Cardiac responses activated by nicotine in canine ganglial plexus between aorta and pulmonary artery

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ABSTRACT   In order to study the location and function of nicotine-sensitive neurons of cardiac ganglial plexuses, microdissections of collections of fat pads were carried out, and nicotine (100 μg) was injected into the ganglial plexus between aorta and pulmonary artery (A-PGP) in 30 anesthetized open-chest dogs. There were numerous ganglia or neurons in A-PGP. Either positive or negative inotropic and chronotropic responses were elicited following injections of nicotine into A-PGP. Control injections of 0.1 ml saline into A-PGP and injections of nicotine (100 or 200 μg) into right marginal ganglial plexus did not elicit any cardiac responses. After acute decentralization, nicotine (100 μg) was again injected into the same locus of A-PGP. Some positive and negative responses could still be induced, but their frequencies were reduced. These suggest that nicotine can directly activate the efferent parasympathetic and sympathetic neurons and indirectly activate them by stimulating the afferent neurons existing on the surface of dog heart.

KEY WORDS   nicotine; heart; parasympathetic ganglia; sympathetic ganglia; neurons; efferent pathways; afferent pathways

Cardiac neurons in canine ganglial plexuses have been assumed to contain only parasympathetic efferent neurons[1-3]. However, afferent, local circuit, and postganglionic sympathetic neurons were also present in

cardiac ganglia[4-7]. Canine intrinsic cardiac neurons have been identified in the fat on both the ventral, and dorsal surfaces of atria[5,8,9], the cranial surface of the ventricles and circumflex coronary arteries, and in the origins of right and left marginal coronary arteries[6], as well as in the ganglial plexus between aorta and pulmonary artery (A-PGP)[10]. It was unclear whether nicotine can activate neurons in cardiac ganglial plexuses so that the cardiodynamics would be changed. Furthermore, little is known about the function of nicotine-sensitive cardiac neurons.

Nicotine activates the efferent postganglionic parasympathetic and sympathetic neurons, but not the intrathoracic autonomic axons[11]. In the present study nicotine was injected into the specific locus within A-PGP to examine the cardiac function of nicotine-sensitive neurons in cardiac ganglia and to identify the cardiac regions innervated by neurons in the ganglial plexus.

METHODS

Anatomical investigations   Hearts from 6 mongrel dogs were fixed by 10 % formalin for 7 d. The collections of the fat pads between aorta and pulmonary artery and right marginal fat pads were stained by 0.02 % methyline blue (pH=7.4) for 15 min and then washed with the buffer for 2 h. Microdissections were made through a Wild M 400 dissecting microscope to identify the location and number of ganglia or neurons. All of the ganglia were collected, dehydrated, cleared up, and covered. The number of ganglia or neurons on slices was counted under the microscope in one focus.

At the end of the experiments, the hearts were excised to identify the ganglia at the sites of injections.

Dog preparation A total of 30 mongrel dogs of either sex, weighting $14.9 \pm s 1.8$ kg, were tranquilized with thiopental sodium (12 mg·kg^{-1}, iv) and anesthetized with alpha chloralose (100 mg·kg^{-1}, iv). During the experiment, chloralose (25 mg·kg^{-1}, iv) was injected at a bolus every $1-2$ h. Following incubation, a positive pressure ventilation was maintained using a Bird Mark 7a ventilator. Bilateral thoracotomy was performed in the 4th intercostal space. Walton-Brodis strain gauge arches were sutured to the right and left atria to record the contraction forces[12]. Miniature solid-state implantable pressure transducers (Konigsberg Instruments, model p6.5a) were inserted into the midwall region of the right ventricular conus and sinus, as well as the ventral and lateral wall of the left ventricle to record regional intramyocardial pressure (IMP)[13]. Left ventricular chamber pressure was measured using a Bently Trantec Model 800 transducer connected to a Cordis #7 catheter inserted into the chamber via a femoral artery. All data, including an electrocardiogram were recorded on an Astro-Med, Model MT 9500 8-channel recorder. The abdomen was opened and the adrenal arteries and veins were occluded bilaterally before injection of nicotine 30 min later.

Nicotine injection Normal saline (0.1 ml) was injected via a 30-gauge needle into A-PGP. After 5 min, nicotine (100 μg in 0.1 ml saline) was injected via the same needle which was maintained in the ganglial plexus during the experiment. In order to mark the injection site, a fine metal wire (0.1 mm wide, 4 mm long) hook, was inserted into the tip of the needle. When the needle was removed after the injection the wire remained end hooked in the tissue. When the right marginal ganglial plexus was studied, 200 μg of nicotine were used (100 μg did not elicit any cardiac responses).

Acute decentralization After nicotine injected into the ganglial plexus, the heart was acutely decentralized by cutting the cervical vagosympathetic complexes and by eliminating all of the connections between both the stellate ganglia and spinal cord and by severing the vertebral nerves, T1, T2 rami and thoracic sympathetic chains immediatly caudal to the stellate ganglia bilaterally. Nicotine was injected again into the same sites via the same needle 30 min later.

Data analyses Heart rate (HR), right atrial force (RAF), left atrial force (LAF), right ventricular conus (RVC) and sinus (RVS) intramyocardial pressure (IMP), left ventricular ventral (LVV) and lateral (LVL) IMP, as well as left ventricular chamber pressure (LVP) were measured and their $\bar{x} \pm s$ were calculated for a period prior to the injection and when maximal responses were elicited by nicotine. The cardiac responses were evaluated for comparing the data obtained immediately prior to each intervention with maximal changes elicited using paried t test. The frequency of responses was evaluated by comparing the enumeration data obtained after decentralization with those prior to it.

RESULTS

Anatomical findings Numerous ganglia and nerves constituting a ganglial plexus were found in the fat between aorta and pulmonary artery in dog hearts. There were at least 35 ± 6 ganglia or 2280 ± 360 neurons in an A-PGP. A very few tiny ganglia (1.7 ± 0.6) or neurons (15 ± 8) were detected in the fat pad adjacent to the origin of the right marginal coronary artery. After the experiments, anatomical microdissections of the collections of fat pad between aorta and pulmonary artery which had been identified through nicotine revealed the presence of ganglia in the vicinity of the locus, as was marked by the wire.

Nicotine injection in heart with innervation Contrastive injections of saline into A-PGP and injection of nicotine (100 or 200 μg) into right marginal ganglial plexus did not elicit any change in the cardiac rate and force. Injections of nicotine (100 μg) into A-PGP induced cardiac responses in all 30 dogs. Either positive or negative responses were seen following different injections. Negative responses on HR and atrial forces and positive ones of inotropic ventricular IMP were elicited with greater consistency (Tab 1).

Usually, when sinus bradycardia was

Tab 1.　Responses elicited by nicotine (100 µg) injected into A-PGP in hearts. $\bar{x}\pm s$.
^aP 0.05, $^bP<0.05$, $^cP<0.01$ vs control; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ vs before decentralization.

	Control	Nicotine	n	Sympathetic		n	Parasympathetic	
				Control	Nicorine		Control	Nicorine
Intact hearts (n=30)								
HR/bpm	166±33	133±47^c	7	162±31	180±37^b	22	168±35	117±40^c
RAF/%	100	80±42^c	7	100	136±30^f	21	100	59±26^b
LAF/%	100	84±41^b	6	100	146±22^c	20	100	62±26^c
RVC/kPa	3.7±1.6	5.7±3.3^c	23	3.7±1.6	6.7±3.3^c	5	4.0±1.3	3.5±1.6^b
RVS/kPa	3.3±1.5	5.3±2.1^c	23	3.3±1.5	6.1±2.8^c	5	3.3±1.5	2.7±1.6^c
LVV/kPa	11±4	16±5^c	24	12±4	18±7^c	5	17.0±2.1	9.7±1.9^c
LVL/kPa	12±4	16±5^c	23	12±4	18±5^c	4	10.1±2.1	9.2±1.7^b
LVP/kPa	13±4	17±7^c	20	13±3	21±6^c	3	11.3±2.7	9.9±2.1^c
Decentralized hearts (n=23)								
HR/bpm	140±20	140±35^a	12^a	140±31	156±40^d	6^f	143±12	105±30^f
RAF/%	100	109±40^a	13^a	100	135±32^c	7^f	100	59±17^c
LAF/%	100	115±44^a	12^a	100	142±46^c	8^f	100	79±17^c
RVC/kPa	3.6±1.2	4.5±1.9^a	17^a	3.6±1.6	5.2±1.7^c	4^d	3.6±1.1	2.5±0.9^c
RVS/kPa	3.1±1.5	3.7±2.0^a	16^a	3.2±1.3	4.7±1.9^c	5^d	2.8±1.3	2.1±1.0^b
LVV/kPa	12±5	15±6^a	18^a	13±5	18±7^c	5^d	12±3	11±3^c
LVL/kPa	11±4	13±5^c	17^a	11±5	15±6^c	4^d	10.2±1.1	9.1±1.5^b
LVP/kPa	12±5	14±7^c	15^d	12±5	16±8^c	3^d	12±5	9±4^a

elicited, atrial force suppression and augmentation of right and left ventricular IMP occurred (Fig 1); when sinus tachycardia was elicited, right and left atrial forces and ventricular IMP were augmented. Therefore, the dominant responses induced by nicotinic activation of the ganglial plexus were sympathetic ones on ventricular IMP and parasympathetic ones on sinus rate and atrial force (Tab 1). However, suppression of ventricular IMP was also accompanied by bradycardia in 4 dogs. The changes of LVP were usually consistent with left ventricular IMP. Furthermore, ventricular fibrillation was induced in 4 dogs, complete atrioventricular block in 3 dogs and Wenckebach's block (Fig 1) in 1 dog after injection of nicotine into A-PGP.

Nicotine injection in decentralized heart
Following acute decentralization, sinus rate was reduced, but the inotropic functions were unaltered. Responses were no longer elicited

in 7 of the 30 dogs when nicotine was reinjected into previously active sites via the same needle, but some positive or negative responses were still induced albeit less frequently. In other words, reponses were no longer elicited in some dogs or some regions of the heart after acute decentralization. Parasympathetic responses on ventricular IMP could still be elicited. The dominant responses were sympathetic changes on ventricular IMP (Tab 1) after decentralization when nicotine was reinjected into A-PGP.

DISCUSSION

Canine intrinsic cardiac ganglia are located in certain specific fat pads on the surfaces of atria and ventricles, including right marginal ganglial plexus[14-9]. However, A-PGP have never been investigated anatomically, although there are numerous ganglia or neurons
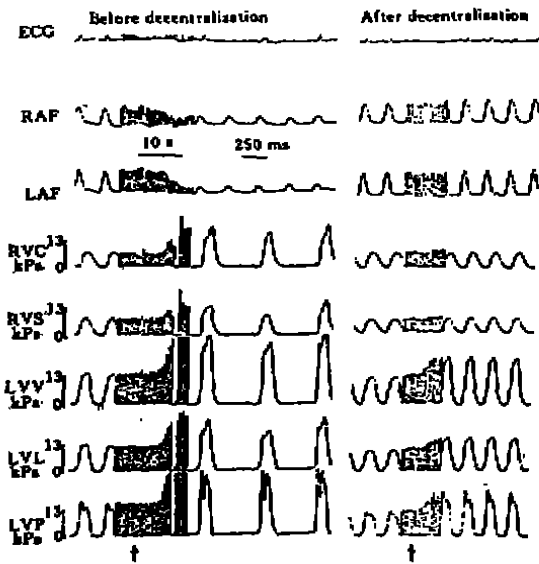
Fig 1. Cardiac responses elicited in a dog after injection (arrow) of nicotine (100 µg) into A-PGP. Before decentralization, sinus bradycardia (172 — 156 beats per minite) with decrease (%) of right and left atrial force (RAF, LAF), and augmentation of right ventricular conus (RVC) and sinus (RVS) IMP (kPa), left ventricular ventral (LVV) and lateral (LVL) IMP (kPa), as well as left chamber pressure (LVP) (kPa). Note Wenckebach's block occurred. After decentralization, signifcant augmentation of left ventricular IMP was still induced, heart rate (HR), RAF, LAF, as well as right ventricular IMP were lightly increased.

in the plexus and activation of these can induce sympathetic or parasympathetic responses. In contrast, much less ganglia or neurons were found in the right marginal ganglial plexus, and nicotine (100 or 200 µg) could not activate sufficient cardiac neurons in the given plexus nor elicit any cardiac responses.

Since electrical stimulation activates both cell bodies and axons, somata, and axons of cardiac neurons can be activated by electric stimuli delivered to the loci within cardiac ganglial plexuses[14] or stellate and middle cervical ganglia[11]. In contrast, nicotine, administered in a dose used in the present study, activates somata but not axons of passage[12].

Hence, local application of nicotine in the vicinity of cardiac neurons can activate efferent postganglionic parasympathetic and sympathetic somata by sitmulating the nicotine receptor and can also induce inotropic and chronotropic responses.

In the present experiments, administration of nicotine adjacent to the ganglia in A-PGP resulted in cardiac responses of different frequencies in all regions of intact and decentralized hearts. It appeared that nicotine-sensitive neurons in A-PGP can directly or indirectly innervate the sinus, atrioventricular node, right and left atria as well as the right and left ventricles in variant intensities. Either positive or negative regional responses were elicited depending on the dominant innervation of local sympathetic or parasympathetic active neurons within an A-PGP. After acute decentralization, which blocked the afferent pathway, the frequency of positive responses of ventricular IMP decreased, while that of tachycardia and positive atrial force intensified. The appearance of some responses after decentralization implied that nicotine can directly activate the efferent sympathetic and parasympathetic neurons in A-PGP. The decrease in frequency of some of the changes indicated that the reactions, which had been elicited in intact hearts by nicotine stimulation of afferent neurons, were abolished by decentralization. The increase in frequency of the positive responses of heart rate and atrial forces may be due to the depression of afferent parasympathetic activation after decentralization.

In conclusion, numerous neurons exist in A-PGP but dose not in the right marginal ganglial plexus. Nicotine can modify the inotropic and chronotropic function by directly activating the efferent sympathetic and parasympathetic neurons in A-PGP or indirectly by activation of afferent neurons. Furthermore,

the nicotine-sensitive neurons in A-PGP may widely innervate cardiac regions in different degrees.

REFERENCES

1 Fields JZ, Roeske WR, Morkin E, Yamamura HI. Cardiac muscarinic cholinergic receptors: biochemical identification and characterization. J Biol Chem 1978; 253: 3251—8.

2 Randall WC, Ardell JL, Wurster RD, Milosavljevic M. Vagal postganglionic innervation of the canine sinoatrial node. J Auton Nerv Syst 1987; 20: 13—23.

3 Blomquist TM, Priola DV, Romero AM. Source of intrinsic innervation of canine ventricles: a functional study. Am J Physiol 1987; 252: H638—H644.

4 Ardell JL, Butler CK, Smith FM, Hopkins DA, Armour JA. Activity of in vivo atrial and ventricular neurons in chronically decentralized canine hearts. Am J Physiol 1991; 260: H713—H721.

5 Armour JA, Hopkins DA. Activity of canine in situ left atrial ganglion neurons. Am J Physiol 1990; 259: H1207—H1215.

6 Armour JA, Hopkins DA. Activity of in vivo canine ventricular neurons. Am J Physiol 1990; 258: H326—H336.

7 Armour JA, Yuan BX, Butler CK. Cardiac responses elicited by peptides administered to canine intrinsic cardiac neurons. Peptides 1990; 11: 753—61.

8 Gagliardi M, Randall WC, Bieger D, Wurster RD, Hopkins DA, Armour JA. Activity of in vivo canine cardiac plexus neurons. Am J Physiol 1988; 255: H789—H800.

9 Ardell JL, Randall WC. Selective vagal innervation of sinoatrial and atrioventricular nodes in canine heart. Am J Physiol 1986; 251: H764—H773.

10 Yuan BX. Cardiac responses induced by bradykinin activation of canine ganglial plexus between aorta and pulmonary artery. Acta Pharmacol Sin 1993; 14: 31—4.

11 Butler CK, Smith FM, Nicholson J, Armour JA. Cardiac effects induced by chemically activated neurons in canine intrathoracic ganglia. Am J Physiol 1990; 259: H1108—H1117.

12 Brandys JC, Hopkins DA, Armour JA. Cardiac responses to stimulation of discrete loci within canine sympathetic ganglia following hexamethonium. J Auton Nerv Syst 1984; 11: 243—55.

13 Armour JA, Randall WC. Canine left ventricular intramyocardial pressures. Am J Physiol 1971; 220: 1833—9.

14 Butler CK, Smith FM, Cardinal R, Murphy DA, Hopkins DA, Armour JA. Cardiac responses to electrical stimulation of discrete loci in canine atrial and ventricular ganglionated plexi. Am J Physiol 1990; 259: H1365—H1373.

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烟碱兴奋犬主动脉和肺动脉间神经节丛引起心脏反应

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摘要 显微解剖发现犬主动脉和肺动脉间神经节丛(A-PGP)有大量神经节或神经元. 注射烟碱100 μg 于30只犬的 A-PGP, 在完整心脏和去神经支配心脏均引出正性或负性变时性和变力性反应. 心脏急性去神经支配后, 反应频数降低. 结论: 烟碱可直接兴奋心脏表面节后交感神经元和副交感神经元, 也可靠刺激传入神经元间接兴奋它们.

关键词 烟碱; 心脏; 副交感神经节; 交感神经节; 神经元; 传出路径; 传入路径