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Cooling and Response to Histamine in Calf Cardiac Vein

K. E. ATALIK[1*] M. KILIÇ[1]

[1]Department of Pharmacology, Faculty of Meram Medicine, University of Selçuk, Konya, 42080, Turkey

[*]**Corresponding Author**
e-posta: esraatalik@hotmail.com

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Abstract

In the present work we studied the responses of calf cardiac vein to histamine (10-9-3x10-4M) and effects of moderate cooling (to 28 ˚C) on these responses with analysis of the role of endothelial mediators and K+ -ions. Concentration-response curves to histamine were isometrically recorded at 37 and 28 ˚C (control). The same procedure was repeated at 28˚C in the presence of NG-nitro-L-arginine methyl ester (L-NAME, 10-4 M), indomethacin (10-5 M), and also in the K+-free medium. During cooling, the sensitivity, but not the maximal response, was significantly lower than 37 ˚C. Cooling to 28˚C after treatment with L-NAME did not modify the effect of cooling, whereas treatment with indomethacin increased, significantly. Furthermore, cooling to 28 ˚C after incubation in K+-free solution increased the sensitivity to histamine. The results of this study suggest the role of cyclooxygenase pathway and also K+ ions in the cooling-induced changes of calf cardiac vein treated with histamine.

Key words: cardiac vein, cooling, histamine, K+ ion, nitric oxide.

INTRODUCTION

The contraction of vascular smooth muscle in response to vasoactive substances can be influenced by moderate cooling [1-3]. Contrary to the contractile responses in cutaneous vessels, the aorta and pulmonary artery dilate when exposed to cooling [4]. Since the description of the essential role of the endothelium in mammalian arteries , it has become obvious that endothelial cells release several relaxing and contracting substances [5]. As vascular relaxants, the endothelium can produce prostacyclin by the metabolic pathway of cyclo-oxygenase [6], and nitric oxide the precursor of which is L-arginine [7], but little information is available on their release during cooling. It has been demonstrated that cooling potentiates the production of nitric oxide from the endothelium of cutaneous vessels [8,9]. Recently, it has been reported that in noncutaneous vessels, endothelial nitric oxide does not play a role in cooling-induced responses [8]. Similarly, we have observed that in rabbit aorta, a noncutaneous vessel, the sensitivity of α-adrenoceptor agonists decreased during cooling by an endothelium-independent mechanism [1]. In addition, we also supported this finding in calf coronary artery, another noncutaneous vessel [10].

K+ ions are vital for maintaining the functionality of K+ channels. In their absence, many K+ channel types enter a long-lasting defunct condition characterized by the absence of conductance and drastic changes in the gating current [11]. In noncutaneous vessels, very little was known about the role of K+ ions underlying the effects of cooling [12].

Histamine is present in essentially all tissues. In isolated vessels, this agent can produce contraction or relaxation via stimulation of H1 and H2 receptors, respectively, and these effects depend upon the animal species and the type of blood vessel within a single species [13]. Calf cardiac vein is a noncutaneous vessel and the effects of cooling on the histaminergic response have not been addressed in this tissue before. Effects of cooling on histamine-induced changes were usually studied with airways smooth muscle [14-16].

Therefore, the purpose of the present study was to determine the effects of cooling (28 ˚C) on the histamine-induced contraction and also the possible role of prostaglandin production , the release of nitric oxide and K+ ions underlying the effects of cooling in calf cardiac vein.

MATERIALS and METHODS

Tissue preparations

Calf hearts were obtained from a slaughterhouse and were immediately placed in Krebs-Henseleit solution. Segments of the great cardiac vein were removed and cut into rings 2.5 mm in length. Care was taken not to damage the endothelium. Each ring was mounted in 25 ml organ baths containing Krebs-Henseleit Solution (KHS), aerated with 95 % O2 and 5% CO2 . KHS was composed of (mM): NaCl 119, KCl 4.70 , MgSO4 1.50, KH_2PO_4 1.20, CaCl2 2.50, NaHCO3 25, Glucose 11. The responses were recorded isometrically by a force-displacement transducer (Grass FT04, Grass Instruments Co, W. Warwick, RI, USA) connected through amplifiers to a polygraph (Grass 7D, Grass Instrument Co). The tissues were allowed to equilibrate for 60 min under a resting tension of 1 g with repeated washing every 15 min.

The endothelial cell integrity was determined in each ring before all experiments. Relaxation responses to acetycholine (10-6 M) in rings preconstricted with 5-HT (10-6 M) were used to test endothelial cell integrity. Preparations which relaxed by more than 70 % of the 5-HT-induced tone after addition of histamine were considered to have undamaged endothelium.

Experimental procedure

First, cumulative concentration-response curves were determined in calf cardiac vein for histamine (10-9 - 3x10-4M) at 37 ˚C. Another set of experiments was designed to determine the effect of cooling on the histamine-induced contractile responses. When preparations stabilized (30 min), bath temperature was decreased to 28 ˚C. Preparations were allowed to equilibrate at this temperature for 1 h before a concentration-response curve was determined. Only one concentration-response curve was generated in each strip to prevent tachyphylaxis to histamine.

In order to analyse the role of endothelial nitric oxide in the vascular response during cooling, concentration response curves to histamine was obtained in the presence of NG nitro-L-arginine methlyl esther (L-NAME, 10-4 M), a nitric oxide synthase inhibitor [17]. L-NAME was added to the organ bath 20 min before concentration-response curves were started. Endothelium did not denude because only the role of endothelial nitric oxide was examined in this study.

In another series of the experiments, the tissues were preincubated with indomethacin (10-5 M) to determine the role of cyclooxygenase. The preparations were incubated for 30 min with 10-5 M indomethacin at 28 oC.

In order to analyse the role of K+ ions in the cooling induced vascular response, concentration-response curves to histamine were obtained in the K+-free medium. The K+-free solution was prepared by removing KCl from KHS and replacing KH_2PO_4 with equimolar NaH2PO4. Preparations were equilibrate in K+-free solution for 30 min.

Paralel experiments were conducted in separate groups of preparations maintained at 37 and 28 ˚C for the duration of the experiment to control for changes in responses due to time.

Only one agent was tested in each preparation.

Statistical analysis

Concentrations of histamine causing 50 % of the maximal response (EC50) were calculated from each individual concentration-response curve and its 95 % confidence interval were obtained for each group of experiments. Maximal responses and EC50 values for curves obtained before and during cooling were compared by using Student's t test. Statistical significance was set at $p<0.05$.

Drugs

Histamine chloride, NG nitro-L-arginine methlyl esther, acetycholine chloride (all dissolved in distilled water) and indomethacin (dissolved in ethanol), were used. All drugs were obtained from Sigma, St. Louis, MO, USA.

RESULTS

Figure 1 shows the effects of histamine (10-9 - 3x10-4 M) on calf cardiac vein rings at 37 ˚C, 28˚C (cooling), and at 28˚C in the presence of L-NAME (10-4 M) , indomethacin (10-5 M) , and K+-free medium. At 37˚C, histamine produced concentration-dependent contractions (EC50 = 4.3 x 10-6 M, 95 % confidence interval = 2.5 x 10-6 – 6.1 x 10-6 M) (Table 1).

During cooling (28˚C) , the sensitivity (EC50 = 5.2 x 10-5 M, 95 % confidence interval = 3.0 x 10-5 – 7.0 x 10-5 M), but not the maximal response, of the cardiac vein was significantly lower (12 times; $p < 0.05$) than at 37 ˚C (Table 1).

Treatment with L-NAME (10-4 M) did not significantly affect the contractile response to histamine during cooling (Fig. 1, Table 1). At 28 ˚C , the sensitivity to histamine was significantly higher in the presence of indomethacin (EC50 = 5.7 x 10-6 M, 95 % confidence interval = 4.8 x 10-6 – 6.6 x 10-6 M) (9 times; $p < 0.05$) and also in K+-free solution (EC50 = 5.8 x 10-6 M, 95 % confidence interval = 5.2 x 10-6 – 6.3 x 10-6 M) (8.9 times; $p < 0.05$) (Table 1).

There was no significant difference in the maximum responses to histamine in the presence of each one of the agents used or in K+-free medium (Table 1) and time had no effects on the responses to histamine ($p> 0.05$, data not given).

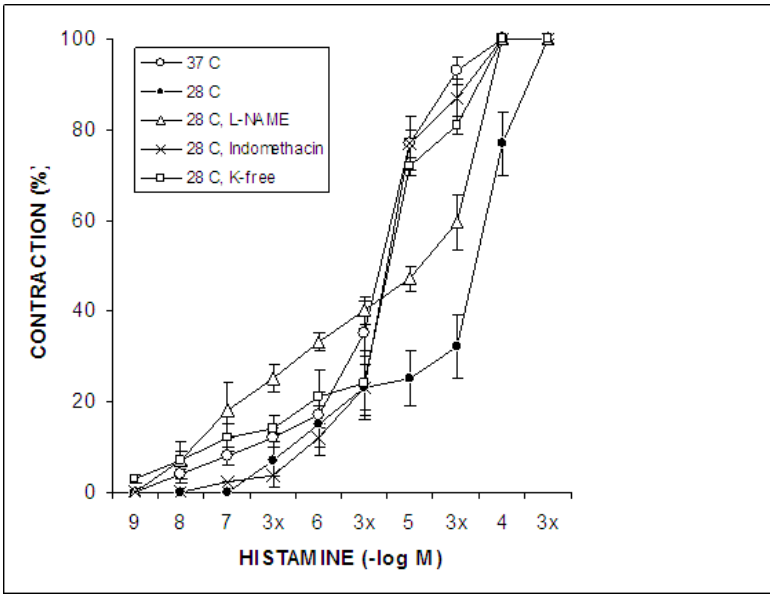

Figure 1) Contractile response to histamine at 37 and 28 °C and in the presence of L-NAME, indomethacin and also K+ free medium at 28 ˚C, in calf cardiac vein. Each point is the mean ± of 6 experiments.

Table 1) EC50 values for histamine in calf cardiac vein at 37, 28 °C and in the presence of L-NAME, indomethacin and also in K+ free medium at 28 ˚C. Maximum responses (Emax) for histamine at 28 °C and at 28 °C in the presence of each one of agents.

	EC_{50}	E_{max}(g)
37 ˚C	4.3×10^{-6} M (2.5×10^{-6} – 6.1×10^{-6} M)	2.2 ± 0.2
28 ˚C	5.2×10^{-5} M (3.0×10^{-5} – 7.0×10^{-5} M)*	2.5 ± 0.1
28 ˚C - L-NAME	1.7×10^{-5} M (0.3×10^{-5} – 3.0×10^{-5} M)	2.2 ± 0.2
28 ˚C - Indomethacin	5.7×10^{-6} M (4.8×10^{-6} – 6.6×10^{-6} M)**	2.8 ± 0.2
28 ˚C - K^+-free medium	5.8×10^{-6} M (5.2×10^{-6} – 6.3×10^{-6} M)**	3.0 ± 0.3

DISCUSSION

In the present work, we studied the responses of calf cardiac vein to histamine, and the effects of cooling on these responses, paying special attention to the role of nitric oxide, prostaglandin and also K+ ions. The cardiac vein is an easily accesible noncutaneous blood vessel and the effects of cooling on histamine-induced contractions on this vessel has not been studied before. Investigations with histamine during cooling were mostly in airways smooth muscle [14-16]. Coronary venous system (cardiac veins) collects one-third blood of the coronary circulation and is considered to be an important site for the blood-tissue exchange of water and nutrients, as well as a possible determinant of ventricular distensibility.

The temperature utilized in this study; 28 ˚C, for cooling was considered to be “moderate cooling” temperature accordingly to our previous studies [1-3,10].

The results of the present study indicate that histamine-induced concentration-dependent contractions at 37 ˚C. As we know, histamine produces contraction by stimulating H1 receptors located in the smooth muscle [18-20]. Compared with the control responses at 37 ˚C , cooling decreased the sensitivity, but not maximal contraction, to histamine in this vessel. Our results with histamine in the calf cardiac vein are in line with our previous findings with different contractile agents in other noncutaneous vessels [1,10]. Based on similar findings by other investigators, it is now generally accepted that although cutaneous vessels constrict on exposure to cold, deeper blood vessels dilate allowing transfer of blood from the superficial to the deep circulation [21]. On the other hand, Fernandez et al. [21]) suggested that cooling reduced the sensitivity of the contraction of the rabbit central ear artery (cutaneous vessel), but it did not affect the response of the femoral artery (noncutaneous vessel) to histamine. Moreover, Allen et al. [22] reported that cooling of the arteries from 37 ˚C to 28 ˚C produces a heterogeneous responsiveness to various agonists. Their data showed that there was an increased sensitivity of isolated rabbit mesenteric artery vessels to exogenous noradrenaline during cooling to 28 ˚C, in contrast a decreased sensitivity to the same agent in the aorta. Allen et al. [22] also reported that cooling had no affects on the sensitivity of either the mesenteric artery or abdominal aorta segments to histamine. This discrepancy with our findings may be related to differences between arteries and veins, and perhaps species. As a matter of fact, cooling specifically alters the vascular sensitivity to various drugs in different parts of the vascular system. It is accepted that cooling depresses the contraction of both cutaneous and noncutaneous vessels to direct activation of the smooth musculature with potassium chloride or BaCl2 [21]. This feature has been also observed before in our laboratory with this vessel (unpublished data).

Cooling can generally alter vascular function and this seems to be the result of effects on many different mechanisms regulating smooth muscle cell tone. We know that vascular endothelium has an important role in the regulation of vascular tonus through the release of endothelium-derived vasoactive substances such as prostaglandins and nitric oxide in various tissues [22]. These mediators are important in controlling local vascular tone [23], including venous tone [24]. A variety of vasoactive substances and stimuli; for example temperature, may promote the release of PGI2 and nitric oxide from endothelial cells. However, despite the well-established role of nitric oxide in the pulmonary [25,26] and saphenous [27] vein, release of nitric oxide in the coronary venous system has not been reported [28]. It’s reported that nitric oxide synthase inhibition by L-NG –nitro-arginine methyl ester (L-NAME) does not affect histamine-induced contraction whereas inhibition of cyclooxygenase induces hyper-responsiveness to histamine in guinea pig trachea [29]. In this study, prior treatment of the cardiac vein with L-NAME was found to be ineffective because the sensitivity of the cardiac vein to histamine was similar in the presence or absence (control) of L-NAME at 28 ˚C. It has been reported that cooling facilitates the stimulated release of nitric oxide from the endothelium in cutaneous, but not in deep vessels [30]. Fernandez et al. [18] reported that the reduced contraction of ear arteries to histamine during cooling was reversed by treatment with L-NAME, suggesting that nitric oxide released from the endothelium could be involved in these effects of cooling. Furthermore, the investigators suggested that changes in temperature might affect the production of nitric oxide in a different way depending on vascular beds [30,31]. Furthermore,

Simonet et al. [31] determined that relaxations of human saphenous veins to moderate cooling were independent of the endothelium and not related to the release of vasoactive substances such as nitric oxide. We have previously observed that cooling (to 28 ˚C) induced subsensitivity to α-adrenoceptor agonists in rabbit aorta [1], and to KCl, 5-HT and carbachol in calf coronary artery [10] and endothelial nitric oxide has no role in these effects. Similarly, it has been demonstrated that the treatment of isolated segments of coronary arteries with cardioplegia at different temperatures (7 ˚C or 37 ˚C) does not alter the production of nitric oxide or the ability of the vascular smooth muscle to contract or relax [32]. The results of this study agree with this finding and others in different noncutaneous vessels [8,33].

A cyclooxygenase inhibitor, indomethacin was used to determine whether prostaglandins played a functional role in the cooling-induced decreased sensitivity to histamine. Cooling to 28 ˚C after treatment with indomethacin increased the sensitivity to histamine suggesting that endothelial cyclooxygenase products of arachidonic acid (presumably prostacyclin) are involved in this effect. Evora et al.[34] reported that in dog coronary, renal and femoral arteries, indomethacin pretreatment blocked the cooling-induced relaxation, and were more pronounced below 30 ˚C. Furthermore, it is reported that in ovine trachea pretreatment with indomethacin potentiated the cooling induced contractions [16]. These results support our observation.

In another part of this study, we examined the role of potassium ions in histamine-induced responses during cooling. The Na+/K+ pump plays an essential role in the maintenance of a relatively low intracellular sodium and high potassium concentration. In smooth muscles this pump can directly contribute to the cell resting membrane potential by actively pumping more sodium ions out than potassium ions into the cells [35,36]. K+-depletion is known to inhibit the Na+-K+-ATPase coupling Na+- Ca2+ exchange system reversibly [37]. In this study, cooling to 28 ˚C after incubation in K+-free solution increased the sensitivity to histamine, indicating that Na+ / K+ and Na+ / Ca2+ exchange mechanisms were important on cooling-induced changes of histamine contractions in calf cardiac vein. In a different study, we also observed that cooling to 28 ˚C after incubation in K+-free solution increased the sensitivity to both coronary artery and cardiac vein to carbachol [12]. It is known that inhibition of the Na+ / K+ pump potentiates contractions elicited by a variety of activating agents [38]. Furthermore, it is reported that depression of the Na+-pump results in hypersensitivity and hyperreactivity of the airway smooth muscle to cooling [39]. These results support this observation.

CONCLUSIONS

In conclusion, the present results indicate that cooling decreases the sensitivity to histamine in calf cardiac vein and suggest a role for cyclooxygenase pathway, and also K+ ions in cooling-induced changes.

REFERENCES

[1]. Atalık KE, Şahin AS, Kılıç M, Doğan N. 2000. Role of the endothelium on the response to adrenoceptor agonists of rabbit aorta during cooling. Fundam Clin Pharmacol 14: 25-30.

[2]. Atalık KE, Kılıç M, Nurullahoğlu ZÜ, Doğan N. 2007. Effects of cooling on histamine-induced contractions of human umbilical artery: the role of ion channels. Methods Find Clin Exp Pharmacol 29: 619-623.

[3]. Atalık KE, Kılıç M, Nurullahoğlu ZÜ, Doğan N. 2008. Effects of cooling and warming on 5-hydroxytryptamine and acetylcholine - induced contractions of human umbilical vessels: role of nitric oxide. Fundam Clin Pharmacol 22: 37-44.

[4]. Seham M, Thulesius O. 2001. Cooling is a potent vasodilator of deep vessels in the rat. Can J Physiol Pharmacol 79: 899-904.

[5]. Vanhoutte PM, Eber B. 1991. Endothelium-derived relaxing and contracting factors. Wien Clin Wochenschr 103: 404-411.

[6]. Moncada, S. 1982. Biological importance of prostacyclin. Br J Pharmacol 76 : 3-31.

[7]. Palmer RMJ, Ferrige AG, Moncada S. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor (EDRF). J Pharmacol Exp Ther 237: 529-538.

[8]. Fernandez N, Monge L, Garcia-Villalon AL, Garcia JL, Gomez B, Dieguez G. 1994. Cooling effects on nitric oxide production by rabbit ear and femoral arteries during cholinergic stimulation. Br J Pharmacol 113: 550-554.

[9]. Monge L, Garcia-Villalon AL, Montoya JJ, Garcia JL, Gomez B, Dieguez G. 1991. Response of rabbit ear artery to endothelin-1 during cooling. Br J Pharmacol 104: 609-612.

[10]. Atalık KE, Şahin AS, Doğan N. 2001. Effects of cooling on contractions of calf coronary artery: Role of the endothelium. Asia Pac J Pharmacol 15: 33-36.

[11]. Loboda A, Melishchuk A, Armstrong C. 2001. Dilated and defunct K+ channels in the absence of K+. Biophys J 80: 2704-2714.

[12]. Atalık KE, Şahin AS, Ulusoy HB, Doğan N. 2001. The role of K+ ions on the response to carbachol of calf coronary artery and cardiac vein during cooling. Methods Find Clin Exp Pharmacol 23: 231-234.

[13]. Douglas WW.1985. Histamine and 5-hydroxytryptamine and their antagonists. In: The Pharmacological Basis of Therapeutics (ed. Goodman Gilman A, Goodman LS, Rall TW and Murad F), pp.605-638 MacMillan, New York.

[14]. Chou D S, Chen TF, Lin-Shiau SY. 1996. Mechanism of cooling-induced relaxation in the isolated guinea-pig trachea. Respir Physiol 106: 179-186.

[15]. Cortijo J, Gonzalez M, Ortiz JL, Morcillo E. 1992. Effects of low temperature and and pharmacological interventions on the responses of the isolated guinea-pig trachea. Naunyn-Schmiedeberg's Arch Pharmacol 346: 573-580.

[16]. Mustafa SM, Pilcher CW, Williams KI. 1999. Cooling-induced contraction in ovine airways smooth muscle. Pharmacol Res 39 :113-123.

[17]. Rees DD, Palmer RMJ, Schulz R, Hudson HF, Moncada S. 1990: Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br J Pharmacol 101: 146-152.

[18]. Fernandez N, Garcia-Villalon AL, Borbujo J, Monge L, Garcia JL, Gomez B, Dieguez G. 1994. Cooling effects on the histaminergic response of rabbit ear and femoral arteries: role of the endothelium. Acta Physiol Scand 151: 441-451.

[19]. Liu S, Yacoub M, Barnes PJ. 1990. Effect of histamine on human bronchial arteries in vitro. Naunyn-Schmiedeberg's Arch Pharmacol 342 : 90-93.

[20]. Toda, N. 1990. Mechanism underlying responses to histamine of isolated monkey and human cerebral arteries. Am J Physiol 258: H311-H317.

[21]. Vanhoutte, P.M.1980. Physical factors of regulation, In: Handbook of Physiology, Section 2: Circulation, vol. II, Vascular Smooth Muscle (ed. Bohr DF, Somlyo AP, Sparks HV.), pp.473-4, American Physiological Society, Washington.

[22]. Allen S, Kumar M, Arnfeh B, Chester A, Dzimiri N, Duran CM, Yacoub M. 1996. Influence of cooling on mesenteric vascular reactivity. Eur J Cardiothorac Surg 10: 1015-1020.

[23]. Furchgott RF, Zawadzki JV. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetycholine. Nature 288: 373-376.

[24]. Furchgott RF, Vanhoutte PM. 1989. Endothelium-derived relaxing and contracting factors. FASEB J 3 : 2007-2018.

[25]. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci 84: 9265-9269.

[26]. Monos E, Berczi V, Nadasy G. 1995. Local control of veins: biomechanical, metabolic, and humoral aspects. Physiol Rev 75 : 611-666.

[27]. Ignarro LJ, Byms RE, Buga GM, Wood KS. 1987. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. Circ Res 61: 866-879.

[28]. Liu ZG, Ge ZD, He GW. 2000. Difference in endothelium-derived hyperpolarizing factor-mediated hyperpolarization and nitric oxide release between human internal mammary artery and saphenous vein. Circulation 102: 296-301.

[29]. Nieri P, Martinelli C, Blandizzi C, Bernardini N, Greco R, Ippolito C, Del Taca M, Breschi MC. 2006. Role of cyclooxygenase isoforms and nitric-oxide synthase in the modulation of tracheal motor responsiveness in normal and antigen-sensitized Guinea pigs. J Pharmacol Exp Ther 319 : 648-656.

[30]. Monge L, Garcia-Villalon AL, Montaya JJ, Garcia JL, Fernandez B, Gomez B, Dieguez G. 1993. Role of the endothelium in the response to cholinoceptor stimulation of rabbit ear and femoral arteries during cooling, Br J Pharmacol 109: 61-63.

[31]. Simonet S, Bonhomme E, Fabiani JN, Verbeuren T. 2000. Temperature-dependent basal tone in isolated human saphenous veins: implication of TP-receptors, Fund Clin Pharmacol 14 : 461-467.

[32]. Evora PR, Pearson PJ, Schaff HV. 1992. Crystalloid cardioplegia and hypothermia do not impair endothelium-dependent relaxation or demage vascular smooth muscle of epicardial coronary arteries. J Thorac Cardiovasc Surg 104: 1365-1374.

[33]. García-Villalón AL, Monge L, Montoya JJ, García JL, Fernández N, Gómez B, Diéguez G

[34]. G. 1992. Cooling and response to adrenoceptor agonists of rabbit ear and femoral artery: Role of endothelium. Br J Pharmacol 106: 727-732.

[35]. Evora PR, Cable DG, Chua YL, Rodrigues AJ, Pearson PJ, Schaff HZ. 2007. Nitric oxide and prostacyclin-dependent pathways involvement on in vitro induced hypothermia. Cryobiology 54:106-113.

[36]. Burke EP, Reed JP, Sanders KM.1988. Role of the sodium pump in membrane potential gradient of canine proximal colon. Am J Physiol 254: 475-483.

[37]. Lee CO.1985. 200 years of digitalis: The emerging central role of the sodium ion in the control of cardiac force. Am J Physiol 249 : 367- 378.

[38]. Haddy, F.J. 1983. Potassium effects on contraction in arterial smooth muscle mediated by Na+-K+-ATPase. Fed Proc 42: 239-245.

[39]. Van Breemen, C., Aaronson, P., Loutzenhiser, R. 1979. Sodium-calcium interactions in mammalian smooth muscle. Pharmacol Rev 30: 167-208.

[40]. Chen WY.1989. Effects of Na+-pump suppression on reactivity of rat trachealis to cooling. Clin Exp Pharmacol Physiol 16: 375-381.