 2000). Calcium-dependent potassium channels (K_{Ca}) were thus characterized due to their activation by an increase in the intracellular concentration of Ca^{2+}. In addition, K_{Ca} channels also increase their activity according to membrane depolarization and can also be affected by other vasodilator stimuli (NELSON, QUAYLE, 1995; JACKSON, 2000).

The K_{Ca} are of significant physiological importance, as they play a role in regulating myogenic tone. Beta adrenergic stimulation activates the K_{Ca} channels in vascular and bronchial smooth muscle, causing vasodilation. This process in the smooth muscle of the coronary arteries occurs through protein kinases that depend on cAMP, as well as the G protein pathway (NELSON, QUAYLE, 1995; JACKSON, 2000).

In hypertension, there is an increase in the activity of calcium-dependent potassium channels as a result of the increase in blood pressure. This situation can be reversed by the administration of antihypertensive treatment. The increase in the activity of these channels in vascular smooth muscle cells can function as a compensatory mechanism for a progressive increase in blood pressure, and can thus provide a negative feedback mechanism helping to restrict the increase in pressure and vascular tone (NELSON, QUAYLE, 1995; MICHELAKIS, et al., 2000; JACKSON, 2000).

Some researchers have shown through electrophysiological studies in arterial myocytes isolated from hypertensive animals that K^+ currents through calcium-dependent potassium channels are increased compared to normotensive myocytes (NELSON, QUAYLE, 1995; MICHELAKIS, et al., 2000; JACKSON, 2000), With this knowledge applied to the results obtained with β-oxime in the presence of 1 mM TEA, it is possible to suggest that these channels are not involved in the relaxation process promoted by test oxime.

4-AP is a blocker of voltage-gated potassium channels (K_v). Kv channels can be selectively inhibited by 4-aminopyridine, which is often used to distinguish these channels from calcium-dependent potassium channels (K_{Ca}) that are also activated by depolarization. These channels are present in the cell membrane of vascular smooth muscle and are activated by depolarization of the membrane from the moment it reaches the ideal potential between -35mv and -55mv, which supports the idea that K-type channels$_v$ are essential in regulating depolarization and consequent vasoconstriction (NELSON, QUAYLE, 1995; JACKSON, 2000).

The K_v channels also play an important role in the repolarization phase of the membrane potential in many excitable cells and also in vascular muscle cells which respond to stimuli through gradual membrane potentials. In addition to their importance in repolarizing the action potential, these channels are also opened by vasodilators acting via cAMP, while vasoconstrictors close these channels through mechanisms that increase the intracellular concentration of Ca^{2+} and activation of protein kinase C.

It is believed that the reduction in the availability of NO derived from the endothelium in chronic hypertension can lead to depolarization of the vascular muscle cells and consequent contraction due to inhibition or closure of the K_v. After analyzing the results and knowledge about K-type channels$_v$, it is suggested that there is no participation of these channels in the relaxing effect promoted by this oxime as there was no significant difference between the EC values$_{50}$, which can be seen in the concentration x response curve.

K_{ATP} (ATP-sensitive potassium channels) are involved in the relaxing effect of a wide range of substances, such as adenosine (FURIAN, 2009), PGI2 (JACKSON et. al.,1993), acetylcholine, whose vasorelaxant action is dependent on the endothelial production of NO (GARLAND; MCPHERSON, 1992), but a few vasorelaxant substances have direct activity on these channels as their main characteristic, such as nicorandil, pinacidil and levocromacaline (LUCKHOFF; BUSSE, 1990; GOJKOVIC-BUKARICA; KAZIC, 1999; SATO, 2000). Most of these relaxing substances act through molecular mechanisms capable of modulating the opening of K_{ATP}, such as the phosphorylation of this channel as a result of the activation of the cAMP/PKA pathway, in the case of adenosine and PGI2 (EGUCHI et al., 2007), or cGMP/PKG, in the case of NO (SOUZA; BOUSKELA, 2013).

ATP-dependent or ATP-sensitive potassium channels in vascular smooth muscle are characterized into two types, the first being those of small or immediate conductance,

which have been identified in the specific smooth muscle cells present in the portal vein, coronary arteries and bladder, and the second type being those of high conductance which have been identified in the vascular smooth muscle cells of the mesenteric arteries and canine aorta. Scientific evidence suggests that the heterogeneity in the conductance of this type of channel may be due to the existence of multiple isoforms (NELSON, QUAYLE, 1995; MICHELAKIS, et al., 2000).

These channels can be blocked pharmacologically by drugs such as Glibenclamide as well as by barium ions (Ba^{2+}). And they can be opened by some antihypertensive drugs such as minoxidil sulphate (NELSON, QUAYLE, 1995; MICHELAKIS, et al., 2000.

The results thus suggest that the relaxing effect promoted by β-oxime involves K-type potassium channels$_{ATP}$. By analyzing the graph of the concentration x response curve, it can be seen that not only was the curve shifted to the right, but also the $E_{máx}$ was 48.6%, which was achieved at a concentration of $3x10^{-4}$ M.

Inward rectifier potassium channels (K_{ir} - inward rectifier) are found in vascular smooth muscle cells, as well as other cell types. These channels are characterized by constantly having an inflow of K ions^{+} greater than the outflow for any given voltage value. Although the current of these ions out of the cell through the K_{ir} channels is small, under physiological conditions the cell's membrane potential is positive in relation to the electrochemical equilibrium potential of K^{+} which generates an electrochemical gradient leading potassium to leave the cell by passive diffusion.

As seen above, K_{ir} are characterized as a type of K_{ATP} and can be blocked by $BaCl_2$. In other words, the participation of K_{ir} channels in the relaxing effect promoted by oxime proves the possible participation of K_{ATP} proven by blocking the channels with glibenclamide.

In other words, after presenting the data and analyzing the displacement of the curve of the concentration x response graph, it can be suggested that this type of channel does participate in the relaxing effect promoted by oxime. In the control condition and in the presence of $BaCl_2$ at a concentration of 10^{-4} M, the $E_{máx}$ value was 62.3% and was reached at a concentration of $3x10^{-4}$ M of β-oxime. When evaluating the average time needed to obtain the maximum effect, it can be seen that there is no significant difference.

CONCLUSION

In view of the data presented, it is possible to conclude that pharmacological evidence has been gathered to support the hypothesis that β-oxime has a concentration-dependent and endothelium-independent relaxing effect on isolated rat aorta;

Apparently, the nitric oxide pathway is not involved in the relaxing effect of β-oxime; the relaxation of aortic smooth muscle promoted by β-oxime depends on potassium channels of the K_{ATP} and K_{IR} types.