Research Results in Pharmacology

Research Results in Pharmacology 9(4): 31–41
UDC: 615.212.7
DOI 10.18413/rrpharmacology.9.10054

Research Article

The influence of the vagus nerve and indole derivative SS-68 on excitation processes in the SA node

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Academic editor: Oleg Gudyrev ♦ **Received** 22 July 2023 ♦ **Accepted** 07 October 2023 ♦ **Published** 11 November 2023

Citation: Nechepurenko AA, Galenko-Yaroshevsky PA, Pokrovskiy VM, Zelenskaya AV, Suzdalev KF, Lebedeva SA, Makhnova NM, Maksemyuk AV, Minenko IA, Nikitin RV, Abushkevich VG (2023) The influence of the vagus nerve and indole derivative SS-68 on excitation processes in the SA node 9(4): 31–41. https://doi.org/10.18413/rrpharmacology.9.10054

Abstract

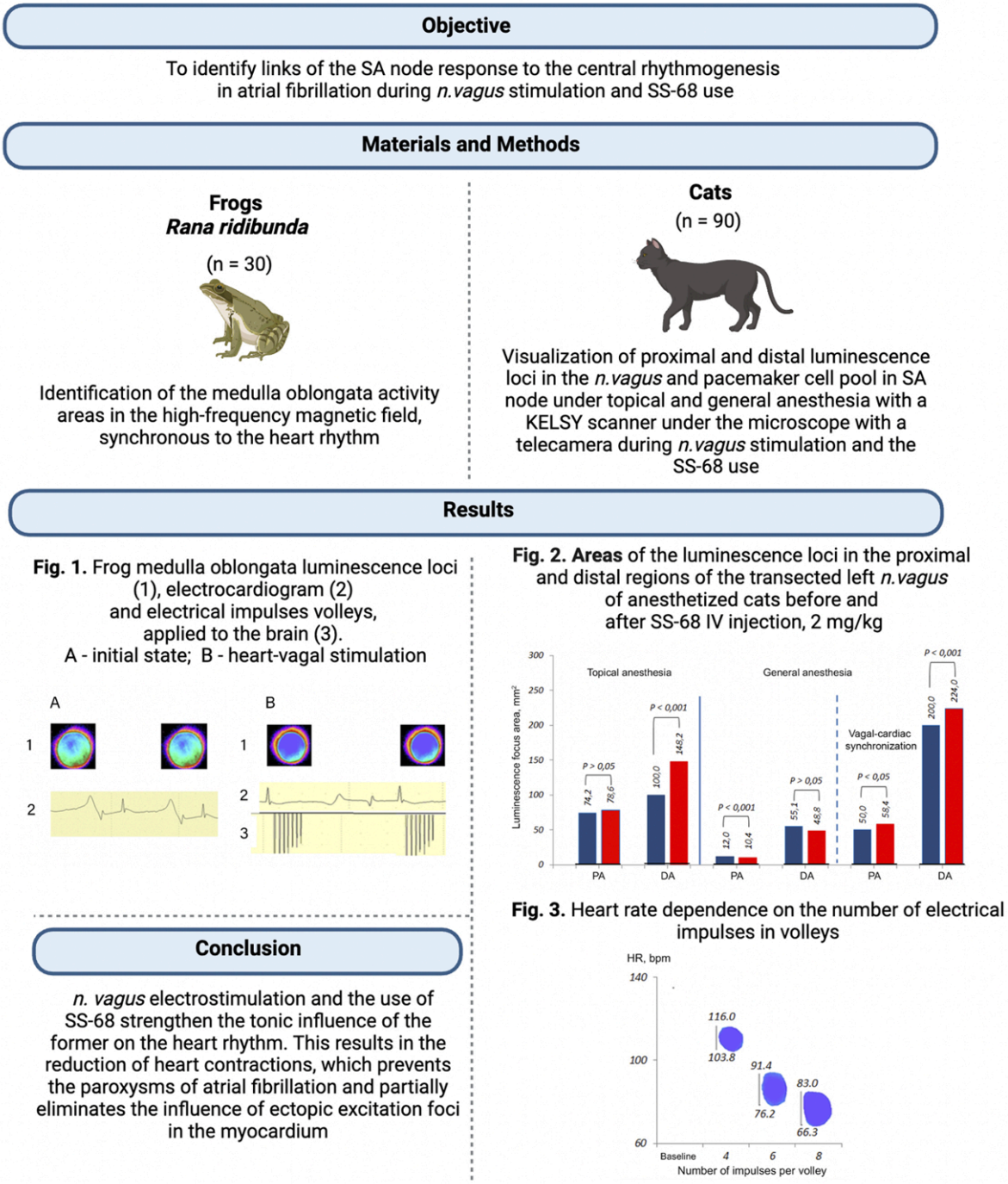
Introduction: Atrial fibrillation (AF) is the most common form of cardiac arrhythmias. Studying the pathogenesis of this pathological process will make it possible to look for new methods of treating AF and to predict its occurrence in a more targeted way. **The aim of the study** was to identify the components of the takeover process of central rhythmogenesis by the SA node in the conditions of atrial fibrillation when stimulating the vagus nerve and using substance SS-68.

Materials and Methods: The experiments were conducted on 30 frogs and 90 cats. In frogs, the activity of the regions of the medulla oblongata synchronous with the heart rhythm was determined in a high-frequency electromagnetic field. In cats, proximal and distal foci of luminescence in the vagus nerve (VN) and pools of pacemaker cells (PCs) in the sinoatrial node were visualized under topical and general anesthesia, using a KELSY scanner with a microscope video capture unit while stimulating VN and using SS-68.

Results and Discussion: The stimulation of VN with volleys of electrical impulses and the introduction of SS-68 increase the foci of luminescence in the nerve and unite the PC pools. This way, under general anesthesia in comparison with topical anesthesia, the area of the proximal focus of VN luminescence decreased by 83.8%, and the distal focus – by 44.9%. Against the background of general anesthesia, the area of the proximal focus of luminescence when stimulating VN with volleys of electrical impulses was by 76.0% larger than before stimulation, and the distal focus – by 72.5%. After the administration of SS-68, there was an increase in the foci of luminescence: under general anesthesia, when compared with topical anesthesia, the area of the proximal foci of luminescence decreased by 86.8%, and the distal one – by 67.1%. Under general anesthesia, the area of the proximal focus of luminescence under conditions of stimulating VN with volleys of electrical impulses was by 82.2% larger than before stimulation and the distal one – by 78.2%. When signals from the brain arrive simultaneously through VN at the PC pools, they are absorbed by the PC pools; the focus of early depolarization becomes wide, which prevents the development of AF. The increased synchronizing influence of VN may be one of the methods for treating autonomic AF, and if its influence decreases, it can be a prognostic factor for the occurrence of recurrent AF.

Conclusion: The tonic effect of VN on the heart rhythm through electrical stimulation of the former and the use of SS-68 is manifested in a decreased heart rate: the difference between the initial heart rhythm and the minimal synchronization range boundary. A decrease in the heart rate under the influence of VN prevents paroxysms of AF, but does not completely eliminate the influence of ectopic foci on it.

Graphical Abstract



Keywords

substance SS-68, pacemaker cells, sinoatrial node, brain rhythm

Introduction

Heart rhythm disturbances, including atrial fibrillation (AF), can develop under the influence of the nervous system (Brotman et al. 2007; Ardell 2011; Filatov and Tarashvili 2012; Legallois et al. 2013; Svensson et al. 2017; Garg et al. 2019; Galenko-Yaroshevsky et al. 2023). A major role in this process is played by the autonomic nervous system (Chen and Tan 2007; Lorincz et al. 2008; Chernyavsky et al. 2013; Chen et al. 2014; Linz et al. 2014; Yaniv et al. 2014, 2014a; Karemaker 2015; Mironova et al. 2018).

Earlier, when computer mapping the depolarization wave in the sinoatrial region in patients with paroxysmal AF using the CARTO-3 system, with non-acute AF, in sinus rhythm, outside anesthesia, using a Lasso multi-electrode probe, a wide focus of initial excitation was identified. Under anesthesia, the focus decreased (Pokrovsky et al. 2018).

A relationship was established between the area of the focus of initial excitation and the duration of the disease. The shorter the duration of the disease, the larger was the focus of the initial excitation (Nechepurenko and Romantsov 2020).

In experiments on animals, when visualizing the focus of initial excitation in a high-frequency electromagnetic field, it was shown that the area of the focus of initial excitation was determined by combining pacemaker cell (PC) pools of the sinoatrial node (SAN) (Pokrovsky et al. 2019).

As generally believed, the heart rhythm is initiated by autogenous structures of the SA node. Indisputable evidence for this is the intrinsic rhythmical excitation of the heart (Baruscotti and Robinson 2007; Abramochkin et al. 2009; Bradd 2012; Grigoriev and Babich 2015; Murphy and Lazzara 2016; van Weerd and Christoffels 2016; Filyukova 2018). However, from the standpoint of the traditional theory (intercellular interaction due to nexuses and electrotonic influence), it is unlikely to give reasons for combining PC pools (Mazurov 2006, 2009).

At the same time, this can be explained from the perspective of the concept by V.M. Pokrovsky about the hierarchical system of rhythmogenesis, according to which, the heart rhythm in a whole body under natural conditions originates in the brain in the form of volleys of nerve impulses arriving along the vagus nerves (VN) to the SAN, and, when interacting with the autogenous structures of the SAN, the heart rhythm is formed (Pokrovsky 2007).

Taking into account the above concept, it can be assumed that the simultaneous arrival of trigger signals along efferent cardiac parasympathetic fibers from the brain to the heart leads to synchronous excitation of PC pools.

Based on the above, it was of interest to identify the components of the takeover process of central rhythmogenesis by the SA node in the conditions of atrial fibrillation by stimulating the vagus nerve and parenteral administration of indole derivative SS-68, which has a pronounced antiarrhythmic effect, as well as a number of positive pleiotropic properties – anticonvulsant, central analgesic, antianginal, antiplatelet and anti-inflammatory (Galenko-Yaroshevsky et al. 2023).

The aim of the study was to identify the components of the takeober process of central rhythmogenesis by the SAN in the conditions of atrial fibrillation when stimulating the vagus nerve and using substance SS-68.

Materials and Methods

Experimental animals

The experiments were conducted on 30 frogs *Rana ridibunda* weighing 180-200 g and 90 mongrel cats (*Domestic cat*) weighing 2.3-3.5 kg. The study protocol was approved by the independent ethical committee of Kuban State Medical University of the Ministry of Health of the Russian Federation (4 Mitrofan Sedin St., Krasnodar, Russia), Minutes No. 102 dated 15 October 2021. The animal care and handling complied with the principles of the Declaration of Helsinki on the humane treatment of animals, Directive of the European Parliament and the Council of the European Union 2010/63/EU dated 22 September 2010 on the protection of animals used for scientific purposes, GOST 33044–2014 "Principles of Proper Laboratory Practice", approved by Order of the Federal Agency for Technical Regulation and Metrology No. 1700-st dated 20 November 2014.

Pharmaceutical substances

The substance under study was the following: indole derivative № 63 [2-phenyl-1-(3-pyrrolidin-1-ylpropyl)-1H-indole hydrochloride; laboratory code SS-68; synthesized at Southern Federal University, Rostov-on-Don]. Sodium thiopental was used to anesthetize the cats.

Experimental design

The experiments were conducted on three groups of animals: the first group included frogs and the other two groups included cats, with the second group of cats consisting of two subgroups of animals.

In the experiments on frogs, luminescence foci in the medulla oblongata were studied using a KELSY scanner (ELSYS Corp., St. Petersburg, Russia) and an electronic level control unit ESU-1 (Promprylad, Moscow, Russia).

In the second part of experiments on cats, in the first subgroup of animals, luminescence foci were studied without the introduction of SS-68 under superficial and general sodium thiopental-induced anesthesia, followed by transection of VN and the use of a KELSY scanner. At the same time, under general anesthesia, luminescence foci were recorded against the background of vagal-cardiac synchronization. In the second subgroup of cats, luminescent foci were examined in the same way as in the first subgroup of animals, but against the background of the administration of SS-68.

In the third part of experiments on cats, the studies were conducted on SA nodes under general sodium-thiopental-induced anesthesia, with transecting VN, giving a tracheotomy (for artificial lung ventilation), connecting the KELSY scanner with subsequent registration of the electrocardiogram (ECG) and luminescence foci in the SAN at the initial heart rate and vagal-cardiac synchronization caused by stimulation of VN with volleys of 4, 6 and 8 electrical impulses (Figure 1).

Research methods

The first part of experiments was conducted on 30 frogs, which were immobilized by destroying the spinal cord. An ECG was recorded (lead I) on an electromyograph

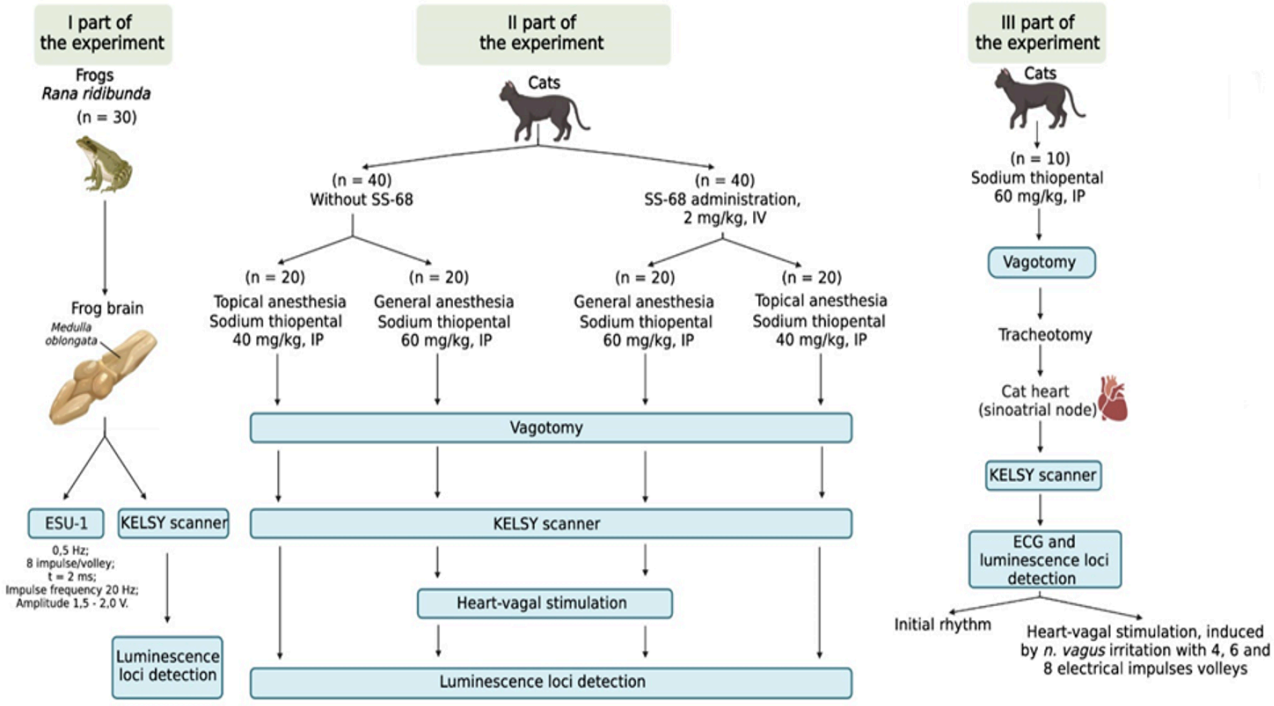


Figure 1. Study design.

Neuro-MVP-4 (Neurosoft, Ivanovo, Russia). The skull was opened. Above the medulla oblongata, a KELSY scanner, a gas discharge visualization device based on Kirlian's effect with a microscope and a video capture unit (ELSYS Corp., St. Petersburg, Russia), was placed, creating a high-frequency electromagnetic field (1024 Hz). Using the scanner with a highly sensitive video capture device, a video was shot through a microscope (25 frames per second), during which the area of luminescence in the medulla oblongata region linked to the heart rhythm was recorded. The medulla oblongata was stimulated with volleys of electrical impulses from the ESU-1 stimulator (Promprylad, Moscow, Russia) through an isolating unit with a frequency 30% lower than the initial rhythm (on average 0.5 Hz; 8 impulses per volley, pulse duration 2 ms, pulse frequency 20 Hz, amplitude 1.5-2.0 V).

The second part of experiments was performed on 80 cats. The animals were randomized into 2 groups of 40 animals each: the cats of the 1st group were studied without administration of SS-68, whereas the cats of the 2nd group were studied five minutes after intravenous (i.v.) administration of SS-68 at a dose of 2 mg/kg. Both the 1st and 2nd groups of animals were further divided into two subgroups of 20 animals each, which were put under topical (by injecting sodium thiopental 40 mg/kg intraperitoneally – i.p.) and general (by injecting sodium thiopental 60 mg/kg i.p.) anesthesia. In the animals of all four subgroups, under sterile conditions, the left VN was isolated from the neurovascular bundle on the neck and, 4 cm from the lower edge of the thyroid cartilage, it was cut off. The central end of the cut nerve was placed into a glass tube with a diameter commensurate with the diameter of the nerve and positioned vertically to the scanner. The KELSY scanner was placed opposite the end of the cut nerve. The excitation process in the transverse plane of the nerve was recorded in the form of spreading luminescence foci, synchronous with the heart rhythm. Against the background of general anesthesia, both with and without injecting SS-68, vagal-cardiac synchronization was induced by stimulating the central VN end with volleys of electrical impulses above the site of the VN transection, while registering an ECG (lead II) with an electromyograph Neuro-MVP-4 and VN foci of luminescence.

The third part of experiments was conducted on 10 anesthetized (sodium thiopental at a dose of 60 mg/kg i.p.) cats. The VN was dissected free in the neck, placed on ligatures and transected. The animals underwent tracheotomy and were maintained under artificial respiration. A transsternal incision was made to open the chest and then the pericardium. On the sinoatrial region of the beating heart, there was placed a device to visualize luminescence of the excitation process in the SAN in a high-frequency electromagnetic field (KELSY scanner) with a microscope and a video capture unit. ECG and luminescence foci were registered at the initial rhythm and under conditions of vagal cardiac synchronization caused by stimulation of the peripheral end of the cut VN with volleys of 4, 6 and 8 electrical impulses from the electrical stimulator ESU-1.

Upon completion of the experiments, the animals were euthanized by administering a lethal dose of sodium thiopental. The animal corpses were then disposed of at the vivarium.

Statistical analysis

Statistical processing was carried out using STATISTICA 10 software. The normality of the distribution was determined, which made it possible to use parametric methods of statistical processing. Based on the variants of the obtained results, the arithmetic mean M and the error

of the arithmetic mean ±m were calculated. The significance value p was determined by the Student's t-test value. When comparing the average values, the difference between them was considered significant at $p<0.05$. The Pearson correlation coefficient was determined.

Results and Discussion

In the first part of experiments, at the initial rhythm in the medulla oblongata of the frog, a focus of luminescence was visualized, synchronous with the heart rhythm, which preceded the V wave (excitation of the venous sinus of the frog's heart) on the ECG (Fig. 2).

When stimulating the medulla oblongata with volleys of electrical impulses, vagal-cardiac synchronization occurred – for each volley, the heart contracted once. Changing the volley frequency within a certain range caused a synchronous change in the heart rate (HR).

The area of the visualized luminescence zone at vagal-cardiac synchronization was 60.0% greater than that at the initial rhythm (Fig. 2).

In the second part of experiments on cats, in a high-frequency electromagnetic field at the central end of the cut VN in the transverse plane, the excitation process linked to the ECG cycle showed against the background of neuronal "noise" as some small and two large foci of luminescence, which are indicated as proximal and distal heartward (Fig. 3).

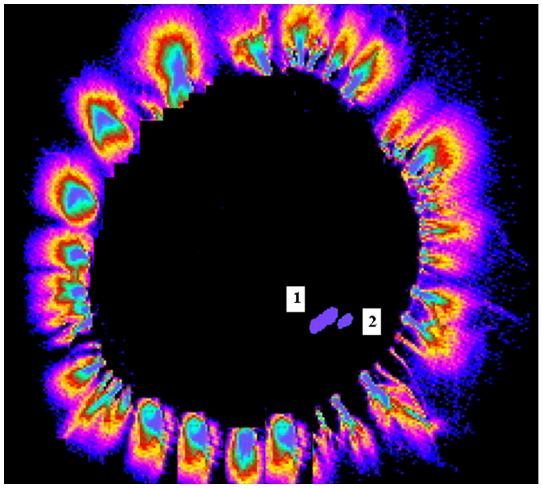


Figure 3. Distal (**1**) and proximal (**2**) foci of luminescence at the central end of the cat's VN under topical anesthesia.

Foci of luminescence were localized deep in the nerve in one sector. They were located nearby, but not on the same axis, which means that the excitation process moves from the brain to the heart along different, but adjacent bundles of nerve fibers.

Under general anesthesia, when compared to topical anesthesia, the area of the proximal luminescence focus decreased by 83.8%, and the area of the distal focus – by 44.9% (Table 1, Fig. 4).

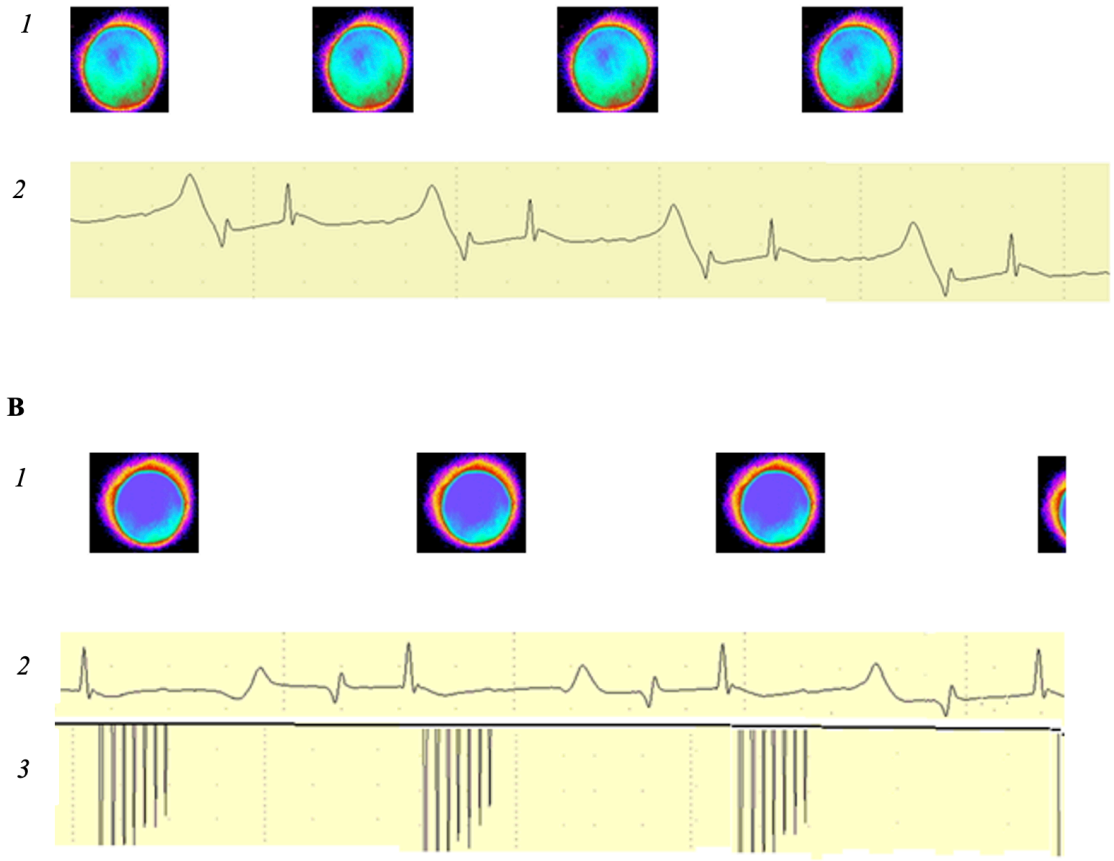


Figure 2. Luminescence foci in the medulla oblongata of a frog (**1**), ECG (**2**) and volleys of electrical impulses applied to the brain (**3**). ***Note:*** **A** – initial state; **B** – vagal-cardiac synchronization.

Under general anesthesia, the area of the proximal focus of luminescence with vagal-cardiac synchronization was by 76.0% larger than without it, and the area of the distal focus – by 72.5% (Table 1).

After intravenous administration of SS-68 at a dose of 2 mg/kg, there was an increase in the foci of luminescence (Fig. 5). Under general anesthesia, when compared to topical anesthesia, the area of the proximal

Table 1. Parameters of luminescent foci in the proximal and distal areas of the cut left VN of anesthetized cats before and after intravenous administration of SS-68 at a dose of 2 mg/kg (M±m, n=20)

Parameters	Baseline data	Proximal area	Distal area
Cross-sectional area of the nerve on the scanogram, mm²	8656.6±12.8		
Without administration of SS-68			
Topical anesthesia			
Focus area on the scanogram, mm² [1]	3.0±0.1	74.2±1.9	100.0±2.3
General anesthesia			
Focus area on the scanogram, mm² [2]	3.2±0.1	12.0±0.2 p_{1-2}<0.001	55.1±3.2 p_{1-2}<0.001
With vagal-cardiac synchronization			
Focus area on the scanogram, mm² [3]	3.3±0.1	50.0±2.1 p_{2-3}<0.001	200.0±3.8 p_{2-3}<0.001
After administration of SS-68			
Topical anesthesia			
Focus area on the scanogram, mm² [4]	3.1±0.1	78.6±1.8 p_{1-4}>0.05	148.2±2.5 p_{1-4}<0.001
General anesthesia			
Focus area on the scanogram, mm² [5]	3.3±0.1	10.4±0.3 p_{4-5}<0.001 p_{2-5}<0.001	48.8±2.6 p_{4-5}<0.001 p_{2-5}>0.05
With vagal-cardiac synchronization			
Focus area on the scanogram, mm² [6]	3.2±0.1	58.4±2.0 p_{5-6}<0.001 p_{3-6}<0.05	224.0±2.9 p_{5-6}<0.001 p_{3-6}<0.001

Note: In square brackets are the numbers of indicators of VN luminescence foci.

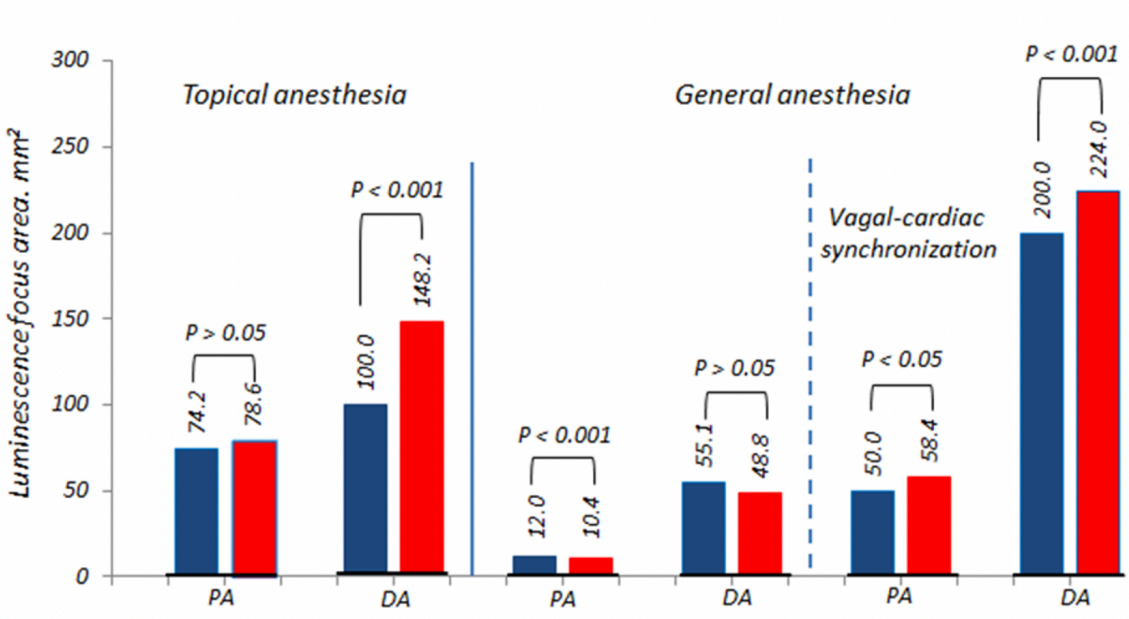


Figure 4. Areas of luminescence foci in the proximal and distal areas of the cut left VN of anesthetized cats before and after intravenous administration of SS-68 at a dose of 2 mg/kg. ***Note*:** PA – proximal area of VN, DA – distal area of VN.

luminescence focus decreased by 86.8%, and the distal one – by 67.1%. Under general anesthesia, the area of the proximal focus of luminescence with vagal-cardiac synchronization was by 82.2% larger than without it, and the area of the distal focus – by 78.2% (Table 1).

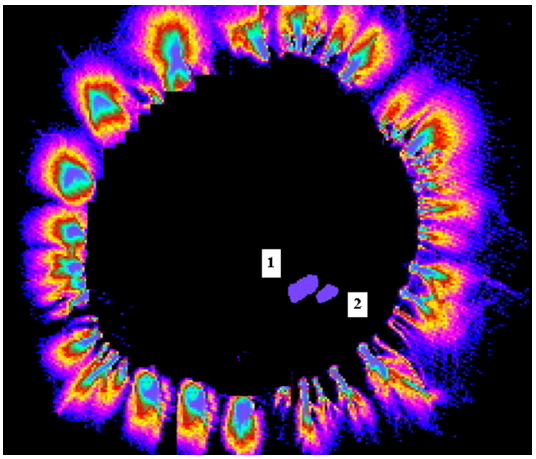


Figure 5. Distal (**1**) and proximal (**2**) foci of luminescence in the central end of the VN of cat under topical anesthesia after administration of 2 mg/kg of SS-68.

When comparing the studied indicators of the areas of luminescence foci both separately and under conditions of vagal-cardiac synchronization without the administration of SS-68 and after injecting it, under topical and general anesthesia, it turned out that, under topical anesthesia, the areas of luminescence foci of the proximal sections of the VN without the administration of SS-68 68 and after injecting it were practically comparable; a similar situation was observed under general anesthesia in the distal areas of the VN; and in other cases, the indicators of the areas of luminescence foci are statistically more significant with the administration of SS-68 than without it (Table 1).

The presented data confirm the previously obtained results concerning the ability of SS-68 to stop cardiac arrhythmias of central origin induced by the introduction of aconitine, strophanthin and cesium chloride into the fourth ventricle of the cat's brain (Galenko-Yaroshevsky et al. 2023).

Since at the central end in the cross-sectional plane of the VN after intravenous administration of SS-68, there increased the visualized focus of luminescence towards the SAN, reflecting the triggering influence of the central nervous system, it emanated from the brain and was a marker of a nerve impulse volley. This assumption can be made from the literature data on the membranotropic activity of SS-68 on mollusk neurons (Vislobokov et al. 2012).

It can be assumed that under the influence of SS-68, conformational changes occur in the membrane receptors of neurotransmitters coupled with ion channels and their transition to the conformation of an open channel, which promotes ion currents. The mechanism for exerting the central effect of SS-68 is also possible due to the emergence of new options for binding endogenous ligands to the most optimal subtypes of receptors and for improving the coupling of the latter and G-proteins, which affects the functional activity of receptors that change the system of second intracellular messengers.

So, SS-68, which has a pronounced antiarrhythmic activity, exhibits high selectivity for central neurochemical mechanisms which may be involved in the regulation of the heart rate (Galenko-Yaroshevsky et al. 2023).

In the third part of experiments, in the cats with the cut VN, the initial heart rate was arrhythmic (Fig. 6).

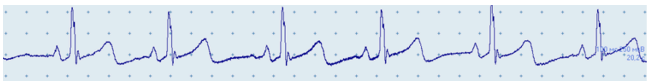


Figure 6. ECG of an anesthetized cat with the cut VN.

Luminescence of the SAN cell pools indicated a diffuse arrangement of the pools (Fig. 7).

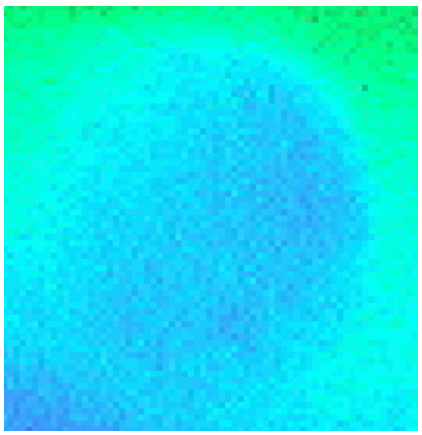


Figure 7. Luminescence of cell pools in the SAN of an anesthetized cat with the cut VN.

When stimulating the peripheral end of the cut VN with volleys of 4, 6 and 8 electrical impulses, there was observed vagal-cardiac synchronization (Figs 8-11).

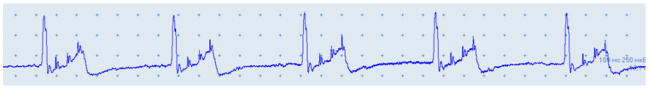


Figure 8. ECG of an anesthetized cat in the conditions of vagal-cardiac synchronization at 4-impulse volleys of electrical stimulation of the VN.

In the conditions of vagal-cardiac synchronization, the majority of SAN cell pools merged into a single monolithic focus.

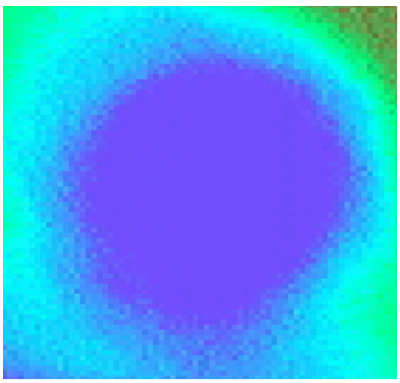


Figure 9. Luminescence of cell pools in the SAN in the conditions of vagal-cardiac synchronization at 4-impulse volleys of electrical stimulation of the VN.

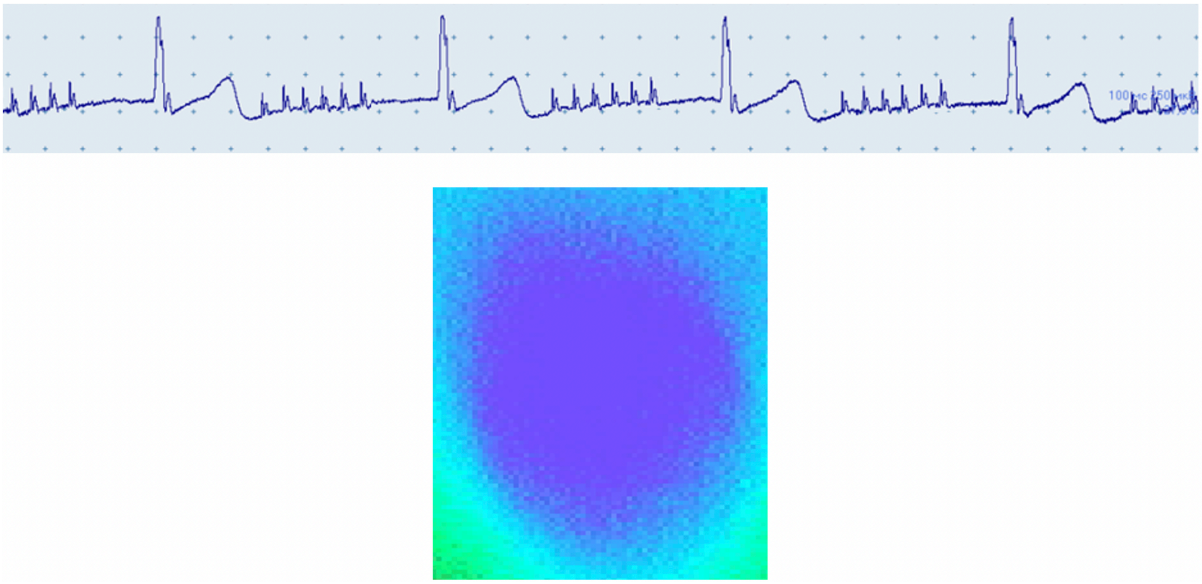


Figure 10. Luminescence of cell pools in the SAN in the conditions of vagal-cardiac synchronization at 6-impulse volleys of electrical stimulation of the VN.

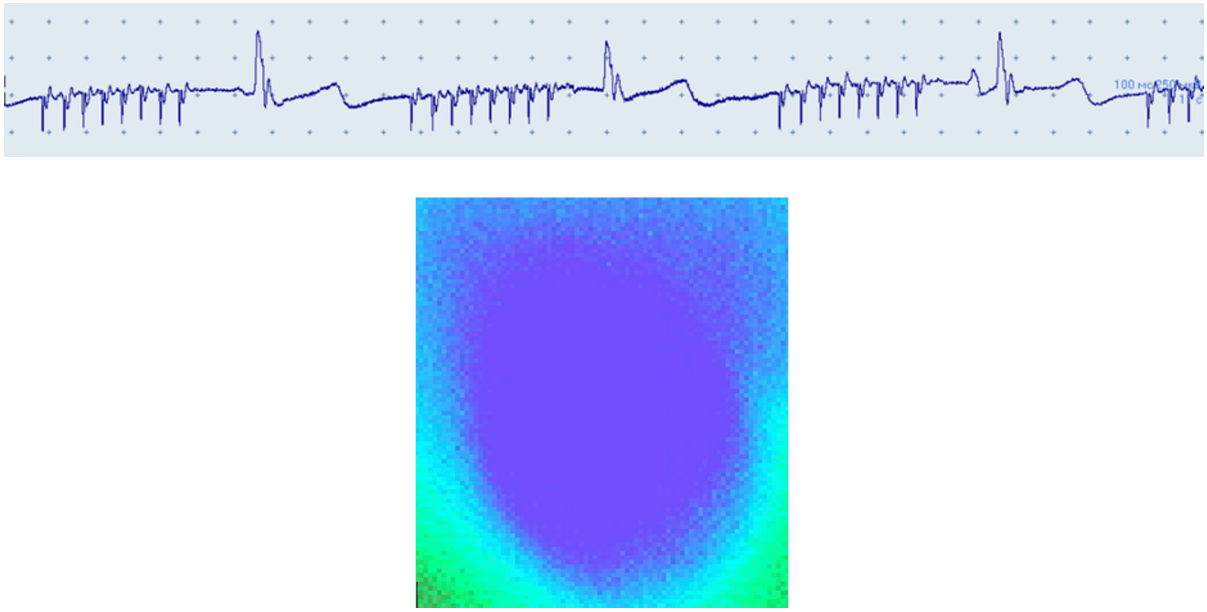


Figure 11. Luminescence of cell pools in the SAN in the conditions of vagal-cardiac synchronization at 8-impulse volleys of electrical stimulation of the VN.

With vagal-cardiac synchronization caused by the stimulation of the VN with 4, 6 and 8 electrical impulse volleys, the changes were observed (compared to the initial data) in the following indicators: luminescence brightness increased by 24.3%, 37.0% and 56.6%; the lower boundaries of the wavelength range of SAN cell luminescence at all the selected volleys of electrical impulses underwent no significant changes; the upper boundaries of the wavelength range of SAN cell luminescence increased by 5.9%, 8.6% and 29.7%; the luminescence wavelength ranges increased by 32.5%, 47.0% and 149.2%; the median luminescence wavelength at 4 electric impulse volleys decreased by 2.2%, and, with 6 and 8 impulse volleys, it increased by 1.8% and 7.1% (in the last but one case – statistically insignificantly), respectively (Table 2).

The areas of the cell pool zone and the zone area of cell pull fusion in the SAN when stimulating the VN with 4, 6 and 8 electrical impulse volleys increased by 281.0% and 175.0%, 525.7% and 437.5%, 874.1%, and 815.0%, respectively (Table 2).

The upper and lower heart rates, while stimulating the VN with 4, 6 and 8 electrical impulse volleys, were 116.0 and 103.8, 91.4 and 76.2, 83.0 and 66.3 beats per minute, respectively, versus 134.3 beats per minute at the initial stage, with differences being statistically significant only at 6 and 8 impulse volleys; the heart rate range in vagal-cardiac synchronization when stimulating the VN with 4, 6 and 8 electrical impulse volleys was 12.2, 15.2 and 16.7 beats per minute respectively (Table 2).

The values of heart rate ranges in vagal-cardiac synchronization correlated with the area of fusion zones

of cell pools in the SAN (Fig. 12). At the same time, the Pearson correlation coefficient was 0.8. From numerous studies on vagal-cardiac t the synchronizing effect of the VN on the heart rhythm – the vagal rhythm being taken over by the heart. Under these conditions, arrhythmia (AF) does not occur.

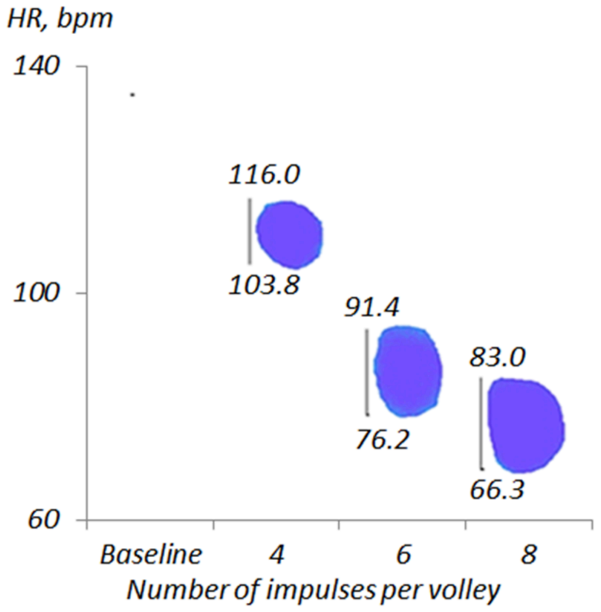


Figure 12. Correlation between heart rate and the number of electrical impulses per volley. ***Note*:** HR – heart rate, bpm – beats per minute. The areas of SAN cell pool fusion zones during controlled bradycardia are colored blue.

Therefore, increasing the synchronizing effect of the VN may be one of the methods for treating autonomic AF, and if the synchronizing impact decreases, it can be a prognostic factor for the occurrence of AF relapse.

Conclusion

In the conditions of vagal-cardiac synchronization, the focus of early depolarization increases and becomes monolithic due to the unified activity of PC pools.

The obtained facts suggest that, in a whole body, the formation of the heart rhythm results from the interaction of discrete signals coming from the brain via the VN with the rhythmogenic structures of the SAN. The result of this process is the heart reproducing the rhythm of signals generated in the brain. With a signal arriving from the nerve simultaneously to different PC pools, it is taken over by them, and the focus of early depolarization becomes wide, thus preventing the development of AF.

Electrical stimulation of the VN and the administration of substance SS-68 enhance the tonic effect of the former on the heart rhythm, which shows in a slowdown in heart contractions, which prevents paroxysms of AF and partially eliminates the influence of ectopic foci of excitation in the myocardium on AF.

Conflict of interests

The authors declare no conflict of interests.

Table 2. Dynamics of luminescence of cell pools of the SAN in anesthetized cats in vagal-cardiac synchronization induced by stimulation of the VN with 4, 6 and 8 electrical impulse volleys (M±m, n=10)

Parameters	Baseline data	Vagal-cardiac synchronization (number of impulses in a volley)		
		4	6	8
Heart rate, bpm				
Upper heart rate, bpm	134.6±2.3	116.0±1.5 $p<0.001$	91.4±3.0 $p<0.001$	83.0±2.2 $p < 0.001$
Lower hear rate, bpm		103.8±2.8 $p<0.001$	76.2±2.2 $p<0.001$	66.3±1.7 $p < 0.001$
Heart rate range in vagal-cardiac synchronization, bpm		12.2±0.5	15.2±1.0	16.7±0.3
Histogram of brightness of luminescence focus, bit	86.5±0.6	107.5±1.2* $p<0.001$	118.5±0.9* $p<0.001$	135.5±0.8* $p < 0.001$
Lower boundary of wavelength, nm	405.2±0.2	404.3±0.3 $p>0.05$	404.0±0.3 $p>0.05$	405.4±0.4 $p > 0.05$
Upper boundary of wavelength, nm	497.0±0.5	526.5±0.6 $p<0.001$	539.5±0.6 $p<0.001$	644.5±0.8 $p < 0.001$
Range, nm	91.8±0.7	122.2±0.8	135.5±0.5	239.1±3.4
Median wavelength, nm	452.0±0.4	442.0±1.9 $p<0.001$	460.0±1.2 $p<0.001$	484.0±1.3 $p < 0.001$
Area of pool zone, mm²	5.8±0.1	22.1±0.3* $p<0.001$	36.3±0.4* $p<0.001$	56.5±0.7* $p < 0.001$
Area of pool fusion zone, mm²	4.0±0.2	11.0±0.2 $p<0.001$	21.5±0.2 $p<0.001$	36.6±0.6 $p < 0.001$

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