Nicotine actions on the action potentials and the contractile force in the rat atrial muscles : Comparison between the right and left atria

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Abstract : Electrophysiological and mechanical effects of nicotine in the right and left atrial muscles of rat were investigated. Nicotine (300μM and 1mM) decreased the action potential amplitude (APA). The resting potential (RP) was depolarized. The action potential duration (APD, at 90% repolarization) of the right atrium was prolonged, whereas the APD of the left atrium was shortened. The responses were concentration-dependent. In the right atrium, nicotine prolonged the cycle length. Nicotine (10μg to 1mg) caused a negative inotropic effect in both the right and left atria in a concentration-dependent manner. The responses were potentiated with an increase in the frequencies of stimulation (0.5 to 3Hz). Nicotine caused more potent depressant effects on the right atrium than on the left atrium. The effects were not modified by atropine (1μM) and hexamethonium (1mM). Addition of isoproterenol (2μM) recovered the depressed action potentials induced by nicotine (300μM). The responses were reversible. These results indicate that nicotine causes only the negative responses on rat atria due to its direct action, and suggest that the differences between the electrophysiological and mechanical effects of nicotine on the right and left atria would result from physiological and anatomical properties of rat atria.

抄録：ウイスター系ラット左右心房筋を用いて，ニコチンによる電気生理学的作用と機械的作用について調べた。ニコチン(300μm-1mM)は活動電位振幅を抑制し，静止膜電位を脱分極した。活動電位持続時間は右心房筋では延長し，左心房筋では短縮した。右心房筋では自発興奮の頻度を抑制した。また，左右心房筋で陰性変力作用を示し，左心房筋の抑制作用は刺激頻度（0.5-1Hz）に依存した。3Hz で刺激の左心房筋と比較して，右心房筋（約200回/分）の方が強い抑制作用を出現した。これらの反応は濃度依存性に出現し，正常タイロード液に戻すと完全に回復した。アトロピン（1μM）やヘキサメトニウム（1mM）前処置によるニコチン反応への影響はなかった。さらに，ニコチンによる抑制作用はイソプロテレノール（2μM）投与によって拮抗された。以上の結果により，ニコチンは陰性変時変力作用をその心筋膜への直接作用によって引き起こすことを明らかにした。また，左右心房筋に対する反応の違いはその生理学解剖学的特性の差異から発現されるのではないかと考えられた。

Introduction

Physiological functions of nicotine on cardiovascular system via ganglia of autonomic nerve, as well as central nerve system and skeletal muscles, have been reported. Nicotine initially produces a positive action and subsequently a negative action on cardiac muscles[1-3]. The initial positive chronotropic and inotropic responses evoked by nicotine have been related to an adrenergic mechanism[4,5]. The norepinephrine (NE) release was actually demonstrated by the analyses using fluorescent dye and radioactive ^{3}H-NE[6,7]. On the other hand, the negative chronotropic and inotropic effects of nicotine are due to activation of cholinergic pathways by stimulation of parasympathetic ganglia within the heart[2,3]. In ventricular muscles with little or no innervation of parasympathetic nerve, nicotine produced only the positive responses[2,8]. In contrast, it has also been reported that nicotine caused only the negative effects in rat heart[9].

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Thus, the effects of nicotine on cardiac muscles are controversial. In our laboratory, most recently, nicotine had only the negative responses accompanied with a down regulation in both right and left atria of rat. In the present experiments, we compared the electrical and mechanical effects of nicotine on the action potentials and the developed tension in right and left atria of rat, and also examined them at different frequencies of stimulation (0.5 to 3Hz).

Methods

Wistar rats (male) of 6- to 12-weeks-old, weighing 250-650g, were anaesthetized with sodium pentobarbital (30mg/kg, intraperitoneally). The details of the methods have been described in our recent reports[10,11]. In brief, the heart was quickly excised. One end of the preparation was fixed on the paraffin base of the bath, and the other end was connected with a force displacement transducer (Nihon Kohden, TB-652T, Tokyo, Japan) using a fine nylon thread. The right atrium possessed spontaneously beating with about 200-250 beats/min. The left atrium was driven at different frequencies (0.5 to 3Hz) of field stimulation (Dia Medical System, DPS-160B, Tokyo, Japan). The duration of stimulation was 3-5 msec, and twice the voltage threshold in strength was used. The action potentials and the contractile force were recorded by a conventional glass microelectrode technique (it's resistance was 5-10MΩ) on an oscilloscope (Nihon Kohden VC-11) and the thermal array recorder (Nihon Kohden, WS-641G).

Solution

The composition of modified Tyrode solution (mM) was as follows: NaCl 137, KCl 2.7, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.45 and glucose 5.5. The pH was adjusted to 7.4 with NaOH. The preparations were superfused in a bath with oxygenated (95% O_2 plus 5% CO_2) Tyrode solution. The temperature was maintained at 36℃.

The following drugs were used; l-nicotine (Sigma Chemical, St. Louis, MO, U. S. A.), l-isoproterenol (Sigma Chemical), atropine sulfate (Wako Pure Chemical, Osaka, Japan), and hexamethonium hydrochloride (C_6, Sigma Chemical). After the magnitude of contractile force became steady state (approximately 20 to 30 min later), the drugs were cumulatively added to the bath solution.

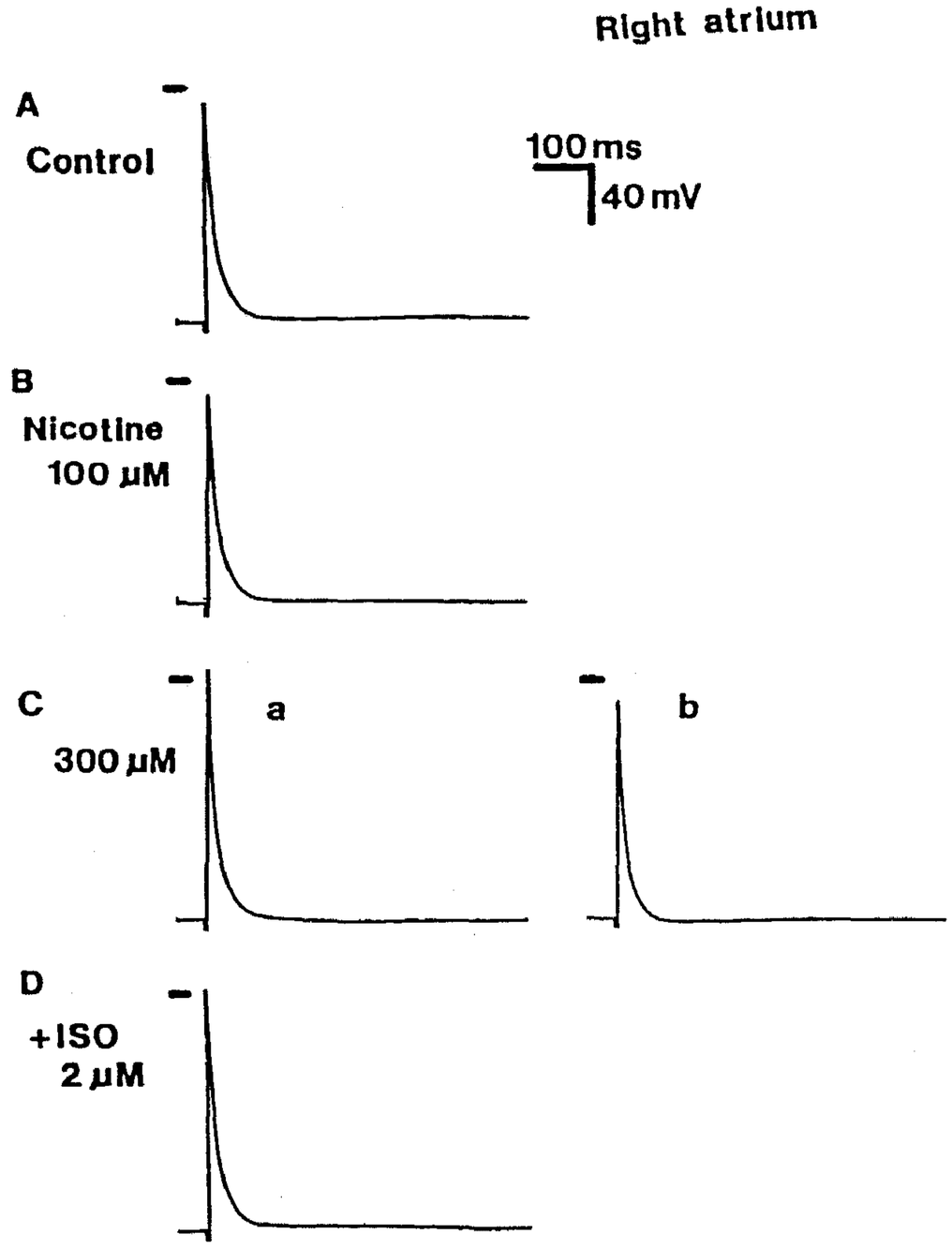

Fig. 1. Effects of nicotine on the action potentials in rat right atrium. **A** : Control. **B** : Nicotine 100μM. **C-a** : 3 min after nicotine (300μM) application. **C-b** : 7 min after nicotine (300μM) application. **D** : Addition of 2μM isoproterenol (ISO) to nicotine (300μM). Short line at the left of the action potential recording represents zero mV.

Statistical analysis

Values are given as mean±S. E. M. The comparisons were analyzed by ANOVA. Probability levels of less than 0.05 were taken as indicating significant differences.

Results

Effects on the right atrium

At low concentrations (10 and 30μM), nicotine did not affect the action potential configuration to significant extent. Nicotine (100μM to 1mM) decreased the action potential amplitude (APA) and prolonged the action potential duration (APD) (Fig. 1). These changes are summarized in Table 1. Nicotine (10μM to 1mM) was administrated cumulatively. The cycle length (CL) was prolonged, and the resting

Table 1
Electrophysiological effects of nicotine on the spontaneously beating right atrial muscle of rat

	n	APA (mV)	APD (msec)	RP (mV)	CL (msec)
Control	8	123 ± 3	39 ± 2	−86 ± 3	282 ± 13
Nicotine					
10μM	8	123 ± 2	39 ± 3	−86 ± 3	286 ± 14
30μM	8	111 ± 2	40 ± 2	−84 ± 4	289 ± 13
100μM	8	99 ± 2**	42 ± 2*	−80 ± 4	296 ± 14
300μM	8	88 ± 2***	46 ± 2***	−78 ± 3	342 ± 15**
1mM	8	82 ± 3***	46 ± 2***	−65 ± 4**	396 ± 16***
+ Isoproterenol					
2 μM	6	98 ± 3†	41 ± 3†	−81 ± 3	288 ± 11†

Values represent mean ± S. E. M. APA : Action potential amplitude. APD : Action potential duration at 90% repolarization. RP : Resting potential. CL : Cycle length. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, with respect to control value. †: $P<0.05$, with respect to the values at 300μM nicotine.

Table 2
Effects of nicotine on the contractile force in the right and left atrial muscles of rat

	n	Right atrium	Left atrium		
		1 Hz	2 Hz	3 Hz	
Control	8	122 ± 11mg	132 ± 21mg	116 ± 19mg	102 ± 18mg
Nicotine					
10 μM	8	1.9 ± 1.4	0.4 ± 2.9	0.4 ± 3.2	0.8 ± 1.5
30 μM	8	2.7 ± 2.2	−1.1 ± 1.3	1.2 ± 1.6	3.6 ± 1.6
100 μM	8	−7.5 ± 2.8	−10.2 ± 2.4*	−9.8 ± 2.0*	−7.6 ± 2.8
300 μM	8	−18.8 ± 3.2**	−18.3 ± 2.6***	−16.3 ± 3.3*	−14.2 ± 1.9***
1 mM	8	−54.1 ± 3.5***	−41.8 ± 1.9***	−34.7 ± 3.5***	−32.6 ± 4.6***

Values (%) represent mean ± S. E. M. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, with respect to control value.

potential (RP) was depolarized. The averaged sinus rate was 213±14 beats/min (n=8) in normal Tyrode solution (equivalent to 282±13msec in the CL). As shown in Fig. 1C-a and b, nicotine at 300μM slightly enhanced, and subsequently (at approximately 7 min later) decreased the APA. The percentage decreases were 28.1±1.8% (n=8, $P<0.01$) in the APA, and 21.8±3.8% (n=8, $P<0.05$) in the APD. The initial positive response was not significant statistically (by 9.1±3.6%), and the data are not shown in Table 1.

Addition of isoproterenol (2μM) attenuated the nicotine (300μM)-induced depression in the action potentials (Fig. 1D). The recovery in 6 preparations was 11.3±1.1% ($P<0.05$) in the APA, 10.8±3.9% ($P<0.05$) in the APD, and 15.8±4.7% ($P<0.05$) in the CL, but not significant in the RP (Table 1).

Nicotine also caused only a negative inotropic effect in a concentration-dependent manner. The percentage changes are summarized in Table 2. Nicotine at over 300μM decreased the contractile force significantly. After a washout, the responses were reversible. About 15min-washout with 300μM nicotine was required to recover to the control value, and about 35min-washout with 1mM.

Pretreatment with atropine (1μM), a muscarinic ACh receptor blocker, did not affect the negative chronotropic and inotropic effects induced by nicotine. Nicotine (1mM) caused the negative chronotropic and inotropic effects by 39.6±3.5% and 54.1±3.5% in the absence (n=7) and by 38.9±3.3% and 53.6±2.3% in

Table 3

Effects of nicotine on the action potentials in rat left atrial muscle at different frequencies of stimulation

	n	APA (mV)	APD (msec)	RP (mV)
At 0.5 Hz				
Control	7	148 ± 3	46 ± 2	-95 ± 3
Nicotine				
10 μM	7	145 ± 2	46 ± 2	-95 ± 3
30 μM	7	150 ± 2	47 ± 2	-95 ± 3
100 μM	7	156 ± 2	48 ± 3	-94 ± 3
300 μM	7	159 ± 3	49 ± 2	-90 ± 3
1 mM	7	157 ± 3	51 ± 2	-89 ± 3
At 1 Hz				
Control	7	138 ± 3	42 ± 2	-93 ± 2
Nicotine				
10 μM	7	130 ± 2	36 ± 2*	-93 ± 3
30 μM	7	129 ± 2	37 ± 3*	-93 ± 3
100 μM	7	128 ± 2	32 ± 2**	-92 ± 4
300 μM	7	123 ± 3**	30 ± 2***	-89 ± 4
1 mM	7	117 ± 2***	30 ± 2***	-87 ± 3
At 2 Hz				
Control	7	131 ± 3	40 ± 3	-93 ± 2
Nicotine				
10 μM	7	124 ± 2	38 ± 3	-92 ± 3
30 μM	7	123 ± 2	38 ± 2	-92 ± 3
100 μM	7	126 ± 2	35 ± 3*	-93 ± 4
300 μM	7	123 ± 2	30 ± 3***	-90 ± 3
1 mM	7	115 ± 2*	30 ± 2***	-89 ± 4
At 3 Hz				
Control	6	130 ± 3	36 ± 3	-94 ± 4
Nicotine				
10 μM	6	123 ± 2	33 ± 3	-94 ± 3
30 μM	6	119 ± 3	32 ± 2*	-93 ± 2
100 μM	6	117 ± 2*	31 ± 3*	-93 ± 3
300 μM	6	123 ± 3	31 ± 2**	-92 ± 3
1 mM	6	116 ± 3**	30 ± 3**	-92 ± 3

Values represent mean ± S. E. M. APA : Action potential amplitude. APD : Action potential duration at 90% repolarization. RP : Resting potential. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, with respect to control value.

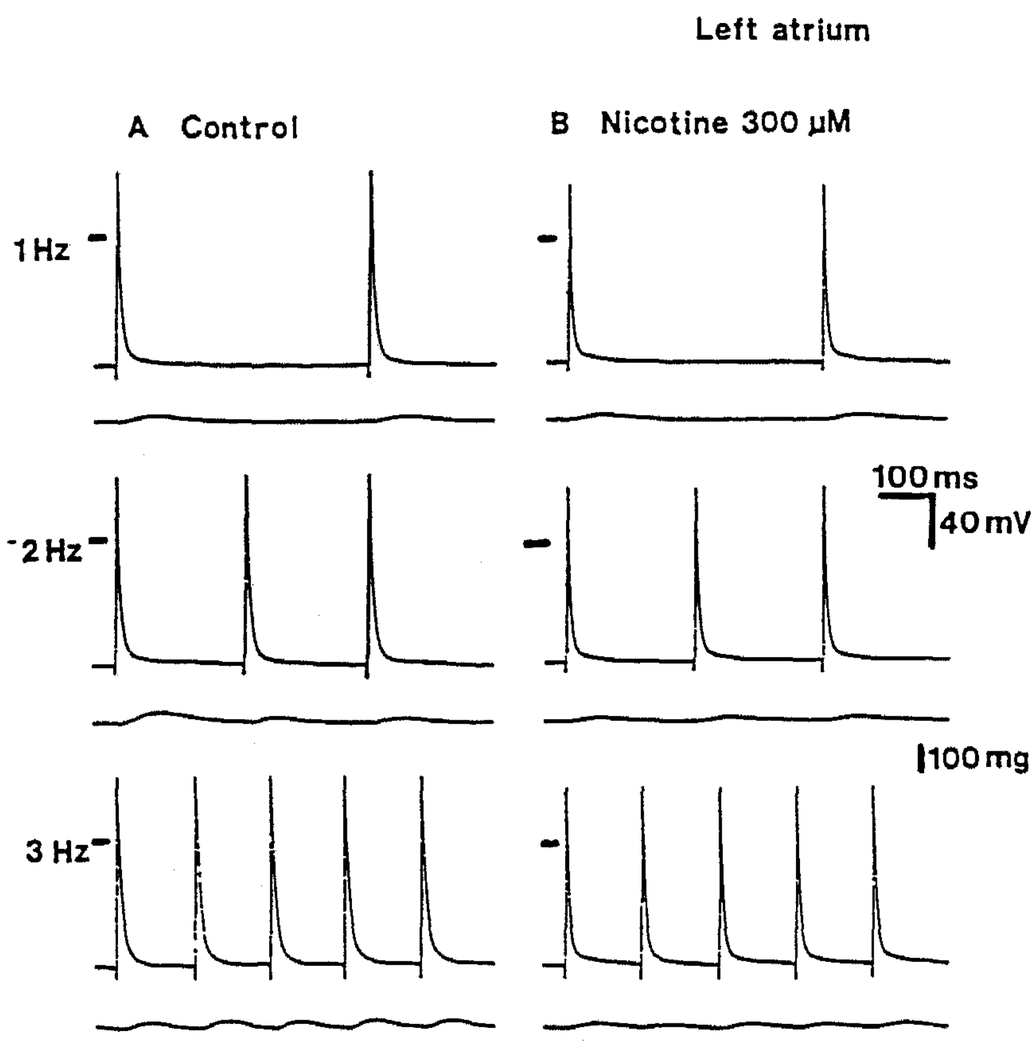

Fig. 2. Effects of nicotine on the action potentials in rat left atrium. **A** : Action potentials and contractile force at 1, 2 or 3Hz stimulation in control. **B** : Action potentials and contractile force at 1, 2 or 3Hz stimulation in nicotine 300μM. Short line at the left of the action potential recording represents zero mV.

the presence (n=7) of atropine (1μM), respectively.

Effects on the left atrium

The effects of nicotine on the left atrial muscle were examined at the different frequencies of stimulation (0.5 to 3Hz) (Fig. 2A and B). Nicotine (10μM to 1mM) was also administrated cumulatively to the bath solution. At 1Hz, nicotine at over 300μM decreased the APA, and at 10μM or higher concentrations it shortened the APD. The average values are summarized in Table 3. The RP was tended to depolarize (but not significantly). The percentage changes in the contractile force of left atrium are also shown in Table 2. Increasing frequency of stimulation produced a negative staircase phenomenon. Nicotine at low concentrations (10 and 30μM) increased the force, but it did not cause to significant extent. At over 100μM, nicotine produced only the negative inotropic effect in a concentration-dependent manner. However, nicotine caused more potent negative inotropic effect at low (1Hz) than at the high frequencies of stimulation (2 or 3Hz).

Although nicotine at low concentrations (10 and

30μM) caused a small positive inotropic effect, the responses to nicotine were not modified by hexamethonium (C_6), a nicotinic ACh receptor inhibitor of autonomic ganglions. Nicotine (1mM) decreased the contractile force by 39.0±4.7% (n=7) in the absence, and by 52.5±6.0% (n=4) in the presence of C_6 (1mM). The responses were reversible. Nicotine caused less potent effects in the left atrium, as compared to the effects in the right atrium.

Discussion

It has been documented that nicotine causes a positive effect followed by a negative effect on the atrial preparations of cats[12], guinea-pigs[5,13,14], rabbits[4] and rats[15]. The biphasic response to nicotine is considered to be due to direct and indirect actions mediated through the pharmacological activation of autonomic nerves. The mechanism of the negative effect may involve with the release of ACh by vagal stimulation (through muscarinic ACh receptors), because it is blocked by atropine or treatment with hemicholinium-3[6]. On the other hand, the mechanism for the initial positive inotropic effect of nicotine is considered as the following. (1) Direct action on calcium release[6,12,16] and on calcium exchange[17,18]. (2) Indirect action on catecholamine release from either post-ganglionic sympathetic nerve endings or chromaffin cells. The positive effect is abolished by reserpine pretreatment or surgical sympathectomy[2,19].

In the present experiments, however, nicotine caused only the negative chronotropic and inotropic effects, and failed to produce the secondary positive effects. This is consistent with recent report by Carrly and co-workers[9]. Also, no positive response occurred in chick atrial and ventricular muscles[20]. Fuder et al.[21] have shown that nicotine causes only small ^{3}H-NE release in rat. These results suggest that nicotine receptors for the release of norepinephrine might be absent. However, other possibilities may also exist. One is that the negative responses were more potent, and the secondary responses were weak or masked. Another is an occurrence of cellular Ca^{2+} overload, because the positive responses evoked by nicotine may be abolished or depressed under the Ca^{2+} overload condition[11,22,23].

On the action potential configuration

Nicotine has been reported to increase the APD and the contractile force in cat and toad ventricular muscles[12] and in rat left atrium[15]. In guinea-pig left atrium, nicotine at low concentrations increased the APA and APD, whereas at high concentrations, it decreased them[14]. This is somewhat consistent with the results of the present experiments; nicotine at 300μM produced the initial stimulation of the action potentials in some cells, and at low concentraions had the tendency of the positive inotropic effect. The APA is dependent on activation of fast Na^+ channels. Nicotine at 300μM actually decreased a maximum rate of depolarization (an indicator of the fast Na^+ current, I_{Na}) by 24.5±2.2% (n=5, $P<0.01$) in our laboratory. Thus, these results indicate that nicotine inhibits the I_{Na}.

The CL is modulated by several factors ; I_{Ca} hypothesis, g_K hypothesis, and I_f hypothesis[24]. In this study, nicotine had only the negative choronotropic effect. The sinus rate is regulated by an interaction of these ionic currents[25]. Therefore, the CL lengthening induced by nicotine would be resulted from the inhibitions of all the currents.

Nicotine prolonged the APD of the right atrium, whereas it shortened the APD of the left atrium. The plateau is regulated by a balance of inward and outward currents. A small change in one of the currents will greatly affect the course of membrane potential on the repolarization[26]. The APD prolongation would be due to mainly decrease in a delayed outward K^+ current (I_K). In contrast, the APD shortening in the left atrium might be due to activation of ACh-activated K^+ current (I_{KACh}) and Ca^{2+}-activated K^+ current[27,28]. From a theoretical point of view, the APD may also be regulated by I_{Na} and I_{Ca} currents, and by their inactivations. Koley et al.[29] concluded that nicotine stimulates the calcium channels, because slow action potential (depolarized by high K^+) was evoked by nicotine and it was blocked by verapamil (4μM). In the present experiments, however, addition of isoproterenol recovered the depressed action potentials induced by nicotine, indicating an inhibitory action of nicotine on I_{Ca}.

Nicotine depolarized the RP. The action of nicotine on the RP is also complex, but the RP would be not regulated by only one ionic current; ex. an inwardly rectifying inward current (I_{Krec}) and a background

current[30]. The nicotine-induced depolarization of RP was more marked in the right atrium, but not in the left atrium, suggesting the pharmacological difference of the nicotine receptors on the right and left atrial cell membranes.

On the inotropic effect

Nicotine had only the negative inotropic effect. At low concentrations, nicotine tended to increase the contractile force. This suggests that nicotine may produce the positive responses like the reports of other species and tissues[4,12,15], and that the positive responses might be masked by more potent negative responses. The mechanisms for the negative chronotropic and inotropic effects are controversial. The inhibition and stimulation induced by nicotine have been reported to suppress by atropine and C_6 in isolated spontaneously beating rabbit atria[4], and in guinea-pig atria[5]. In this study, however, the effects were not affected by both atropine and C_6. This is consistent with some previous reports[7,31]. The discrepancy might be resulted from different species and tissues.

Primary effect of nicotine may be more on the mobilization of calcium at the cell surface rather than intracellularly[17]. Nicotine does not act on the intracellular site, because verapamil selectively antagonized the effect of nicotine without affecting the response to caffeine[18,24]. Therefore, these results indicate that the electrical and mechanical effects induced by nicotine are due to mainly its direct action on the nicotinic ACh receptors (or nicotine receptors) on the cell membrane of rat atria. The responses to nicotine exhibited a down-regulation (unpublished data), suggesting that the nicotine effects would be produced through the receptors. The negative inotropic effect would also be modulated by inhibitions of I_{Ca} and I_{Na}.

Difference between right and left atria

The effects of nicotine on the APD of the right and left atria were quite different. The difference would result from different effects of nicotine on the ionic channels to regulate the APD. The difference may be related with difference in the coupling between myocardial nicotine receptors and ionic channels. There may be differences between the sensitivities and/or binding sites of nicotine in the right and left atria[32].

Nicotine (1mM) caused more potent negative inotropic effect (by approximate 22%) in the right atrium than in the left atrium (at 3Hz). The difference was also produced on the electrophysiological effects. This might be due to the difference of the innervation of autonomic nerves in the right and left atria, and be due to the spontaneous beating in the right atrium. Further single-channel patch-clamp and biochemical experiments required to elucidate the mechanisms of nicotine.

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