Electrophysiological responses to imidazoline/α_2-receptor agonists in rabbit sinoatrial node pacemaker cells[1]

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KEY WORDS moxonidine; clonidine; agmatine; xylazine; adrenergic alpha-1 receptors; adrenergic alpha-2 receptors; action potentials; sinoatrial node; vasoconstriction; rabbits

ABSTRACT

AIM: To compare the effects of moxonidine (Mox), clonidine (Clo), agmatine (Agm), and xylazine (Xyl) on action potentials (AP) of the rabbit sinoatrial node (SAN) pacemaker cells and investigate the contribution of α-adrenoceptors to the cardiac electrophysiologic responses induced by the agonists. **METHODS:** Intracellular microelectrode technique was used to record AP in the rabbit SAN pacemaker cells. Vasoconstrictive responses to norepinephrine (NE), Mox, Clo, Agm and Xyl were observed in the thoracic aorta and ear vein isolated from rabbits. **RESULTS:** (1) In the rabbit thoracic aorta, a rank order of potency producing vasoconstrictive responses was NE>Clo>Mox; and a rank order of potency in ear vein was Clo>NE>Xyl=Mox. Agm did not produce any vascular responses in both preparations, and Xyl did not produce vascular responses in the thoracic aorta. (2) Mox, Clo, Xyl, and Agm concentration-dependently decreased the rate of pacemaker firing (RPF), and prolonged APD_{50} and APD_{90} in the rabbit SAN pacemaker cells. The rank order of decreasing RPF or prolonging APD was Clo>Xyl=Mox. (3) Most effects of Clo were partially inhibited by yohimbine, but those of Xyl and all the effects of Agm on the AP in SAN pacemaker cells were not affected by the treatment with yohimbine. (4) In the presence of propranolol 1 μmol/L, phenylephrine did not cause any effects on AP in the rabbit SAN pacemaker cells. **CONCLUSION:** Like Mox, Clo changes AP of the rabbit SAN pacemaker cells via α_2-adrenoceptors partially, but the effects of Xyl and Agm on the AP are almost not related to α_2-adrenoceptors. Moreover, there are no obviously functional α_1-adrenoceptors in the rabbit SAN pacemaker cells.

INTRODUCTION

Moxonidine (Mox), one of the second generation of centrally acting anti-hypertensive drugs, offers significant protection against the development of arrhythmias induced by regionally myocardial ischemia in the conscious rat[1]. Mox activates I_1-imidazoline receptors (I_1-receptors) more strongly than α_2-adrenoceptors[2,3], however its mechanism as an antiarrhythmic agent is not clear. Clonidine (Clo), one of the first generation of centrally acting anti-hypertensive drugs, induced vasoconstriction in a concentration-dependent manner[4], whereas xylazine (Xyl, a selective α_2-adrenoceptor agonist) did not elicit any vasoconstriction[4]. In radio-

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ligand binding experiments using membranes of the bovine rostral ventrolateral medulla, the affinity ratio of I_1 site /α_2 site was reported to be 4 for Clo and even about 30 for Mox[3,5]. Recently, we found that Mox and Clo decreased the rate of pacemaker firing (RPF), prolonged the duration of repolarization (APD) of the rabbit sinoatrial node (SAN) pacemaker cells in the preliminary experiments. Though I- and α_2-receptors were reported to exist on hearts[6,7], there were no suitable agonists or antagonists for identifying I-receptors clearly in the functional study up to the present. Agmatine (Agm), an endogenous ligand for I-/α_2-receptors, has been shown to act on the vascular system to regulate vascular function[8], and to affect the cardiac electrophysiologic activity[9,10]. However, the agonists or antagonists acting on I-receptors also have higher affinity to α_2-adrenoceptors, and it is difficult to analyze the physiological and pharmacological effects regulated by I-receptors in the heart[9,10]. On the other hand, very little is known regarding the effects of Clo and Xyl on action potentials (AP) of the rabbit SAN pacemaker cells. Consequently, the present study was designed to investigate the effects of Mox, Clo, Agm, and Xyl on AP of the rabbit SAN pacemaker cells using the intracellular microelectrode technique, and compared their (Mox, Clo, Agm, and Xyl) potency order for producing vasoconstriction in the rabbit isolated thoracic aorta (α_1-adrenoceptor preparation)[11] and rabbit isolated ear vein (α_2-adrenoceptor preparation)[12], to elucidate the contribution of I-receptors and α- adrenoceptors to the cardiac electrophysiologic action induced by the four agonists.

MATERIALS AND METHODS

Rabbits Male New Zealand white rabbits (2.5-3.0 kg), provided by the Experimental Animal Center of Hebei Province (Certificate No 0059).

Chemicals Moxonidine hydrochloride was synthesized by North China Pharmaceutical Cooperation Research and Development for Drug Center (Batch No 980324), and clonidine hydrochloride, agmatine sulfate, xylazine hydrochloride, phenylephrine hydrochloride, yohimbine hydrochloride, propranolol hydrochloride, deoxycorticosterone acetate, desipramine hydrochloride, (−)-norepinephrine (NE) bitartrate, and sodium phentobarbital were obtained from Sigma Chemical Co. All drugs were dissolved in distilled water except that deoxycorticosterone acetate was dissolved in 1,2-propanediol.

Thoracic aorta and ear vein preparations Rabbits were killed by an overdose of sodium pentobarbital (25 mg/kg) injected via the ear vein of the left ear, then exsanguinated. The thoracic aorta was excised and cleaned of excess connective tissue and fat. A scored polythene cannula (external diameter slightly smaller than vessel internal diameter) was inserted into aorta, and the lumen was carefully rubbed to remove the endothelium[13]. Ring segments (4 mm in length) without endothelium were mounted horizontally in a 10-mL organ bath by carefully inserting a tungsten wire through the lumen of the vessel ring and anchoring it to a stationary support. Another wire similarly inserted, was connected to an isometric tension transducer, and responses were recorded on a polygraph (ERT-884, Youlin Electron Co, Kaifeng). Preload of 4.0 g was applied to aorta arterial ring. The preparations were allowed to equilibrate for 1 h in physiological solution of the following composition (mmol/L) : NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8, and $CaCl_2$ 2.52, pH 7.2-7.4. The solution was maintained at 37 ºC and aerated with 95 % O_2 and 5 % CO_2. A successful removal of the arterial endothelium was confirmed by the loss of relaxation response to ACh (1 mol/L) in precontracted arterial rings. In all cases, the solution contained propranolol 2 μmol/L, deoxycorticosterone 5 μmol/L, and desipramine 0.2 μmol/L[11].

The ear vein was carefully removed from the right ear. Ring segment preparations with endothelium of the ear vein were made by the method mentioned above except that the preload was 0.4 g in the ear vein preparations. Physiological solution also contained propranolol 2 μmol/L, deoxycorticosterone 5 μmol/L, and desipramine 0.2 μmol/L.

Sinoatrial node preparations The SAN preparation was excised from the right atrium in aerated Krebs-Henseleit (K-H) solution (mmol/L: NaCl 118, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10, pH 7.3-7.4), and then mounted to the silicon rubber placed on the bottom of 1.5-mL tissue bath by stainless steel needles. The preparation was perfused with K-H solution (35.0-35.5 ºC) gassed with 95 % O_2 and 5 % CO_2 at a rate of 4 mL/min. After preparation stabilization in K-H solution for 1 h, the glass microelectrode filled with KCl 3 mol/L was inserted into the primary SAN pacemaker cell to record the electrical signal intracellular. The parameters including maximal diastolic potential (MDP), action potential amplitude

(APA), maximal depolarization rate of phase 0 (V_{max}), velocity of diastolic (phase 4) depolarization (VDD), duration of 50 % and 90 % repolarization (APD_{50} and APD_{90}), and RPF were on-line analyzed by a microcomputer system MAP2, a program designed by the Department of Physiology, Hebei Medical University. The criteria to discriminate the primary pacemaker cells (at the central nodal area) were reported in our previous study[14].

Statistics analysis Vasoconstriction responses to agonists were expressed as a percentage of the NE-induced maximal constriction obtained from the NE concentration-response curve before constructing the concentration-response curves for one of the agonists (Mox, Clo, Agm, and Xyl). The EC_{50} value of each agonist was calculated with the equation: lg $[E/(E_{max}-E)]$=lg$C$-lg$K$ (E, response; E_{max}, maximal response; C, agonist concentration; K, equilibrium dissociation constant). Data presented were mean±SD. The AP responses in SAN to agonists in Fig 2-4 were calculated as percentage of the control value obtained immediately before giving each concentration of the agonist. Paired t-test was used to evaluate the significant difference between the data before and after agonist, and unpaired t-test was used to evaluate the significant difference between the responses to the same concentration of agonist in the preparations treated with and without yohimbine. $P<0.05$ were considered statistically significant.

RESULTS

Contractile responses to Mox, Clo, Agm, and Xyl in rabbit isolated thoracic aorta and ear vein NE (0.001-100 μmol/L) was added cumulatively to the organ bath to construct concentration-response curves of the vasoconstriction of thoracic aorta. The concentration-response curves for NE were repeated two times in each preparation at 35-min intervals, the first set of data was not used in analysis, and the second one was used to standardize the vasoconstrictive responses to Mox, Clo, Xyl, or Agm. In the preparations of ear vein, the concentration-response curves for NE as a standardization agent were repeated three times, and the first and second sets of data were not used in analysis. Thirty-five minutes after NE stimulations, one of Mox, Clo, Agm, Xyl, and NE was added cumulatively (0.001-100 μmol/L) to the organ bath to construct concentration-response curves, and one preparation was only exposed to one agonist except standardization agent NE.

NE, Mox, and Clo produced vasoconstrictive responses in a concentration-dependent manner in the rabbit thoracic aorta, and the maximal vasoconstriction induced by Mox was the same to NE ($P>0.05$), but the maximal vasoconstrictive response to Clo was only 38.1 % of that by NE (Fig 1A, Tab 1). A rank order of potency was NE>Clo>Mox. Agm and Xyl did not produce any vascular responses in the thoracic aorta.

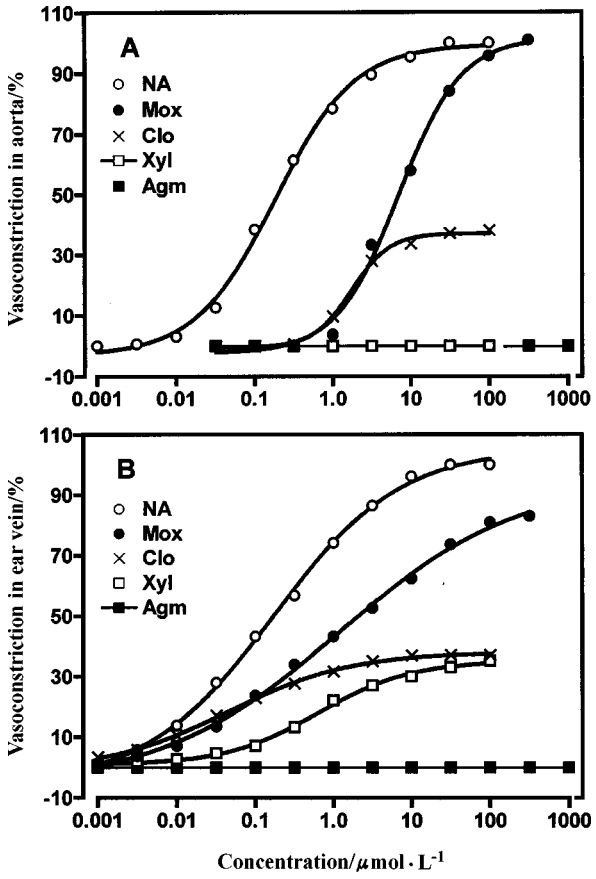

Fig 1. Concentration-dependent response curves for NE, Mox, Clo, Xyl, and Agm in the rabbit thoracic aorta (A, n=6) and ear vein (B, n=11-15). Vasoconstriction responses to agonists were expressed as a percentage of the NE-induced maximal constriction.

In contrast with the rabbit thoracic aorta, NE, Mox, and Clo but also Xyl produced concentration-dependent vasoconstriction in the ring preparations of rabbit ear vein. The maximal response to Xyl was near to that of Clo ($P>0.05$), and was much less ($P<0.05$) than that induced by Mox or NE (Fig 1B, Tab 1). A rank order of potency was Clo>NE>Xyl=Mox (Tab 1). Agm did not produce any vascular responses in the ear vein preparations.

Tab 1. Vasoconstrictive responses to several agonists in the rabbit aorta and ear vein. Aorta: *n*=6; Ear vein: *n*=11-15. Mean±SD.

Compound	E_{max}/%		-lg EC_{50}	
	Aorta	Ear vein	Aorta	Ear vein
Noradrenaline	100	100	6.71±0.07	6.41±0.11
Moxonidine	101±3	81±14	4.83±0.16	5.8±0.3
Clonidine	38±8	37±7	5.90±0.16	7.0±0.4
Xylazine	n.a.	35±8	n.a.	6.0±0.5
Agmatine	n.a.	n.a.	n.a.	n.a.

n.a.=not applicable since no contractile response was observed.

Effects of Mox, Clo, Agm, and Xyl on AP of SAN pacemaker cells Each agonist was injected with a microsyringe (50 μL) into the tissue bath directly at final concentrations of 0.1, 0.3, 1, and 3 mmol/L except Agm, the final concentrations of which were 1, 3, 10, and 30 mmol/L, and the changes in AP were recorded before and at 10 s, 20 s, 30 s, 45 s, 1 min, 2 min, 3 min, 4 min, 6 min, 8 min, 15 min, and 30 min after the administration, respectively. The interval of agonist administration at each concentration was 30 min. One preparation was used for examining only one agonist. Mox, Clo, Xyl, and Agm concentration-dependently decreased RPF (Fig 2), and prolonged APD_{50} and APD_{90} (Fig 3). The rank order of decreasing RPF or prolonging APD was Clo>Xyl=Mox>Agm. Only Mox and Clo significantly changed MDP to more negative direction (Fig 4A), and Mox, Clo, and Xyl decreased VDD concentration-dependently and greatly (Fig 4B).

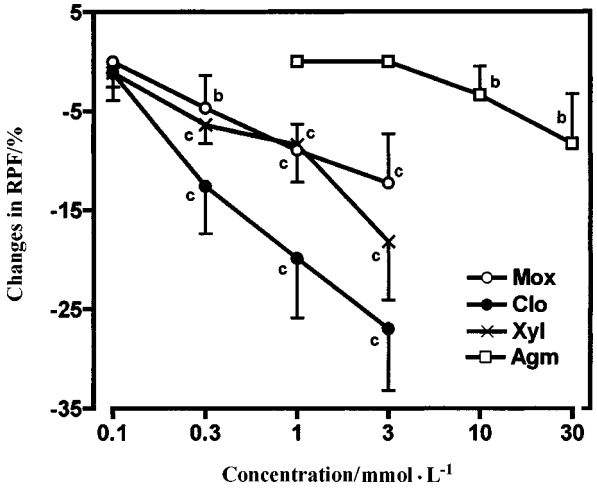

Fig 2. Concentration-dependent response curves for Mox, Clo, Xyl, and Agm on RPF of AP in the rabbit SAN pacemaker cells. *n*=5-6. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* before agonists.

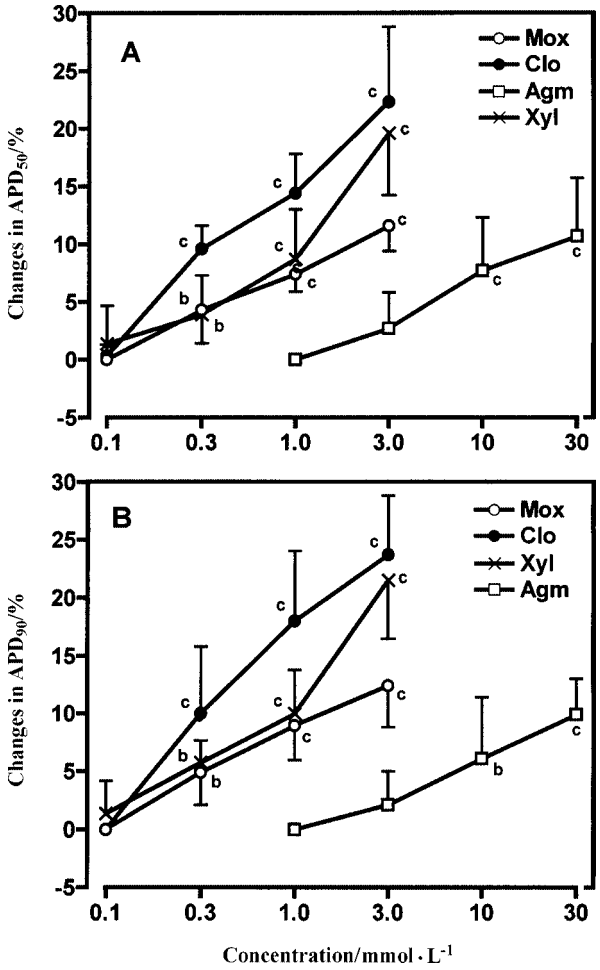

Fig 3. Concentration-dependent response curves for Mox, Clo, Xyl, and Agm on APD_{50} (A) and APD_{90} (B) of action potential in the rabbit SAN pacemaker cells. *n*=5-6. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* before agonists.

Effects of phenylephrine on AP of SAN pacemaker cells Propranolol (1 μmol/L) given with constant perfusion for 20 min did not affect the parameters of AP of the rabbit SAN pacemaker cells markedly (*n*= 5, data not shown). After 20-min incubation with propranolol, the preparations were exposed to phenylephrine at 0.01, 0.03, 0.1, and 0.3 mmol/L, which did not produce any effects on parameters of AP (*n*=5, data not shown).

Effects of Mox , Clo, Agm, and Xyl on AP of SAN pacemaker cells treated with yohimbine Yohimbine 1 μmol/L given with constant perfusion for 20 min did not affect the parameters of AP in the rabbit SAN pacemaker cells markedly (*n*=5, data not shown).

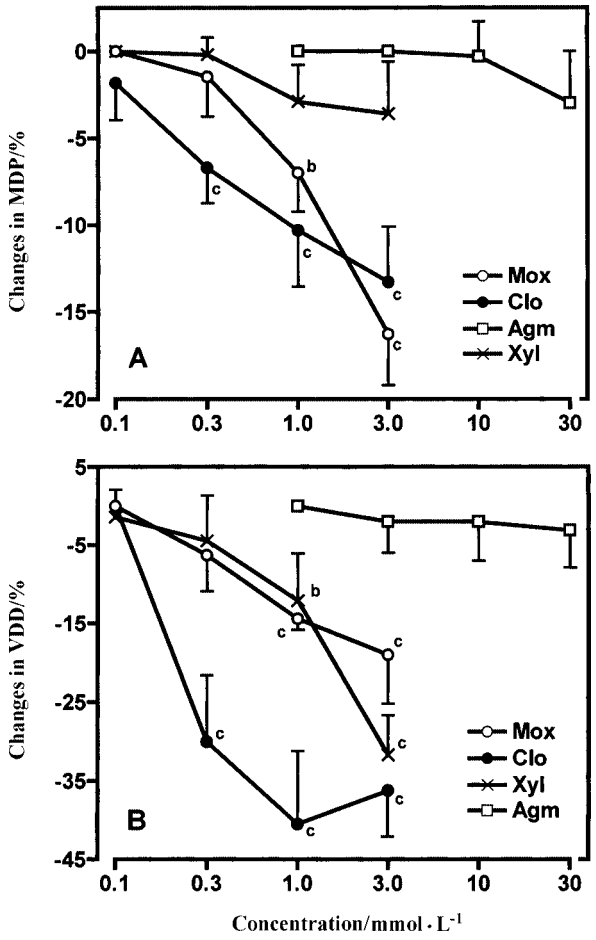

Fig 4. Concentration-dependent response curves for Mox, Clo, Xyl, and Agm on MDP (A) and VDD (B) of AP in the rabbit SAN pacemaker cells. *n*=5-6. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* before agonists.

After 20-min incubation with yohimbine, the preparations were exposed to Mox, Clo, Xyl, or Agm administered into the tissue bath at 0.1, 0.3, 1, and 3 mmol/L except Agm used at higher concentrations. Most effects of Mox on the AP in SAN pacemaker cells of the rabbit were abolished or inhibited by the treatment with yohimbine[13], and those of Clo were partially inhibited by yohimbine except its influence on MDP, which was almost abolished by yohimbine (Tab 2). On the other hand, the most effects of Xyl (Tab 3) and all the effects of Agm (*n*=6, data not shown) on the AP in SAN pacemaker cells were not affected by the treatment with yohimbine.

DISCUSSION

Recently, we speculated that Mox decreased VDD and RPF, and prolonged APD of the rabbit SAN pacemaker cells mainly via α_2-adrenoceptors[14], even though the agent is known as a highly selective agonist of I_1-receptors. In this study, we offered new evidence to show that Clo was similar to Mox to affect the AP of the rabbit SAN pacemaker cells via α_2-adrenoceptors partially, but the effects of Xyl and Agm on the AP were almost not related to α_2-adrenoceptors, and there were no obviously functional α_1-adrenoceptors regulating the AP in the rabbit SAN pacemaker cells.

It is well known that the functional α-adrenoceptors to respond to the vasoconstriction induced by sympathetic agents in the rabbit thoracic aorta belong to α_1-adrenoceptors only[11]. The vasoconstrictive responses to NE, Mox, and Clo of the rabbit thoracic aorta in the present experiments indicated that Mox and Clo activated α_1-adrenoceptors to produce vasoconstriction in the preparations, but Clo might not be a full agonist because its maximal response was much less than that of Mox or NE. Clo behaved as a partial agonist in rabbit pulmonary artery[15]. We did not find any stimulating activities by Agm (an endogenous ligand for I-/α_2-receptors) and Xyl (a selective agonist of α_2-adrenoceptors) in the rabbit thoracic aorta. For comparison, another study was carried out on the rabbit ear vein, which contained predominantly functional α_2-adrenoceptors[12]. NE, Mox, Clo, and Xyl produced concentration-dependent vasoconstriction in the vein preparations, and the rank order of potency was Clo> NE>Xyl=Mox. Mox, Clo, and Agm used in the present study were imidazoline-like agents, but Agm even up to the concentration of 10 mmol/L did not cause any vascular responses, which was quite different from Clo and Mox.

Mox, Clo, Agm, and Xyl concentration-dependently decreased RPF, and prolonged APD_{50} and APD_{90} in the rabbit SAN pacemaker cells. Mox, Clo, and Xyl also concentration-dependently decreased VDD. The rank order of decreasing RPF or prolonging APD was Clo>Xyl=Mox, which was similar to that in the rabbit ear vein. However, a particular point to note is that the threshold concentrations of Mox, Clo and Xyl to produce obvious effects on AP in the rabbit SAN pacemaker cells were much higher than their maximal concentrations to produce vasoconstriction in the rabbit thoracic aorta and ear vein. On the other hand, the effects of Clo on MDP, APD and RPF were partially inhibited by pretreatment with yohimbine, but yohimbine did not affect the electrophysiological responses to Xyl almost, indicating that the effects of Xyl on the

Tab 2. Effects of clonidine (Clo) on action potential in the rabbit sinoatrial node pacemaker cells untreated or treated with yohimbin 1.0 mol/L. *n*=6. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* corresponding control. [e]*P*<0.05, [f]*P*<0.01 *vs* Clo at the same concentration in the preparations untreated with yohimbine (unpaired *t*-test used to compare the percentage changes).

Clo/mmol·L^{-1}	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	APD_{50}/ms	APD_{90}/ms	RPF/min
Untreated preparations							
Control	-56±6	63±9	4.1±1.6	56±9	112±15	163±16	160±15
Clo (0.1)	-57±8	64±8	4.4±1.6	55±11	112±14	163±16	157±18
Control	-58±4	63±5	4.2±1.1	54±8	111±9	164±22	160±19
Clo (0.3)	-62±4[c]	67±4[c]	5.3±0.9[c]	37±7[c]	122±10[c]	180±21[c]	140±10[c]
Control	-55±4	65±5	4.0±1.3	55±10	109±6	164±11	163±17
Clo (1.0)	-60±5[b]	73±4[c]	6.4±2.0[c]	32±3[c]	125±8[c]	194±23[c]	131±23[c]
Control	-56±5	65±5	4.1±1.1	55±12	111±6	165±12	162±12
Clo (3.0)	-63±6[c]	73±5[c]	6.1±2.1[c]	35±9[c]	136±8[c]	203±11[c]	128±14[c]
Preparations treated with yohimbine							
Control	-58±3	61±6	4.1±1.6	54±12	119±10	170±17	155±13
Clo (0.1)	-58±4	61±6	4.1±1.6	54±12	119±10	170±17	153±13
Control	-59±3	62±6	4.1±1.6	54±11	120±13	171±17	157±18
Clo (0.3)	-59±3	64±6[c]	4.7±1.8[c]	40±11[c]	125±17[bf]	180±17[ce]	140±18[c]
Control	-60±2	62±5	4.3±1.4	54±13	121±12	170±13	156±15
Clo (1.0)	-60±4	66±6[c]	4.9±2.0[be]	33±11[c]	137±11[c]	200±18[c]	128±18[c]
Control	-59±4	60±6	4.2±0.8	53±8	120±10	170±14	151±19
Clo (3.0)	-61±5[b]	68±4[be]	5.1±1.1[b]	31±8[c]	140±15[c]	204±9[c]	132±15[ce]

MDP: maximal diastolic potential; APA: action potential amplitude; VDD: velocity of diastolic (phase 4) depolarization.

Tab 3. Effects of xylazine (Xyl) on action potential in the rabbit sinoatrial node pacemaker cells untreated or treated with yohimbin 1.0 mol/L. *n*=6. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* corresponding control. [e]*P*<0.05 *vs* Xyl at the same concentration in the preparations untreated with yohimbine (unpaired *t*-test used to compare the percentage changes).

Xyl/mmol·L^{-1}	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	APD_{50}/ms	APD_{90}/ms	RPF/min
Untreated preparations							
Control	-58±4	70±7	5.8±1.3	51±7	119±15	172±22	159±23
Xyl (0.1)	-59±3	70±7	5.8±1.4	50±6	120±14	174±20	158±26
Control	-59±4	70±7	5.7±1.3	51±7	117±15	170±23	162±25
Xyl (0.3)	-60±4	69±8	5.4±1.4[b]	49±5	122±16[b]	180±24[b]	152±26[c]
Control	-59±4	69±8	5.7±1.5	53±9	118±15	170±24	160±22
Xyl (1.0)	-60±3	68±9	5.3±1.6[c]	47±10[b]	128±15[c]	186±23[c]	147±25[c]
Control	-58±5	69±8	6.1±1.6	53±10	119±16	171±22	158±23
Xyl (3.0)	-60±2	67±7[b]	4.8±1.8[c]	36±11[c]	141±12[c]	207±16[c]	130±22[c]
Preparations treated with yohimbine							
Control	-61±3	67±5	5.1±1.9	56±13	128±14	180±20	141±16
Xyl (0.1)	-62±3	67±5	5.1±1.8	55±13	129±16	180±20	141±15
Control	-61±3	67±4	5.1±1.5	56±13	130±15	180±20	142±17
Xyl (0.3)	-61±3	67±4	5.0±1.6	52±13[b]	138±20[b]	191±26[b]	136±19
Control	-62±3	68±5	5.1±1.4	58±15	128±15	180±21	143±16
Xyl (1.0)	-63±3	67±4	4.4±1.2[c]	50±15[c]	143±20[c]	202±28[c]	133±14[c]
Control	-62±3	67±5	5.0±1.2	58±12	130±16	181±22	141±14
Xyl (3.0)	-63±3	66±6	4.0±1.2[c]	44±16[c]	154±22[c]	220±34[c]	128±10[ce]

AP were not related to α_2-adrenoceptors. It has been reported[16] that Xyl had similar effects to Clo in the rat isolated working heart, and yohimbine did not antagonize the negative chronotropic effect of Xyl.

Yohimbine did not affect the electrophysiological responses to Agm in the rabbit SAN pacemaker cells, indicating that the action of Agm on AP did not relate to α_2-adrenoceptors directly. Even though Agm is a ligand to α_2-adrenoceptors, it does not appear to have any directly post-junctional effects in the rat tail artery that contains functional α_2-adrenoceptors[8]. Li *et al* reported[9] that Agm not only decreased RPF, but also prolonged APD in a concentration-dependent manner in the rabbit SAN pacemaker cells, and considered that the electrophysiological responses to Agm were mediated by I-/α_2-receptor because those responses to Agm were significantly inhibited by idazoxan, a mixed I_1-/α_2-receptor antagonist. EI-Ayoubi *et al*[17] used membrane binding assays, autoradiography, immunolocalization and immunoblotting to identify I_1-receptors in the hearts obtained from the animals with hypertension, heart failure or from normal animals, and their results demonstrated the presence of I_1-receptors in the hearts and upregulated I_1-receptors in the hearts suffered from cardiovascular diseases. Yohimbine-insensitive electrophysiological responses to imidazolines and Agm remains to be identified further.

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