

Effect of stimulation of α_2 -Adrenoceptor on the isolated rat heart after limitation of motor activity

Efecto de la estimulación del adrenoceptor α_2 en el corazón de rata aislado después de la limitación de la actividad motora

¹ Milyausha Ildousovna Sungatullina¹, Postgraduate (Department of Human Health Protection) Institute of Fundamental Medicine and Biology, e-mail: violet-diva@mail.ru

¹ Railya Irekova Zaripova¹, Senior Lecturer (Department of Human Health Protection) Institute of Fundamental Medicine and Biology, ID Scopus 56372235300, e-mail: ratno1992@mail.ru

^{1,2} Khalil Iatipovich Gainutdinov^{1,2}, Professor Institute of Fundamental Medicine and Biology, ID Scopus 7004692198, e-mail: kh_gainutdinov@mail.ru

¹ Alina Maratovna Galieva¹, Postgraduate (Department of Human Health Protection) Institute of Fundamental Medicine and Biology, e-mail: galieva_alina94@mail.ru

¹ Nafisa Ilgizovna Ziyatdinova¹, Professor Department of Human Health Protection) Institute of Fundamental Medicine and Biology, ID Scopus 6602571223, e-mail: nafisaz@mail.ru

¹ Timur Lvovich Zefirov¹, Professor Department of Human Health Protection) Institute of Fundamental Medicine and Biology, ID Scopus 6701534991, e-mail: zefirovt@mail.ru

¹Kazan Federal University

²Zavoisky Physical-Technical Institute of the Russian Academy of Sciences. Tel.: +79520394405

Received/Recibido: 08/28/2020 Accepted/Aceptado: 09/15/2020 Published/Publicado: 11/09/2020

DOI: 10.5281/zenodo.4421964

Abstract

The study of the effect of limiting motor activity on the body and organ systems is an urgent task. The importance of this issue is due to not only the modern lifestyle but also the high prevalence of hypokinesia in medical practice (prolonged bed rest due to fractures, diseases of the cardiovascular system, and other diseases). The functional role of α -adrenergic receptors in adapting the cardiovascular system to changes in the motor regime requires further study. The study of the mechanisms of hypokinetic disorders, the development of methods, and means of prevention and correction of their consequences are of great social importance. Based on the foregoing, the question of the functional significance of α_2 -adrenergic receptors in the heart with a change in the motor regime remains relevant. This experiment was carried out on outbred white rats growing under conditions of limited physical activity for 30 days. A Langendorff preparation study of the effect of a α_2 -adrenergic receptor agonist on the functional parameters of the isolated heart of rats (LVP, HR, CF) was carried out. To stimulate α_2 -adrenergic receptors, a pharmacological agent, clonidine hydrochloride, was used at concentrations from 10^{-9} M to 10^{-6} M. Stimulation of α_2 -adrenergic receptors led to an increase in the contraction force of the isolated heart, except for the concentration 10^{-7} M, which had the opposite effect. Application of clonidine, in hypokinetic rats developed bradycardia and a decrease in the coronary blood flow of the isolated heart.

Keywords: isolated heart, hypokinesia, rat, α_2 -adrenergic receptors, left ventricle pressure, heart rate, coronary flow.

Resumen

El estudio del efecto de limitar la actividad motora sobre el cuerpo y los sistemas de órganos es una tarea urgente. La importancia de este tema se debe no solo al estilo de vida moderno, sino también a la alta prevalencia de hipocinesia en la práctica médica (reposo prolongado en cama debido a fracturas, enfermedades del sistema cardiovascular y otras enfermedades). El papel funcional de los receptores α -adrenérgicos en la adaptación del sistema cardiovascular a los cambios en el régimen motor requiere evaluación. El estudio de los mecanismos de los trastornos hipocinéticos, el desarrollo de métodos y medios de prevención y corrección de sus consecuencias son de gran importancia social. En base a lo anterior, la cuestión de la importancia funcional de los receptores α_2 -adrenérgicos en el corazón con un cambio en el régimen motor sigue siendo relevante. Este experimento se llevó a cabo en ratas blancas consanguíneas que crecieron en condiciones de actividad física limitada durante 30 días. Se llevó a cabo un estudio en la preparación de Langendorff sobre el efecto de un agonista del receptor adrenérgico α_2 sobre los parámetros funcionales del corazón aislado de ratas (PVI, FC, FC). Para estimular los receptores α_2 -adrenérgicos, se utilizó un agente farmacológico, el clorhidrato de clonidina, en concentraciones de 10^{-9} M a 10^{-6} M. La estimulación de los receptores α_2 -adrenérgicos condujo a un aumento en la fuerza de contracción del corazón aislado, a excepción de la concentración 10^{-7} M, que tuvo el efecto contrario. En respuesta a la aplicación de clonidina, las ratas hipocinesizadas mostraron desarrollar bradicardia y una disminución en el flujo sanguíneo coronario del corazón aislado.

Palabras clave: corazón aislado, hipocinesia, rata, receptores α_2 -adrenérgicos, presión del ventrículo izquierdo, frecuencia cardíaca, flujo coronario.

Introduction

Limitation (deficiency) of muscle activity leads to the disruption of all organ systems of the human body. Namely, in the cardiovascular system, prolonged limitation of motor activity leads to the following changes: the contractile function of the myocardium changes, the heart muscle, coronary vessels are weakened, the energy potential of the heart decreases, the minute volume decreases. Hypokinesia of varying duration suppresses the body's defense systems, reducing the synthesis of antioxidant protective enzymes: inducible and constitutive heme oxygenases. At the same time, it reduces the resistance of the circulatory system to damaging factors, thereby causing cardiovascular diseases¹.

During sympathetic regulation, nerve terminals release epinephrine and norepinephrine, which stimulating cardiomyocytes G-proteins associated adrenergic receptors, affect the myocardium. To date, the presence in the heart of α_1 , α_2 and $\beta_1, \beta_2, \beta_3$ adrenergic receptors is confirmed. In rats, the β_1 - and α_1 - adrenergic receptors are mainly involved in the regulation of the cardiovascular system^{2,3}.

All types of α -adrenergic receptors are expressed in the heart. The function of α_2 -adrenergic receptors in the heart is not well understood, there is scanty, often contradictory information: their significance in the development of a negative inotropic effect due to the inhibition of norepinephrine release from sympathetic nerves terminals, associated to bradycardia, has been studied. Stimulation of α_2 -adrenergic receptors reduces the force of contraction and blood pressure³. In addition, a positive inotropic effect upon stimulation of α_2 -adrenergic receptors has been reported. Indeed, during myocardial hypoperfusion, α_1 - and α_2 -adrenergic stimulation, increase inotropy and cardiac activity. Moreover, α_2 -adrenergic receptors are responsible for epicardial vasoconstriction in the hypoperfused myocardium⁴. The experiments performed myocardium strips of the atria and ventricles show multidirectional effects of the force of contraction after activation of α_2 -adrenergic receptors³; while clonidine administration in isolated hearts of 6-week-old rats, produces a multidirectional inotropic effect. Clonidine at a concentration of 10^{-9} M caused an increase in the left ventricle pressure; and at higher concentrations, a decrease in the force of contraction was observed⁵. When α_2 -adrenergic receptors are blocked, a short-term increase in the force of contraction of the heart muscle was observed, and then a decrease⁶.

Studies of the nature of heart rate changes during stimulation of α_2 -adrenergic receptors are few and ambiguous. A number of researchers confirm that stimulation of α_2 -adrenergic receptors results in a decrease in the frequency of contractions^{5,7}. The experiments established the age-related features of the chronotropic effect. For example, it was shown that the α_2 -adrenergic receptor blocker, yohimbine, reduces heart rate, in 6 and 20-week-old rats; while in heart of 1 and 3-week-old rats does not affect heart rate⁸. The α_2 -adrenergic receptor agonist, clonidine, in isolated heart of 6-week-old rats led to a decrease in heart rate and a decrease in coronary flow⁵.

α_2 -adrenergic receptors affect the contraction force of myocardiocytes when this receptor interacts with the inhibitory Gi protein. This interaction leads to a decrease in the activity of the adenylate cyclase cascade and causes a decrease in the stimulation of cAMP-dependent protein kinase. α_2 -adrenergic receptor agonists also promote NO release in the coronary arteries and an increase in coronary blood flow through the mechanisms of endogenous and exogenous adenosine in an *in vivo* model⁹.

The evidence indicate shifts in adrenergic, cholinergic regulation of heart activity under hypokinesia^{10,11}. *In vitro* experiments have shown that stimulation of β -adrenergic receptors with isoproterenol in hypokinesized rats can have multidirectional effects (a decrease in the contractility of myocardial strips at low doses and an increase at a concentration of 10^{-6} M). This action can be explained by stimulation of different subtypes of β -adrenergic receptors, the quantitative ratio of which is possible changes during hypokinesia¹¹. In hypokinesia, chronotropic effects on the heart are weakened; the activity of acetylcholinesterase and monoamine oxidase decreases, the efficiency of stimulation of α -adrenergic receptors of the myocardium is significantly reduced¹⁰.

This experimental work aimed to study the effects of α_2 -adrenergic receptors stimulation with clonidine, at various concentrations, on heart rate (HR), the pressure developed by the left ventricle (LVP), and coronary flow (CF) of the Langendorff-isolated heart of rat pups under limited muscle activity.

Methods

White outbred laboratory rats were studied under limited motor activity for 30 days (hypokinesia) in the age range from 3 to 7 weeks. To simulate the hypokinetic effect, the rats were kept in individual organic glass cages for 30 days. A method was used that creates conditions for the formation of hypokinesia developed by Abzalov¹². For the first two days, the animals were subjected to hypokinesia for 1 hour, on days 2 and 3 – for two hours, and also every 2 days the time of restriction of physical activity increased by 2 hours¹². The peculiarities of this model of hypokinesia (in a horizontal position) is a decrease in the influence of the stress factor, as a result of a gradual increase in the time spent by experimental animals under limited motor activity, and the likelihood of studying animals without additional changes in the hydrostatic blood pressure. In anesthetized rats (urethane intraperitoneally at a concentration of 800 mg/kg), the thorax was opened, the heart was removed and placed in a cold working Krebs-Henseleit solution (pH=7.4). The heart was fixed on a Langendorff cannula and perfused with a working solution. The solution was saturated with oxygen and the temperature was maintained at 37°C. Intraventricular pressure was recorded using a latex balloon, which was introduced into the left ventricular cavity.

The percentage of changes in the functional indicators of the activity of an isolated heart (HR, LVP, CF) on the effect of a pharmacological agent from the baseline was assessed.

Ex vivo experiments were carried out on an isolated heart using a Power Lab 8/35 device (AD Instruments); data were recorded using the LabChart Pro software. Statistical processing was carried out in Excel. The significance of the data was determined using the Student's t-test. The data were considered statistically significant at $p < 0.05$.

Results

After adding clonidine to the perfusion solution, at a concentration of 10^{-9} M, we observe an increase in LVP from 42.34 ± 5.6 mmHg to 50.75 ± 4.3 mmHg ($p < 0.01$), which is 19.8% of the baseline (Figure 1). HR in hypokinesized rats was 142.9 ± 5.6 bpm. After adding the agonist, the maximum decrease in heart rate was observed by the 20th minute of the experiment, heart rate decreased to 90.25 ± 9.5 bpm ($p < 0.01$). Thus, after adding clonidine at a concentration of 10^{-9} M, the heart rate in hypokinesized rat pups decreases on average by 24% from the baseline (Figure 2). The initial CF of hypokinesized rats was 5.07 ± 0.8 ml/min. By the 20th minute after adding clonidine at a concentration of 10^{-9} M, a gradual decrease in this value by 25% was observed, that is, to 3.8 ± 0.7 ml/min ($p < 0.05$) (Figure 3).

Stimulation of α_2 -adrenergic receptors with clonidine at a concentration of 10^{-8} M caused an increase in LVP in hypokinesized rats from 35.9 ± 4.5 mmHg. up to 40.7 ± 6.13 mmHg ($p < 0.05$), which was 13% from the baseline (Figure 1). The heart rate of hypokinesized rats by the 20th minute after adding clonidine decreased from 173.4 ± 18.12 bpm to 118.6 ± 11.2 bpm ($p < 0.01$), a decrease in heart rate by 32% from the initial value was observed (Figure 2). CF after adding clonidine by the end of the experiment decreased to 5.56 ± 0.6 ml/min ($p < 0.01$) from the initial 6.88 ± 0.8 ml/min, which was 19.2% (Figure 3)

Clonidine at a concentration of 10^{-7} M in an isolated heart had the opposite effect on LVP compared to *from* 10^{-8} M *and* 10^{-9} M and reduced the force of contraction. A decrease in LVP by 30% (Figure 1) from 45.67 ± 1.8 mmHg up to 31.99 ± 3.95 mmHg ($p < 0.01$) was observed. After perfusion of an isolated heart of hypokinesized rats with an agonist (10^{-7} 10^{-7} M), a decrease in heart rate was recorded on average by 40% from the initial value, from 172.05 ± 17.9 bpm to 103.64 ± 20.4 bpm, respectively ($p < 0.01$) (Figure 2). We also observed a decrease in the CF of an isolated heart by an average of 42% from the initial value (Figure 3).

Clonidine at a concentration of 10^{-6} M led to an increase in LVP in an isolated heart of hypokinesized rats by 16% (Figure 1); changes in values were observed from 38.68 ± 3.09 mm Hg to 44.9 ± 3.5 mm Hg ($p < 0.05$). An average increase of 16% was observed (Figures 1 and 4). The chronotropic reaction of the isolated rat heart with limited motor activity in response to the action of clonidine at a concentration of 10^{-6} M was negative, as there was a decrease in heart rate from 204.7 ± 17.9 bpm to 158.3 ± 24.6 bpm ($p < 0.01$), the decrease was 22.7% (Figures 2 and 4). After the administration of a α_2 -adrenergic receptor agonist, CF in the isolated heart of hypokinesized

rats decreased to 5.45 ± 0.6 ml/min from the initial value by the 20th minute (from 6.29 ± 0.4 ml/min) ($p < 0.01$), and a decrease in CF by 13.3% was observed (Figures 3 and 4).

Figure 1. Average values of the dose-dependent effect of clonidine hydrochloride on LVP of an isolated heart of hypokinesized rats. LVP – left ventricle pressure, * $p < 0.05$, ** $p < 0.01$.

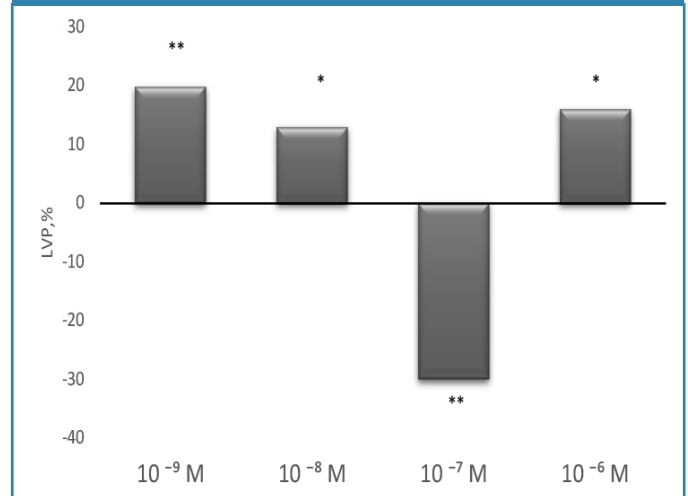


Figure 2. Average values of the dose-dependent effect of clonidine hydrochloride on HR of an isolated heart of hypokinesized rats. HR – heart rate, ** $p < 0.01$.

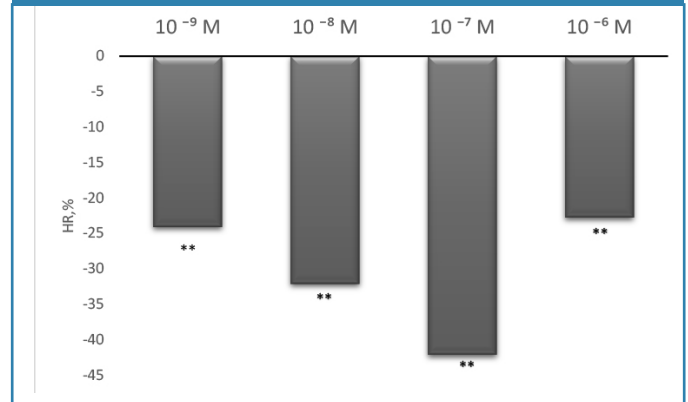


Figure 3. Average values of the dose-dependent effect of clonidine hydrochloride on CF of an isolated heart of hypokinesized rats. CF – coronary flow, * $p < 0.05$, ** $p < 0.01$.

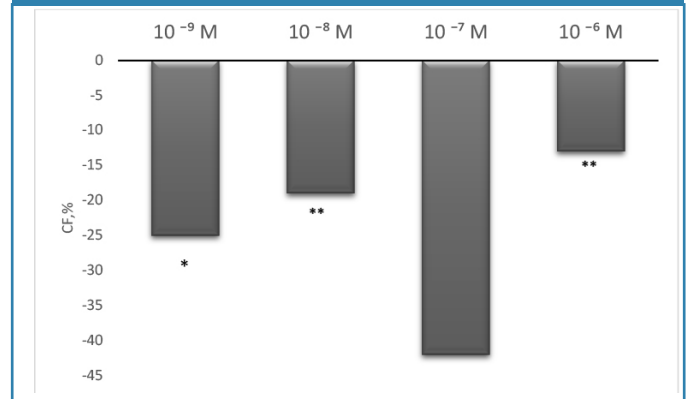
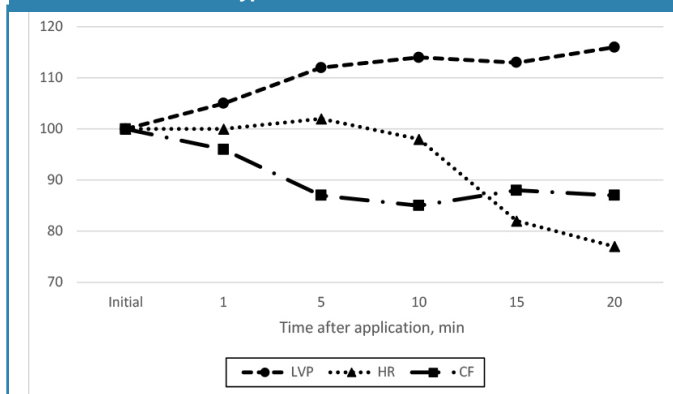


Figure 4. Changes of the dose-dependent effect of clonidine hydrochloride at a concentration of 10^{-6} M on LVP, HR, and CF of an isolated heart of hypokinesized rats.



Our data indicate that the α_2 -adrenergic receptor agonist, clonidine, at a concentration of 10^{-9} M, 10^{-8} M, 10^{-6} M caused an increase in the force of contraction in an isolated heart of rats under limited motor activity. Clonidine at a concentration of 10^{-7} M reduced LVP in an isolated heart. Clonidine hydrochloride in all concentrations led to a significant decrease in the heart rate and coronary flow of an isolated heart in hypokinesized rat pups.

Stimulation of α_2 -adrenergic receptors with clonidine is known to cause negative chronotropic and inotropic effects³. Our results are consistent with the data on the effect of clonidine on heart rate since a negative chronotropic effect was observed at all concentrations of the substance. Perhaps this is due to the adaptive nature of the hypokinetic period, that is, there is a need to increase this parameter to ensure effective heart activity¹³. It is also possible that the effect of stimulating α_2 -adrenergic receptors in hypokinesia depends on the expressed subtype of these adrenergic receptors^{11,13}.

Clonidine hydrochloride at all concentrations caused a decrease in coronary flow, which indicates coronary constrictive properties associated with the excitation of α_2 -adrenergic receptors. Our data are consistent with the experimental data obtained on 6-week-old rats, where a decrease in CF was observed after the addition of an agonist⁵. The hypokinetic period was revealed to contribute to disturbances in the tone of the heart vessels. The factor that changes the vascular tone is the stress response of the body, in which the release of nitric oxide (NO) is observed. This is confirmed by studies in which the amount of NO in the heart tissues doubles during prolonged hypokinesia^{14,15}. However, although NO causes vasodilation, we observe a decrease in coronary flow. This is possibly due to the presence of two types of α_2 -adrenergic receptors in the coronary vessels - smooth muscle and endothelial. Where the excitation of the former produces vasodilation, and endothelial dependent vasoconstriction and the excitation of the former predominates over the latter type^{16,17}.

Having analyzed our data, we can assume the possible cardioprotective function of α_2 -adrenergic receptors. In this regard, it should be noted that the role of α_2 -adrenergic receptors in the cardiovascular system is still underestimated clinically and requires further research.

Acknowledgments

This study was prepared in accordance with the Russian state program of competitive growth of Kazan Federal University and supported by the RFBR and Government of the Republic of № 18-44-160022.

References

- Kikugawa, K., Kojima, T., Yamaki, S., Kosugi, H. (1992). Interaction of the thiobarbituric acid recovery of rat liver and brain homogenates in the presence of ferric ion and ethylenediaminetetraacetic acid. *Anal. Biochem*, 202(2):249-255.
- Sinclair, M. (2003). A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. *Can. Vet J.*, 44(11):885-897.
- Zefirov, T. L., Khisamieva, L. I., Ziyatdinova, N. I., & Zefirov, A. L. (2015). Peculiar Effects of Selective Blockade of α_2 -Adrenoceptor Subtypes on Cardiac Chronotropy in Newborn Rats. *Bulletin of experimental biology and medicine*, 160(1), 6-8.
- Westby, J. O. E. R. G. E. N., Birkeland, S. I. G. U. R. D., Rynning, S. E., Myking, O. L., Lekven, J., & Grong, K. E. T. I. L. (1992). Alpha-adrenergic vasoconstriction in normal and hypoperfused myocardium during sympathetic nerve stimulation. *American Journal of Physiology-Heart and Circulatory Physiology*, 263(6), H1682-H1688.
- Kuptsova, A. M., Ziyatdinova, N. I., & Zefirov, T. L. (2018). Dose-dependent influence of clonidine hydrochloride to the 6 week old rats isolated heart activity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 9(6), 479-485.
- Kuptsova, A. M., Zaripova, R. I., Hisamieva, L. I., Biktemirova, R. G., Ziyatdinova, N. I., & Zefirov, T. L. (2016). Yohimbine influence on myocardium contractile activity among newborn rats. *International journal of advanced biotechnology and research*, 7(4), 1305-1309.
- Boblewski, K., Lehmann, A., Sączewski, F., Kornicka, A., & Rybczyńska, A. (2014). Vagotomy reveals the importance of the imidazoline receptors in the cardiovascular effects of marsanidine and 7-ME-marsanidine in rats. *Pharmacological Reports*, 66(5), 874-879.
- Zefirov, T. L., Ziyatdinova, N. I., & Khisamieva, L. I. & Zefirov, A.L. (2011). Effect of α_2 -Adrenoceptor Stimulation on Cardiac Activity in Rats», *Bull. Exp. Biol. Med.*, 151(6): pp. 664-666.
- Mizobe, T., Maze, M. (1995). α_2 -Adrenoceptor agonists and anesthesia. *International Anesthesiology Clinics.*, 33(1):81-102.
- Chinkin, A.S. (1995). Motor activity and heart. Kazan: Publishing house of KSU, p. 192.
- Zaripova, R. I., Ziyatdinova, N. I., & Zefirov, T. L. (2016). Effect of NO Synthase Blockade on Myocardial Contractility of Hypokinetic Rats during Stimulation of [beta]-Adrenoreceptors. *Bulletin of experimental biology and medicine*, 161(2), 215-217.
- Abzalov, R.A. (1985). Movement and the developing heart. Textbook. - M.: V.I. Lenin Moscow State Pedagogical Institute, P. 90.
- Nuzhny, V. P., Kibler, N. A., Tsvetkova, A. S., Shmakov, D. N., Nuzhny, P. V., & Panyko, S. V. (2011). The combined analysis of the interaction of electrocardiostimulating system and the heart. *Izv. Komi Nauch. Tsentra Ural. Otdel. Ross. Akad. Nauk*, (6), 45-52.
- Zaripova, R. I., Andrianov, V. V., Iafarova, G. G., Gañnutdinov, K.,

Khabibrakhmanov, I. I., & Zefirov, T. L. (2014). Influence of hypokinezia of varying duration on the nitric oxide productions dynamics in rat's heart, spinal cord and liver. *Rossiiskii fiziologicheskii zhurnal imeni IM Sechenova*, 100(8), 926-935.

15. Kozlovskii, V. I., Zinchuk, V. V., Khlopitskii, S. (2010). Investigation of the role of NO in the effects of agonists of various subtypes of adrenergic receptors in coronary vessels. *Experimental and Clinical Pharmacology*, 73(2):10-13.
16. Turabian, J. L. (2019). Psychosocial aspects of drug prescription: recognizing these phenomena to improve the quality of clinical practice. *Archives of Pharmacology and Therapeutics*, 1(2).
17. Lamelas, P., Diaz, R., Orlandini, A., Avezum, A., Oliveira, G., Mattos, A., ... & Otero, J. (2019). Prevalence, awareness, treatment and control of hypertension in rural and urban communities in Latin American countries. *Journal of hypertension*, 37(9), 1813-1821.



www.revhipertension.com
www.revdiabetes.com
www.revsindrome.com
www.revistaavft.com

Manuel Velasco (Venezuela) **Editor en Jefe** - Felipe Alberto Espino Comercialización y Producción

Reg Registrada en los siguientes índices y bases de datos:

SCOPUS, EMBASE, Compendex, GEOBASE, EMBiology, Elsevier BIOBASE, FLUIDEX, World Textiles,

OPEN JOURNAL SYSTEMS (OJS), REDALYC (Red de Revistas Científicas de América Latina, el Caribe, España y Portugal),

Google Scholar, ZERONO, OPENAIR

LATINDEX (Sistema Regional de Información en Línea para Revistas Científicas de América Latina, el Caribe, España y Portugal)

LIVECS (Literatura Venezolana para la Ciencias de la Salud), LILACS (Literatura Latinoamericana y del Caribe en Ciencias de la Salud)

PERIÓDICA (Índices de Revistas Latinoamericanas en Ciencias), REVENCYT (Índice y Biblioteca Electrónica de Revistas Venezolanas de Ciencias y Tecnología)

SABER UCV, DRJI (Directory of Research Journal Indexing)

CLaCALIA (Conocimiento Latinoamericano y Caribeño de Libre Acceso), EBSCO Publishing, PROQUEST



Esta Revista se publica bajo el auspicio del Consejo de Desarrollo Científico y Humanístico Universidad Central de Venezuela.



cdch-ucv.net

publicaciones@cdch-ucv.net

www.revistahipertension.com.ve
www.revistadiabetes.com.ve
www.revistasindrome.com.ve
www.revistaavft.com.ve