Somatostatin decreases the calcium inward current in guinea-pig atria

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- 1 The effects of somatostatin on mechanical and electrophysiological responses were studied in guinea-pig atrial muscle preparations and single cells.
- 2 Somatostatin ($\geq 10^{-8}$ M) decreased the twitch contraction in a concentration-dependent manner in electrically driven left atria and spontaneously beating right atria. However, the beating rate was not affected.
- 3 The negative inotropic effect of somatostatin was transient. Desensitization to this agent developed slowly during continuous exposure to the peptide.
- 4 In single atrial cells, somatostatin significantly shortened the action potential duration, but the resting potential and action potential amplitude were not affected.
- 5 Under whole cell voltage-clamp conditions, somatostatin decreased the calcium inward current without affecting the sodium and potassium currents.
- 6 These results suggest that somatostatin selectively acts on the calcium channel of guinea-pig atrial cells to reduce the calcium inward current, which in turn gives rise to the negative inotropic effect.

Introduction

Somatostatin is present in sympathetic and/or sensory nerves in many peripheral tissues including heart (Pradayrol *et al.*, 1980; Hökfelt *et al.*, 1980; Day *et al.*, 1985). Although the functional significance of somatostatin in the cardiovascular system is still unclear, the peptide may be implicated in a variety of cardiac disorders including arrhythmia. In a clinical trial, intravenous administration of somatostatin has been shown to restore sinus rhythm in patients with paroxysmal supraventricular tachycardia and junctional tachycardia (Greco *et al.*, 1984; Ghirlanda *et al.*, 1985). Somatostatin also has a negative inotropic effect in the human isolated atria (Franco-Cereceda *et al.*, 1987), guinea-pig atria (Quirion *et al.*, 1979; Diez *et al.*, 1985; Lin *et al.*, 1988) and dog Purkinje fibres (Lin *et al.*, 1988). Since somatostatin inhibited calcium influx in the experiment with ^{45}Ca (Diez & Tamargo, 1987), the negative inotropic and/or chronotropic effects may be related to the decrease in calcium inward current.

In the present study, we analysed the effects of somatostatin on guinea-pig cardiac cells with special reference to calcium current. The results obtained here suggest that the negative inotropic action of somatostatin may be attributable to a decrease in the calcium inward current.

Methods

Contractility measurement

Male guinea-pigs weighing 250–350 g were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} , i.v.), exsanguinated from the carotid arteries and the hearts were quickly isolated. Right and left atria and papillary muscles were dissected and mounted vertically in 20 ml organ baths containing modified Tyrode solution of the following composition (mM): NaCl 136.9, KCl 2.7, CaCl_2 1.8, MgCl_2 1.0, NaHCO_3 23.8, NaH_2PO_4 0.21 and glucose 5.6, gassed with a mixture of 95% O_2 and 5% CO_2 at 30°C . Square wave pulses of 1 ms duration

and suprathreshold voltage were applied at frequencies ranging from 0.1 Hz to 2 Hz in left atria and papillary muscles. In right atria, spontaneous beating was recorded. Contractile force was measured isometrically by a force displacement transducer (T7-30-240, Toyo boldwin, Japan) and recorded on a strip chart recorder (RJG-4128, Nihon Kohden, Japan). The preparations were equilibrated at a resting tension of 1 g (in atria) or 0.5 g (in papillary muscle) for 90 min before the experiments were started. Propranolol (10^{-6} M) and atropine (10^{-6} M) were added to the baths 15 min before the treatment with somatostatin in order to eliminate possible neurogenic responses.

Isolation of single atrial cells

Guinea-pig heart was isolated and mounted on the Langendorff apparatus to perfuse the coronary artery with oxygenated and pre-warmed (37°C) normal external solution (Table 1) at a pressure of 65 cmH_2O . After the heart had been cleared of blood, the perfusate was changed to Ca-free external solution containing 0.4 mg ml^{-1} collagenase (Type I, Sigma, U.S.A.) and the solution recirculated by a peristaltic pump for 25 min. Thereafter, the preparation was washed out with 100 ml of high K-low Cl solution (storage solution) and was stored in this solution at 4°C until the experiments were started.

Whole-cell clamp experiment

A piece of tissue was dissected from the collagenase-perfused left atrium mentioned above and the cells dispersed in the experimental chamber (0.5 ml in volume) filled with the storage solution. After the cells had settled on the floor of the chamber, the perfusion of normal external solution was started at a rate of $2\text{--}3 \text{ ml min}^{-1}$. The temperature of the perfusate in the experimental chamber was maintained at 33°C .

Patch electrodes were fabricated from glass capillaries of 1.5 mm in outer diameter by a double step microelectrode puller (PP-83, Narishige, Japan). After the tip was polished by heat, the electrode was filled with internal solution (Table 1).

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Table 1 Composition of external and internal solutions (mM)

	External solution								
	NaCl	KCl	CaCl ₂	MgCl ₂	Glucose	HEPES			
Normal	136.9	5.4	1.8	0.5	10	5			
K-free	136.9	—	1.8	0.5	10	5			
	Internal solution								
	K		Cs				Phospho- creatine*	ATP**	EGTA
	aspartate	KCl	aspartate	CsCl ₂	MgCl ₂				
K-rich	100	20	—	—	2.5	5	5	5	5
Cs-rich	—	—	100	20	2.5	5	5	5	5

The pH of the external solution was adjusted to 7.2 by NaOH. In internal solutions, pH was adjusted to 7.2 by KOH or CsOH.

* Phosphocreatine (disodium salt); **adenosine-5'-triphosphate (dipotassium salt).

Outer diameter of the electrode tip was 2–5 μm and the electrode resistance ranged between 1 and 3 M Ω . Patch clamp amplifier (S-3666, Nihon Kohden) was used to record the whole-cell membrane currents. After the electrode tip touched the cell surface, a negative pressure of 20–40 cmH₂O was applied to the interior of the electrode. Within a few seconds, a G Ω seal was established between the electrode tip and the cell surface. Then the membrane patch at the tip of the electrode was ruptured by applying further suction of –50 to –100 cmH₂O. Rupture was indicated by a large increase in the capacitive current. The current signals and membrane potentials were displayed on an oscilloscope (VC-10, Nihon Kohden) and the signals were digitized by a PCM recording system (NF Circuit Design Block, RP-880) and stored on videotape for later analysis.

Solutions and drugs

The compositions of the external and internal solutions used are listed in Table 1. The composition of high K-low Cl storage solution was (mM): taurine 10, oxalic acid 14, glutamic acid 80, KCl 25, KH₂PO₄ 10, N-(2-hydroxyethyl)piperazine-N'-3-propanesulphonic acid (HEPES) 10, glucose 11, ethylene glycol bis (β -aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) 0.5, pH was adjusted to 7.2 by adding KOH (Isenberg & Klöckner, 1982). Somatostatin (Peptide Institute Inc., Osaka, Japan) was dissolved in saline solution containing 2% gelatin. ($\pm$)-Propranolol hydrochloride (Nacalai Tesque Inc., Kyoto, Japan) and atropine sulphate (Wako Pure Chemical Industries Ltd., Osaka, Japan) were dissolved in distilled water.

Data analysis

Experimental values are given as mean $\pm$ s.e.mean. The effects of drugs were considered significant if $P < 0.05$ by Student's *t* test for unpaired data.

Results

Effects of somatostatin on mechanical responses in atrial and papillary muscle preparations

Somatostatin attenuated the twitch contraction both in electrically driven left atria (0.33 Hz) and spontaneously beating right atria (Figure 1). A decrease in twitch contraction by 10^{–6} M somatostatin was produced immediately after the application and attained the maximum in a few minutes, then contraction recovered to the control level gradually. Subsequent cumulative application of 10^{–6} M somatostatin failed to cause further reduction in twitch contraction (Figure 1). These results suggested the occurrence of a comparatively slow but apparent desensitization phenomenon in the response of somatostatin. Figure 2 shows the concentration-response curves of somatostatin, in which somatostatin was applied non-cumulatively and the maximum response at each concentration was plotted. The effects of somatostatin were concentration-dependent and no difference was observed in the potencies between left and right atria. Beating-rate of the right atria was not significantly affected by 10^{–8} M to 10^{–6} M

Figure 1 Representative recordings showing the negative inotropic effect of somatostatin (10^{–6} M) in isolated right atrium (a), left atrium (b) and papillary muscle (c). Right atrium beat spontaneously. Left atrium and papillary muscle were electrically driven at a frequency of 0.33 Hz. Somatostatin (10^{–6} M) was applied cumulatively in (a) and (b).

Figure 2 Concentration-inhibition curves of somatostatin in the right atria (●), left atria (▲) and papillary muscles (■). Left atria and papillary muscle were driven at a frequency of 0.33 Hz. The extent of inhibition of twitch contraction was plotted against the concentrations of somatostatin. Each value indicates the mean ($n = 4-8$). Vertical lines show s.e.mean. * $P < 0.05$, ** $P < 0.01$ (significant difference from control).

somatostatin (131 ± 15 beats min^{-1} and 134 ± 17 beats min^{-1} , before and after application of 10^{-6} M somatostatin, respectively; $n = 5$).

In contrast to the atria, somatostatin (10^{-8} M– 10^{-6} M) did not affect the twitch contraction in the papillary muscles stimulated at a constant rate of 0.33 Hz (Figure 1) or 1 Hz (data not shown).

Frequency-dependent effects on mechanical response in the left atria

Figure 3 shows the effect of the stimulus frequencies on the negative inotropic action of somatostatin in electrically driven left atria. Since the effect of somatostatin was transient, as mentioned above, 10^{-6} M somatostatin was applied under conditions where twitch contractions reached a steady state at each frequency. Significant reduction of twitch response by somatostatin was produced at all frequencies tested (0.1–2 Hz). However, the effects varied with stimulus frequency; the inhibition was more marked at lower frequencies. Therefore, we used a frequency of 0.33 Hz in the following experiments.

Figure 3 Effects of stimulus frequency on the negative inotropic effect of somatostatin in left atria. Each value indicates mean ($n = 4-7$). Vertical lines show s.e.mean. * $P < 0.05$, ** $P < 0.01$ (significant difference from control).

Effects on the resting and action potentials in single atrial cells

Somatostatin at concentration of 10^{-7} M or 10^{-6} M shortened the action potential duration in the single atrial cells. Representative recordings of the effects of 10^{-7} M and 10^{-6} M somatostatin are shown in Figure 4. The cells were stimulated at a frequency of 0.33 Hz. The shortening of action potential

Figure 4 Effects of somatostatin on the action potentials in single left atrial cells. The cells were stimulated at a frequency of 0.33 Hz. (a) Shows action potentials before (C) and 5 min after the treatment with 10^{-7} M somatostatin (S). (b) Shows action potentials before (C) and 5 min after the treatment with 10^{-6} M somatostatin (S). Recordings from two different cells.

Table 2 Effects of somatostatin on resting and action potentials of guinea-pig single atrial myocytes

Treatment	n	RP	APA	APD ₃₀	APD ₅₀	APD ₉₀
Control	6	100% (-75.5 ± 1.2 mV)	100% (107.7 ± 2.9 mV)	100% (67.8 ± 13.1 ms)	100% (90.7 ± 12.6 ms)	100% (109.8 ± 15.5 ms)
Somatostatin 10^{-7} M	3	$98.8 \pm 0.7\%$	$100.6 \pm 0.9\%$	$80.9 \pm 4.2\%^*$	$86.7 \pm 5.2\%$	$94.1 \pm 6.3\%$
10^{-6} M	3	$100.8 \pm 0.2\%$	$99.1 \pm 0.5\%$	$69.6 \pm 4.0\%^{**}$	$72.6 \pm 3.5\%^{**}$	$78.0 \pm 2.0\%^{**}$

RP, resting potential; APA, action potential amplitude; APD₃₀, APD₅₀ and APD₉₀, action potential duration at 30%, 50% and 90% repolarization. n: number of cells. Values after somatostatin treatment are expressed as % of control. Absolute values in control are also shown in parentheses.

* $P < 0.05$, ** $P < 0.01$ (significant difference from control).

appeared immediately after the perfusion of somatostatin was commenced and lasted for more than 5 min. The effects of somatostatin on membrane potential parameters are summarized in Table 2, where each parameter is expressed as percentage of the control value because of the large variation in absolute values between the cells. Somatostatin (10^{-7} M and 10^{-6} M) significantly shortened the action potential duration, an effect which was more prominent at 30% repolarization (APD₃₀) than at 50% repolarization (APD₅₀) or 90% repolarization (APD₉₀). The action potential amplitude and resting membrane potential were unaffected by somatostatin.

Effects of somatostatin on ionic currents in single atrial cells

Whole-cell voltage clamp experiments were carried out using single atrial cells of guinea-pig. In experiments with normal external and potassium-rich internal solutions (Table 1), the membrane potential was held at the resting potential (-70 mV in Figure 5) and the depolarizing or hyperpolarizing pulses for 300 ms to various potentials were delivered every 3 s. Somatostatin (10^{-6} M) did not affect the currents flowing at the end of the 300 ms test pulses (Figure 5). However, the inward currents at the early phase of depolarizing pulses were somewhat attenuated by somatostatin. In order to analyse the effects on the calcium inward current, we dialyzed the cells internally with 120 mM caesium (Lee & Tsien, 1982; Nishio *et al.*, 1986) and superfused externally with potassium-free solution (Table 1). Furthermore, the membrane potential was held at -50 mV to inactivate sodium channels. Thus, calcium inward current was isolated from overlapping sodium and potassium currents (Muramatsu *et al.*, 1987). Figure 6 shows

Figure 5 Effects of somatostatin on ionic currents in a single atrial cell. (a) Representative currents recorded upon clamp steps from -70 mV (holding potential) to -10 mV and -100 mV before and after application of 10^{-6} M somatostatin. The sodium currents at a depolarization to -10 mV could not be recorded exactly. (b) Current-voltage relationships. The current amplitude was measured at the end of 300 ms clamp pulses before and 5 min after application of somatostatin.

Figure 6 Effect of somatostatin on calcium currents. The atrial cell was dialysed with caesium-rich internal solution and perfused with potassium-free external solution. (a) The membrane was held at -50 mV under voltage clamp conditions and step depolarizing pulses to 0 mV were applied every 3 s. (i) Before and (ii) 5 min after application of 10^{-6} M somatostatin. (b) Current-voltage relationships before (●) and 5 min after the application of 10^{-6} M somatostatin (○).

the calcium inward currents elicited by the depolarizing test pulses to 0 mV, before and 4 min after application of somatostatin, and the current-voltage relationships, in which the current amplitude was measured at the peak of the inward current. The inward calcium currents were elicited by test pulses to membrane potentials more positive than -40 mV. The calcium inward current was significantly reduced after perfusion of 10^{-6} M somatostatin ($45.1 \pm 8.2\%$ reduction in the current amplitude at 0 mV, $n = 6$).

Finally, we examined the effect of somatostatin on sodium current. The cell was perfused with normal external solution and the membrane was held at the resting potential (-75 mV). The experimental temperature was 22°C to slow down the sodium channel kinetics. Figure 7 shows representative recordings, in which the sodium current was elicited by a depolarization from -75 mV to -30 mV. Somatostatin (10^{-6} M) did not affect the sodium current ($n = 3$).

Discussion

Somatostatin produced a negative inotropic effect in guinea-pig atria and shortened the action potential duration (APD) of

Figure 7 Effects of somatostatin (10^{-6} M) on sodium current elicited by 50 ms clamp pulses to -30 mV from a holding potential of -75 mV in a single atrial cell. Temperature 22°C .

single atrial cells. The shortening of APD was more dominant at the plateau phase rather than at the end of repolarization. Since the calcium inward current contributes to maintain the plateau phase of action potential in the atrial myocytes (Hume & Uehara, 1985), it is likely that somatostatin causes the shortening of action potential duration by inhibiting calcium inward current. In fact, somatostatin inhibited the calcium inward current in single atrial cells without affecting the potassium and sodium currents under voltage clamp conditions. It is well known that calcium inward current is responsible for excitation-contraction coupling (Reuter, 1973). Therefore, the negative inotropic effect of somatostatin seems to be accounted for by the decrease of calcium inward current.

In contrast to the atria, somatostatin failed to produce any significant effects in the ventricular muscles (Figure 1c). The reasons for such regional differences in the effects of somatostatin are not known. However, it is interesting to note that somatostatin is present in the sinoatrial and atrioventricular node but not in the ventricular muscle (Day *et al.*, 1985). Such

heterogeneous distribution of somatostatin suggests that the peptide may play a physiological role in the conduction system of the heart. In fact, intravenous administration of somatostatin has been shown to restore sinus rhythm in paroxysmal supraventricular tachycardia and junctional tachycardia in man (Greco *et al.*, 1984; Webb *et al.*, 1986; Ghirlanda *et al.*, 1989). The present study also indicates that the antiarrhythmic effect of somatostatin may result from an inhibition of calcium channels.

Somatostatin has been shown to act on various ionic channels in various tissues. For example, somatostatin decreases the calcium current in neuroblastoma cells (Tsunoo *et al.*, 1986) and increases an inwardly rectifying potassium current in guinea-pig submucous plexus neurones (Mihara *et al.*, 1987). The present study indicates that somatostatin inhibits calcium channel current, without affecting the other ionic channels, in guinea-pig heart and that its negative inotropic effect can be attributed to this decrease in calcium inward current.

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