α-Adrenergic inhibition of the β-adrenoceptor-dependent chloride current in guinea-pig ventricular myocytes

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1. α_1-Adrenoceptor-mediated inhibition of the β-adrenoceptor-dependent Cl^- current was investigated in guinea-pig ventricular myocytes using the patch clamp technique. The Cl^- conductance activated by noradrenaline (0·1–10 μM) with an α_1-blocker (prazosin, 5 μM) was significantly greater than that activated by noradrenaline alone. Phenylephrine and methoxamine, α_1-agonists, exerted an inhibitory effect on the Cl^- conductance activated by isoprenaline. The dose–response relationship for isoprenaline and the Cl^- current activation was shifted to higher doses in the presence of phenylephrine (30 μM).

2. The interaction of α_1- and β-agonists on Cl^- current was also observed on the single channel level; in some of the outside-out membrane patches, phenylephrine (50 μM) depressed the activity of the single Cl^- channel which was induced by 5 μM adrenaline.

3. Phenylephrine had no effect on the Cl^- conductance induced by forskolin (0·5–5 μM), an activator of adenylate cyclase. The Cl^- conductance activated persistently by isoprenaline in GTPγS-loaded cells was also insensitive to phenylephrine. The results suggest that the observed α_1-adrenergic attenuation of the β-adrenergic response is not primarily due to inhibition of adenylate cyclase activity. The α_1-adrenergic action may interfere with the processes leading to enzyme activation in the β-adrenergic pathway.

In cardiac tissue, sympathetic activity plays an important role in controlling the cellular electrical activity by modulating several kinds of ionic channels (for review see Hartzell, 1988; Gadsby, 1990). β-Adrenergic stimulation has been found to induce a novel background current in cardiac cells (Egan, Noble, Noble, Powell & Twist, 1987), and this current was identified as a Cl^- current (Bahinski, Nairn, Greengard & Gadsby, 1989; Harvey & Hume, 1989; Matsuoka, Ehara & Noma, 1990; Ehara & Ishihara, 1990). Cl^- current may play an important modulatory role for cardiac action potentials. Activation of this current can lead to abbreviation of the action potential duration and may minimize the action potential prolongation associated with β-adrenergic enhancement of Ca^{2+} current. In relation to this effect, a possible feature of the Cl^- current to act as a target site for antiarrhythmic agents has also been given attention (Hume & Harvey, 1991; Ackerman & Clapham, 1993).

The β-adrenergic pathway responsible for activation of cardiac Cl^- current has been studied extensively (Hwang, Horie, Nairn & Gadsby, 1992; Nagel, Hwang, Nastiuk, Nairn & Gadsby, 1992). According to these studies, activation of the Cl^- current involves phosphorylation of the channel protein mediated by cyclic AMP-dependent protein kinase (PKA). However, the β-adrenergic mechanism may not be the sole physiological one controlling the Cl^- current. Histamine also activates Cl^- current, presumably via a similar PKA-dependent pathway (Harvey & Hume, 1990). Acetylcholine inhibits the β-adrenergically activated Cl^- current through muscarinic receptors, and this effect is attributed to antagonistic inhibition of adenylate cyclase activity (Tareen, Ono, Noma & Ehara, 1991; Hwang *et al.* 1992).

α_1-Adrenergic stimulation is known to modulate several types of cationic currents in cardiac cells (for review see Endoh, 1991; Terzic, Pucéat, Vassort & Vogel, 1993), and these currents include even those sensitive to β-adrenergic stimuli (e.g. delayed rectifier K^+ current; Tohse, Nakaya & Kanno, 1992). In the case of cardiac Cl^- current, the relationship between the α-adrenergic pathway and this current system has not been thoroughly examined. In the present study, we examined α- and β-adrenergic interactions on the Cl^- current in guinea-pig heart cells. The results show that α_1-adrenergic stimulation inhibits the β-adrenergic activation of Cl^- current. This inhibitory action, unlike the muscarinic inhibition, appears to be upstream in the adenylate cyclase cascade.

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METHODS

Preparation of single cells

Single ventricular cells were obtained from guinea-pig hearts using an enzymatic dissociation technique similar to that described elsewhere (Isenberg & Klöckner, 1982). Briefly, guinea-pigs (250–330 g) were killed by sodium pentobarbitone overdose (70–90 mg kg^{-1} I.P.). The chest was opened and the heart was quickly excised. The heart was hung on a Langendorff-type perfusion system and was perfused first with Tyrode solution and subsequently with Ca^{2+}-free Tyrode solution. When the heart beat ceased, the perfusate was changed to Ca^{2+}-free Tyrode solution containing collagenase (0·05 mg ml^{-1}; Yakult, Tokyo, Japan). After this enzyme treatment, the cells were dissociated in a high-K^+, low-Cl^- solution ('KB medium', Isenberg & Klöckner, 1982) and stored in this medium before use.

Patch clamp and recording technique

Whole-cell recordings were performed following the technique described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). The recording pipettes had a resistance of 2–3 MΩ when filled with the pipette solution (internal solution). The cell was voltage clamped at −40 mV, and ramp pulses (triangle wave, −140 to +60 mV, $dV/dt = \pm 1$ V s^{-1}) were applied every 6 s to monitor the membrane conductance. The hyperpolarizing portion of the ramp pulses was used for measurement of the I–V relation. The membrane capacitance was measured by dividing the half-amplitude of the current jump at the peak of the ramp by the slope of the ramp pulse. The membrane conductance was calculated from the whole-cell I–V relation at a voltage (usually −30 mV) near the reversal potential of Cl^- current. The depolarizing portion of the ramp pulses always activated the fast Na^+ current (I_{Na}). For clarity, in the chart records shown in this paper the spiky traces of I_{Na} were eliminated by retouching. In some experiments, outside-out patch recordings (Hamill *et al.* 1981) were performed to record single Cl^- channel currents activated by the agonists. The pipette type and the external and internal solutions for these experiments were the same as those used for the whole-cell recordings.

Whole-cell currents were recorded using a whole-cell clamp system (TM-1000, ACT ME, Tokyo, Japan), and single-channel currents, with a patch clamp amplifier (EPC-7, List, Darmstadt, FRG). Data were stored on a digital audiotape using a PCM data recorder (RD101T, TEAC, Tokyo, Japan) for later computer analysis (PC98 RL, NEC, Tokyo, Japan).

Solutions and drugs

The normal Tyrode solution contained (mM): NaCl, 140; KCl, 5·4; $MgCl_2$ 0·5; $CaCl_2$ 1·8; glucose, 10; Hepes, 5 (pH 7·4 with NaOH). The external solution used for recording the membrane conductance was a K^+- and Ca^{2+}-free solution containing (mM): NaCl, 140; $MgCl_2$ 2; glucose, 10; Hepes, 10 (pH 7·4 with NaOH). Ouabain (10 μM), $BaCl_2$ (2 mM) and nicardipine-HCl (1 μM, Sigma) were added to suppress the Na^+–K^+ pump, K^+ and Ca^{2+} channels, respectively. The composition of the internal solution was (mM): CsOH, 90; aspartate, 90; CsCl, 30; $MgCl_2$ 2; TEA-Cl, 20; EGTA, 5; Tris-ATP, 5; GTP, 0·1; glucose, 10; Hepes, 5 (pH 7·2 with CsOH). Free Ca^{2+} concentration in this solution was estimated to be approximately 0·1 nM, according to Fabiato (1988). In some experiments, EGTA (5 mM) was replaced with 20 mM BAPTA. In this case, free Ca^{2+} concentration was estimated to be below 0·01 nM, with dissociation constants of BAPTA (Tsien, 1980). All experiments were performed at 35·0 ± 0·5 °C.

Drugs used were: D,L-noradrenaline (Sankyo, Tokyo), L-adrenaline (Daiichi, Tokyo), L-isoprenaline-HCl (Nikken, Tokyo), L-phenylephrine-HCl (Sigma), methoxamine-HCl (Nippon Shinyaku, Tokyo), D,L-propranolol-HCl (Sumitomo, Tokyo), prazosin-HCl (Sigma), acetylcholine-Cl (Daiichi), and forskolin (Sigma).

RESULTS

Under our experimental conditions with selected external and internal media, Ca^{2+} and K^+ currents and other exchange currents were almost totally suppressed, and the β-adrenoceptor-dependent Cl^- conductance could be measured by applying ramp pulses to the whole cell (Matsuoka *et al.* 1990; Tareen *et al.* 1991). Adrenaline, noradrenaline and isoprenaline were all effective in producing the Cl^- current, as expected. However, when adrenaline or noradrenaline was used as a current activator, we often (in about 30% of the experiments) observed a slight and transient increase in the Cl^- conductance immediately after washing the agonist, as shown in Fig. 1. Since such a 'rebound' phenomenon was never observed with isoprenaline, a pure β-stimulant, we hypothesized that α-action, which adrenaline and noradrenaline possess, might have some role in this conductance transient. If α-adrenergic action were to have an inhibitory effect on the β-adrenergic activation of Cl^- current, and if this inhibition would subside more quickly than β-action with elimination of agonist, a transient increase in the Cl^- conductance would manifest upon removal of the mixed α- and β-adrenergic agonists. Therefore, we evaluated the action of noradrenaline with and without α-blocker.

Figure 2 shows the result of an experiment in which the effect of 0·1 and 5 μM noradrenaline on the membrane conductance was examined in the presence and absence of 5 μM prazosin, an α_1-blocker. The agonist-induced Cl^- conductance was definitely greater in the presence of prazosin than in its absence at both agonist concentrations. Table 1 summarizes the results obtained from a number of similar experiments. Here, the conductance value is expressed relative to the capacitive membrane area (pS pF^{-1}). At all activating doses (0·1, 1 and 10 μM) of noradrenaline, the co-presence of prazosin significantly facilitated the Cl^- conductance activation.

The above results suggest that α_1-adrenergic action can attenuate the β-adrenergic mechanism. Therefore, we directly examined the effect of α_1-agonists on the β-adrenergically activated Cl^- conductance. Figure 3 shows an example of such experiments. In line with the above view, 40 μM phenylephrine substantially reduced the Cl^- conductance which was activated by 0·2 μM isoprenaline. The inhibitory effect of phenylephrine was reversible (Fig. 3) and was abolished by 5–10 μM prazosin (not shown). Figure 4 shows the dose–response relationship for

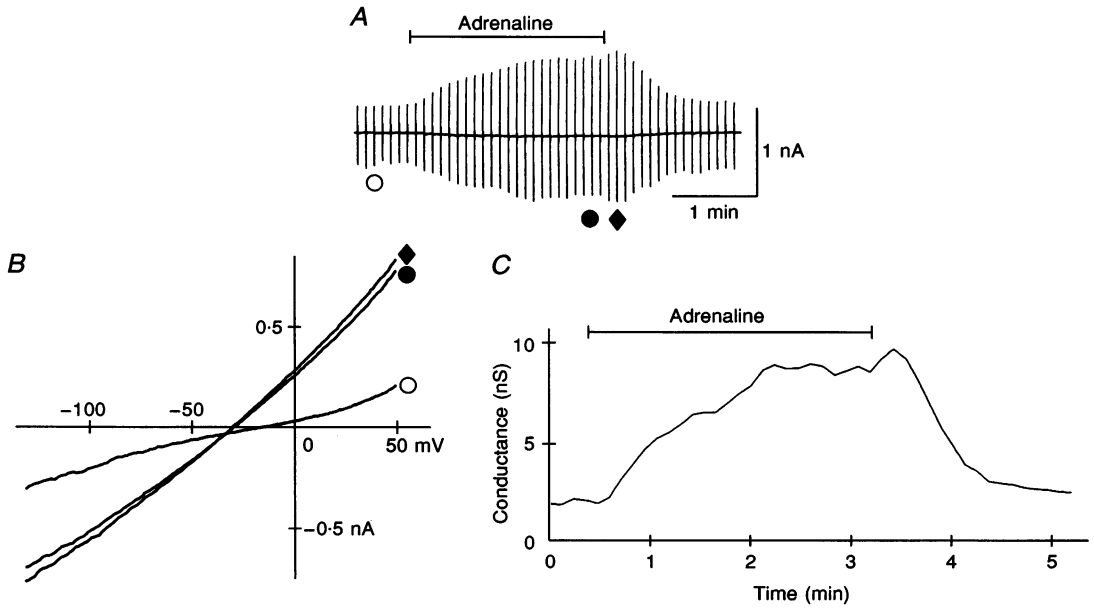

Figure 1. Activation of Cl^- current by adrenaline (0·5 μM) and its rebound increase after wash-out

A, chart record of membrane current. In this and subsequent similar figures, vertical deflections of the trace represent current responses produced by ramp pulses which were applied every 6 s from a holding potential of −40 mV. Adrenaline was applied for the period indicated by bar. Current traces indicated by the symbols are the sources for *I–V* relations shown in *B*. *B*, *I–V* relations obtained at the times indicated by the corresponding symbols in *A*. *C*, time course of membrane conductance measured in the experiment shown in *A*. Conductance was calculated from the *I–V* relation at −30 mV, a voltage near the reversal potential of Cl^- current. Note that the conductance showed a transient increase immediately after agonist wash-out.

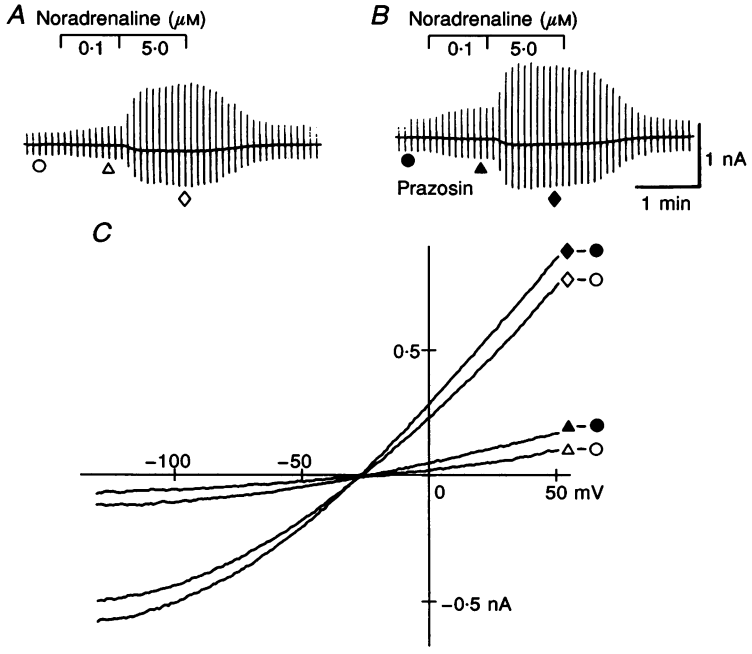

Figure 2. Enhancement of noradrenaline-activated Cl^- current by an α_1-blocker, prazosin

A and *B*, chart records of membrane current in response to ramp pulses obtained in the absence (*A*) and presence (*B*) of prazosin (5 μM). Noradrenaline (0·1 and 5 μM) was applied for the period indicated by bars. *C*, *I–V* relations of noradrenaline-induced current obtained in the absence (△ — ○ and ◇ — ○) and presence (▲ — ● and ◆ — ●) of prazosin. Noradrenaline-induced currents were obtained by subtracting the control *I–V* relation from the *I–V* relation with 0·1 and 5 μM noradrenaline, respectively.

Table 1. Conductance changes induced by noradrenaline in the absence and presence of 5 μM prazosin

[Noradrenaline] (μM)	N	Conductance changes: Control (pS pF^{-1})	Conductance changes: After prazosin (pS pF^{-1})
0·1	9	18·9 ± 12·7	32·6 ± 22·1*
1	9	50·2 ± 30·1	88·6 ± 62·8*
10	6	83·1 ± 41·2	148·6 ± 70·6**

N is the number of experiments. Values are means ± S.D. The values after prazosin are significantly greater than control (* $P < 0·02$ and ** $P < 0·05$, according to Student's t test for paired data).

isoprenaline activation of Cl^- conductance determined with and without phenylephrine (30 μM). The presence of phenylephrine shifted the dose–response curve to the right, while the maximum isoprenaline response, obtained at 1 μM isoprenaline, was little attenuated by phenylephrine. The half-maximally activating concentration of isoprenaline was 28 nM in control and increased to 86 nM in the presence of the α_1-agonist.

The same type of α_1- and β-adrenergic interaction was also observed when methoxamine (up to 100 μM) was used as an α_1-agonist (5 experiments). On the other hand, application of phenylephrine or methoxamine alone to the myocardial cell had no effect on the background conductance (not shown). We concluded that α_1-adrenergic stimulation inhibited the β-adrenergic activation of membrane Cl^- conductance in guinea-pig ventricular cells.

A previous study showed that activity of single Cl^- channels can be recorded in the outside-out membrane patches excised from cyclic AMP-loaded cardiac cells (Ehara & Matsuura, 1993). Using a similar system, we attempted to examine the α_1- and β-adrenergic interaction on Cl^- current at the single channel level. Here the outside-out membrane patches were excised from cells with a pipette containing the usual internal solution, and the external side of the membrane was exposed to adrenaline and phenylephrine.

It was difficult to obtain an electrically stable outside-out membrane patch. Furthermore, even the stable patches did not necessarily develop a Cl^- channel activity in response to adrenaline (5 μM). However, we could detect an adrenaline-induced Cl^- channel activity in seven patches, and in two out of these we could observe a clear α_1- and β-adrenergic interaction on the channel activity, as shown in Fig. 5. The adrenaline-induced activity of the Cl^- channel declined upon application of phenylephrine (50 μM), and this decline was due to a drastic decrease in the open probability of the channel (Fig. 5*Bb*), though the open probability showed some fluctuations during phenylephrine application (Fig. 5*Bc*). The unresponsiveness of the patch membrane to agonist and antagonist seen in many patches remains unexplained, but our results may indicate that α_1-adrenergic suppression of the macroscopic Cl^- current is due to a decreased activity of at least a fraction of the activated Cl^- channels on the membrane.

The Cl^- current can also be induced by exposing the cell to forskolin which directly activates adenylate cyclase independently of the β-receptor (Tareen *et al.* 1991; Hwang *et al.* 1992). If α_1-inhibition of the β-adrenergic activation of Cl^- current, like muscarinic inhibition (Tareen *et al.* 1991; Hwang *et al.* 1992), is due to antagonistic inhibition of adenylate cyclase activity, α_1-agonist would also be expected to suppress the forskolin-induced Cl^- conductance. Therefore, we tested whether phenylephrine could affect the forskolin-induced response. In the experiment shown in Fig. 6, Cl^- conductance was first activated by application of 2·5 μM forskolin, and then the cell was exposed to 2 μM acetylcholine or 40 μM phenylephrine. Acetylcholine remarkably reduced the activated Cl^- conductance, as expected, while phenylephrine was without effect. In this experiment, the bath solutions always contained propranolol (1 μM), a β-blocker, and hence any β-adrenergic action of phenylephrine was suppressed. We performed a number of the experiments similar to the above, using various concentrations (0·5–5 μM) of forskolin to vary the magnitude of pre-activation. It was important to see the effect of phenylephrine on the small forskolin response, because, for evaluation of the inhibition, pre-activation of adenylate cyclase should not be 'oversaturated'. In these experiments, it was consistently observed that phenylephrine (up to 100 μM) failed to inhibit the forskolin-induced Cl^- conductance, irrespective of magnitude of the pre-activated response. These results can be taken to indicate that the α_1-adrenergic pathway does not directly act on adenylate cyclase.

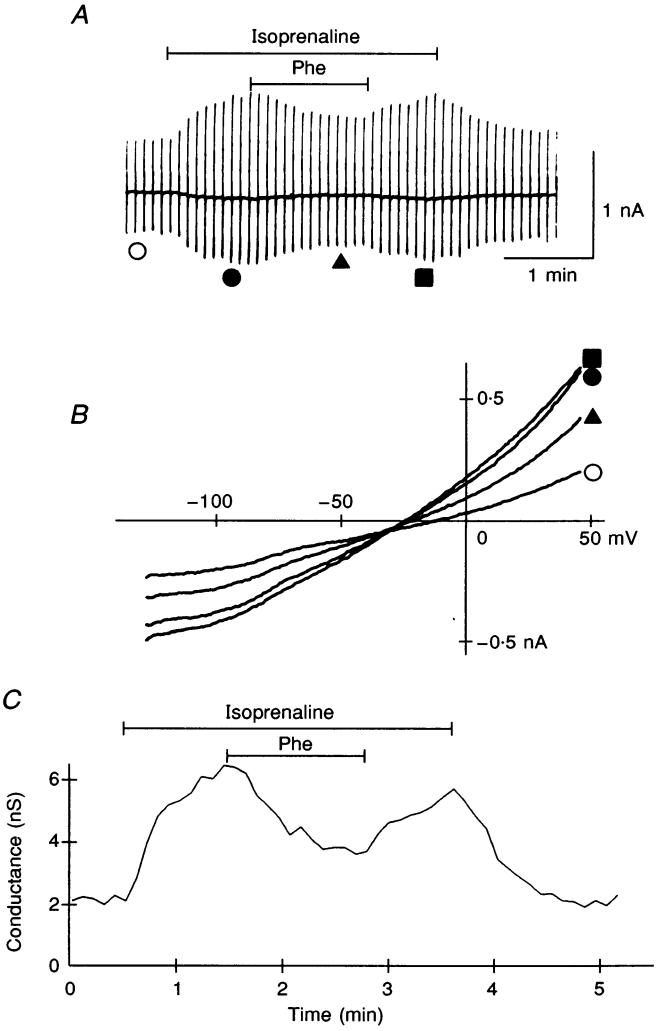

Figure 3. Suppression of isoprenaline-activated Cl^- current by phenylephrine

A, chart record of membrane current in response to ramp pulses. Isoprenaline (0·2 μM) and phenylephrine (Phe, 40 μM) were applied for the period indicated by bars. Current traces indicated by the symbols are the sources for *I–V* relations shown in *B*. *I–V* relations obtained in control solution (○) and in the presence of isoprenaline without (● and ■) and with (▲) phenylephrine. *C*, time course of membrane conductance obtained in the experiment shown in *A*.

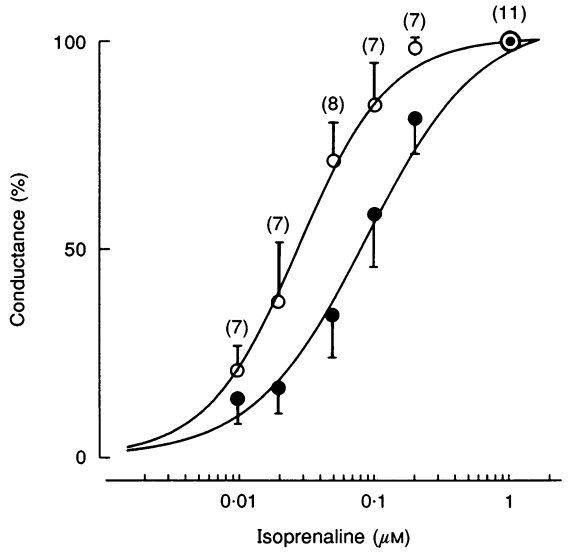

Figure 4. Dose–response relations for the Cl^- conductance and isoprenaline as influenced by phenylephrine

Cl^- conductance was measured in the absence (○) and presence (●) of phenylephrine (30 μM) at different isoprenaline concentrations. In every experiment, Cl^- conductance induced by 1 μM isoprenaline was determined at least once in the absence of phenylephrine, and conductance data for lower isoprenaline concentrations, with or without phenylephrine, were expressed as relative to this value. In the graph, each data point represents mean ± S.D., with S.D. for the data at 1 μM isoprenaline with phenylephrine within the symbol. Numbers in parentheses are the number of paired data at each isoprenaline concentration. The smooth curves were drawn by the least-squares fit of the Hill equation:

$$\text{normalized conductance} = 1/(1 + (K_{½}[\text{drug}]^{-1})^{n}),$$

where $K_{½}$ is the half-maximally activating drug concentration, and n is the Hill coefficient. Control: $K_{½} = 28$ nM, $n = 1{\cdot}3$. With phenylephrine: $K_{½} = 86$ nM, $n = 1{\cdot}1$.

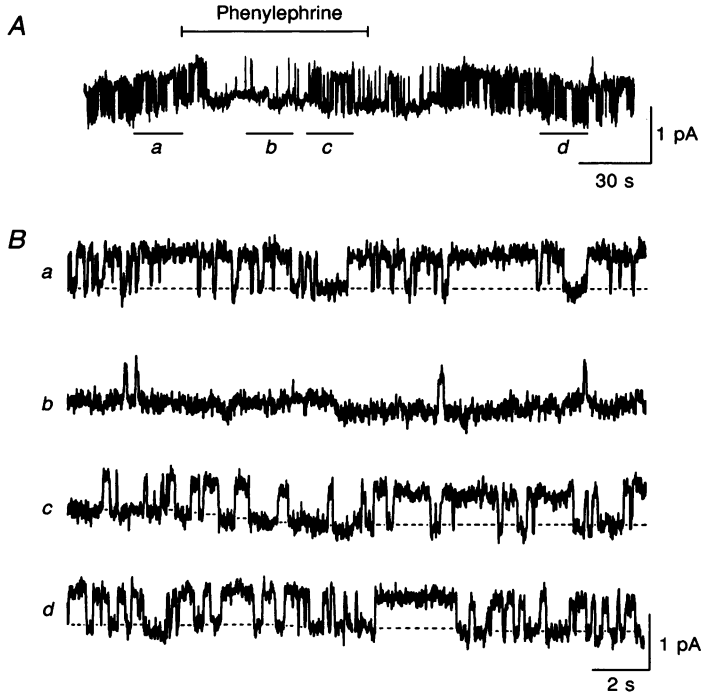

Figure 5. Outside-out patch recording showing the effect of phenylephrine on the single Cl^- channel currents

Activity of the Cl^- channel was induced in an outside-out patch by exposing the patch membrane to 5 μM adrenaline, and 50 μM phenylephrine was then added to the bath (bar above the current trace in *A*) in the continuous presence of adrenaline. *B*, expanded records of the parts of *A* indicated by bars (*a*, *b*, *c* and *d*). The patch membrane was held at +40 mV throughout. Dashed lines indicate closed levels.

Another way to obtain an apparently β-receptor-independent Cl^- conductance is to stimulate the cells that are loaded with GTPγS, a non-hydrolysable GTP analogue. Since GTP hydrolysis is essential to terminate the signal transduction processes linking β-adrenoceptor occupation and activation of effector enzyme (Gilman, 1987), once the adenylate cyclase activity has been stimulated by a β-agonist, it persists even after removal of the agonist if the cells are loaded with GTPγS, and this results in a persistent activation of Cl^- conductance (Hwang *et al.* 1992). As seen in Fig. 7, phenylephrine failed to inhibit this persistently activated Cl^- conductance. Similar

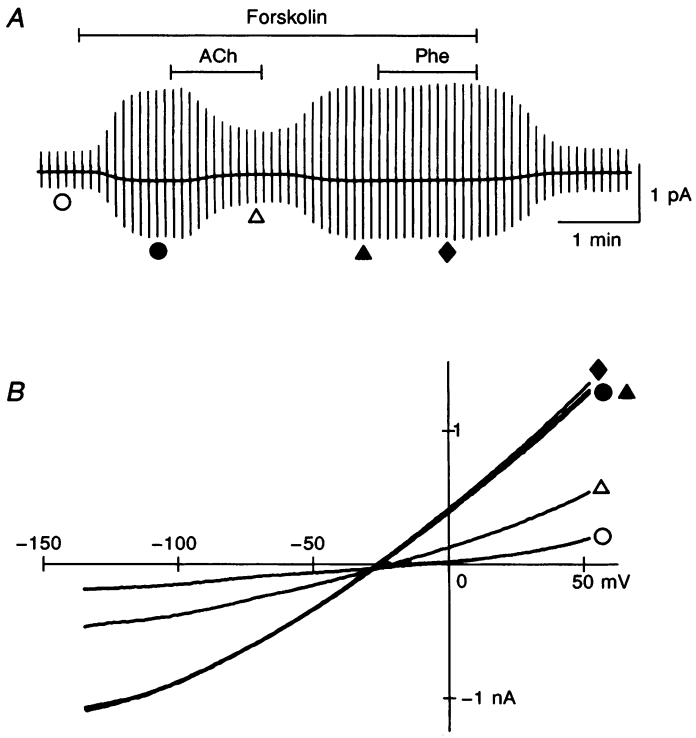

Figure 6. Effects of acetylcholine and phenylephrine on forskolin-induced Cl^- conductance

The cell was first exposed to 2·5 μM forskolin and then to 2 μM acetylcholine (ACh) and 50 μM phenylephrine (Phe), as shown in *A* which shows a chart record of membrane currents. *B*, *I–V* relations obtained at the times indicated by the corresponding symbols in *A*. Propranolol (1 μM) was present throughout.

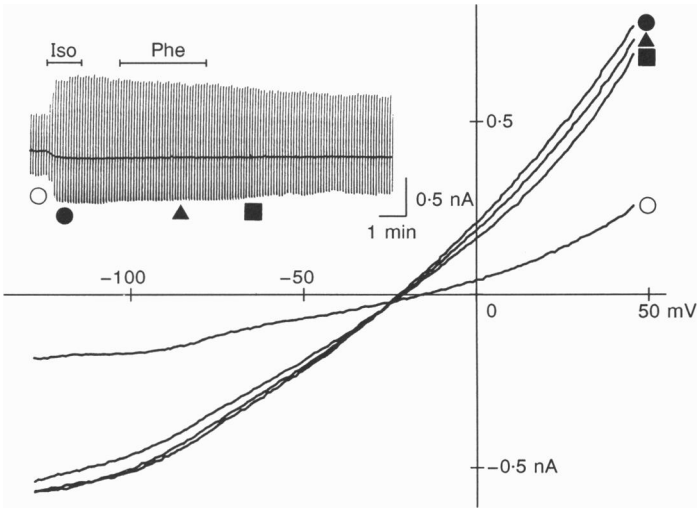

Figure 7. Effects of phenylephrine on the Cl^- conductance activated persistently by isoprenaline in the presence of internal GTPγS
The pipette solution contained 100 μM GTPγS. The cell was exposed to 0·2 μM isoprenaline (Iso) for 1 min, and later to 50 μM phenylephrine (Phe), as shown in the inset which shows chart record of membrane currents. Note that isoprenaline-activated Cl^- conductance declined little or only slowly after removal of the agonist. The graph shows *I–V* relations obtained at the times indicated by the corresponding symbols in the inset. Phenylephrine was applied with propranolol (1 μM).

observations were made in three other experiments. This finding, together with the result of forskolin experiments (Fig. 6), appears to imply that α_1-adrenergic stimulation cannot exert its effect on Cl^- conductance, if the activity of adenylate cyclase is maintained through mechanisms independent of the β-receptor. We concluded that α_1-adrenergic inhibition of the β-adrenergic activation of Cl^- conductance was not due to a direct inhibition of adenylate cyclase activity, and that the inhibition probably occurred in the processes leading to enzyme activation in the β-adrenergic pathway. In addition, the results from GTPγS-loaded cells (Fig. 7) may indicate that α_1-action does not affect the β-adrenergically induced communication between GTP binding proteins and the effector enzyme.

In some cardiac preparations, stimulation of α_1-adrenoceptor has been shown to increase diastolic intracellular Ca^{2+} level (Iwakura *et al.* 1990; Sen, Liang, Colucci & Smith, 1990; Eckel, Gerlach-Eskuchen & Reinauer, 1991; Jahnel, Jakob & Nawrath, 1992). Under our experimental conditions, the internal solution contained 5 mM EGTA, which was expected to maintain a low level of $[Ca^{2+}]_i$. Therefore, it is unlikely that an increase in $[Ca^{2+}]_i$ played a role in the observed α_1-adrenergic action. This view was further tested by examining the α_1- and β-adrenergic interactions when the capacity of intracellular Ca^{2+} buffer was greatly increased by loading the cells with 20 mM BAPTA. As shown in Fig. 8, the inhibitory effect of phenylephrine on the isoprenaline-induced Cl^- conductance persisted in these cells, supporting the above view.

DISCUSSION

The present study showed that, in guinea-pig ventricular myocytes, α_1-adrenoceptor stimulation inhibited the β-adrenergically activated whole-cell Cl^- conductance. The α_1- and β-adrenergic interaction on the Cl^- current was also

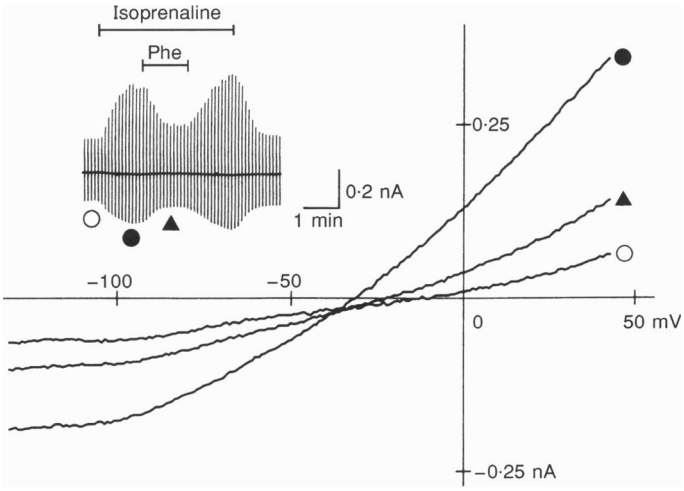

Figure 8. Effects of phenylephrine on isoprenaline-induced Cl^- conductance in a cell in which internal Ca^{2+} was massively chelated
The pipette solution contained 20 mM BAPTA. Isoprenaline (0·02 μM) and phenylephrine (Phe, 30 μM) were applied as shown in the inset which shows a chart record of membrane currents. The graph shows *I–V* relations obtained at the times indicated by the corresponding symbols in the inset.

detected on the single channel level. The mechanism underlying α_1- and β-adrenergic interaction on cardiac muscle has been studied in some detail in rat heart. Boutjdir, Restivo, Wei & El-Sherif (1992) showed that, in rat ventricular myocytes, α_1-adrenoceptor stimulation exerted an inhibitory effect on the Ca^{2+} current that was enhanced by β-adrenergic stimulation. In this case, however, α_1-adrenoceptor stimulation also suppressed the facilitatory action of forskolin on Ca^{2+} current, though it was ineffective on the current that was enhanced directly by intracellular dialysis of cyclic AMP. These authors suggested that this α_1-action was mediated by a GTP-binding protein which inhibited adenylate cyclase activity. In accordance with this view, Barrett, Honbo & Karliner (1993) observed that, in neonatal rat ventricular cells, α_1-adrenoceptor stimulation reduced the cyclic AMP accumulation induced by β-adrenergic stimulation, and this effect appeared to result from an antagonistic suppression of the adenylate cyclase activity, not from an activation of phosphodiesterase which would degrade cyclic AMP (Buxton & Brunton, 1985). Thus, in rat heart, regulation of adenylate cyclase activity through GTP-binding proteins appears to play a key role in the α_1-adrenergic inhibition of the β-adrenergic response.

The above scheme may not simply apply to guinea-pig heart, although the mechanism underlying muscarinic and β-adrenergic interaction on the Cl^- conductance in this tissue is explained in a way similar to the above (Tareen *et al.* 1991; Hwang *et al.* 1992). In the present study, phenylephrine caused little suppression of the forskolin-induced Cl^- conductance (Fig. 6), suggesting that α_1-adrenergic stimulation did not directly act on adenylate cyclase. Instead, α_1-adrenergic action was considered to attenuate the β-adrenergic pathway by affecting some reaction step(s) involved prior to enzyme activation. The finding that phenylephrine failed to suppress the Cl^- conductance which was persistently activated in GTPγS-loaded cells (Fig. 7) may also support this view. In this hypothesis, the enzyme activity decreases secondarily to the preceding interaction.

In guinea-pig cardiac tissue, to our knowledge, no biochemical study has been done concerning α_1-adrenergic action on the adenylate cyclase cascade. However, Keung & Karliner (1990) were able to show that α_1-adrenergic stimulation suppressed the Ca^{2+} current in guinea-pig ventricular myocytes under the condition in which the inhibitory GTP-binding proteins were inactivated by pertussis toxin treatment. This finding implies that α_1-adrenergic action can modulate an adenylate cyclase-dependent membrane current independently of enzyme activity. Although the biochemical mechanism underlying this modulation must be different from that responsible for the α_1- and β-adrenergic interaction observed in the present study, the above finding is consistent with the view that the primary target site of the α_1-adrenergic pathway in guinea-pig heart cells is not the enzyme adenylate cyclase, which is in line with our hypothesis.

The precise signal transduction pathway of this α_1-adrenergic action remains to be resolved. In cardiac tissue, α_1-adrenergic stimulation is known to activate phospholipase C, which leads to generation of inositol 1,4,5-trisphosphate (IP_3; Poggioli, Sulpice & Vassort, 1986; Otani, Otani & Das, 1988) and diacylglycerol (Okumura, Kawai, Hashimoto, Ito, Ogawa & Satake, 1988; Bordoni, Biagi, Rossi & Hrelia, 1991), an activator of protein kinase C (PKC). α_1-Adrenergic stimulation, by activating phospholipase A_2, may also induce arachidonic acid, which then leads to generation of various arachidonic metabolites (Axelrod, Burch & Jelsema, 1988). Thus there is the possibility that these second messengers play a role in the α_1-adrenergic action. In the present study, the inhibitory action of phenylephrine on the β-adrenergically activated Cl^- conductance persisted under the condition in which intracellular Ca^{2+} was strongly buffered with high concentrations of BAPTA (Fig. 8), suggesting that changes in internal Ca^{2+} concentration, which might be induced by the Ca^{2+}-mobilizing action of IP_3, do not mediate the inhibition described.

It has been shown that PKC also activates a background Cl^- current in ventricular cells (Walsh, 1991). The PKC-activated Cl^- current exhibits properties very similar to those of the PKA-activated one on the whole cell level (Walsh & Long, 1994; Zhang, Barrington, Martin & Ten Eick, 1994) and on the single channel level (Collier & Hume, 1995). Furthermore, the Cl^- current maximally activated by the PKC-dependent pathway cannot further be enhanced by the PKA-dependent pathway, and vice versa, while effects of simultaneous activation of both pathways at submaximal levels are roughly additive (Zhang *et al.* 1994). Thus, it has been suggested that both PKA and PKC activate the same population of Cl^- channels in ventricular cells (Zhang *et al.* 1994; Collier & Hume, 1995; but see Walsh & Long, 1994). In view of these studies, it seems highly unlikely that PKC was involved in the observed inhibitory effect of α_1-adrenergic stimulation on the β-adrenergically activated Cl^- conductance. The present finding that α_1-adrenergic effects persisted in BAPTA-loaded cells (Fig. 8) would also support this view, since activity of PKC may depend on the Ca^{2+} concentration. Obviously, further experiments using specific activators and inhibitors of the possible second messengers are needed to clarify the signal transduction pathway involved in the observed interactions.

On the other hand, Walsh (1991) observed that application of noradrenaline in the presence of β-blocker induced a background current in guinea-pig ventricular cells. This current component had a reversal potential near the predicted Cl^- equilibrium potential and showed no time-

dependent change during the clamp pulses (40 ms in duration), and it was proposed that α_1-adrenoceptor stimulation activated a Cl^- conductance via the PKC-dependent pathway, though the relationship between reversal potential and Cl^- concentration gradient across the membrane was not systematically examined. In our study, α_1-adrenergic agonists alone did not influence the background conductance of ventricular cells. The reason for this discrepancy is unclear. However, the study of Walsh (1991) was done at room temperature with a K^+-rich internal solution containing approximately 1 nM Ca^{2+}, whereas our study was done at around 35 °C with a Cs^+-rich internal solution containing about 0·1 nM Ca^{2+}. It is possible that these differences led to development of different membrane responses to α_1-adrenergic stimulation. Further studies seem to be necessary to resolve whether α_1-adrenergic stimulation by itself activates the Cl^- conductance in cardiac cells.

Regardless of the mechanism involved, the α_1-adrenergic inhibition of the β-adrenergic response in cardiac tissue could be one important feature of the autonomic control of cardiac function. Since adrenergic stimuli are mediated predominantly by the naturally occurring transmitter noradrenaline, a mixed α- and β-adrenoceptor agonist, sympathetic control of cardiac Cl^- current, as well as Ca^{2+} current (Boutjdir *et al.* 1992), is expected to operate along with an intrinsic negative feedback mechanism. This may provide protection against deleterious catecholamine surges, and enable a fine control in the moment-to-moment regulation of cardiac electrical activity.

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