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Effects of dopamine on L-type Ca^{2+} current in single atrial and ventricular myocytes of the rat

*H. Zhao, [1]S. Matsuoka, **Y. Fujioka & A. Noma

Departments of Physiology and **Cardiovascular Surgery, Faculty of Medicine, Kyoto University, Kyoto 606-01, Japan and *Department of Anatomy and K.K. Leung Brain Research Centre, The 4th Military Medical University, Xian 710032, People's Republic of China

1 The effects of dopamine on the L-type Ca^{2+} current ($I_{Ca,L}$) of both atrial and ventricular single myocytes and on the force of contraction of atrial trabeculae in rat heart were investigated.

2 Dopamine increased atrial $I_{Ca,L}$ at concentrations higher than 1 μM, but had little or no effect on $I_{Ca,L}$ at lower concentrations. The increase in $I_{Ca,L}$ at high concentrations was reversed by propranolol and acetylcholine, but not by phentolamine. Activation and inactivation kinetics of $I_{Ca,L}$ were not altered by dopamine.

3 In rat ventricular myocytes in which the D_4 receptor mRNA does not express, dopamine (20–100 μM) also increased the $I_{Ca,L}$ amplitude and propranolol reversed this effect.

4 Clozapine, a potent D_4 receptor antagonist, blocked the augmenting effect of dopamine on $I_{Ca,L}$. However, this effect could be explained by β-antagonism, since clozapine also inhibited the isoprenaline effect.

5 In the atrial trabeculae, the increase in contraction by dopamine (1 to 30 μM) was reversed by 1 μM propranolol, but not by 2 μM phentolamine. Low doses of dopamine (0.01 to 0.3 μM) did not affect the contraction in the controls or during a modest stimulation of the β-adrenoceptor with 0.01 μM isoprenaline.

6 These results indicate that the positive inotropic action of dopamine is mediated through direct stimulation of the β-adrenoceptor in both atrial and ventricular myocytes. Involvement of D_4 receptor appears unlikely in the regulation of the atrial contraction.

Keywords: Cardiac myocytes; dopamine; β-adrenoceptor; positive inotropy; D_4 receptor

Introduction

It is well known that myocardial contractility is greatly increased by high concentrations ($>10^{-5}$ M) of dopamine. This positive inotropic effect has been ascribed to either direct β-adrenoceptor agonism in myocytes or indirect stimulation of β-adrenoceptors via release of noradrenaline from nerve endings within the cardiac tissue (guinea-pig; Mugelli *et al*., 1977; Brown, 1990), and/or α-adrenoceptor agonism (rabbits and man; Brodde *et al*., 1980; Wagner *et al*., 1980). The positive chronotropic effect of dopamine has also been described and attributed to β_1-adrenoceptors in guinea-pig cardiac myocytes (Lumley *et al*., 1977). However, the involvement of dopamine receptors has not been identified in either inotropy or chronotropy (Lumley *et al*., 1977; Motomura *et al*., 1978; Martinez-Mir *et al*., 1987).

Recently, molecular cloning techniques have revealed a diversity of dopamine receptor subtypes (D_1, D_2, D_3, D_4 and D_5) in the central nervous system (see review by Civelli *et al*., 1993; Gingrich *et al*., 1993). In peripheral tissues, the mRNA of D_4 receptor is expressed in rat atrium (O'Malley *et al*., 1992). Since dopamine binds to the D_4 receptor with a high affinity (dissociation constant (K_d) = 30 nM, Seeman & Van Tol, 1994), the D_4 receptor may mediate dopamine action in the cardiovascular system as well as in the central nervous system.

The aim of the present study was to examine if the direct effects of dopamine on cardiac myocytes are mediated only through β- or α-adrenoceptors, and also to determine the involvement of the D_4 receptor. Interestingly, the mRNA of the D_4 receptor in rat heart has been shown to exist in the atrium, but not in the ventricle (O'Malley *et al*., 1992; Amenta *et al*., 1995). In the present study, dopamine effects were investigated mainly by recording the L-type Ca^{2+} current ($I_{Ca,L}$) of rat atrial and ventricular myocytes, because $I_{Ca,L}$ plays a pivotal role in mediating the chronotropic as well as inotropic regulation of the heart. To our knowledge, effects of dopamine on cardiac $I_{Ca,L}$ in dissociated myocytes have not been reported, except for the abstract form by Habuchi *et al*. (1995), who showed that 1 or 10 μM dopamine did not affect $I_{Ca,L}$ in rat ventricular, rabbit atrial and sinoatrial node cells. In mouse photoreceptor and in heterologous expression systems, negative coupling of D_4 receptor to adenylate cyclase has been found (Cohen *et al*., 1992; Chio *et al*., 1994; Seabrook *et al*., 1994; McHale *et al*., 1994; Asghari *et al*., 1995). Therefore, the developed tension of the atrial trabeculae was also recorded to examine possible coupling of the D_4 receptor to adenylate cyclase. It was demonstrated that dopamine increases both rat atrial and ventricular $I_{Ca,L}$ by direct stimulation of the β-adrenoceptor and that the D_4 receptor is not involved in regulation of atrial contraction.

Methods

Isolation of atrial and ventricular myocytes

The atrial and ventricular myocytes were dissociated by treating rat heart with collagenase. Since the atrial tissue could not be well perfused by Langendorff perfusion, we developed an alternative technique. Wistar rats of 200–300 g body weight were deeply anaesthetized by intraperitoneal injection of pentobarbitone sodium (>10 mg 100 g^{-1} body weight). The chest was opened under artificial respiration and a syringe needle (27 G) was inserted into the right atrium to perfuse the atrial cavity with a control Tyrode solution. The perfusion line

[1] Author for correspondence at: Department of Physiology, Faculty of Medicine, Kyoto University, Yoshida-Konoe, Sakyo-ku, Kyoto 606-01 Japan.

connected to the needle was included in a water jacket (37°C). The superior and inferior vena cava were clamped and the ascending aorta was cut to drain the perfusate, which passed through the lung. The perfusion was firstly with the control Tyrode solution, then with a Ca^{2+}-free Tyrode solution until the atrial beat stopped. The atrium was treated for 5–10 min with the Ca^{2+}-free Tyrode solution containing trypsin (5 mg 50 ml^{-1}, Sigma) to remove the endocardial endothelium. Then, the Ca^{2+}-free Tyrode solution containing collagenase (50 mg 50 ml^{-1}, WAKO, Japan) was perfused for 20–30 min. After enzymatic digestion, single cells were dissociated in a high-K^+, low-Cl^- solution and stored at room temperature.

For isolation of single ventricular myocytes, the conventional coronary perfusion method was employed by use of Langendorff type apparatus (Powell *et al*., 1980). The heart was perfused firstly with the control Tyrode solution, then with nominally Ca^{2+}-free Tyrode solution until heart beat stopped, and finally with Ca^{2+}-free Tyrode solution containing collagenase (40–70 mg 100 ml^{-1}, WAKO) for 15 min. The heart was placed in the high-K^+, low-Cl^- solution, then isolated cells were stored in the 5 mM HEPES-buffered MEM solution (Dainippon Pharmaceutical Co., Ltd., Japan, pH = 7.4).

Solutions

The control Tyrode solution contained (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES 5; pH was adjusted to 7.4 with NaOH. The high-K^+, low-Cl^- solution used to maintain the dissociated myocytes contained KCl 30, glutamate 70, KH_2PO_4 10, $MgCl_2$ 1, taurine 20, EGTA 0.3, glucose 10 and HEPES 10, adjusted to pH 7.4 with KOH.

The composition of K^+-rich internal (pipette) solution was (mM): aspartate 120, KOH 120, MgATP 5, EGTA 5, HEPES 5, GTP 0.2, TEA-Cl 10, (pH = 7.2 with KOH). To suppress membrane K^+ conductance, a Cs^+-rich internal solution was used, which was prepared by replacing KOH with CsOH in the K^+-rich pipette solution. The control Tyrode solution containing an additional 5 mM CsCl was used as the external solution during the whole cell voltage clamp.

Chemicals used for experiments were: acetylcholine (Sigma), (±)-isoprenaline (Sigma), (±)-propranolol (Sigma), phentolamine (Sigma), clozapine (Research Biochemicals International (RBI)) and dopamine (RBI). One to 5 mM stock solutions for these chemicals were made. Ascorbic acid 1 and 5 mM was added to the isoprenaline and the dopamine stock solutions, respectively, to prevent oxidization.

Whole-cell voltage clamp

The whole-cell voltage clamp was conducted with a patch clamp amplifier (TM-1000, Act Med, Japan or AXOPATCH200A, Axon Instruments, Inc., U.S.A.). The patch electrodes were made from borosilicate glass capillaries and the tip resistance was 2–4 MΩ when filled with the pipette solution. The current signal was filtered by a low-pass filter at 5 kHz. The membrane voltage and currents were recorded by an on-line computer (PC-9821AP, NEC, Japan) through an A/D converter (ADX-98H, Canopus, Japan) and by a digital tape recorder (RD-120T, TEAC, JAPAN).

The measurements of membrane potential were corrected for the liquid junction potential of the low-Cl^- pipette solution in contact with Tyrode solution (~ −10 mV).

Tension measurement

Man-made strands, approximately 1 × 5 mm, were dissected by cutting the atrial wall along a trabecula, which showed no obvious bifurcation. One end of the strand was fixed in a recording chamber, and the other end was ligated and connected to a rod of strain gauge transducer. The recording bath was perfused with the control Tyrode solution. The resting muscle length was adjusted to yield ~80% of the maximal contractile

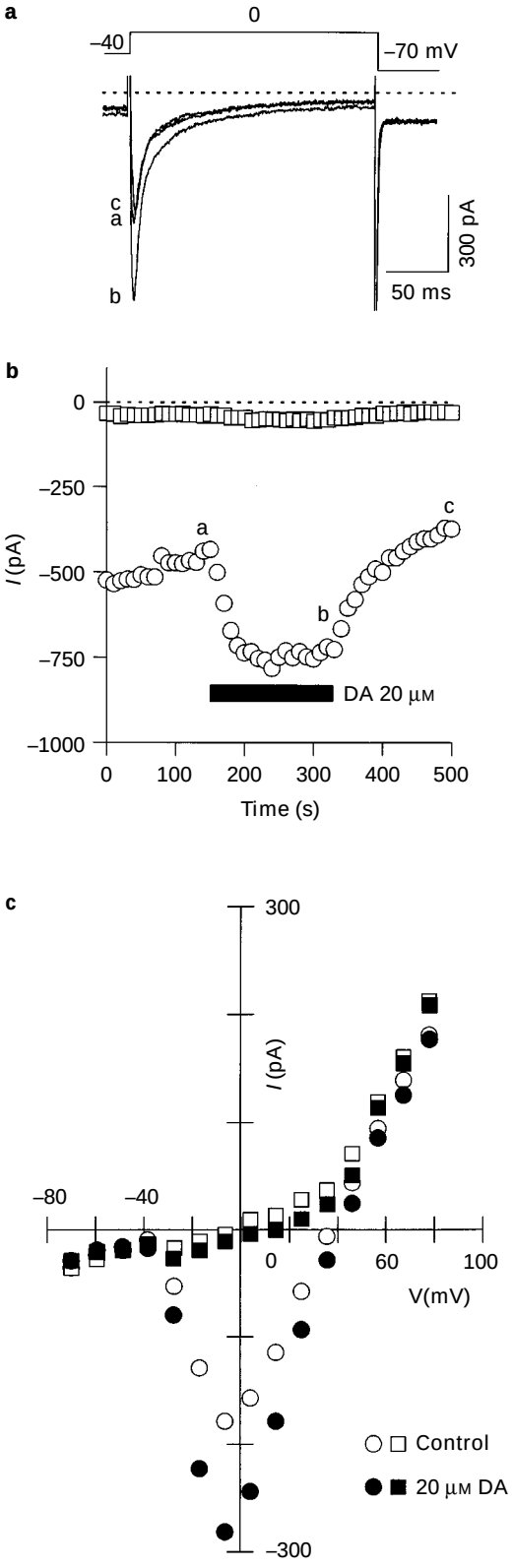

Figure 1 Effects of dopamine on $I_{Ca,L}$ in an atrial myocyte. (**a**) Original recordings of $I_{Ca,L}$ obtained before (a), during (b) and after (c) application of 20 μM dopamine are superimposed. Holding potential was −70 mV and test pulse to 0 mV was applied after a prepulse to −40 mV (100 ms in duration). Dotted line indicates zero current level (**b**). Time course of changes in amplitude of $I_{Ca,L}$ (○), which was measured as a difference between peak and steady current level (200 ms after onset of test pulse). Changes in steady current level (□) are also shown. (a), (b) and (c) indicate time of recording corresponding currents shown in (**a**). Application of dopamine (DA) is indicated by a thick bar. (**c**) *I*-V relationship of peak (○, ●) and steady current (□, ■). Open and filled symbols indicate control and current in the presence of 20 μM dopamine, respectively.

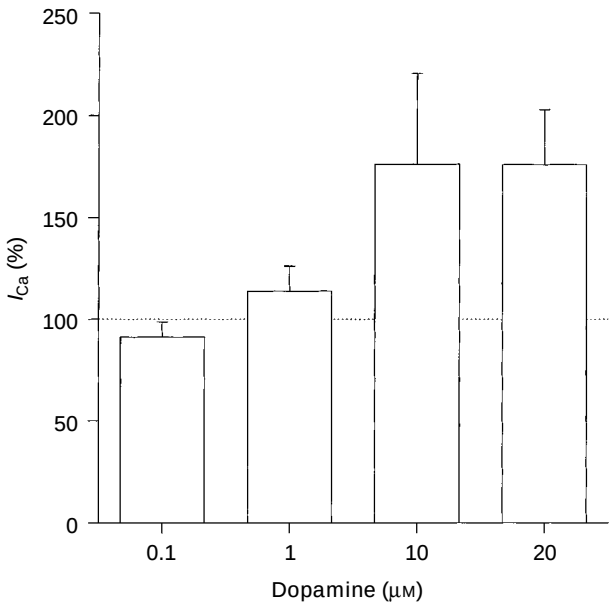

Figure 2 Dose-dependence of dopamine effect on atrial $I_{Ca,L}$. Amplitude of $I_{Ca,L}$ was normalized by control record obtained before application of the drug in each experiment. Data were (mean ± s.d.), 91 ± 7.5% ($n=4$), 114 ± 12.3% ($n=6$), 176 ± 44% ($n=8$) and 176 ± 26.8% ($n=14$) at 0.1, 1, 10 and 20 μM dopamine, respectively.

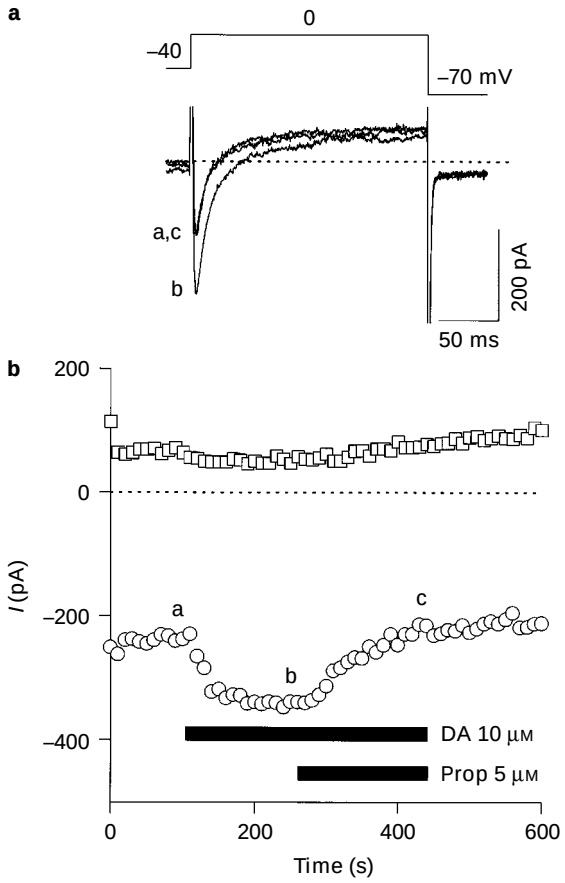

Figure 3 Effect of propranolol on atrial $I_{Ca,L}$ enhanced by dopamine. (**a**) Original current traces obtained in control (a), during 10 μM dopamine (b) and 10 μM dopamine + 5 μM propranolol (c). (**b**) Time course of inhibition of dopamine (DA)-enhanced $I_{Ca,L}$ by propranolol (Prop). As in Figure 1b, (○) the amplitude of $I_{Ca,L}$ and (□) steady current.

force and the trabeculae was stimulated as 1 Hz throughout the experiment.

All experiments were carried out at 37°C. Results are presented as mean ± s.d.

Results

Enhancement of $I_{Ca,L}$ *by dopamine*

The effects of dopamine on $I_{Ca,L}$ were first studied in rat single atrial myocytes. In order to isolate $I_{Ca,L}$, the membrane K^+ conductance was minimized by use of the Cs^+-rich pipette solution and by adding 5 mM Cs^+ to the bath solution. The Na^+ current and the transient outward current were inactivated by a prepulse to −40 mV from −70 mV (100 ms in duration) before applying the test pulse (0 mV). Figure 1 demonstrates a set of representative current recordings (Figure 1a) and the time course of the dopamine effect (Figure 1b). The amplitude of $I_{Ca,L}$, measured as the distance from the peak to the current level 200 ms after the test pulses, gradually increased after exposure to 20 μM dopamine and saturated within approximately 1 min. Simultaneously, the steady current level at 200 ms shifted slightly inward. These effects were reversed upon washing out of the dopamine.

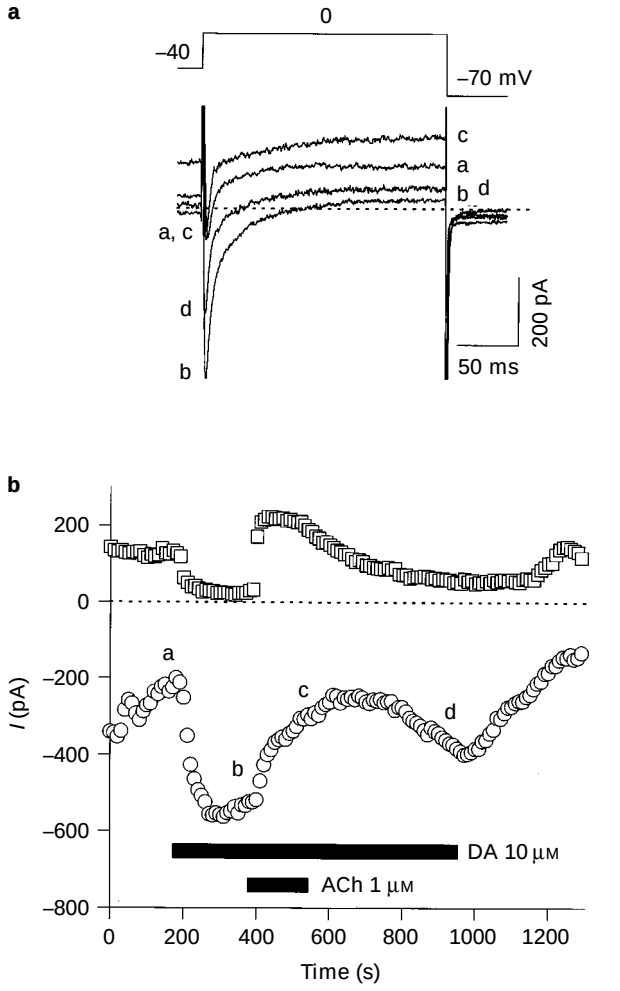

Figure 4 Effects of ACh on atrial $I_{Ca,L}$ enhanced by dopamine. (**a**) Original current traces obtained in control period (a), during 10 μM dopamine (b), 10 μM dopamine + 1 μM ACh (c) and with 10 μM dopamine after washing out ACh (d). (**b**) Time course of inhibition by ACh of dopamine (DA)-enhanced $I_{Ca,L}$. The K^+-rich pipette solution was used to record $I_{K,ACh}$. As in Figure 1b, (○) the amplitude of $I_{Ca,L}$ and (□) steady current.

With respect to the current-voltage (*I*-V) relationship measured before and during the application of dopamine (Figure 1c), voltage-dependent activation of $I_{Ca,L}$ was observed at more positive potentials than −40 mV and the peak amplitude was obtained between −10 and 0 mV in both the absence and the presence of dopamine. No obvious change was observed in either the background current at more negative potentials than −40 mV or in the outward rectification of the late current. Essentially the same findings were obtained in 6 experiments. In addition, the time-dependent activation and inactivation of $I_{Ca,L}$ were not significantly modified by dopamine ($n=4$, data not shown). These findings are consistent with the changes in $I_{Ca,L}$ observed on stimulation of β-adrenoceptors.

The dose-dependency of the dopamine effect is summarized in Figure 2. Enhancement of $I_{Ca,L}$ was observed only at concentrations higher than 1 μM. Thus, the concentrations of dopamine used to augment $I_{Ca,L}$ were much higher than the dissociation constant of dopamine for the D_4 receptor (30 nM, Seeman & Van Tol, 1994).

Receptor types involved in the effect of dopamine

The effect of dopamine on $I_{Ca,L}$ was antagonized by propranolol, a blocker of β-adrenoceptors. Figure 3 shows a representative experiment, in which 5 μM propranolol applied in addition to 10 μM dopamine completely reversed the preceding enhancement of $I_{Ca,L}$ by dopamine. Essentially the same findings were obtained in three experiments. On the other hand, 1 μM phentolamine, a blocker of α-adrenoceptors, failed to modify the enhancement of $I_{Ca,L}$ by 10 μM dopamine ($n=3$, data not shown).

These findings support the view that the increase in $I_{Ca,L}$ induced by high concentrations of dopamine is mediated by the β-adrenoceptor. This view was further confirmed by the accentuated antagonism of β-adrenoceptor stimulation by ACh. In the experiment shown in Figure 4, 1 μM ACh was applied in the presence of 10 μM dopamine. The dopamine-induced increase in $I_{Ca,L}$ was antagonized by ACh almost completely and in a reversible manner on washing out ACh

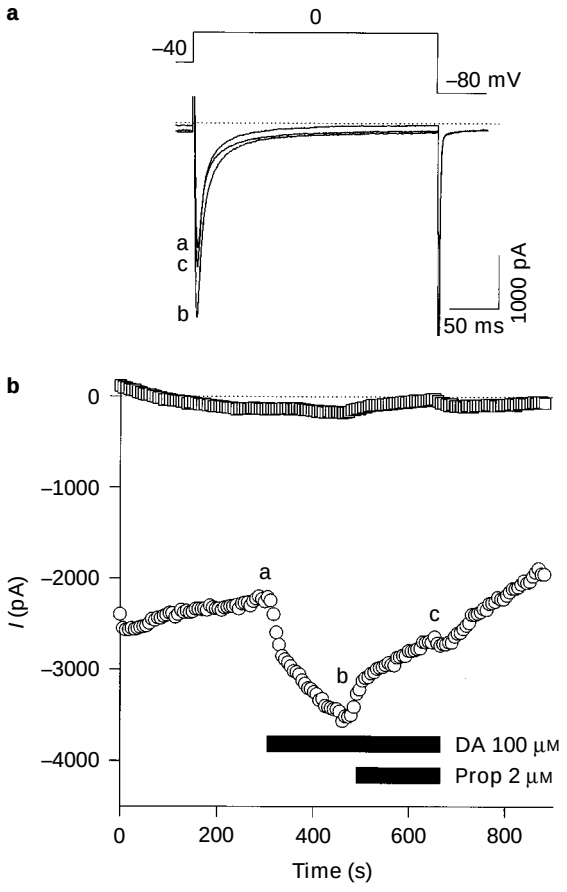

Figure 5 Enhancement of ventricular $I_{Ca,L}$ by dopamine and inhibition of the dopamine effect by propranolol. (**a**) Original current traces obtained in control (a), during 100 μM dopamine (b) and 100 μM dopamine +2 μM propranolol (c). (**b**) Time course of inhibition of dopamine (DA)-enhanced $I_{Ca,L}$ by propranolol (Prop). The amplitude of $I_{Ca,L}$ (○) and steady current (□).

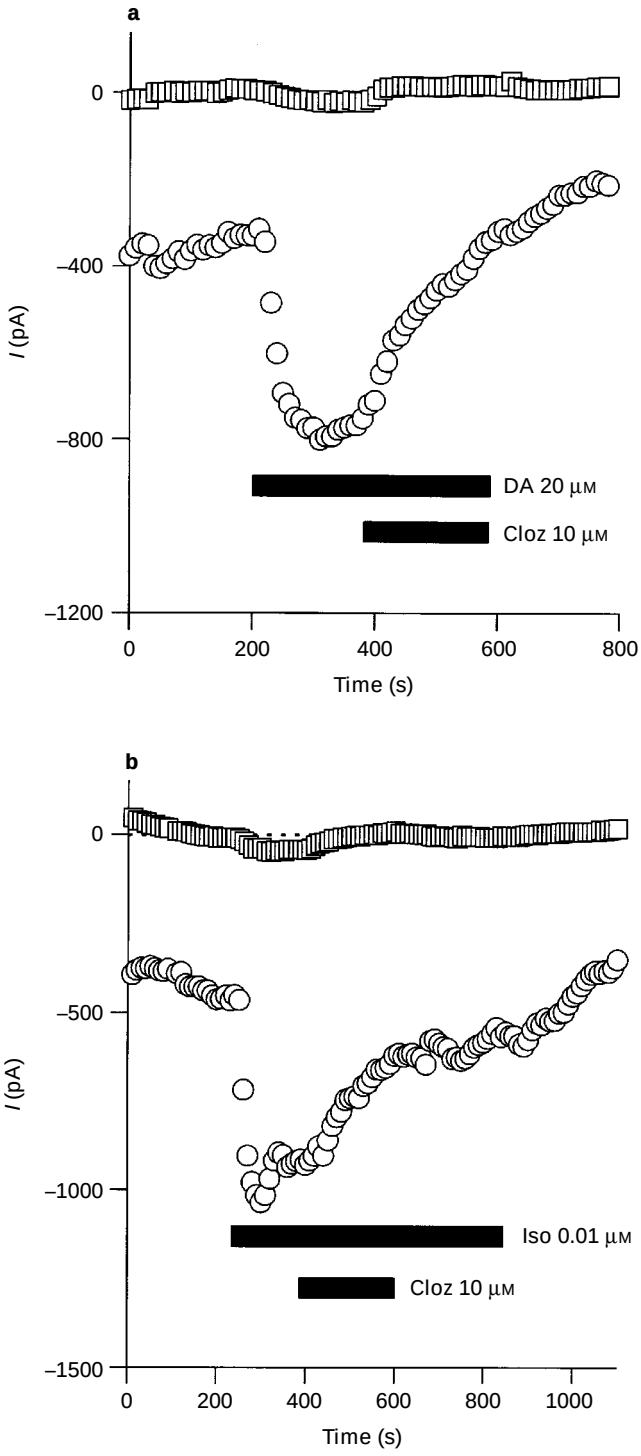

Figure 6 Effects of clozapine on atrial $I_{Ca,L}$ enhanced by dopamine (a) or isoprenaline (b). (a) Time course of change in amplitude $I_{Ca,L}$ (○) and late current (□) by 20 μM dopamine and 10 μM clozapine (Cloz). (b) Time course of change in amplitude of $I_{Ca,L}$ and late current by 0.01 μM isoprenaline (Iso) and 10 μM clozapine.

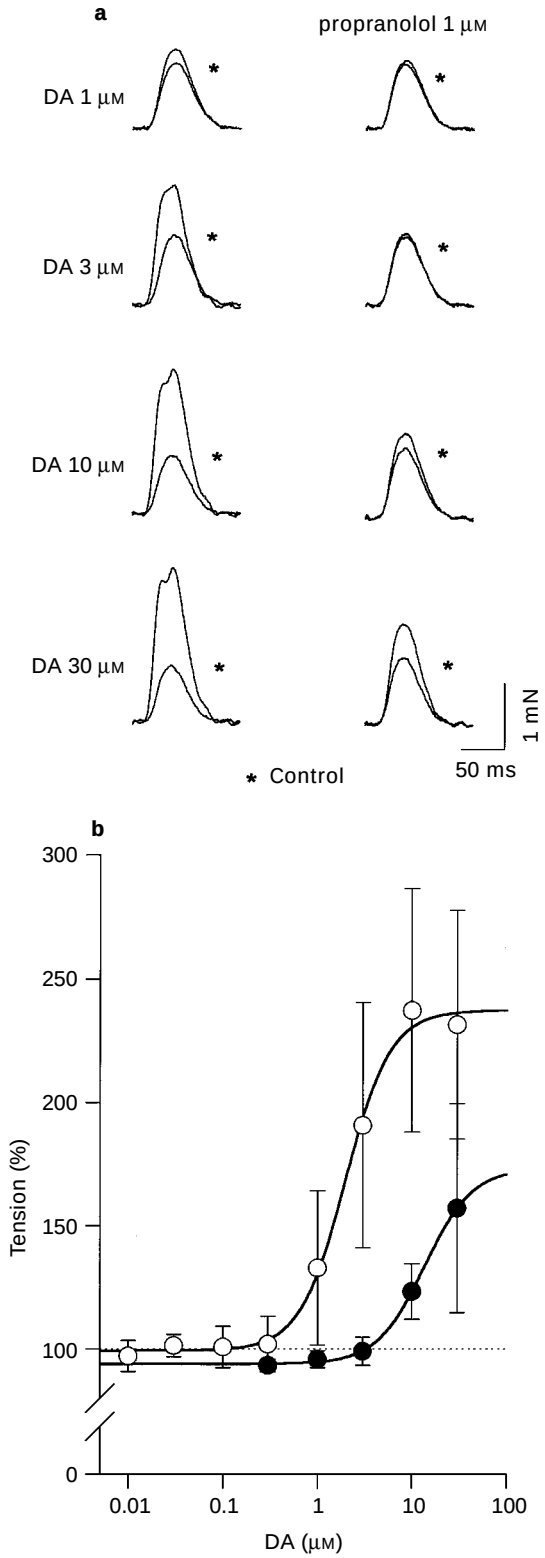

Figure 7 Enhancement of contraction of atrial trabecula by dopamine. (a) Original recordings of developed tension. Records in control (*) and in the presence of 1–30 μM dopamine are superimposed in left column. Same protocol was repeated in the continuous presence of 1 μM propranolol (right column). (b) Dopamine dose-contractile response relationship. The magnitude of developed tension was normalized to that obtained before applying dopamine in the absence (○, $n=4$) and presence (●, $n=3$) of 1 μM propranolol. Curves were fitted to Hill equations,

$$T = \frac{T_{max}}{1 + \left(\frac{K_{1/2}}{[Dopamine]}\right)^{n_H}}$$

where T is normalized magnitude of developed tension, T_{max} is maximum tension, $K_{1/2}$ is concentration of drug giving a half-maximal response, and n_H is Hill coefficient. In control, $K_{1/2}$ and n_H were 2.0 μM and 1.8, respectively. In the presence of propranolol, $K_{1/2}$ and n_H were 14 μM and 1.7, respectively. A dotted line shows the control level (100%).

($n=4$). To confirm the intactness of the muscarinic receptor, the K^+-rich pipette solution was used in this series of experiments in place of the Cs^+-rich solution. As evident in Figure 4, the application of ACh shifted the current at the end of the test pulse in the outward direction, indicating activation of the muscarinic K^+ current ($I_{K,ACh}$).

The results presented so far strongly indicate that dopamine at high doses ($\geqslant 1$ μM) directly stimulates β-adrenoceptors in rat atrial cells. Thus, the D_4 receptor does not seem to be involved in the regulation of $I_{Ca,L}$. The effect of dopamine on $I_{Ca,L}$ was further studied in rat ventricular cells, in which the mRNA of the D_4 receptor is not expressed (O'Malley *et al.*, 1992; Amenta *et al.*, 1995). As shown in Figure 5, dopamine (20–100 μM) increased $I_{Ca,L}$ and 2 μM propranolol antagonized the effect of 100 μM dopamine. This result also supports the notion of direct β-adrenoceptor stimulation by high doses of dopamine. Similar results were obtained in three experiments.

Effects of clozapine

The possible involvement of the D_4 receptor in the effect of dopamine was studied by use of clozapine which is a potent antagonist of the D_4 receptor with a high affinity (Coward, 1992; Seeman & Van Tol, 1994). As seen in Figure 6a, the increase in rat atrial $I_{Ca,L}$ by dopamine was reversed by the additional application of 10 μM clozapine ($n=3$). Clozapine at 1 μM failed to modify the effect of 20 μM dopamine and 5 μM clozapine induced an incomplete block of the dopamine effect ($n=3$). However, we found that clozapine at these high concentrations can also block the effect of isoprenaline on atrial $I_{Ca,L}$, as shown in Figure 6b. The application of 10 μM clozapine depressed atrial $I_{Ca,L}$, which had been increased by pretreatment with 0.01 μM isoprenaline ($n=3$). Thus, clozapine most likely blocks the interaction of dopamine with the β-adrenoceptor.

Effects of dopamine on atrial contraction

In order to search for any possible D_4 receptor mediated dopamine action, the muscle contraction was measured in the atrial trabeculae. Dopamine increased the force of contraction at concentrations higher than 1 μM as shown in Figure 7. However, the enhancement of contraction was blocked by pretreatment with 1 μM propranolol. Figure 7b summarizes the effects of dopamine on the contraction in the absence and presence of 1 μM propranolol. Notably, the force of contraction was not affected by 0.01 to 0.3 μM dopamine, which should be high enough to activate the D_4 receptor. Whereas 1 to 30 μM dopamine increased the contraction in a dose-dependent manner, propranolol induced a right-ward shift of this dose-response curve. These findings are in good agreement with the β-adrenoceptor antagonism observed in $I_{Ca,L}$.

Phentolamine 2 μM had little effect on the increase in contraction induced by dopamine (Figure 8a), suggesting that any contribution by the α-adrenoceptor in the positive inotropic action of dopamine is negligible. This is also consistent with the results obtained with $I_{Ca,L}$.

If release of noradrenaline (NA) from nerve endings is involved in the inotropic effect of dopamine, imipramine would be expected to enhance the effect. Imipramine blocks re-uptake of NA, but has little effect on re-uptake of dopamine (Hardman *et al.*, 1996). However, in our experiments superfusion of the preparation with 5 μM imipramine for up to 60 min did not enhance the dopamine action (data are not shown). On the

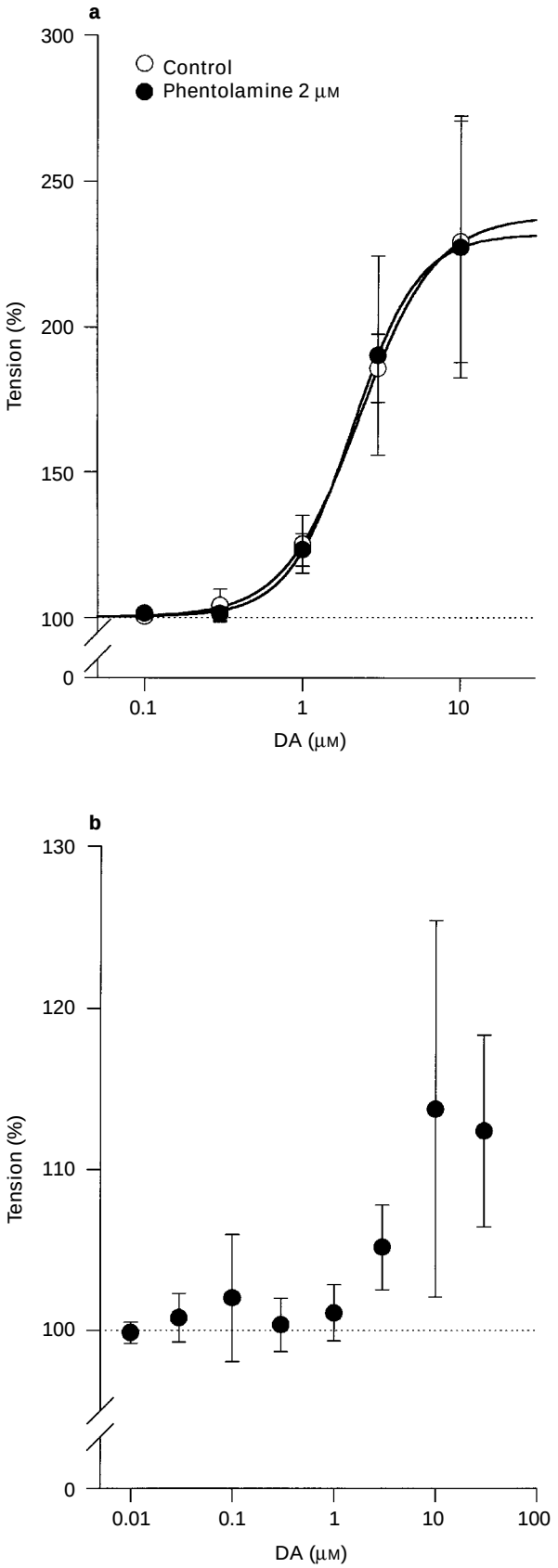

Figure 8 Effects of phentolamine and isoprenaline on the positive inotropic action of dopamine. Magnitudes of contraction at different concentrations of dopamine were normalized to those measured immediately before dopamine application (100%, dotted lines), and the normalized values were plotted against dopamine concentrations. (a) Effect of the α-adrenoceptor antagonist, phentolamine, on the positive inotropic effect of dopamine. Data were obtained in the absence (○, $n=4$) and presence (●, $n=3$) of 2 μM phentolamine. In control, $K_{1/2}$ and n_H from the Hill equation were 2.3 μM and 1.8, respectively, and in the presence of 2 μM phentolamine, 2.1 μM and 2.1, respectively. Curves were fitted to the Hill equations. (b) Inotropic effect of dopamine under continuous stimulation of adenylate cyclase; dopamine was applied in the presence of 0.01 μM isoprenaline throughout. Note that no significant change was evoked by less than 1 μM dopamine.

contrary, imipramine decreased the apparent affinity of the contraction for dopamine by approximately 10 fold. Therefore, it is unlikely that dopamine increases NA release from nerve endings under out experimental conditions. The decrease in the apparent affinity for dopamine may be due to different effects of imipramine, such as inhibition of ionic channels (Kotake *et al*., 1987; Isenberg & Tamargo, 1985).

In mouse photoreceptors, activation of the D_4 receptor eliminates the light-sensitive pool of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Cohen *et al*., 1992). Inhibitory coupling of the D_4 receptors to adenylate cyclase has also been described in Chinese hamster ovary (CHO) cells (Chio *et al*., 1994; Asghari *et al*., 1995), GH_4C_1 pituitary cells (Seabrook *et al*., 1994) and HEK293 cells (McHale *et al*., 1994), in all of which the human D_4 receptor was heterologously expressed. In these expression systems, activation of the D_4 receptor decreased forskolin-stimulated cyclic AMP level. Thus, if the atrial D_4 receptor is also coupled to adenylate cyclase, a low dose of dopamine may have an effect on the muscle contraction only when adenylate cyclase is partially activated. To examine this possibility, different concentrations of dopamine were applied when the contraction was slightly enhanced by a continuous application of 0.01 μM isoprenaline (Figure 8b). However, low concentrations (0.01–0.3 μM) of dopamine hardly affected the contraction. Therefore, the D_4 receptor does not appear to be involved in the regulation of atrial contraction, at least on a beat to beat time base.

Discussion

Direct β-adrenoceptor stimulation by dopamine

The present study demonstrated that dopamine causes an increase in $I_{Ca,L}$ in both atrial and ventricular myocytes via direct stimulation of the β-adrenoceptor. The range of dopamine concentrations required to activate $I_{Ca,L}$ was almost identical to that required for the positive inotropic effect. Therefore, the increase in $I_{Ca,L}$ most likely plays a pivotal role in the positive inotropic action of dopamine. The involvement of the α-adrenoceptor in the dopamine action is negligible, because phentolamine did not affect dopamine action on both $I_{Ca,L}$ and muscle contraction. Dopamine-induced release of noradrenaline from nerve endings, if any, should be small, because imipramine did not enhance the effect of dopamine.

To the best of our knowledge, the effect of dopamine on ion channels has not been definitively clarified in single cardiac myocytes. Habuchi *et al*. (1995) showed that 1 or 10 μM dopamine did not affect $I_{Ca,L}$ in rat ventricular cells. This differs from the results of the present study in which dopamine at high concentrations increased $I_{Ca,L}$ and the effect was reversed by propranolol. Furthermore, in our preliminary experiments in guinea-pig ventricular cells, high concentrations of dopamine activated $I_{Ca,L}$ as well as the cyclic AMP-dependent Cl^- current (unpublished observations).

Lack of involvement of D_4 receptor in rat atrial myocytes

The magnitude of the developed tension is influenced not only by the magnitude of $I_{Ca,L}$, but also by several other factors, such as the Ca^{2+} stores within the sarcoplasmic reticulum and the Ca^{2+} sensitivity of myofilaments. Therefore, it was neces-

sary to examine the effects of dopamine on the developed tension over a relatively low concentration range (<1 μM), which covers the binding constant of dopamine to the D_4 receptor. However, the findings demonstrated in Figures 7 and 8 excluded any functional involvement of the D_4 receptor in the regulation of contraction by dopamine in the rat atrium. Involvement of the D_4 receptor in the positive inotropy at high concentrations of dopamine ($\geqslant 1$ μM) also seems unlikely, since the positive inotropy was antagonized by propranolol. Our results are consistent with previous findings showing negligible involvement of dopamine receptors in the cardiac effects of dopamine (Lumley *et al*., 1977; Motomura *et al*., 1978; Martinez-Mir *et al*., 1987).

Negative coupling of the D_4 receptor to adenylate cyclase, demonstrated in mouse photoreceptor and in heterologous expression systems of the human D_4 receptor (Cohen *et al*., 1992; Chio *et al*., 1994; Seabrook *et al*., 1994; McHale *et al*., 1994; Asghari *et al*., 1995), was not identified in rat atrium under our experimental conditions. The cloned rat D_4 gene (O'Malley *et al*., 1992) shares only 73% amino acid and 77% nucleic acid sequence homology with the human D_4 gene (Van Tol *et al*., 1991). Most of the differences between the two genes occur in the third intracytoplasmic loop where there is only 50% amino acid identity. This structural difference may cause different signal transduction of the D_4 receptor in rats and man.

Although clozapine is a potent antagonist of the D_4 receptor with a high affinity ($K_d = 21$ nM, Seeman & Van Tol, 1994), the present study demonstrated that clozapine cannot selectively differentiate the action of dopamine through the D_4 receptor. Clozapine is known to bind with a high affinity to many other types of receptors, such as 5-hydroxytryptamine$_{1C}$ (5-HT$_{1C}$), 5-HT$_2$, muscarinic and histamine H_1 receptors (Coward, 1992). Baldessarini *et al*. (1992) showed that clozapine interacts with adrenoceptors; with a very high affinity to the α_1-adrenoceptor (inhibitory constant (K_i) = 6 nM), and with moderate affinity to the β-adrenoceptor ($K_i = 60$ nM) or to the α_2-adrenoceptor ($K_i = 160$ nM). The blocking of isoprenaline effects by clozapine in the present study (Figure 6) is consistent with the binding study of clozapine, and functionally demonstrates the β-antagonistic action of clozapine.

In conclusion, we demonstrated that dopamine at concentrations greater than 1 μM increases $I_{Ca,L}$ of both rat atrial and ventricular myocytes via direct stimulation of the β-adrenoceptor, and that dopamine at the same concentration range augments muscle contraction of rat atrium by directly stimulating the β-adrenoceptor. No functional involvement of the D_4 receptor was identified in the cyclic AMP-dependent regulation of $I_{Ca,L}$ or in the contraction of rat atrial myocytes. In addition, clozapine was shown to possess a β-antagonistic effect.

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