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RESEARCH

The regulatory effects of mesedin and beditin alpha2-adrenoblockers on the functional activity of the nervous, cardiovascular, and endocrine systems in rats under the hypoxic conditions

Ashkhen L. Manukyan[1,2] · Magdalina M. Melkonyan[1,2] · Lilit M. Sukiasyan[3] · Svetlana O. Vardanyan[4] · Lilit S. Hunanyan[1,2] · Konstantin B. Yenkoyan[5,6] · Seda H. Harutyunyan[4]

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Abstract

One of the reasons of the development of pathologies causing death is hypoxia. The purposes of this study were (1) to study some physiological and biochemical mechanisms of α_2-adrenoblockers, which ensure the tissue resistance increase to hypoxia; (2) to offer new drugs contributing to the increase of tissues' stability towards the hypoxic affection; and (3) to submit new medications to surpass by their anti-hypoxic activity of those already used in modern medicine and have some advantages. The reactivity of postsynaptic vascular α_2-adrenoceptors was determined on the damaged spinal cord expressed by the blood pressure increase in response to intravenous administration of azepexole that selectively binds to α_2-adrenoceptors. Determination of the systemic hemodynamic values and the vascular resistance to the blood flow was performed by the method with plastic microspheres of marked isotopes. pO_2 in the blood and the oxygen-transporting function were determined in a sample of 0.1 ml of blood in 30, 90, and 180 min after the α_2-adrenoblockers' injections. It has been found that one of the major hemodynamic effects of mesedin and beditin was an improvement in cardiac output, as well as a prolonged increase in coronary blood flow and vasodilation of the heart vessels. Some anti-hypoxic mechanisms of the studied α_2-adrenoblockers are an improvement of blood oxygen-transporting function followed by tissue oxygenation and the increased level of corticosterone and resistance to hypoxia. Revealing the mechanisms of action of the postsynaptic α_2-adrenoceptors suggests that mesedin and beditin are potentially effective therapeutic means for many hypoxic conditions.

Keywords α_2-Adrenoceptors · α_2-Adrenoblockers · Hypoxia

Introduction

From the point of homeostasis, the physiological regulatory systems of mammalians ensure the optimal oxygenation of cells in each organism (Michiels 2004) through the evolution of complex mechanisms for O_2 delivery which include an entry (lungs), transport vehicle (erythrocytes), a highway and secondary pathway system (vasculature), and a propulsion device (heart) (Semenza 2010; Samanta et al. 2017). The respiratory chain function is optimized for physiological arterial oxygen partial pressure (pO_2) levels and sustained deviations from normoxia and also increased production of the reactive oxygen species (ROS) by the electron transport chain (Smith and Schumacker 2018). ROS cause the oxidation of lipids, proteins, and nucleic acids leading to cellular dysfunction or death (Manukyan 2020; Manukyan et al. 2020a, 2020b; Manukyan 2022). Thus, homeostatic mechanisms regulate O_2 levels within the cells and tissues

✉ Ashkhen L. Manukyan
manukyanashkhen@mail.ru; ashkhmanuk@mail.ru

1 Department of Medical Chemistry, Yerevan State Medical University After M. Heratsi, Koryun 2, 0025 Yerevan, Armenia

2 Cobrain Center, Yerevan State Medical University After M. Heratsi, 0025, 2 Koryun Str., Yerevan, Armenia

3 Laboratory of Morphological Studies, SRS, Yerevan State Medical University After M. Heratsi, 0025 Yerevan, Armenia

4 Scientific-Technological Center of Organic-Pharmaceutical Chemistry of NAS RA, Institute of Fine Organic Chemistry After A.L. Mnjoyan, 0014 Yerevan, Armenia

5 Neuroscience Laboratory, Cobrain Center, Yerevan State Medical University After M. Heratsi, 0025, 2 Koryun Str., Yerevan, Armenia

6 Department of Biochemistry, Yerevan State Medical University M. Heratsi, 0025 Yerevan, Armenia

(Semenza 2010). For the critical role of oxygen in functional homeostasis, eukaryotes have developed an efficient and rapid oxygen sensing system, the hypoxia-inducible factor which stimulates the transcription factor production (Beall et al. 2010; Kapitsinou et al. 2010).

It is known that in patients with the hypoxic and hemic hypoxia (Sarkar et al. 2017; Sainburg et al. 2012), as well as in cerebral ischemia (DeSai and Shapshak 2021), hereafter already in patients infected with COVID-19 (Rahman et al. 2021), the processes of tissue oxygenation are disrupted. As in any stress condition, hypoxia elicits the sympathoadrenal contribution to cardiorespiratory response. As a result, at a given oxygen uptake, all plasma catecholamines were increased in hypoxia (Favier et al. 1985). In such situations, drugs that increase the affinity of hemoglobin to oxygen, or the process of oxygen transfer from the blood to the tissues, or increase the blood supply are proven to be effective. The goal of the hypoxia-induced experiments was initially to search for antihypoxants among the benzodioxanes, as well as a comparison with the known antihypoxant, the benzodioxane idazoxan, which has a limitation in use because of its high toxicity. That is why a search for other α_2-adrenoblocking agents of less toxicity and even more efficacy gains new actuality today. In this regard, two other derivatives, mesedin and beditin, became a target of our research due to their lower toxicity and prevalence of high affinity to the postsynaptic receptors (Fig. 1). Additionally, their preventive effect on lipid peroxidation as antioxidants was reported in some studies on plasma proteins and erythrocytes' membranes (Melkonyan et al. 2015); also, the ability to increase the animal's survival and reduce symptoms of cognitive decline was observed in the stress conditions (Manukyan 2017; Melkonyan et al. 2018; Manukyan et al. 2020a, 2020b). At the same time, only a few studies have indicated benzodioxane derivatives' high antihypoxic effect (Vartanyan et al. 1993). One of the main tasks of our research was to study the changes in central and peripheral α_2-adrenoceptors (α_2-AR) under the hypoxic conditions, as well as the pattern of their neurohumoral regulation. On the other hand, to study original compounds that combine anti-hypoxic and α_2-adrenoblocking properties and therefore rapid reversal of agonist effects by selective antagonists was of specific interest.

Material and methods

Animals

All experiments have been carried out on the mongrel albino male rats weighing 150–200 g (aged 14–15 weeks). The animals were bred in the animal facility of the Scientific-Technological Center of Organic-Pharmaceutical Chemistry of NAS RA, maintained on a 12-h light/dark cycle with food and water ad libitum. The protocol was approved by the Institutional Animal Care and Ethics Committee of the Yerevan State Medical University and Scientific-Technological Center of Organic-Pharmaceutical Chemistry of NAS RA in accordance with the European Communities Council Directive (86/609/EEC) on the care and use of animals for experimental procedures. The animals were allowed to adapt to our laboratory environment for 7 days before the experiment. For the purposes of this study, the rats were assigned into four groups. All groups were clustered into four subgroups ($n = 8$ per subgroup). The first group's subgroups were (1) naive control group; (2) mesedin-injected group; (3) beditin-injected group; and (4) idazoxan-injected group. All the first group subgroups' animals underwent cannulation. The second group's subgroups were (1) control – azepexole group; (2) azepexole + mesedin group; (3) azepexole + beditin group; and (4) azepexole + idazoxan group. All the second group subgroups' animals underwent spinal cord destruction. The third group's subgroups were (1) control – clonidine group; (2) clonidine + mesedin group; (3) clonidine + beditin group; and (4) clonidine + idazoxan group. The fourth group was clustered into four subgroups: (1) naive control group; (2) mesedin-injected group; (3) beditin-injected group; (4) idazoxan-injected group. All the subgroups of the fourth group of animals underwent hypoxia.

As a result of the studies, the chemical compounds, mesedin and beditin, were isolated as benzodioxane derivatives, which were synthesized for the first time in the Scientific-Technological Center of Organic-Pharmaceutical Chemistry of NAS RA.

During the experiment, the animals of subgroup 2 received an intravenous injection (i.v.) of mesedin (10 mg/

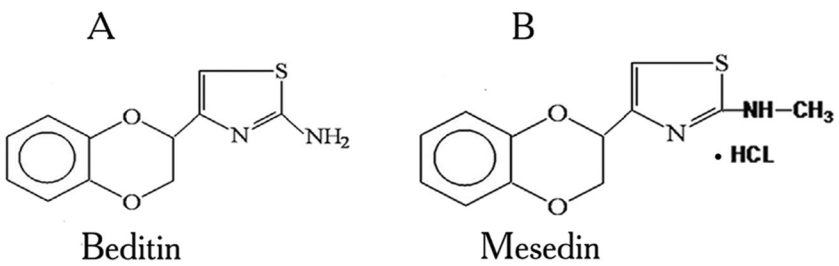

Fig. 1 Chemical structure of 2-aminothiozolyl-1,4-benzodioxane (beditin) and 2-(2-methyl-amino-thiozolyl)-1,4-benzodioxane hydrochloride (mesedin) containing five-membered heterocycle with two heteroatoms (nitrogen and sulfur) in the second position

kg of weight of animal) (Manukyan et al. 2017; Melkonyan et al. 2021), those of subgroup 3 received beditin (2 mg/kg of weight of animal) (Melkonyan et al. 2010; Manukyan et al. 2021), and those of subgroup 4—idazoxan (RX-781094, Rickitt & Colman, England) (2 mg/kg of weight of animal) (Hunanyan et al. 2010).

Hypoxia was reproduced in animals by placing them in a chamber that was continuously purged with a hypoxic air mixture consisting of N_2 and O_2. Herein, the partial pressure of O_2 in the chamber was decreased from 123.7 ± 2 to 27 ± 3 mm Hg. The hypoxia chamber has been prepared by the technical staff of the Institute of Fine Organic Chemistry and verified by long-term testing and standardization. The life span of the control animals under the given conditions made up 28 ± 2.5 min.

Preoperative preparation

Immediately prior to the operation, the femoral artery area was shaved for two times larger than the estimated area of the surgical wound. The animals were deprived of food a few hours before the start of the operation. The operation room complied with the standard requirements was warm and silent, and the light was moderately bright. To disinfect the surgical field, the animal's skin was treated with a disinfectant according to the "from the center to the periphery" principle. The animals were put under the injectable intra-peritoneal ketamine anesthesia (0.5–0.75 mg/kg i.p.) according to anesthetic protocols. An arterial/vein catheter made of a soft silicon tube with a needle inside (of a 22 to 26 G and 1.5 to 4 cm long depending on the rat size) was used for the femoral artery/vein after the topographic assessment of landmarks. It was necessary for the selection of operative access to the catheterized vessel and exteriorization of it at a site inaccessible to the animal upon the back under the skin through the shovels and attaching to the skin so that the animal is out of reach to avoid injury. The used catheter at the end was treated with sodium heparin in a saline solution (López-Briz et al. 2018). The final concentration of heparin was 150 IU/ml. To prevent occlusion of the catheter, it was flushed regularly (before and after infusion or blood sampling) with saline, including the addition of heparin (5 U/ml) (Vose et al. 2019) at least every 30 min.

Femoral artery/vein catheterization procedure

Anesthetized rats were placed in dorsal recumbence for the inguinal surgical preparation. A 1- to 1.5-cm incision of the skin was performed perpendicular to the limb and parallel to the abdominal line in the inguinal region to access the proximal segment of the femoral artery and leave the distal part of the limb for catheter securing. The end of the catheter was gently grasped with the forceps and pulled through the cavity made ultimately out of the incision. Then, the catheter was connected to the pressure transducer for the recording of the values. To protect the catheter, a long extender filled with heparinized saline was placed between the catheter and the transducer to allow the rat movement in the cage. Then, two ligatures were placed: one loose on the cardiac end and one tight on the opposite proximal part which enlarged the artery by obliterating the blood flow. For a better approach, the artery was suspended with the wire from the second ligature. Then, a small incision on the artery between two ligatures was made to insert the catheter. The latter was inserted around 2 cm until it reached the abdominal aorta and was fixed with the proximal ligature. The procedure was completed by suturing the skin (Jespersen et al. 2012).

Measurements were performed on anesthetized and catheterized animals 24–48 h after the animals had emerged from the anesthesia and awakened with the capability to active movement. Similarly, blood was taken from the catheters placed in the venous and arterial vessels while the animals were awake and in free movement.

Determination of reactivity of the peripheral postsynaptic vascular α_2-ARs and central mydriasis-responsible α_2-ARs

The assessment of the peripheral postsynaptic vascular reactivity can be achieved by measuring α_2-adrenergic effects on BP. In this respect, the assessment of the reactivity of central mydriasis-responsible effects was achieved by measuring α_2-adrenergic effects on pupil reaction based on the pupil's dilation with the help of a microscope.

The reactivity of postsynaptic vascular α_2-adrenergic receptors was determined in rats with a damaged spinal cord (Zou et al. 2006) expressed by an increase in the blood pressure value in response to intravenous administration of substances that selectively bind to α_2-adrenoceptors (B-HT 933, the so-called azepexole (Boehringer, Germany)). At the same time, the grade of inhibition of these pressor reactions in experimental animals indicated the grade of suppression of the α_2-AR reactivity, which was also observed in the introduction of selective blockers of α_2-ARs. All compounds were injected into the femoral vein (after dissolution in saline). Each administration was carried out after the initial blood pressure restoration (approximately 15 min later). The value of adrenoceptor reactivity was calculated relatively to the initial blood pressure value established before the administration of α-adrenergic agonist.

The spinal cord was destroyed in order to disable the presynapse activity, which proves the selective nature of beditin and mesedin in contrast to non-selective idazoxan which affects both presynapse and postsynapse α_2-ARs. This procedure was done by passing the metal rod through the eye socket along the entire spinal cord to damage the central

nervous system (in 2–3 min after the injection of drugs into the femoral artery BP was measured through the femoral artery). This determines the effect of the substance on the postsynaptic α_2-ARs. During the entire experiment, animals were anesthetized and kept under the artificial ventilation.

The state of the central α_2-ARs was judged by the expansion of the pupil diameter in the control and experimental rats in response to intravenous administration of selective α_2-receptor activator, clonidine (Sigma, USA), and the removal of this effect by introduction of the known selective α_2-adrenoblocker. The essence of this method is the fact of α-adrenomimetics' easy penetration into the CNS; in particular, clonidine along with its hypotensive effect, thereby activating α_2-ARs in various areas of the medulla oblongata, also leads to pupil dilation. The latter effect is due to the activation of α_2-ARs of the Edinger-Westphal nuclei leading to inhibition of the preganglionic fibers of the iris parasympathetic innervation (Wu et al. 2022). The pupil diameter of rats was measured under constant illumination with an MBS-9 microscope (magnification 1×10). Pupil reaction was recorded at the 15th minute after intravenous administration of medication.

The control reactivity of vascular α_2-AR was calculated by the pressor effect strength of azepexole (i.v.; 0.16 mg/kg) in relation to the initial blood pressure. The control reactivity of central α_2-ARs was calculated upon the mydriatic effect expressed by clonidine at a dose of 0.1 mg/kg, i.v.

Determination of pO_2 in blood

On the experiment day, a control blood test was drawn from the vein and artery of rats by means of a glassy capillary, and then, the examined substances were injected intravenously. pO_2 in the blood and the oxygen-transporting function were determined in a sample of 0.1 ml of blood collected before injection: the control and following injections of mesedin, beditin, and idazoxan in the 30, 90, and 180 min after injection using Bayer Rapidlab 348 Blood Gas Analyzer.

Determination of the systemic hemodynamic values and the resistance of vessels to the blood flow

The method is based on the use of plastic microspheres with isotopes marked 7–15 μm (slightly larger than erythrocytes) (Lepran et al. 1983), which are injected into the bloodstream, mixed with blood, spread throughout the whole organism, and accumulated in the arteries and capillaries. The number of microspheres driven into tissues or organs was directly proportional to the rate of blood flow in that area; thus, by determining the microspheres' number, it is possible to make sense of the blood supply to the given area of an organ. Rats were injected with 1.4 million microspheres without any side effects. The number of microspheres used (Co-57, Sc-46, Sr-85, Sn-113; made in "NEN" USA) (no more than 400 thousand per rat) did not disturb the blood circulation and have any toxic effect. The experiment was conducted 24–48 h after the animals had woken up and came to free movement (vigor). The injection of microspheres took 10 s. Simultaneously, blood was collected from the femoral artery (for measurement with a hermetic plastic vial by a gamma counter). After the experiment, the animals were dropped, and the examined organs and tissues were isolated, weighed, and then placed in plastic containers. All microspheres were measured with a Compu-Gamma 1282 gamma counter (LKB Wallack, Sweden) to determine the activity of each type of microsphere. The activity level of the used microspheres was not lower than 0.8 CPM/microsphere (Shirinyan et al. 2004).

The time course of the hemodynamic studies was followed in 30, 90, and 180 min after injection.

Invasive blood pressure (IBP) monitoring by artery cannulation

Invasive blood pressure measurements were performed on awakened from anesthesia animals using the femoral artery previously brought out in the dorsal cervical region during anesthetization. To record BP after the catheterization, the arterial catheter was connected to a pressure flexible transducer (calibrated to 0 mm Hg) through a fluid extender filled with heparinized saline and then monitored with a data acquisition system for the signal collecting, followed by their amplification and conversion into digital numeric values in real time on the screen. BP was measured by TAM-A Transducer Amplifier Module (Hugo Sachs, Germany), LabScribe recording software (iWorx, v4). To describe the cardiac activity in medicine, the systolic and diastolic pressures were determined, and the mean arterial pressure was evaluated to find out how well the organs stayed supplied with blood. This value can be easily calculated using the following formula $(2(\text{DBP}) + \text{SBP})/3$, where DBP is the diastolic pressure, and SBP is the systolic pressure (Parasuraman and Raveendran 2012).

Determination of concentration of corticosterone in the adrenal glands and blood

The concentration of corticosterone in the blood plasma and adrenal glands was determined with the help of enzyme-linked immunosorbent assay using "Corticosterone ELISA kit" in accordance with the manufacturer's protocol.

Statistical processing of the obtained data was carried out by the following standard formulas:

1. Cardiac output (CO), in ml/min—$\text{CO} = A \times C/a$, where A is the number of single introduced microspheres; C is the blood sampling rate, in ml/min; and a is the number of microspheres in the taken portion of blood

2. Cardiac index (CI), in ml/min/100 g—CI = CO/*M*, where *M* is the mass of the animal
3. Total peripheral vascular resistance (TPVR), in mmHg/ml/min/100 g—TPVR = BP/CI, where BP is the mean arterial pressure in mmHg
4. Regional blood flow (RBF), in ml/min/g tissue—RBF = C × v/a, where *C* is the rate of blood sampling in ml/min and *c* is the number of microspheres per 1 g of tissue
5. Regional vascular resistance (RVR), mm Hg/ml/min/g—RVR = BP/RBF

Statistical analysis

All analysis was performed using the BIOSTAT system. All measurements were represented as mean ± SEM. The significance of the means' difference was evaluated using the paired Student–Newman–Keuls test. Statistical significance, determined by one-way ANOVA, was set at $p<0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Results

The action of α_2-adrenoblockers on the reactivity of peripheral postsynaptic vascular α_2-ARs and the central clonidine-mediated α_2-ARs of the brain

Assessment of reactivity of the peripheral postsynaptic vascular and the central mydriasis-inducible α_2-ARs showed that mesedin caused a stable and pronounced blocking effect on the reactivity of peripheral postsynaptic vascular α_2-adrenoceptors in comparison to the agonist azepexole control and azepexole + idazoxan groups (Fig. 2a).

Additionally, in regard to the clonidine control group, mesedin did not lead to inhibition of the mydriatic reaction mediated by central α_2-ARs, while idazoxan under the same conditions of the experiment showed a central α_2-adrenoblocking effect. The initial value of the pupil diameter before the administration of clonidine and the studied α_2-adrenoblockers was about 4 mm (which was considered to be as 100%). After 15 min of clonidine injection, it became 9.1 mm (4 + 5.1), mesedin 4 mm, beditin 4.07 mm (4 + 0.7), and idazoxan 8.95 mm (4 + 4.95).

Regarding the beditin group, a little presynaptic and much more expressed postsynaptic blocking activity on the α_2-ARs were detected. In contrast to mesedin and beditin, idazoxan expressed both presynaptic and postsynaptic blockage effects (Fig. 2b).

The effect of α_2-adrenoblockers on the level of pO_2 in the arterial and venous blood of vigorous rats

Based on the analysis of pO_2 in the arterial and venous blood, mesedin and beditin significantly increased pO_2 in the arterial blood in the first 30 min in comparison to untreated control and idazoxan control groups (Fig. 3a). Additionally, mesedin evoked an increase in pO_2 in the arterial blood in 30, 90, and 180 min (Fig. 3a–c) and a significantly noticeable and prolonged drop of the pO_2 in the venous blood

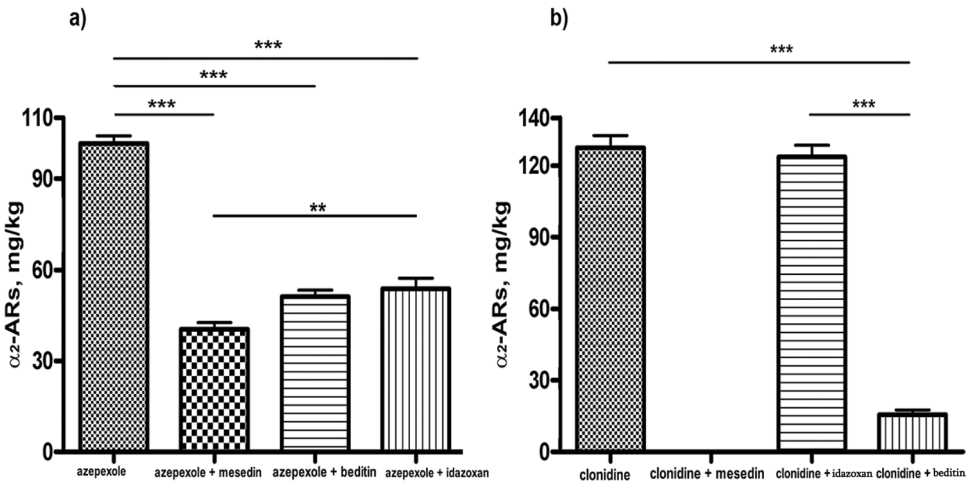

Fig. 2 Blocking action of the mesedin, beditin, and idazoxan on the reactivity of peripheral postsynaptic vascular and the central mydriasis-responsible α_2-adrenergic receptors (α_2-AR). Animals ($n = 8$) were injected with azepexole serving as a control. Mesedin, beditin, and idazoxan were injected i.v. into the azepexole-treated rats. One-way ANOVA comparison test, $^{**}p < 0.01$, *** $p < 0.001$. The mean ± SEM values of blocking action on the reactivity of peripheral postsynaptic vascular α_2-AR of mesedin, beditin, and idazoxan are shown (**a**). Animals ($n = 8$) were injected with clonidine serving as a control. Mesedin, beditin, and idazoxan were injected i.v. into the clonidine-treated rats. One-way ANOVA comparison test, $^{**}p < 0.01$, *** $p < 0.001$. The mean ± SEM values of blocking action on the reactivity of the central mydriasis-responsible α_2-AR of mesedin, beditin, and idazoxan are shown (**b**)

(Fig. 3d–f). As a result, the arterial-venous difference in oxygen in the blood increased the level of pO_2 over a long period (3 h) (Fig. 3g–i). In contrast to mesedin, beditin did not reach a level of significance regarding increasing pO_2 in the arterial blood in 90 min, but it failed to reach statistical significance in 30 and 180 min. The same pattern (in 180 min) was noticed regarding the pO_2 decrease in the venous blood; however, there was no significance in 30 and 90 min. The difference between beditin-/mesedin-treated animals and idazoxan control groups was the fact of pO_2 increase in both arterial and venous blood and the arterial-venous oxygen difference decrease.

The effect of α_2-adrenoblockers on the systemic hemodynamic values and the vascular resistance towards the blood flow of the rats

The identified changes in vascular resistance in organs were in line with the position that vascular resistance to blood flow is in close inverse correlation with the volumetric blood flow velocity. Thus, in the heart and adrenal glands, a significant increase in volumetric blood flow was observed under the influence of beditin and mesedin (Fig. 4a, b), as well as there was a significant decrease in vascular resistance (Fig. 4a, b). The effect of beditin and mesedin on other organs' (muscles, intestine) changes in the systemic hemodynamic values and the vascular resistance to blood flow were not significant (Figs. 4, 5c, d). Regarding the effect of the studied adrenergic blockers on the hemodynamic parameters of the heart and considering the inhibitory properties of mesedin and beditin on the vascular α_2-adrenoceptors and their high anti-hypoxic efficacy, it has been suggested that the latter have a favorable effect on the blood circulation (Figs. 4, 5e). In accordance with these indicators, a reduced level of vascular resistance to the blood flow remained; i.e., there was a prolonged dilatation of the coronary vessels. Idazoxan, in comparison to mesedin and beditin, had an effect on the hemodynamics of all the studied organs; namely, it increased the blood flow and reduced vascular resistance in the heart, adrenal glands, muscles, and lungs (Figs. 4, 5a, b, d, e) versus the intestine where the oppositely directed hemodynamic shifts took place (Figs. 4, 5c).

Changes in systemic hemodynamics in vigorous rats in 15 min after injection of α_2-adrenoblockers

Mesedin and beditin did not alter BP and the heart rate (HR) in comparison to idazoxan, which lowered BP with almost no effect on HR (Fig. 6a, b). Despite this, α_2-adrenoblockers remarkably increased the cardiac output in comparison to the untreated control, whereas the effect of mesedin and beditin was superior to that of idazoxan (Fig. 6c). Similarly, α_2-adrenoblockers decreased the total peripheral resistance of the blood vessels in comparison to the untreated control. Evidentially, the effect of mesedin on vascular resistance was higher than that of beditin and idazoxan (Fig. 6d).

Influence of α_2-adrenoblockers on the concentration of corticosterone in the adrenal glands and blood and their anti-hypoxic effect

The observed anti-hypoxic effect of mesedin and beditin was in line with the data indicating the rats' resistance changes to the acute hypoxic hypoxia due to the sympathoadrenal system's different link effects. A drastically increased level of corticosterone was observed in the adrenal glands and blood in the mesedin group in comparison to the naive and idazoxan controls (Fig. 7a, b). The beditin group increased the corticosterone level, but in comparison to the mesedin-injected changes, it was at a lower extent. In contrast to mesedin, the difference in the level of corticosterone between the beditin group and idazoxan did not reach a level of significance in the adrenal glands. A similar effect was noticed by the evaluation of resistance to hypoxia. Administration of α_2-adrenoblockers led to an increase in resistance to hypoxia even more than it was in the untreated control group (Fig. 7c). The highest efficacy of mesedin was in correspondence with the highest resistance to hypoxia, which prevailed over that of the beditin group and idazoxan.

Discussion

It is known that along with localization in peripheral organs and tissues, α_2-ARs are also present in the central nervous system. Excitation of these receptors in the central nervous system leads to inhibition of the activity of peripheral sympathetic nerves. Blocking of the central α_2-ARs leads to the "uncontrolled" release of the neurotransmitters, which is expressed in reverse, i.e., with a stimulating effect (Philipp et al. 2022; Guimarães and Moura 2001).

α_2-Adrenoceptors were found in both the central and peripheral nervous systems, located both pre- and postsynaptically. In the CNS, these receptors can regulate a neurotransmitter release acting as autoreceptors when located on noradrenergic nerve terminals, as well as acting as heteroreceptors when bounding with nonnoradrenergic nerve terminals. The pharmacological classification of α_2-ARs is based on interaction with selective agonists and antagonists (Fava and Papakostas 2008). The endogenous agonists adrenaline and noradrenaline are approximately equipotent at both a_1- and α_2-ARs. This enabled the development of a number of selective α_1- and α_2-ARs agonists and antagonists both as pharmacological tools in tissue and animal studies and as potential pharmacotherapeutic

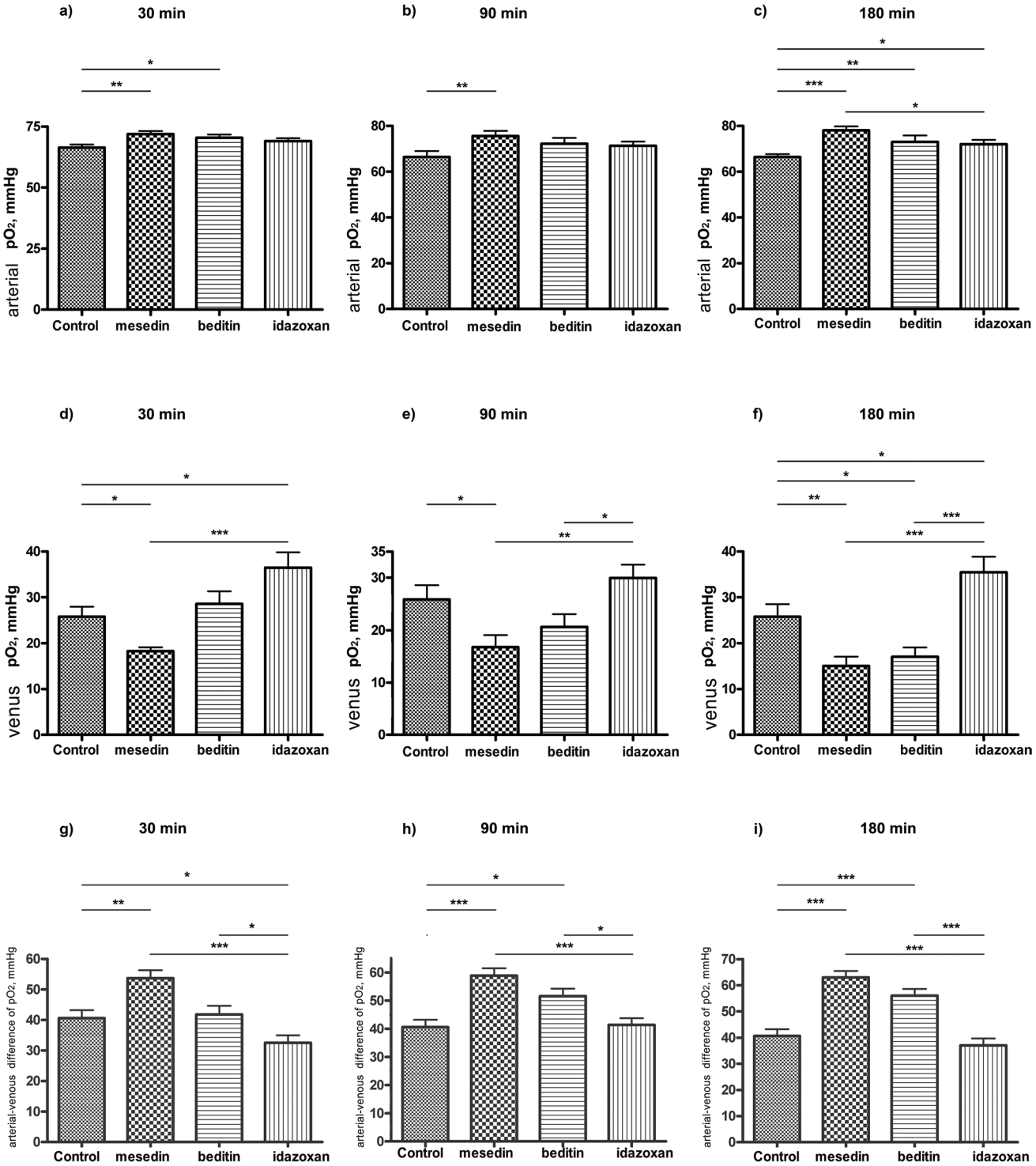

Fig. 3 The effect of mesedin, beditin, and idazoxan on the level of pO_2 in the arterial-venous blood and in the arterial-venous difference of rats. Animals ($n=8$) were pre-catheterized and untreated serving as a control (C). Mesedin (M), beditin (B), and idazoxan (I) were injected i.v. into the catheterized rats. One-way ANOVA comparison test, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. The mean ± SEM value effects of mesedin, beditin, and idazoxan on the level of pO_2 in the arterial blood in the 30, 90, and 180 min are shown (**a**, **b**, **c**), respectively. The mean ± SEM value effects of mesedin, beditin, and idazoxan on the level of pO_2 in the venous blood in the 30, 90, and 180 min are shown (**d**, **e**, **f**), respectively. The mean ± SEM values of mesedin, beditin, and idazoxan on the arterial-venous difference in the 30, 90, and 180 min are shown (**g**, **h**, **i**), respectively

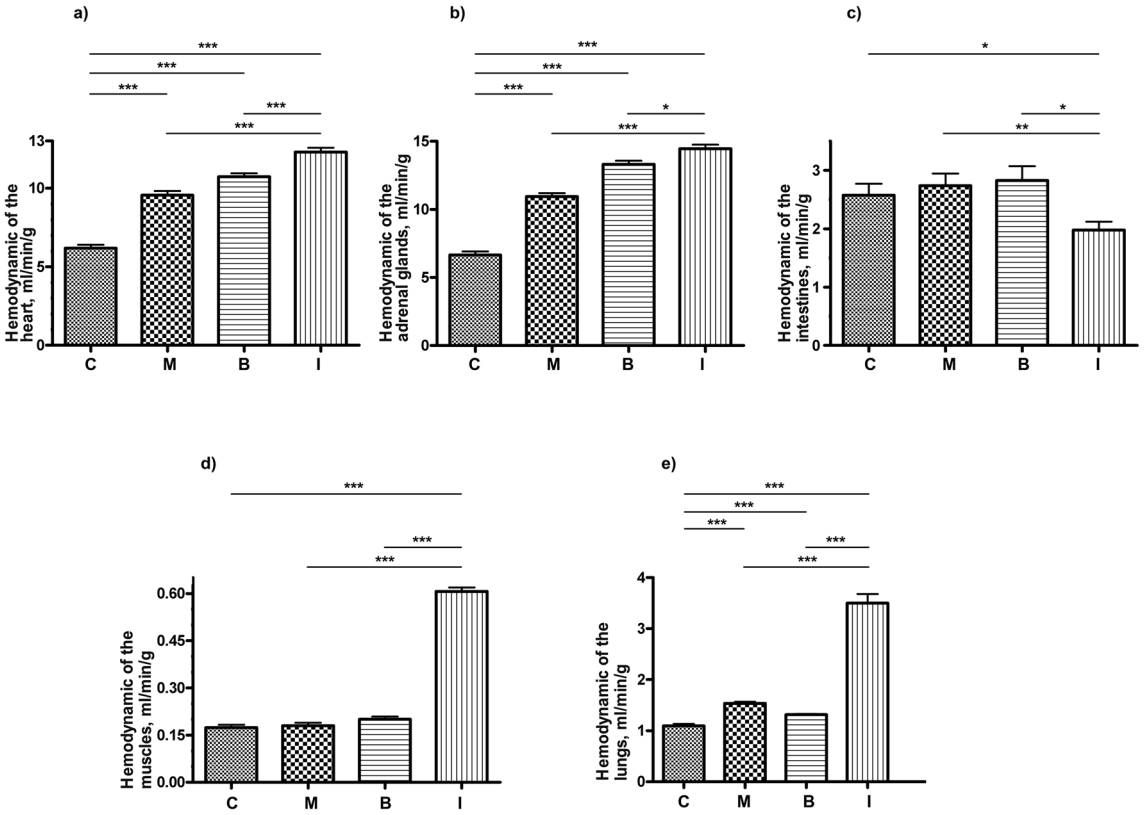

Fig. 4 The effect of mesedin, beditin, and idazoxan on the systemic hemodynamic values in the internal organs of the rats. Animals ($n=8$) were pre-catheterized and untreated serving as a control (C). Mesedin (M), beditin (B), and idazoxan (I) were injected into the catheterized rats i.v. One-way ANOVA comparison test, $*p<0.05$, $**p<0.01$, $***p<0.001$. The mean ± SEM value effects of mesedin, beditin, and idazoxan on the level of hemodynamic values in the internal organs, heart, adrenal glands, intestines, muscles, and lungs, are shown (**a**–**e**), respectively

agents. Reportedly, clonidine is shown to have a central effect on α_2-ARs, resulting in inhibition of the sympathetic cardio-accelerator and vasoconstrictive properties. Namely, it increases baroreceptor activity and acts on the peripheral postsynaptic α_2-ARs and also decreases sympathetic outflow from the central nervous system, and so the peripheral resistance of vessels as well (Gilden 2012).

In our in vitro and in vivo experiments, when animals were subjected to hypoxic hypoxia, usage of the α_2-ARs agonists, azepexole and clonidine, was compared with beditin, mesedin, and idazoxan α_2-adrenoblockers. On the basis of screening studies of biheterocyclic derivatives of 1,4-benzodioxane, mesedin was identified as selectively blocking mainly the peripheral α_2-ARs.

Our data have shown that mesedin and beditin do not lead to inhibition of the mydriatic reaction mediated by the central α_2-ARs; at the same time, idazoxan under the same experimental conditions showed maximally a central α_2-adrenoblocking effect. Thus, the data obtained indicate a high α_2-adrenoblocking activity and high selectivity (unlike idazoxan) of the action of mesedin and beditin on the peripheral α_2-ARs. At the same time, the action of mesedin comparably was stable, and what is of no less importance, of relatively low toxicity which was accounted when choosing this compound. Non-selective idazoxan, in terms of strength and α_2-selectivity of blocking action, blocks both the central and the peripheral α_2-ARs (i.e., the pre- and postsynaptic ones).

The clinical signs and symptoms of hypoxia include dyspnea, increased respiratory effort, nasal flaring, and mouth breathing (Rothan and Byrareddy 2020). It can be inferred from our study results that a shift in the arterial-venous difference of pO_2 indicates that mesedin had a significant and prolonged improvement in oxygen delivery to tissues, i.e., increase the tissue oxygenation level, which can be extremely important for the COVID-19-infected patients' treatment (Benner et al. 2022). This may explain the strong anti-hypoxic effect of the compound. Regarding this indicator, the effect of idazoxan was different. This well-known α_2-adrenoblocker improved the affinity of hemoglobin to oxygen; i.e., it binds more oxygen; anyway, the release and supply of oxygen to tissues did not significantly better. Thus, in a study of the blood oxygen content in venous and

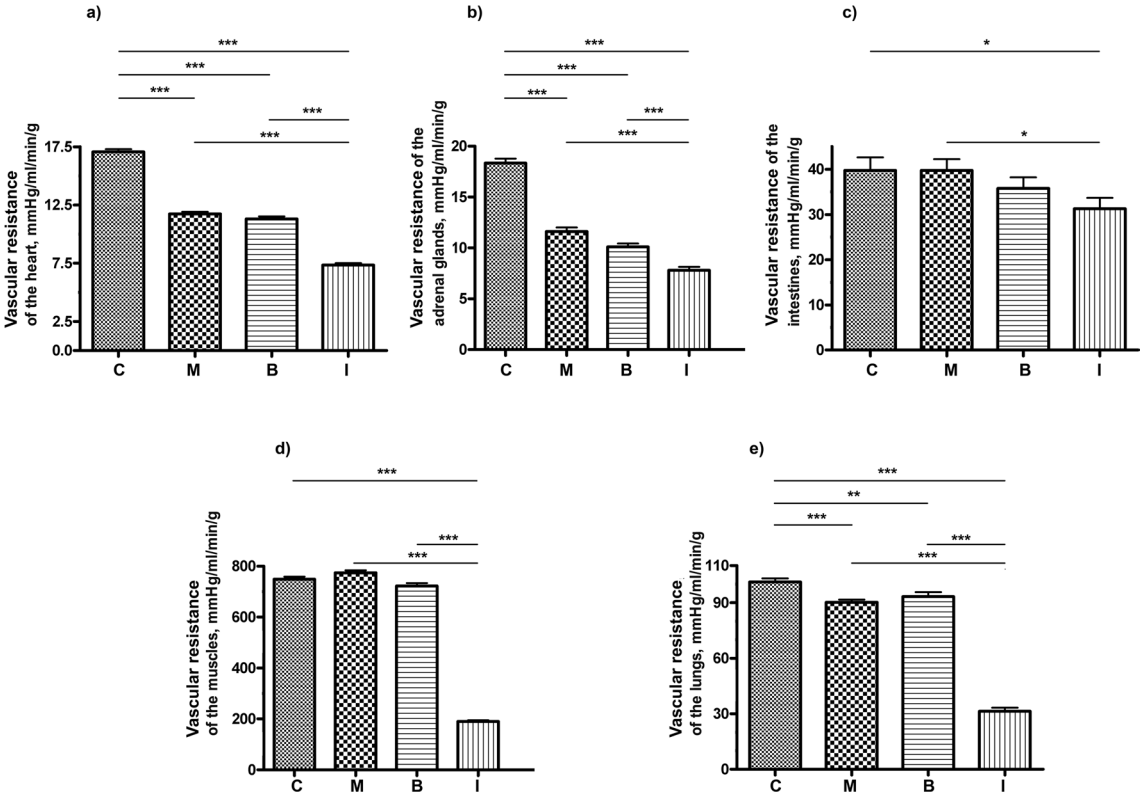

Fig. 5 The effect of mesedin, beditin, and idazoxan on the vascular resistance towards the blood flow of the rats. Animals ($n=8$) were pre-catheterized and untreated serving as a control (C). Mesedin (M), beditin (B), and idazoxan (I) were injected into the catheterized rats i.v. One-way ANOVA comparison test, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. The mean ± SEM value effects of mesedin, beditin, and idazoxan on the vascular resistance towards the blood flow in the internal organs, heart, adrenal glands, intestines, muscles, and lungs, are shown (**a**–**e**), respectively

arterial catheters, we found that mesedin slightly increased the pO_2 in the arterial blood, causing a significant and prolonged reduction of pO_2 in the venous blood. In this regard, a similar effect of beditin was mild and comparably short-lived. Completely different shifts were observed with the use of idazoxan: that is, an increase in pO_2 in both arterial and venous blood. Similar shifts in pO_2, which characterize an increase in the affinity of hemoglobin for oxygen, may indicate the property of idazoxan to increase the uptake of oxygen by the blood, which can be of vital importance, especially in conditions of hypoxic hypoxia (Benner et al. 2022). Thus, the increased extraction of O_2 by the tissues from the inflowing arterial blood, apparently, is one of the factors contributing to the manifestation of the powerful anti-hypoxic effect of mesedin and, to some extent, beditin.

Thus, studying the blood oxygen level and acid–base balance in the venous and arterial catheters, we found that mesedin slightly increased the pO_2 in the arterial blood, and decreased it in the venous blood. One of the major modes of action of the selective postsynaptic a_2-ARs detected in hypoxic conditions is their benevolent effect on organs' hemodynamics and cellular metabolism due to the cascade mechanism of the postsynaptic α_2-AR blockade. That leads to vasodilation and increased blood flow to organs, thereby increasing their oxygenation rate. In some peripheral organs, the compounds either do not change the hemodynamic parameters or lead to a decrease in blood flow by vasoconstriction (in intestines and muscles).

Hypoxic conditions are known to lead to catastrophic and unavoidable disruption of system-organ (local) hemodynamics. Given that mesedin and beditin have a pronounced anti-hypoxic effect, it is naturally assumed that one of their mechanisms of the protective effect is the favorable effect of the latter on the body's blood circulation. Studies in pre-catheterized rats using labeled microspheres have shown that an increase in initial blood flow velocity (15 min) is seen as a consequence of the mesedin-induced vasodilating effect (adrenal, heart), which is due to the presence of the α_2-ARs in these organs. As for the intestine and muscles, there are mainly β-adrenoceptors presented (Upadhyaya et al. 2020), which resulted in a vasoconstrictor effect due to the realization of compensatory mechanisms and the subsequent appearance of blood redistribution. In the other examined peripheral organs (muscles, intestines), this effect is not

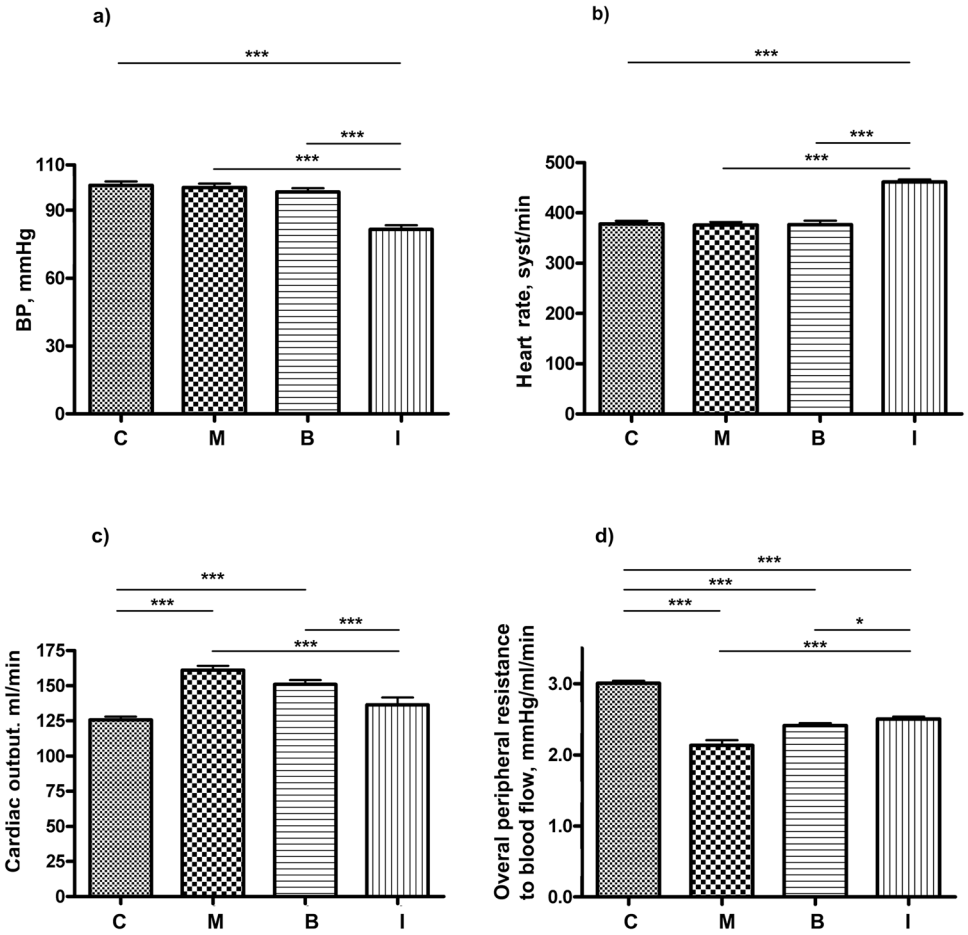

Fig. 6 Changes in systemic hemodynamics in vigorous rats in 15 min after injection of mesedin, beditin, and idazoxan. Animals ($n=8$) were pre-catheterized and untreated serving as a control (C). Mesedin (M), beditin (B), and idazoxan (I) were injected into the catheterized rats i.v. One-way ANOVA comparison test, $*p<0.05$, $***p<0.001$. The mean ± SEM value effects of mesedin, beditin, and idazoxan on the BP, heart rate, cardiac output, and overall peripheral resistance blood flow (**a**–**d**), respectively

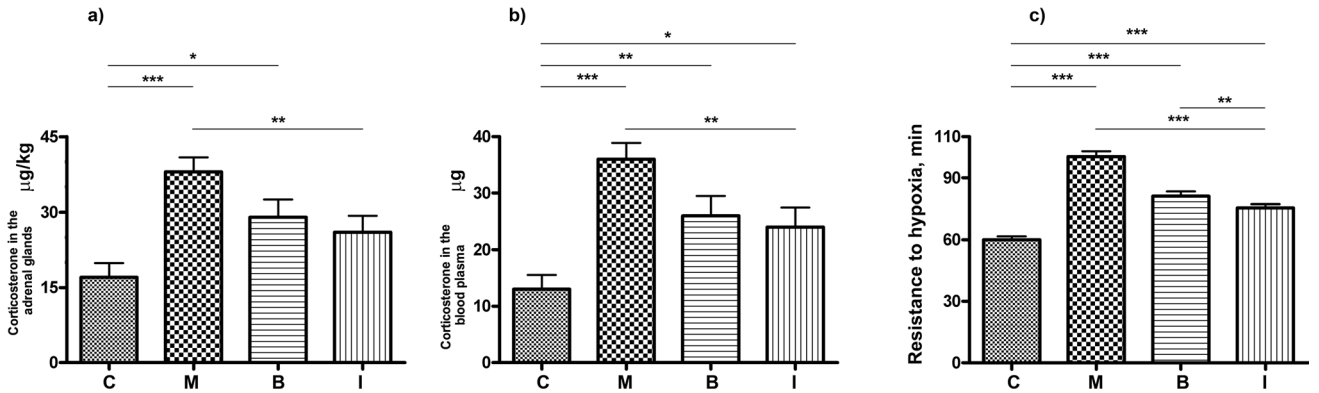

Fig. 7 The effect of mesedin, beditin, and idazoxan on the concentration of corticosterone in the adrenal glands and blood and the resistance to hypoxia. Animals ($n=8$) were untreated serving as a control (C). Mesedin (M), beditin (B), and idazoxan (I) were injected into the rats i.v. One-way ANOVA comparison test, $*p<0.05$, $**p<0.01$, $***p<0.001$. The mean ± SEM value effects of mesedin, beditin, and idazoxan on the level of corticosterone in the adrenal glands and blood are shown (**a**, **b**), respectively. The mean ± SEM value effects of mesedin, beditin, and idazoxan on the resistance to hypoxia are shown (**c**)

observed, as the adrenoceptor field is mainly represented by the type β_2-adrenoceptors (Farzam and Jan 2022). A study of the cardiovascular effects of peripheral postsynaptic α_2-adrenoblockers in rats allows concluding that the compound mesedin and beditin are vasoactive substances that do not change BP and HR in animals. Herein, one of the main effects of these drugs is a significant and selective improvement in blood circulation and vasodilation, mainly

in the central circulatory organ—the heart, as well as in the adrenal glands, an organ having the dominant roles in the urgent organization of protective and adaptive reactions in extreme conditions. The non-selective idazoxan does not have such selectivity of action and causes a total change in systemic and regional hemodynamic parameters in most organs and tissues of the body.

Blockade of α_2-ARs was shown to result in a significant 60% attenuation of isolated artery flap contracture and a 90% prevention of platelet aggregation. During brain ischemia and general hypoxia of the body, which is accompanied by a massive release of catecholamines, the sensitivity of α_2-ARs increases sharply, by 100% and 40%, respectively, and their selective blockade leads to a significant decrease in vascular resistance, a simultaneous increase in general and local blood flow to the brain (Shirinyan et al. 1989). These changes in the vascular system, together with the metabolic changes caused by α_2-AR blockade, significantly increase the survival rate of animals in hypoxic and ischemic conditions. The obtained data will be used in the perspective of correcting angio-hematic interactions by acting on α_2-ARs. Our data have shown that the adrenal glands are one of the few internal organs in which the α_2-ARs have been found to interfere in significantly increasing the blood flow volume velocity. Most likely, the functional role of such an improvement in adrenal blood supply is to increase the synthesis of corticosteroids in the glands, and then release them into the bloodstream. The role of the sympathoadrenal and the pituitary-adrenal cortex systems in the organization of adaptation reactions and in increasing organism resistance is well-known (Herman et al. 2016). We have already mentioned that the main target of influence of the discovered and under the study substances is the postsynaptic α_2-ARs.

The data on recording the organism's endurance to acute hypoxia and the reactivity of peripheral postsynaptic α_2-ARs obtained in animals with selectively inhibited links of the sympathetic part of the sympathoadrenal system enables the identification of the primary dependence of durability to severe hypoxia on the state of peripheral adrenergic structures. Our experimental data had shown that increased corticosterone hypersensitivity of postsynaptic α_2-ARs develops: their number increases and, consequently, the mediated reactions are being triggered (Giralt and Garcia-Sevilla 1989). In the given experiments, such an increase in the reactivity of postsynaptic α_2-ARs with the insufficiency of peripheral adrenergic innervation and a sharp decrease in hypoxia tolerance suggest that the main reason for the disruption of the body's adaptive capabilities is an increase in cascade prohypoxic reactions triggered by α_2-ARs of effector cells. The validity of this assumption is evidenced both by our data on the antihypoxic properties of the used selective α_2-ARs and the literature data on the participation of peripheral α_2-ARs in hypoxia counteracting reactions providing cardiovascular and hemo-vassal shifts in the organism.

Conclusion

Revealing the mechanisms of action of the postsynaptic α_2-adrenoceptors suggests that mesedin and beditin are potentially effective therapeutic means for many hypoxic conditions and diseases. Thus, the studied mechanisms allow to diversify and expand the scope of these compound's applications. However, we do not exclude that in the metabolic and functional shifts realized by 1,4-benzodioxane derivatives mesedin and beditin, increasing the body's resistance to hypoxic affections may also involve other systems of organisms. Thus, based on the analysis of the obtained data, we suggested that the selective blockade of effects mediated by postsynaptic α_2-adrenergic receptors with low-toxic compounds can become one of the promising directions in increasing the resistance of tissues and the whole organism when exposed to hypoxic factors.

Author contributions All authors read and approved the final manuscript. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The protocol was approved by the Institutional Animal Care and Ethics Committee of the Yerevan State Medical University and Scientific-Technological Center of Organic-Pharmaceutical Chemistry of NAS RA in accordance with the European Communities Council Directive (86/609/EEC) on the care and use of animals for experimental procedures. Approval was granted by the Ethics Committee of the Yerevan State Medical University (Date 21.03.2022/No. 8).

Competing interests The authors declare no competing interests.

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