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A ganglionic stimulant, 1,1-dimethyl-4-phenylpiperazinium, caused both cholinergic and adrenergic responses in the isolated mouse atrium

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ABSTRACT

An isolated atrial preparation of the mouse is useful for analyzing the actions of drugs on the myocardium, autonomic neurons and endocardial endothelium. The aim of the present study was to examine the functions of intrinsic neurons of the atrium using a ganglionic stimulant, 1,1-dimethyl-4-phenylpiperazinium (DMPP). DMPP (1–100 μM) caused a negative chronotropic action followed by a positive chronotropic action in spontaneously beating right atria and also caused biphasic inotropic actions consisting of initial inhibition followed by potentiation of electrical field stimulation (EFS)-induced contraction in the left atria. Inotropic actions in the left atria induced by DMPP were characterized using some autonomic drugs and M_2 and/or M_3 muscarinic receptor knockout (M_2R-KO, M_3R-KO and M_2M_3R-KO) mice. Atropine and hexamethonium decreased the initial negative inotropic actions of DMPP. In the atria from pertussis toxin-treated, M_2R-KO and M_2/M_3R-KO mice, the negative inotropic actions were abolished. On the other hand, the following positive inotropic actions were decreased by hexamethonium, atropine and atenolol. In the atria from reserpine-treated mice, positive inotropic actions were also decreased. The positive inotropic action induced by DMPP was almost the same in M_2R-KO mice but was reduced in both M_3R-KO mice and M_2/M_3R-KO mice. In conclusion, DMPP caused biphasic inotropic/chronotropic actions in the mouse atrium through activation of intrinsic cholinergic and adrenergic neurons. M_2 and M_3 muscarinic receptors and β_1-adrenoceptor are thought to be involved in these actions.

1. Introduction

Cardiac functions (contraction force, heart rate and conduction velocity) are regulated by sympathetic and parasympathetic neurons. Activation of sympathetic neurons stimulates cardiac function through release of noradrenaline, while activation of parasympathetic neurons decreases cardiac contraction and heart rate through release of acetylcholine. In general, sympathetic preganglionic fibers make their synapses near the spinal cord and postganglionic fibers arising from the ganglia innervate cardiac tissue, whereas the parasympathetic nervous system has its ganglia within organs (intrinsic cardiac ganglia, Levy and Martin, 1981; Loffelholz and Pappano, 1985). Intrinsic cardiac ganglia were initially regarded as simple relay stations in parasympathetic preganglionic cholinergic neurons to postganglionic cholinergic neurons innervating the myocardium. However, cardiac ganglia have been demonstrated to have a complex neurochemical phenotype different from the classical view of postganglionic cholinergic neurons. Morphological study indicated that some cholinergic nerve cell bodies contained noradrenergic neurons markers, such as tyrosine hydroxylase, dopamine-β-hydroxylase and noradrenaline transporters. The presence of such hybrid intrinsic cardiac neurons has been reported in several animal species (Baluk and Gabella, 1990; Hoard et al., 2007, 2008; Hoover et al., 2009; Rysevaite et al., 2011; Weihe et al., 2005). Therefore, regulation of cardiac contractility through these kinds of intrinsic neurons might be interesting, but the functional role of these neurons has not been clarified in detail.

The isolated atrium is widely used to examine inotropic and chronotropic actions of bioactive substances. Using mice atria, we demonstrated that carbachol caused biphasic inotropic actions consisting of initial negative actions (M_2 muscarinic receptor) followed by slowly developed positive actions (M_3 muscarinic

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receptor, Kitazawa et al., 2009). M_3 receptors were demonstrated to be expressed on the endocardial endothelium, and cyclooxygenase-2/prostaglandins pathways were shown to be involved in downstream of M_3 receptor activation (Harada et al., 2012). Analysis of electrical field stimulation (EFS)-induced responses of isolated visceral organs has been used to identify functional innervation. For example, analysis of EFS-induced responses indicated functional innervation of the gastrointestinal tract (Burnstock, 1972). Similarly, nitrergic nerves were demonstrated in blood vessels using EFS (Toda and Okamura, 2003). EFS has also been applied to isolated atria of rats and guinea-pigs, and characterization of evoked responses suggested inhibitory cholinergic and excitatory adrenergic innervation of cardiac tissues (Goto et al., 1987; Saito et al., 1986). However, EFS can excite all neural components in cardiac tissues (intrinsic neurons, efferent and sensory neurons) comprehensively and, therefore, EFS might not be a suitable stimulation for analyzing the function of intrinsic neurons in the mouse heart. Effects of ganglionic stimulants on the heart rate of rodents in vitro have been reported. However, the responses have not been characterized in detail (Nakatani et al., 1994; Wong, 1994).

The present study was designed to elucidate the function of cardiac intrinsic neurons in the mouse atrium. To accomplish the objectives, intrinsic neurons expressing the nicotinic receptor were excited by a ganglionic stimulant, 1,1-dimethyl-4-phenylpiperazinium (DMPP), and the evoked mechanical actions were characterized. Atria from reserpine- or pertussis toxin-treated mice and muscarinic receptor knockout mice were also used for further characterization of the DMPP-induced responses.

2. Materials and methods

2.1. Animals and tissue preparations

All experiments were performed in accordance with the institutional guidelines approved by the Animal Ethics Committee of Rakuno Gakuen University, Ebetsu, Hokkaido, Japan.

Wild-type mice (DDY strain; Sankyo Lab Service, Sapporo, Japan) and mice lacking either the M_2 muscarinic receptor (M_2R-KO) or the M_3 muscarinic receptor (M_3R-KO) or both M_2 and M_3 muscarinic receptors (M_2/M_3R-KO) were used in the present experiments (either male or female). The generation of M_2R-KO, M_3R-KO and M_2/M_3R-KO mice has been described previously (Gomeza et al., 1999; Struckmann et al., 2003; Yamada et al., 2001). The genetic backgrounds of the mice used in the present study were 129J1 (50%) × CF1 (50%) for M_2R-KO mice, 129vEv (50%) × CF1 (50%) for M_3R-KO mice, and 129J1 (25%) × 129SvEv (25%) × CF1 (50%) for M_2/M_3R-KO mice. The animals were housed in ventilated-polycarbonate cages. The temperature of the animal room was maintained at 23 ± 1 °C with relative humidity of 40%–60% and a daily light/dark cycle (7:00 am–7:00 pm). Food (CRF-1, Oriental Yeast Co Ltd, Japan) and water were given ad libitum.

Adult mice that were more than 3 months old (weight: 23–30 g) were killed by cervical dislocation. The beating heart was isolated from each animal and immersed in warmed and bubbling Krebs solution (NaCl, 118 mM; KCl, 4.75 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; $NaHCO_2$, 25 mM; and glucose, 11.5 mM, 37 °C, gassed with 95% O_2 + 5% CO_2). To induce myocardial contraction, the left atrium was placed between a pair of platinum rod electrodes and suspended vertically in an organ bath. The end of the preparation was tied and connected to a force–displacement transducer. EFS (1 Hz, 2 ms in duration, 1.5 × threshold voltage; Kitazawa et al., 2009; Harada et al., 2012) was applied by an electrical stimulator. After steady EFS-induced contraction had been established, several drugs including DMPP were applied to the organ bath and the evoked responses were observed. In experiments for observation of chronotropic action, one end of the beating right atrium was attached with thread to a stationary glass rod, and the opposite end was tied with thread to a force–displacement transducer to record spontaneous contraction.

2.2. Experimental protocols

Inotropic actions: After establishment of steady EFS-induced contraction (generally 60–70 min of equilibration time) of the left atrium, DMPP (1–100 μM) was added to the organ bath and its effects on EFS-induced contraction was observed for 15 min. After determining the concentration–response relationships of DMPP, pharmacological properties of the DMPP-induced inotropic actions were characterized using several autonomic drugs. Since the inotropic actions induced by DMPP (100 μM) were not reproducible even at 1-h-interval application in this experimental condition, DMPP was applied only one time in one preparation. In brief, atrial preparations were treated with respective autonomic drugs for 15 min and then DMPP was applied and its inotropic action was compared with that in non-treated atria (control). To examine the involvement of the M_2 muscarinic receptor in DMPP-induced actions, DDY mice were treated with pertussis toxin (300 μg/kg, i.p.) 96 h prior to experiments. Pertussis toxin has been shown to abolish M_2 receptor-mediated negative chronotropic and inotropic actions in the mouse atrium though inhibition of G_i protein function (Kitazawa et al., 2009). Treatment with pertussis toxin did not cause any obvious behavioral changes or toxic effects. After 96 h, atria were isolated and the inotropic actions induced by DMPP were compared with those in control atria from non-treated mice. Involvement of adrenergic neurons in DMPP-induced actions was examined using atria from reserpine-treated mice. Reserpine inhibits adrenergic neural function through depletion of noradrenaline contents (Martínez-Olivares et al., 2006). Reserpine was injected (10 mg/kg, i.p.) and atria were isolated 18 h later, and the effects of DMPP were compared with the control. Catalepsy was observed in all mice treated with reserpine. DMPP-induced inotropic actions were also examined in atria from muscarinic receptor knockout mice (M_2R-KO, M_3R-KO and M_2/M_3R-KO mice), and muscarinic receptor subtypes involved in the actions were characterized.

Chronotropic actions: Spontaneously beating right atria from control (non-treated) mice, pertussis toxin-treated mice, reserpine-treated mice and muscarinic receptor knockout mice were suspended vertically in an organ bath and equilibrated for 1 h. DMPP (1–100 μM) was applied, and its effects on both heart rate and contraction amplitude were analyzed as chronotropic and inotropic actions. Similar to the case of the left atrium, the DMPP-induced chronotropic action at 100 μM was not reproducible at 1-h-interval application. Therefore, 100 μM DMPP was applied one time in each atrium for characterization of pharmacological properties. In brief, atrial preparations were treated with respective autonomic drugs for 15 min and then DMPP (100 μM) was applied to compare the induced actions in the presence and absence of drugs.

2.3. Chemicals

The following chemicals were used: Atenolol (Sigma), atropine sulfate (Wako Pure Chemicals, Osaka, Japan), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, Sigma), hexamethonium chloride dehydrate (Wako), physostigmine sulfate (Wako), reserpine (Apoplon, Daiichi Sankyo, Tokyo, Japan) and pertussis toxin (Wako). Pertussis toxin was dissolved in sterilized saline and was