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Acute Effect of Selective Chemical Inactivation of Sympathetic or Parasympathetic Atrial Ganglionated Plexus Structures on Atrial Fibrillation Inducibility in Pigs

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We studied the role of both parts of the autonomic intracardiac nervous system in the pathogenesis of atrial fibrillation (AF). In 12 pigs weighing 39±3 kg, AF was induced by burst stimulation. Chemical inactivation of intrinsic cardiac neurons within the right atria was performed by transendocardial injections of liposomal neuromodulators into the dorsal part of the right atrial wall. Sympathetic and parasympathetic terminals were inactivated with 6-hydroxydopamine (6-OHDA, *n*=6) and ethylcholine aziridinium ion (AF64A, *n*=6), respectively. Neuromodulators were encapsulated in liposomes (LS) with diameters of 310±50 nm for OHDA and 290±50 nm for AF64A. LS-6-OHDA and LS-AF64A were injected into the ganglionated plexuses after measuring the baseline effective refractory period and assessing myocardial resistance to AF. These measurements were repeated 90 min after the injections. The optimal doses were 0.2 mg/kg for LS-6-OHDA and 0.4 mg/kg for LS-AF64A (in 4 ml of suspension). Immediately after injections of liposomal neuromodulators, almost all pigs showed an increase in HR, and a short-term BP elevation was observed in the LS-AF64A group. At the end of the experiment, similar decrease in the effective refractory period and similar increase in the resistance to AF were observed in all animals. Thus, selective chemical inactivation of cholinergic and adrenergic terminals of the intracardiac nervous system with liposomal neuromodulators increased the resistance to AF in an acute experiment. However, the short observation period does not allow making a definite conclusion about the role of the autonomic nervous system in the pathogenesis of AF, which requires verification of the obtained data in a chronic experiment.

Key Words: *autonomic nervous system; atrial fibrillation; ganglionic plexuses; liposomes; neurotoxins*

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Ganglionated plexuses (GP) of the heart are interconnected clusters of neurons that form autonomic ganglia on the surface of the heart, predominantly in the epicardial adipose tissue. GP include both afferent and efferent neurons of the parasympathetic nervous

system and, presumably, adrenergic neurons [1-3]. It has been proven that increased neuronal activity of GP contributes to both the initiation and maintenance of atrial fibrillation (AF) [4]. AF is a supraventricular tachyarrhythmia characterized by chaotic atrial electrical activity with a contraction rate of 300-700 bpm and irregular ventricular rhythm (in the absence of complete atrioventricular block). AF is the most common tachyarrhythmia in clinical practice, with an incidence of 1-2%. Intracardiac hemodynamic disorders in AF lead to a sharp increase in the risk of thromboembolic complications and, in particular, thromboembolic stroke [5].

Risk factors leading to AF are associated with changes in the tone of the autonomic nervous system and especially with hyperactivation of the sympathetic nervous system [6]. It is known that any autonomic imbalance, namely, vagal or sympathetic activation, predisposes to electrophysiological changes in the atrial myocardium and the occurrence/maintenance of AF [7]. Previous studies have shown the possibility of selective inactivation/destruction of adrenergic or cholinergic structures in the atrial GP to assess the comparative contribution of hyperactivation of local sympathetic or parasympathetic autonomic neurons [3,8].

The aim of this experimental study was a comparative analysis of the role of both parts of the intracardiac autonomic nervous system in the pathogenesis of AF in the corresponding model in pigs. For this purpose, endocardial administration of liposome-incapsulated selective neuromodulators providing inactivation of adrenergic and cholinergic GP neurons was used.

MATERIALS AND METHODS

To block sympathetic influences from the GP, a neuromodulator was used that provides selective destruction of sympathetic terminals, 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine, 6-OHDA) (Sigma-Aldrich) [9,10]. Ethylcholine aziridinium ion (AF64A) was used to block the effect of cholinergic GP neurons. 1-Ethyl-1-(2-hydroxyethyl)aziridinium chloride (SimSon) was used as a precursor [11]. Liposomes (LS) were obtained by hydration of a thin lipid film. LS with 6-OHDA in a concentration of 2 mg/ml (diameter 310±50 nm) and LS with AF64A in a concentration of 4 mg/ml (diameter 290±50 nm) were prepared.

The study involved 12 domestic pigs weighing 39±3 kg, 6 animals in each group: LS-6-OHDA and LS-AF64A. After induction of anesthesia (20 mg/kg Zoletil, 3 mg/kg xylazine), artificial lung ventilation was performed with an air mixture containing 1.5-2.5% isoflurane and 30-40% oxygen. BP and HR were monitored using a system for monitoring physiological parameters (Beneview T5) through an arterial catheter; HR and BP were recorded every 30 min.

After intravenous administration of an anticoagulant (300 U/kg heparin), an electrode for cardiac electrophysiological study (EPS) was positioned into the right atrium using a vascular access to the common femoral vein using a Biotok multichannel complex for EPS and electroanatomical mapping (BIOTOK Medical Electronic Engineering Laboratory). EPS included measuring of the effective refractory period (ERP) and assessing the resistance of the atrial myocardium to AF. When measuring ERP, the electrode was positioned on the free wall of the right atrium (RA), in the RA appendage, and in the interatrial septum. To measure the ERP of the AV node, 9 pulses were applied to the diagnostic electrode, of these 8 cycles were with the same coupling interval (basic stimulation), and the last one was premature (extrastimulus). The coupling interval of the extrastimulus was reduced in increments of 10 msec until the conduction of the action potential to the ventricles ceased.

The frequency and duration of experimentally induced AF episodes were quantitative criteria for sensitivity to AF. AF induction was performed by burst stimulation with 5 blocks with the same set of 4 frequencies of 1200, 1500, 2000 and 3000 pulses per minute with a stimulation duration of 10 sec in the first block, 20 sec in the second, 30 sec in the third, 60 sec in the fourth, and 120 sec in the fifth block (5 blocks×4 stimulations=20 stimulations). The stimulating electrode was placed on the free wall of the RA. The occurrence of an episode of supraventricular arrhythmia with an irregular *R-R* interval lasting more than 1 sec was considered the criterion for the onset of AF [12]; AF episodes lasting >30 sec were considered stable [13]. Myocardial resistance to AF was assessed by the number of AF episodes that occurred after each burst stimulation episode.

Then, through the introducer installed in the femoral vein, the mapping electrode was positioned in the RA to construct a real-time 3D anatomical map of the RA using the Biotok multichannel complex. The next step was transendocardial injections of liposomal neuromodulators into epicardial adipose tissue through the dorsal wall of the RA along the line between the cranial and caudal veins using an injection catheter (MyoStar, Biosense-Webster Inc.) [13]. To this end, the catheter was positioned perpendicular to the endocardial surface of the RA and the needle was advanced by 2 mm. Correct position of the catheter was verified by X-ray control and evaluation of the position of the electrode according to the 3D navigation mapping system. For each animal, from 20 to 30 injections were performed with a distance between injections of 2-5 mm (total volume 4 ml). Second EPS was performed 90 min after the administration of suspensions of liposomal neuromodulators.

To control the correctness of the injection sites at the end of the experiment, an autopsy was performed with macroscopic and histological examination of the RA wall; three fragments of the RA dorsal wall from the area of endocardial injections were taken for this purpose. Sections (4-5 μm) were stained according to Nissl for subsequent morphometry of atrial GP and conducting nerve bundles, which included measuring the distance from the edge of the hemorrhage (injection site) to the nearest nerve bundle or ganglion.

Statistical analysis of the obtained results was carried out using the nonparametric Wilcoxon's test (Statistica 9.0 software; StatSoft, Inc.). For each parameter, the median and interquartile range were calculated. The differences were considered significant at $p<0.05$.

RESULTS

Baseline BP and HR did not differ significantly between the groups. Immediately after endocardial administration of liposomal neuromodulators, HR increased in both groups; in the LS-AF64A group, the mean HR did not return to baseline 90 min after the end of injections (Fig. 1, *a*, *b*). BP significantly increased only in the LS-AF64A group immediately after transendocardial injections and returned to the baseline values in 60 min (Fig. 1, *c*, *d*). The increase in BP and HR can be a result of stimulation of cardiomyocytes by neurotransmitters released from neurons damaged by neuromodulators. The reaction to injury caused by the catheter needle at the injection site also cannot be ruled out. These reactions make it difficult to assess the expected effect of neuromodulators, but are an inevitable consequence of the procedure.

In all animals, shortening of the ERP by the end of the observation period was noted (Fig. 2). In a similar study [13], the effect of botulotoxin was evaluated after at least a week and an increase in ERP was observed. In our case, the shortening of the ERP can be explained by the acute effect of neurotransmitters on GP neurons and the continued release of

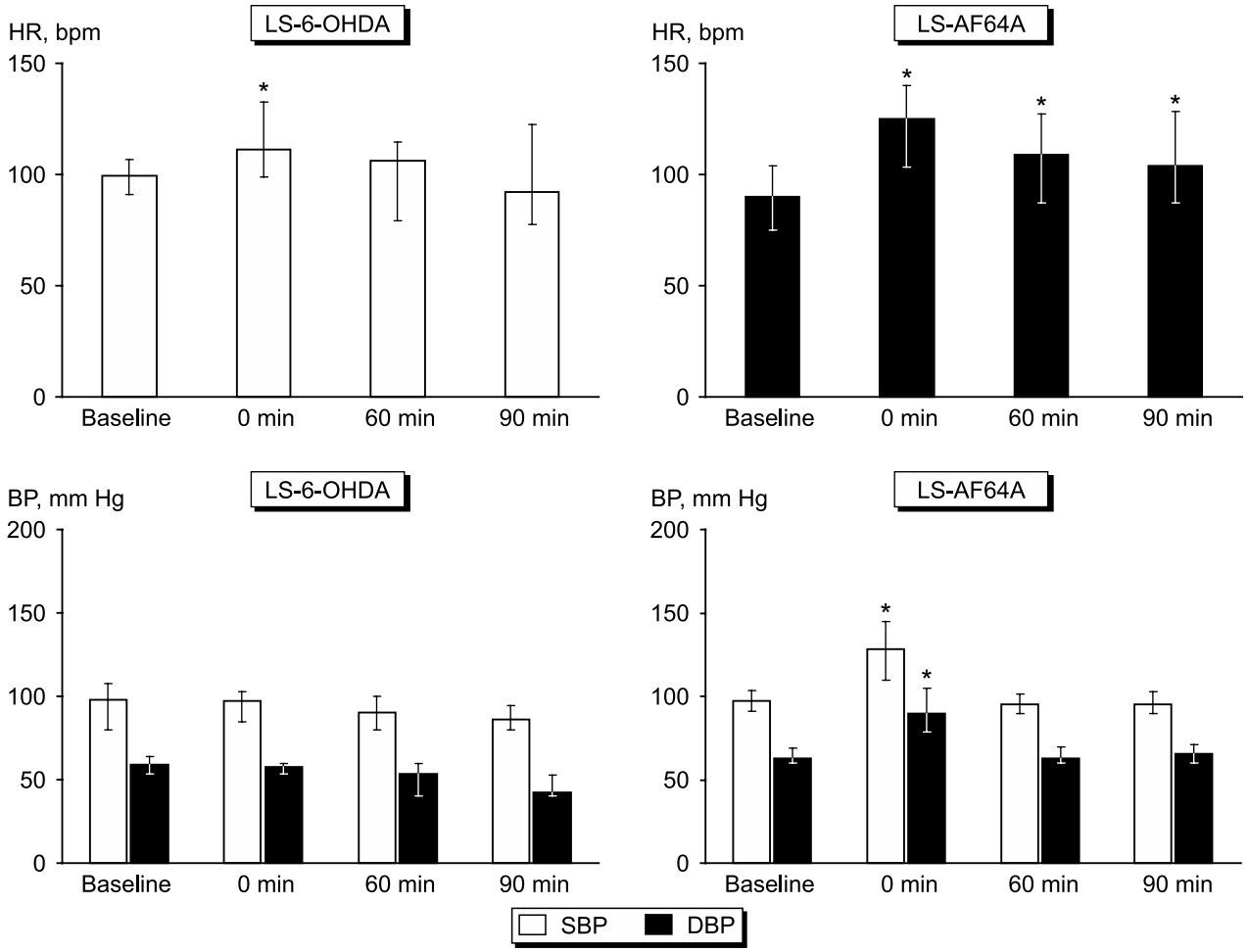

Fig. 1. Dynamics of HR and BP after transendocardial injections of LS-6-OHDA and LS-AF64A. SBP, systolic BP; DBP, diastolic BP. *$p<0.05$ in comparison with the baseline.

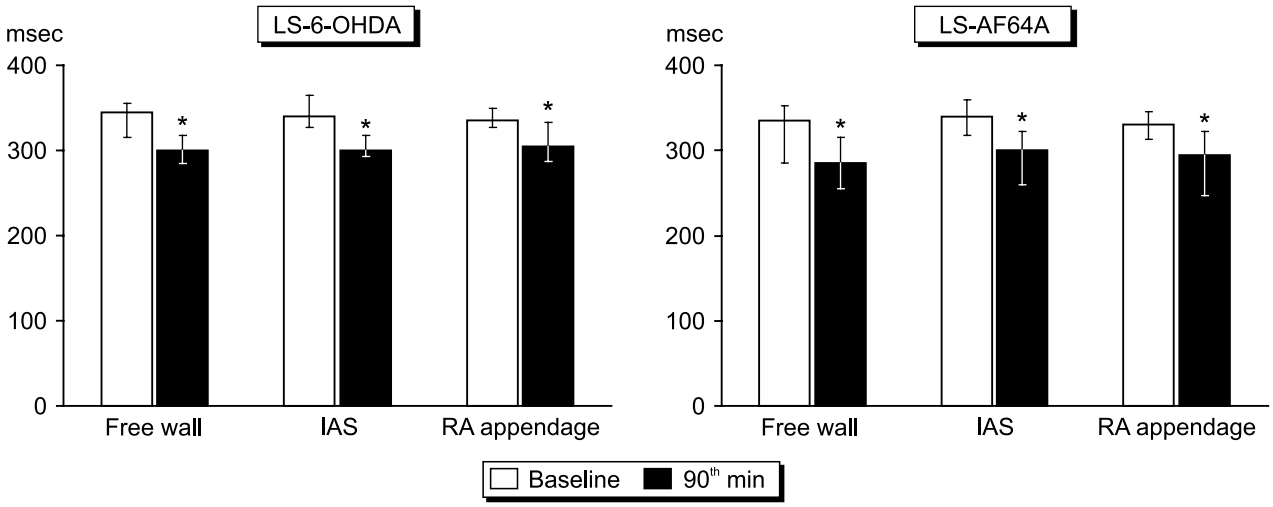

Fig. 2. Decrease in the effective refractory period of RA at the 90th min after the end of transendocardial injections of LS-6-OHDA and LS-AF64A in comparison with the baseline duration. *p<0.05 in comparison with baseline. IAS, interatrial septum.

acetylcholine throughout the observation period after neurotransmitter injections, especially in the LS-AF64A group. At the same time, changes in metabolic processes in the atria during the experiment leading to electrolyte imbalance in cardiomyocytes cannot be excluded.

In the baseline, the resistance of pigs to AF widely varied, ranging from the inability to induce AF (1 case) to intractable arrhythmia with subsequent termination of the experiment (2 cases in the pilot study). Due to the high risk of developing sustained AF at the beginning of the experiment (before endocardial injections), we decided to perform the first block of the AF induction protocol with a duration of 10 sec in half of the pigs (Table 1), and only in one pig, all five blocks of the AF induction protocol were performed. The influence of both changes in the frequency of burst stimulation and its duration was observed. The duration of arrhythmia increased with increasing the frequency of burst stimulation from 20 to 25 and 33.3 Hz. In the experiment with the full burst stimulation protocol, a decrease in myocardial resistance to AF and an increase in the duration of arrhythmias were observed with increasing the duration of burst stimulation at the baseline.

In all episodes of AF induced by high-frequency stimulation, we observed rapid transition from AF to atrial flutter (AFL), followed by the restoration of the sinus rhythm. The duration of AFL prevailed over the duration of AF. In some cases, ventricular tachycardia episodes were observed, during which sinus rhythm spontaneously recovered.

After endocardial injections of LS-6-OHDA, an increase in myocardial resistance to AF was observed, which manifested itself in the absence of AF episodes lasting more than 3 min (Table 2). All episodes of arrhythmias stopped spontaneously and did not exceed 2 min. Only a quarter of all episodes of AF-AFL were sustained, *i.e.*, lasted more than 30 sec.

In the LS-AF64A group, all episodes of supraventricular arrhythmias also spontaneously stopped. The

TABLE 1. Initial AF Resistance under Conditions of Stimulation with 10-sec Bursts (AF duration after induction, sec)

Pig No.	Burst stimulation rate, pulses/min			
	1200	1500	2000	3000
1	28	65	93	10
2	20	>180	32	2
3	11	7	12	19
4	5	33	10	>180
5	0	10	—	—
6	—	6	—	—
Median, sec	11	33	22	14.5
Percentage of sustained AF (>30 sec), %	0	50	50	25

Note. 0, sinus rhythm after AF induction; "—", burst stimulation was not performed at this frequency.

TABLE 2. Assessment of the Resistance to AF before and after Transendocardial Injections of Liposomal Neuromodulators

Duration of induction, sec	Burst stimulation rate, pulses/min	Duration of AF after induction, sec			
		LS-6-OHDA		LS-AF64A	
		baseline	90 min after injections	baseline	90 min after injections
10	1200	20	4	0	36
	1500	>180	0	6	11
	2000	Termination of AF induction	10	10	19
	3000		62	>180	5
20	1200		32	Termination of AF induction	2
	1500		2		10
	2000		34		6
	3000		60		66
30	1200		0		10
	1500		122		22
	2000		0		186
	3000		20		20
60	1200		35		24
	1500		2		6
	2000		7		26
	3000		9		10
120	1200		0		11
	1500		14		21
	2000		14		53
	3000		6		37

Note. In these representative experiments, a short protocol of AF induction was performed before transendocardial injections because of the development of prolonged episodes of arrhythmia (>180 sec), which was the criterion for stopping burst stimulation.

duration of arrhythmias ranged from 6 to 27 sec for all induction frequencies. The frequency of sustained episodes of AF-AFL did not exceed 25% and it developed at all frequencies and durations of stimulation (Table 2).

The data of morphological analysis of RA dorsal wall samples confirm the accuracy of delivery of liposomal neurotoxins to the RA epicardial fat layer adjacent to the myocardial layer and containing the greatest accumulation of nerve bundles and ganglia is observed [1]. There were no cases of bleeding or hemopericardium. Planimetry showed that the density of ganglia is higher in the zone of the cranial vena cava (0.22 ganglions per 1 μm^2), as well as in the zone between the cranial and caudal vena cava (0.17 ganglions per 1 μm^2), in contrast to the zone of the caudal vena cava (0.03 ganglions per 1 μm^2). One ganglion contained from 1 to 30 neurons. The nervous system elements are located at different distances from the endocardium: from 261.72 to 6192.52 μm. The distance from the endocardium to the injection sites (hemorrhages) ranged from 142.28 to 1716.52 μm. The distance from the edge of the hemorrhage to the nearest nerve bundle or ganglion ranged from 37.87 to 1396.71 μm, which indicates that the tip of the needle had reached the GP.

Thus, the resistance of the atrial myocardium to AF increased and the duration of AF-AFL episodes induced by high-frequency stimulation decreased 90 min after the injections of LS-6-OHDA and LS-AF64A into the epicardial adipose tissue of the dorsal wall of the RA. These results prove the possibility of selective modulation of adrenergic or cholinergic neurons in the GP by liposomal neuromodulators. However, short follow-up period in the acute experiment, prolonged release of neurotoxins, and aseptic inflammation at the injection site limit our conclusions about the role of the sympathetic and parasympathetic parts of the intracardiac nervous system in the mechanism of AF onset and maintenance. At the next stage of the study, it is planned to perform an EPS at the end of a 3-week follow-up after the injections of liposomal neuromodulators.

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