Pflügers Archiv - European Journal of Physiology (2023) 475:1089–1095
https://doi.org/10.1007/s00424-023-02835-7

ORGAN PHYSIOLOGY

The effect of temperature on vascular smooth muscle: cooling-induced vasodilation in deep arteries and veins

Seham Mustafa[1]

Received: 11 May 2023 / Revised: 13 June 2023 / Accepted: 26 June 2023 / Published online: 5 July 2023

Abstract

Blood vessels in the cardiovascular system include arteries and veins, which are responsible for moving blood to and from tissues around the body. Our previous studies showed that cooling induces relaxation of arteries. The aim of this study is to investigate the effect of cooling on both arteries and veins pairs. Isometric tension was recorded in rat artery ring preparations (aorta, carotid, pulmonary arteries) and their vein pairs (vena cava, jugular, pulmonary veins) in organ baths during stepwise cooling from 37 to 4 °C. Cooling responses were tested before and after the addition of various standard agents. The possibility of the presence of a cooling-relaxed substance and the influence of endothelium were also examined. Cooling-induced relaxation of both arteries and veins and the degree of relaxation were inversely proportional to the temperature. The cooling response was highest in arteries than their respective paired veins. The relaxation response was not endothelium dependent or affected with neurogenic mechanism (autonomic blockers or tetrodotoxin). Additionally, it was not changed by alterations of extra- or intra-cellular calcium transfer, and no relaxant substance was released from vascular smooth muscles during cooling. The study showed that cooling induces relaxation of both arteries and veins. Our results suggested that the effect of cooling could be through a thermal receptor in the vascular smooth muscle. Therefore, cold temperature can act as an agonist and increase in cooling temperature behaves as increase in concentration of the agonist. This study contributes to a better understanding of the mechanisms behind cooling-induced relaxation of blood vessels, which may have implications for the treatment of cardiovascular diseases.

Keywords Blood vessels · Cooling · Hypothermia · Carotid · Jugular · Pulmonary artery · Pulmonary vein · Aorta · Vena cava

Introduction

The cardiovascular system is one of the most complex and vital organ systems in the human body. It is responsible for the transport of blood, gases, hormones, and nutrients throughout the body, and consists of the heart, blood, and blood vessels. The cardiovascular system is a closed system in which blood circulates only within the network of tubes which are blood vessels. The system is divided into three main types of blood vessels: arteries, capillaries, and veins [8].

Arteries are blood vessels responsible for carrying oxygenated blood from the heart to all the other tissues of the body. Capillaries are the tiny blood vessels that facilitate the exchange of oxygen, nutrients, and wastes between the blood and the tissues. Veins, on the other hand, carry oxygen-depleted blood from the body tissues to the heart. As the blood travels through the arteries, it loses oxygen, and the veins carry it back to the heart to absorb more oxygen. The veins usually hold about 75% of all the blood flowing through the body. Unlike arteries, veins generally need to work against gravity to push blood back to the heart, and they have valves to help with this. These are one-way pairs of flaps inside a vein that open for blood that's heading upwards toward the heart, and close to keep blood from flowing back downwards. There are two blood circulations in the body, systemic circulation, and pulmonary circulation. Pulmonary circulation is where fresh oxygen enters the

✉ Seham Mustafa
mustafaseham@yahoo.com; sm.mustafa@paaet.edu.kw

[1] Department of Biomedical Sciences, College of Nursing, Public Authority for Applied Education & Training, Kuwait City, Kuwait

blood. Systemic circulation supplies oxygen and other vital substances to organs, tissues, and cells [3].

The aorta and the vena cava are the two main blood vessels in the body. The aorta is the main artery that carries oxygenated blood from the left ventricle of the heart to the tissues of the body, while the vena cava is the main vein that brings the oxygen-poor blood to the right atrium of the heart from the upper half and lower half of the body. The carotid arteries extend out from the aorta, which carry blood through the neck up to the brain and face, while jugular veins drain blood from brain, face, and neck returning it to the heart via the superior vena cava.

The pulmonary artery is the artery that carries deoxygenated blood pumped from the right ventricle of the heart to the lungs, while the pulmonary veins are the veins that carry oxygenated blood from the lungs to the left atrium. The pulmonary circulation is different than systemic in context that within the pulmonary circulation, veins carry oxygenated blood, whereas arteries carry deoxygenated blood [7].

While previous research has demonstrated that isolated artery preparations exhibit relaxation in response to cooling, the effect on veins remains unclear [4, 5]. As veins play a critical role in the circulatory system, it is important to understand how they respond to cooling. Therefore, this study aims to determine the stepwise effect of cooling on the smooth muscle of both arteries and their corresponding veins. By elucidating the mechanisms underlying the vascular response to cooling, this research may have implications for the treatment of vascular disorders and the development of new therapeutic interventions.

Materials and methods

Preparation of samples

Male Sprague Dawley rats weighing approximately 200 g were used for this study. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Aorta, pulmonary, carotid arteries, and vena cava, pulmonary, jugular veins were removed and dissected free of extraneous fat and connective tissue. The vessels were then cut into 5-mm ring segments, which were mounted on triangular wire supports and suspended in 10 mL organ baths containing Krebs' solution of the following composition (mmol/L): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.2, KH_2PO_4 1.2, $NaHCO_3$ 26, glucose 11.1, pH 7.4, maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Tension was continuously recorded using a computerized, automated isometric transducer system (Schuler organ bath 809; Hugo Sachs Electronik) connected to a Gould recorder. The segments were initially loaded to a tension of 2 g for aorta and jugular and 1 g for the other blood vessels, then allowed to equilibrate for 60 min, during which time they were washed twice. The presence of an intact endothelium was confirmed by adding acetylcholine (1 mol/L), which resulted in relaxation of norepinephrine (1 mol/L)-precontracted rings. All the experiments were performed on blood vessel preparations in which the endothelium was intact, except when examining the effect of the endothelium (removal by gentle rubbing of the blood vessel lumen with cotton buds). At the end of each experiment, the muscle was weighed, and responses were calculated as mg/mg tissue weight and in some figures we used mg tension.

Cooling protocol

The cooling protocol involved reducing the organ bath temperature using a cooling circulator bath (Haake F3, Fisons), which had been set to the desired temperature. The temperature was lowered in steps from 37 to 4 °C (37 °C, 30 °C, 25 °C, 20 °C, 15 °C, 10 °C, 4 °C), with each cooling period maintained until a peak response had leveled off before further temperature reduction.

Bioassay to examine a cooling-released relaxing substance.

One ring segment of aorta or jugular was suspended in an organ bath at 37 °C, under 2 g tension and connected to a Gould recorder. The temperature was reduced to 20 °C, and the peak response was obtained; then, the temperature was reset to 37 °C. Eight ring segments of aorta or jugular were added to the organ bath. After 15 min, the temperature was reduced to 20 °C and maintained until a peak response had leveled off.

Drugs

Norepinephrine hydrochloride, acetylcholine hydrochloride, sodium nitroprusside, phentolamine hydrochloride, cocaine, propranolol hydrochloride, atropine sulphate, capsaicin, methylene blue and ethylene glycol bis (b-aminoethylether)-N,N,N,N-tetra-acetic acid (EGTA), indomethacin, tetrodotoxin, diphenhydramine hydrochloride, verapamil hydrochloride, and tetrodotoxin were obtained from Sigma Chemical Co. Nifedipine, L-NAME, and thapsigargine were from Research Biochemical International (RBI), Natick, Mass. All drugs were dissolved in distilled water except EGTA, was dissolved in 0.1 NaOH, while nifedipine and indomethacin were dissolved in ethanol.

Statistical analysis

Statistical analysis was performed using mean ± S.E.M. of (n) experiments. Student's *t*-test (paired or unpaired as appropriate) was used to compare differences between two

mean values, while one-way ANOVA was used for multiple comparisons, followed by the Student-Newman-Keuls test. The difference was assumed to be significant at $P < 0.05$.

Results

Cooling-induced relaxation

All isolated arterial and venous ring segments showed relaxation upon cooling. Before cooling, all preparations maintained stable baseline tension. The degree of relaxation was inversely proportional to the temperature, with maximum relaxation occurring at 4 °C. Upon returning to 37 °C, tension rapidly returned to basal levels. Arterial ring segments exhibited significantly greater relaxation than their paired venous ring segments. The magnitude of relaxation increased with decreasing temperature, and the difference in relaxation response between arteries and veins increased at lower temperatures. Cooling induces relaxation in veins was 75% ± 6 mg tension of the cooling response in arteries.

As an example, to all blood vessels that used arteries or veins, a typical original tracing representing cooling-induced relaxation of rat pulmonary vein rings from basal tone is shown in Fig. 1.

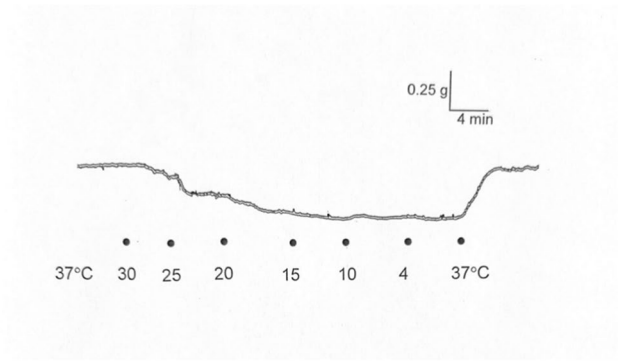

Fig. 1 Original recording of stepwise cooling on basal tone and rewarming of isolated pulmonary vein smooth muscle rings of the rat

Aorta and vena cava

Cooling-induced relaxation in both the isolated aorta and its paired vein, the vena cava. The difference in relaxation magnitude between the two at 4 °C was around 30%, as shown in Fig. 2.

Carotid and jugular vein

Cooling-induced relaxation in both the isolated carotid artery and its paired vein, the jugular vein. The difference in relaxation magnitude between the two at 4 °C was around 25%, as shown in Fig. 3.

Pulmonary artery and pulmonary vein

Cooling-induced relaxation in both the isolated pulmonary artery and its paired vein, the pulmonary vein. The difference in relaxation magnitude between the two at 4 °C was around 25%, as shown in Fig. 4.

Response to cooling is more in all arteries than venous response determined by using either mg tension or mg/mg tissue weight calculations.

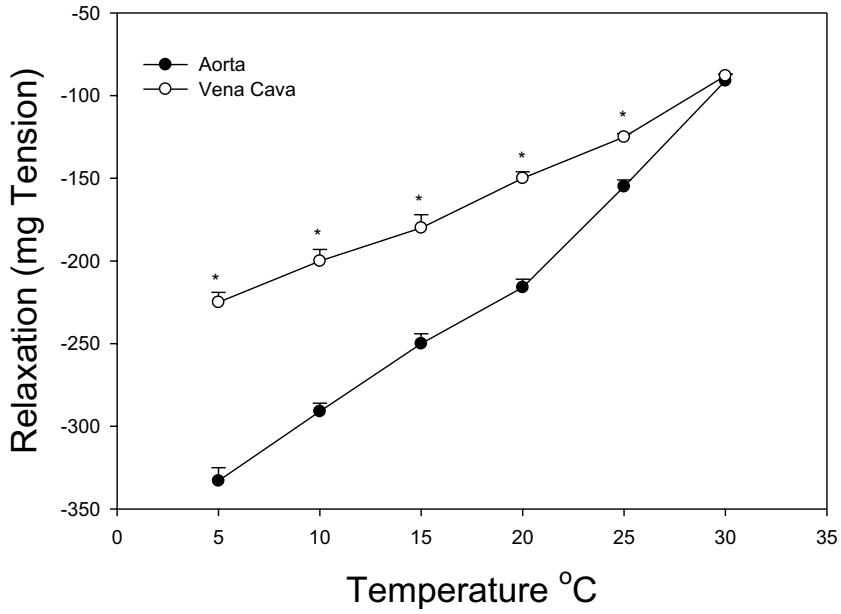

Fig. 2 Effect of cooling from basal tone in isolated aorta and its vein pair vena cava smooth muscle rings of the rat. Values are mean ± S.E.M. of 6 experiments. *$P < 0.05$

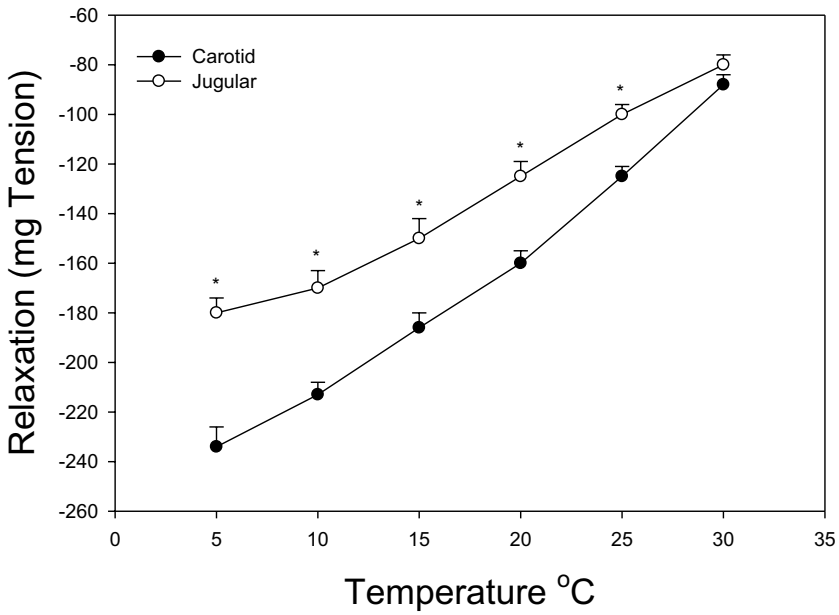

Fig. 3 Effect of cooling from basal tone in isolated carotid artery and its vein pair jugular vein smooth muscle rings of the rat. Values are mean ± S.E.M. of 6 experiments. *$P < 0.05$

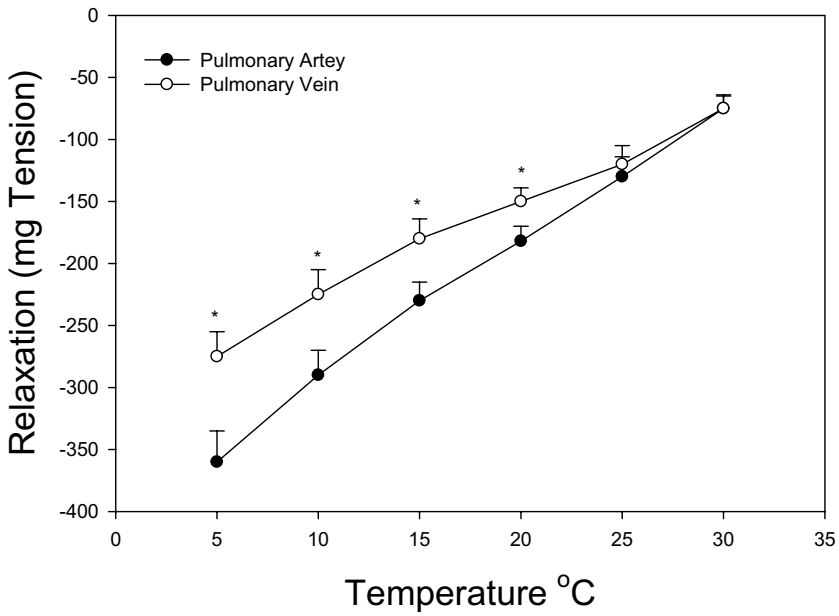

Fig. 4 Effect of cooling from basal tone in isolated pulmonary artery and its vein pair pulmonary vein smooth muscle rings of the rat. Values are mean ± S.E.M. of 6 experiments. *$P < 0.05$

Figure 5 illustrates the relaxation of all isolated arterial and venous ring segments. The cooling response to 20 °C was strongest in the carotid artery, followed by the pulmonary artery, and then the aorta, when calculated by mg tension as shown in Fig. 5A. However, when calculated by mg/mg tissue weight, the highest relaxed artery was the carotid artery, followed by the pulmonary artery, and then the aorta as shown in Fig. 5B.

Influence of endothelium

In the absence of endothelium (confirmed by the absence of any relaxed response to acetylcholine) in the pulmonary artery, sodium nitroprusside relaxed the norepinephrine-induced contraction to the basal level. Similarly, cooling to 20 °C relaxed the arterial smooth muscle to its basal tone, as shown in Fig. 6 indicating that cooling-induced relaxation is not endothelium-dependent.

Effect of blocking agents

In our previous study, we did these experiments using rat artery as pulmonary artery (Mustafa and Thulesius, 2001). In this study, we repeat the same experiment using pulmonary vein. The relaxation responses to

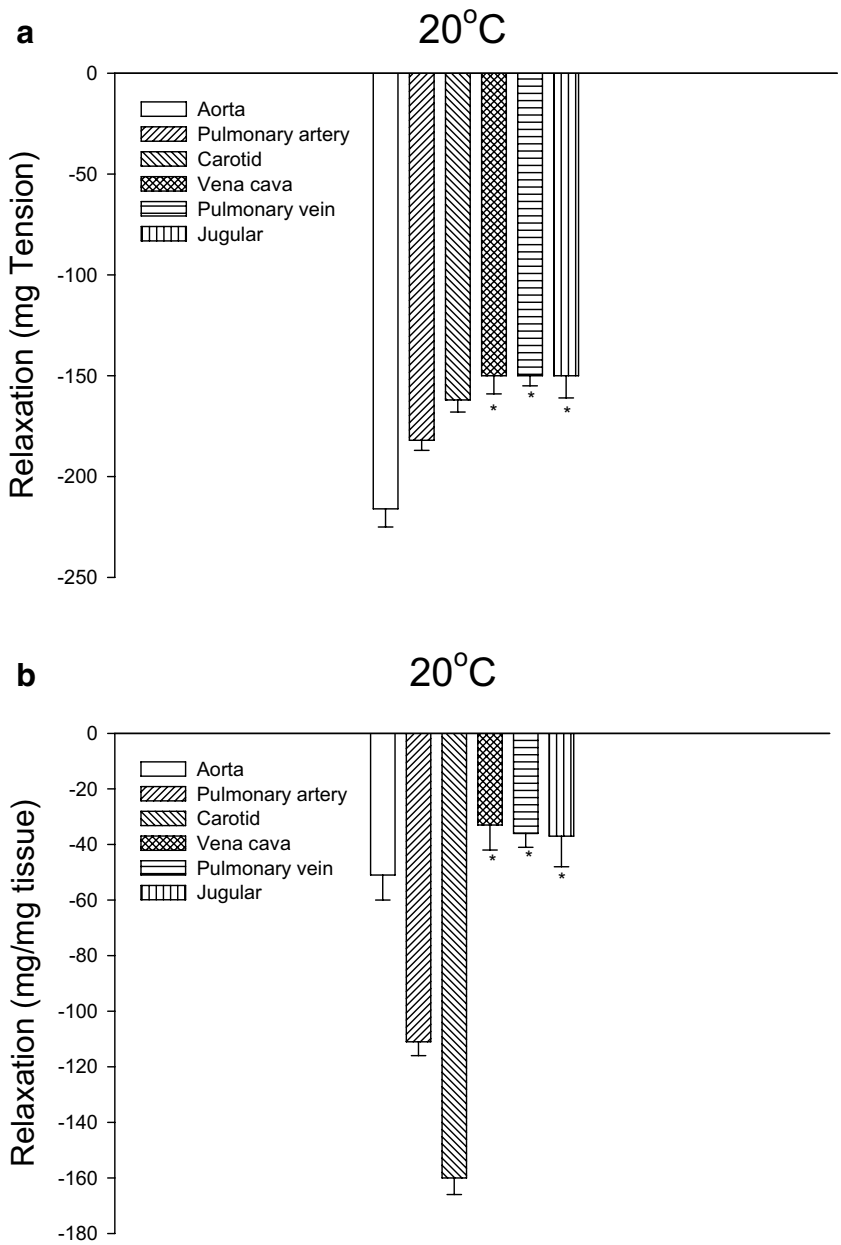

Fig. 5 **A** Effect of cooling at temperature 20 °C in isolated aorta and vena cava; carotid artery and jugular; pulmonary artery and pulmonary vein smooth muscle rings of the rat. Values are mean ± S.E.M. of 6 experiments. *$P < 0.05$. Relaxation in mg tension. **B** Effect of cooling at temperature 20 °C in isolated aorta and vena cava; carotid artery and jugular pulmonary artery and pulmonary vein smooth muscle rings of the rat. Values are mean ± S.E.M. of 6 experiments. *$P < 0.05$. Relaxation in mg/mg tissue weight

cooling were not inhibited by pretreatment with the muscarinic agonist atropine (1 μM), the H_1 antagonist diphenhydramine (1 μM), the α_2-adrenoceptor antagonist yohimbine (0.5 μM), the neural blockers tetrodotoxin (1 μM), or indomethacin (1 μM), prostaglandin synthesis inhibitor. The calcium channel blocking agent nifedipine or incubation for 30 min in calcium-free, EGTA (2 mM)-containing Krebs solution had no effect on relaxation responses. Removal of the endothelium, incubation with L-NAME (100 μM), an inhibitor of nitric oxide synthesis, or incubation with methylene blue (30 μM), an inhibitor of cGMP, did not affect cooling responses.

Cooling did not release a relaxed substance

There was no added relaxant response in the absence or presence of 8 ring segments of aorta or jugular in the organ bath during cooling. We therefore conclude that cooling did not release a vasodilator substance.

Discussion

The present study investigates the effects of cooling on blood vessels in the cardiovascular system. Arteries and veins play important roles in moving blood to and from tissues, with

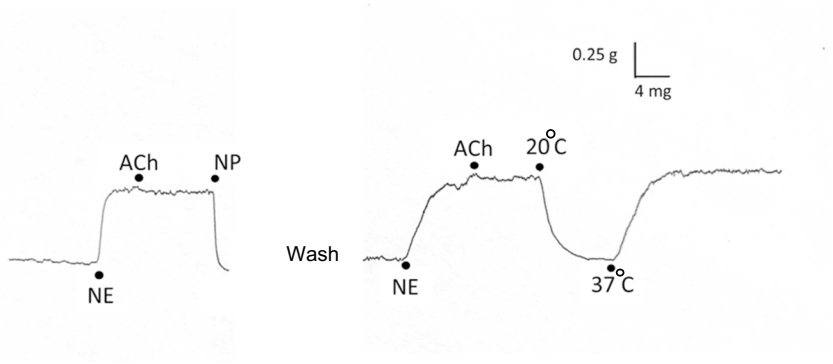

Fig. 6 Original recording of the effect of cooling at temperature 20 °C, sodium nitroprusside (NP) 1 μM, and acetylcholine (ACh) 1 μM on norepinephrine (NE) 10 μM-induced contraction on isolated pulmonary artery ring segment of the rat without endothelium. N.B. Due to removal of endothelium, ACh could not relax the artery, while nitroprusside and cooling (20 °C) relaxed it indicating that endothelium has no role in cooling effect

arteries carrying blood away from the heart and veins carrying blood back to the heart. It has been observed that blood moves more quickly through arteries than veins. This is since arteries are thicker and more elastic, allowing them to withstand the higher pressure of blood flow. Veins, on the other hand, are thinner and less elastic, which helps them to transport larger amounts of blood over longer periods of time [2].

In the present investigation, all arteries and their paired veins from rats were used to prevent differences between species, and the results were found to be in-line with our earlier findings showing cooling-induced relaxation in different arteries obtained from different species [4–6].

The results of this study indicate that cooling induces vasodilation in arteries and their paired veins by smooth muscle relaxation. With stepwise cooling inducing reproducible graded relaxations that were inversely proportional to temperature. The cooling response is highest in the aorta, pulmonary artery, and carotid, with differences in relaxation response observed between arteries and their paired veins. The relaxation response in mg tension was highest in the aorta, followed by the pulmonary artery and carotid artery. However, when the calculations were based on mg/mg tissue weight, the highest relaxed artery was the carotid artery, followed by the pulmonary artery and aorta. This was due to the weight of the arteries' ring segments, with the aorta being the heaviest and the carotid artery being the lightest. Vein ring segments, on the other hand, were found to be heavier than arteries ring segments.

This study confirmed our previous studies on arteries. It was found that the cooling response was not influenced by neurogenic or myogenic mechanisms of relaxation, and there was no relaxant substance released from vascular smooth muscles during cooling [4]. Cooling responses were also not endothelium-dependent [1, 4, 5], and neither NO nor CO was involved [4, 5].

We suggested that the effect of cooling is likely due to the activation of thermal receptors in the vascular smooth muscle, which induces relaxation. The presence of cold receptors may be responsible for this effect.

In conclusion, our study provides evidence that cooling induces relaxation in arteries and veins of rat ring preparations. The vasodilatation induced by cooling may have therapeutic potential in certain medical conditions. Further research is needed to explore the underlying potential clinical and therapeutic applications of cooling-induced vasodilatation.

Based on our findings, we suggest that the effect of cooling-induced relaxation of blood vessels occurs through a specific mechanism involving a thermal receptor in the vascular smooth muscle. The relaxation response of cooling could be due to the presence of cold receptors that can be activated by cold temperature. Cold could act as an agonist, and an increase in cooling temperature would behave like an increase in the concentration of the agonist, and when the temperature was reset to 37 °C, the tone rapidly returned to the basal level as if we washed off the agonist. This may have implications for the development of new therapies for vascular disorders and conditions that affect blood flow. Further studies are going to characterize the thermal receptor to fully understand the mechanism underlying cooling-induced relaxation of blood vessels.

Acknowledgements The author acknowledges the Faculty of Medicine, Kuwait University, for supplying the animals, chemicals, and equipment for performing this research.

Author contribution One author (Seham Mustafa) did all the work and wrote this paper.

Data availability All the data and materials are available in my lab.

Declarations

Ethics approval The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Consent to participate Not applicable

Consent for publication The author gives her consent with the publication of the commentary.

Competing interests The author declares no competing interests.

References

1. Fukaya Y, Ohhashi T (1996) Acetylcholine- and flow-induced production and release of nitric oxide in arterial and venous endothelial cells. Am J Physiol 270(1 Pt 2):H99-106
2. Germann WJ, Stanfield CL (2002) Principles of Human Physiology. Benjamin Cummings, San Francisco
3. Hall JE, Hall ME (2021) Guyton and Hall textbook of medical physiology, 14th edn. Elsevier
4. Mustafa SMD, Thulesius O (2001) Cooling is a potent vasodilator of deep vessels in the rat. Can J Physiol Pharmacol 79(11):899–904
5. Mustafa S, Thulesius O (2002) Cooling-induced carotid artery dilatation, an experimental study in isolated vessels. Stroke 33:256–260
6. Mustafa S (2021) Hypothermia induces opposite response in vascular and non-vascular smooth muscles. J Therm Biol 95(4):102818
7. Sembulingam K, Sembulingam P (2016) Essentials of medical physiology, 8th edn. Jaypee Brothers Medical Publishers
8. Fox SI, Rompolski K (2021) Human physiology, 16th edn. McGrow-Hill Education