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Negative inotropic effect of somatostatin in guinea-pig atrial fibres

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1 The effect of somatostatin (SS, 1×10^{-7} M–5×10^{-6} M) was studied on the electrical and mechanical properties of isolated atria of the guinea-pig.

2 On spontaneously beating right atria, SS produced a dose-dependent negative inotropic effect which was accompanied by a decrease in atrial rate and a prolongation of the sinus node recovery time.

3 In electrically driven left atria, SS produced a dose-dependent negative inotropic effect which occurred concomitantly with a decrease in the amplitude and duration of the plateau phase of the action potential of atrial fibres.

4 SS also decreased the amplitude and maximum rate of depolarization of the slow action potential as well as the amplitude of the slow contractions induced by isoprenaline and caffeine in K-depolarized atrial fibres.

5 The negative inotropic effect of SS varied with the concentration of Ca and Na in the bathing media and the frequency of stimulation.

6 SS, 1×10^{-6} M and 5×10^{-6} M, decreased ^{45}Ca uptake in electrically driven atria.

7 All these results suggest that the negative inotropic effect produced by SS on rat isolated atria is related to its ability to reduce Ca influx via the slow inward Ca current.

Introduction

Somatostatin (SS) is a 14-amino acid-containing cyclic peptide the main function of which is the regulation of growth hormone and thyrotropin secretion (Reichlin, 1983; Gómez-Pan & Rodriguez-Arnao, 1983). SS is widely distributed not only within the nervous system but also in specific secretory cells of the gut and pancreatic islands, in the salivary glands and in the urinary secretory system of some species (Gómez-Pan & Rodriguez-Arnao, 1983). Despite this information very little is known about the cardiac effects of SS. Thus, Quirion *et al.*, (1979) studied the effects of SS in spontaneously beating right atria of different animal species. SS was completely inactive in rat and rabbit atria, whereas it exerted a dose-dependent decrease in contractile force in guinea-pig atria which was not accompanied by a decrease in atrial rate. This negative inotropic effect was attributed to a decrease in Ca entry into atrial fibres. In man, following intravenous administration of SS, Lundbaeck (1978) described a transient rise in blood pressure accompanied by a slight fall in pulse rate, whereas other authors (Rosenthal *et al.*, 1978a,b) found no significant change in either parameter. Moreover, in patients suffering from high renin hypertension it was proposed that SS exerted a negative inotropic effect that became overt only when the drug was administered with furosemide (Rosenthal *et al.* 1978a,b).

Very recently, it has been demonstrated that SS can restore sinus rhythm in patients with paroxysmal supraventricular and junctional tachycardia (Greco *et al.*, 1984), an effect which was attributed to a Ca antagonistic effect of SS on the atrioventricular nodal cells. The possibility that the cardiac effects of SS could be related to an inhibition of Ca entry into cardiac fibres is consistent with the results of different authors who proposed that the antisecretory effects of SS in pancreatic islets (Taminato *et al.*, 1975), gastric antrum (Bolman *et al.*, 1978) and pituitary gland (Schofield *et al.*, 1974) may be related to its ability to inhibit Ca entry into the secretory cells. However, up to now, there has been no study of the effects of SS on ^{45}Ca uptake and on the electrophysiological characteristics of isolated mammalian cardiac preparations. The present study, therefore, was undertaken to study the inotropic and electrophysiological effects of a wide range of concentrations of SS in guinea-pig isolated atrial fibres. The results of this paper confirmed the negative inotropic effect of SS. The role of Ca influx on the SS-induced negative inotropic effect has been also evaluated.

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Methods

General procedure

Guinea-pigs of either sex weighing 350–450 g were stunned by a sharp blow on the head and their hearts were rapidly removed. Right and left atria were dissected and mounted vertically in 10 ml organ baths containing Tyrode solution of the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42 and glucose 5.5. The solution was bubbled with 95% O_2 : 5% CO_2 and maintained at 34°C. In some experiments 30% of the Na concentration of the Tyrode solution was replaced by sucrose isosmotically. In others, the Ca concentration was varied from 0.9 to 7.0 mM. Left atria were electrically stimulated through bipolar platinum electrodes with square-wave pulses (1 ms duration, twice threshold strength) delivered from a multipurpose programmable stimulator. Under basic conditions, atrial muscle was stimulated at a basal rate of 1 Hz unless stated otherwise. Rate and amplitude of contractions were measured isometrically by a force-displacement transducer Grass FT03 and recorded on a Grass polygraph. Resting tension was adjusted to 1 g and a 30 min equilibration period was allowed to elapse before control measurements were taken. The different parameters of isometric contractions, the sinus node recovery time (SNRT) and the strength-duration curves were determined as previously described (Tamargo, 1980; Tamargo *et al.*, 1982). The influence of SS on the amplitude-interval relationship was determined as described by Barrigón *et al.* (1982).

After control values for each parameter were obtained, incremental doses of SS were added to the bath to obtain a complete dose-response curve in each muscle preparation. The interval between doses of SS was 10 min, since preliminary time-response studies indicated that the effects of the drug had stabilized in less than 10 min. The values for the different parameters obtained before SS were used as controls and compared to those obtained 10 min after each increment in drug concentration.

For experiments on the interaction between SS and the positive inotropic responses to $CaCl_2$, the following procedure was used. After control records were taken, increasing concentrations of $CaCl_2$ were added to the bath and a dose-response curve was obtained. Once stable curves were obtained, the same procedure was repeated 10 min after the addition of SS to the bathing media. Dose-response curves were expressed as percentage of maximal response vs. dose of the drug.

Intracellular recordings

Transmembrane action potentials (APs) were recorded as described previously (Rodriguez & Tamargo, 1980; Barrigón *et al.*, 1982). Preparations were set up in a 7 ml Lucite chamber, perfused with Tyrode solution and impaled with glass microelectrodes filled with 3 M KCl (tip resistance of 10–30 megohms). Microelectrodes were connected to high-impedance, capacity neutralizing amplifiers (WPI), displayed on a storage oscilloscope (Tektronix 5104N) and photographed on film. The maximum rate of rise of depolarization (V_{max}) was obtained by electronic differentiation. AP characteristics measured included: resting membrane potential, action potential amplitude, V_{max} and action potential duration measured at both 50% and 90% level of repolarization. The effective refractory period (ERP) was determined by delivering premature test-stimuli every eighth drive stimulus at different intervals from the preceding driving stimulus (Rodriguez & Tamargo, 1980).

Slow APs and contractions were induced in atria equilibrated in Tyrode solution and then rendered inexcitable by depolarizing with high K (27 mM) Tyrode solution (Pappano, 1970). Under those conditions atrial fibres became inexcitable despite intense electrical stimulation. Excitability was restored in atria driven at a basal rate of 0.12 Hz by adding caffeine (5 mM) or isoprenaline (10^{-6} M) to the perfusion solution.

^{45}Ca uptake

To determine ^{45}Ca uptake, left atria driven at a basal rate of 1 Hz were incubated in Tyrode solution for 30 min. In each experiment half of the atrium served as control and the other half as experimental preparation. Following equilibration the experimental half was treated with SS for 10 min. Then both halves were exposed for various time intervals (2, 5 and 10 min) to ^{45}Ca-labelled Tyrode solution (sp. act. 1 μCi ml^{-1}; Radiochemical Centre, Amersham).

At the desired time intervals, atria were removed, blotted on filter paper, dipped into Tyrode solution, reblotted and weighed. The atria were then placed in scintillation vials and 0.5 ml of Soluene-350 (Packard) added and digested overnight at 50°C. Radioactivity was assayed on a liquid scintillation counter (Intertechnique Model SL-3000) as previously described (Barrigón *et al.*, 1982).

Drugs

The following drugs were used: SS (Serono), caffeine (Sigma), isoprenaline hydrochloride (Sigma). Drugs were dissolved in distilled deionized water. Ascorbic acid (1×10^{-4} M) was added to each solution of isoprenaline daily to prevent its oxidation. All concentrations refer to the salt.

Throughout the paper results are expressed as

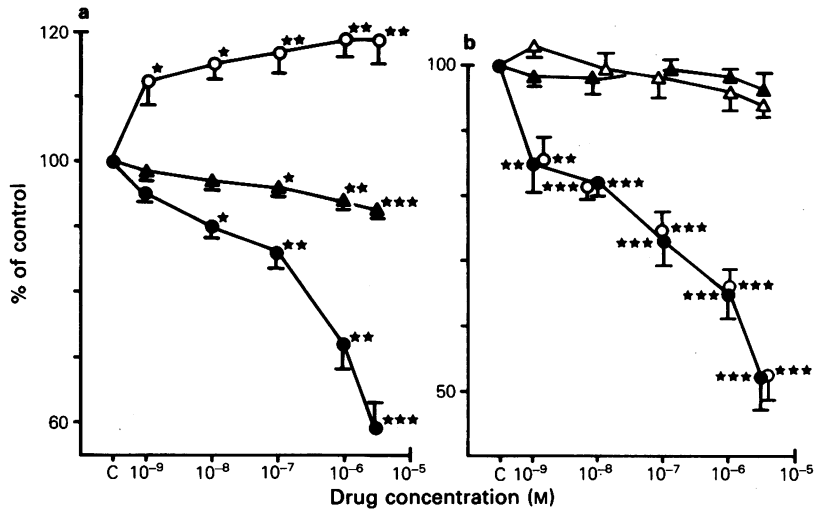

Figure 1 (a) Effect of somatostatin (SS) on peak contractile force (●) and rate (▲) of spontaneous contractions and on the sinus node recovery time (○) in isolated right atria. (b) Effect of SS on peak contractile force (●), df/dt_{max} (○), time to peak tension (▲) and time for total contraction (△) in electrically driven left atria. Ordinate scale: % of control values. Abscissa scale: drug concentration (M). Each point represents the mean of 12 experiments; vertical bars show the s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

mean ± s.e.mean. The statistical significance of differences from the control was estimated by Student's *t* test. A *P* value of less than 0.05 was considered significant.

Results

Effect of somatostatin in right and in left atrial preparations

The effects of SS in a range of concentrations between 1×10^{-9} M and 5×10^{-6} M on rate and amplitude of spontaneous contractions were studied in 12 right atria. Control values for both parameters were 177.0 ± 7.5 beats min^{-1} and 497.0 ± 72.0 mg, respectively. When added to the bathing media, SS produced a dose-dependent decrease in rate and contractile force (Figure 1a). Thus at 5×10^{-6} M, SS decreased contractile force by 41.6 ± 4.0% ($P < 0.001$) whereas atrial rate decreased by 7.5 ± 1.0% ($P < 0.001$). The onset of the cardiodepressant effect of SS occurred within 1 min of its addition to the bath and reached steady-state values within 3–6 min. In another 10 spontaneously beating right atria the control value for the SNRT averaged 394.1 ± 17.0 ms. As is shown in Figure 1a, SS 1×10^{-9} M–5×10^{-6} M, produced a dose-dependent significant prolongation of the SNRT. Thus, at 5×10^{-6} M, SS prolonged the SNRT by 20.0 ± 4.2% ($P < 0.001$) over control values.

The effects of cumulative concentrations of SS on different parameters of isometric contractions were also studied. In 12 left atria driven at a constant rate of 1 Hz, control values of isometric contractions were: peak contractile force = 731.0 ± 11 mg, df/dt_{max} = 15.0 ± 2.6 $mgms^{-1}$, time to peak tension = 67.1 ± 2.1 ms and time for total contraction = 230.0 ± 0.7 ms. At concentrations higher than 1×10^{-9} M SS significantly reduced peak contractile force but to a similar extent to that seen in right atria (Figure 1b). This negative inotropic effect was accompanied by a similar decrease in the df/dt_{max} but no significant changes were found in the time to peak tension or time for total contraction. Increase in basal tension was not observed in either right or left atria at any dose of SS studied. After the atria were rinsed with drug-free Tyrode solution atrial rate and contractile force gradually recovered to values similar to those recorded during the control period. Moreover, the negative inotropic effect of SS was antagonized when the external Ca concentration was increased to 3.6 mM or following the addition of isoprenaline (1×10^{-7} M) to the bathing media.

Influences of somatostatin on frequency-force and amplitude-interval relationships

The effects of SS on the positive inotropic effect elicited by increasing the frequency of stimulation from 0.25 to 3 Hz were studied in 8 left atria. Following the equilibration period, the rate of stimulation was decreased to 0.25 Hz and then in-

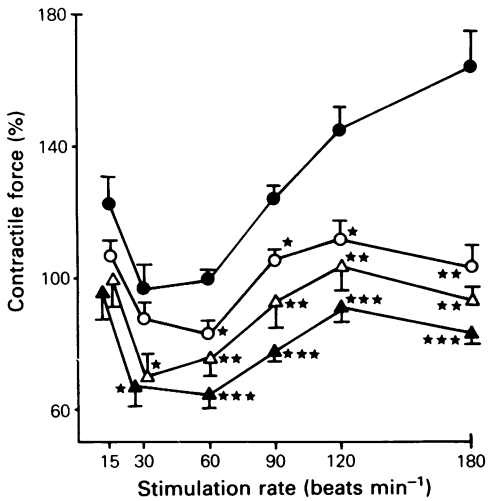

Figure 2 Effect of somatostatin (SS) on the force-frequency relationship in electrically driven left atria. Ordinate scale: contractile force (controls at 1 Hz were taken as 100%). Abscissa scale: stimulation rate (Hz). Each point represents the mean of 6 experiments; vertical bars show s.e.mean. (●) Control; (○) SS, 1×10^{-7} M; (△) SS, 1×10^{-6} M; (▲) SS, 5×10^{-6} M. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

creased stepwise to 3 Hz. Time was allowed after each frequency change for the maximum contractile response to be obtained. As is shown in Figure 2, in the absence of SS a biphasic frequency-force relationship was obtained. SS (1×10^{-7} M, 1×10^{-6} M and 5×10^{-6} M) produced a negative inotropic effect, this effect being more marked at high than at low frequencies. As a consequence, it inhibited the ascending branch of the frequency-force relationship.

In order to detect any interference with the availability of Ca from its intracellular stores, in another group of experiments after the development of steady contractions in left atria stimulated at 1 Hz, a single test contraction was elicited after various time intervals (1–8 s). The force of this contraction was plotted as percentage of the test contraction to obtain the amplitude-interval relationship (Figure 3). SS, 1×10^{-7} M and 1×10^{-6} M, only produced a significant reduction of peak contractile force at the resting interval 1 s, whereas at the other resting intervals the amplitude of the test contraction was not significantly different from that obtained under control conditions. At 5×10^{-6} M, SS significantly decreased ($P < 0.05$) the inotropic effect of the test contraction elicited after short resting intervals, i.e. between 1 and 3 s, whereas at longer intervals no

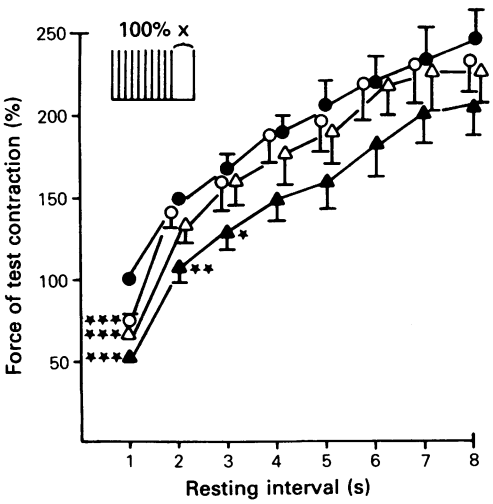

Figure 3 Amplitude-interval relationships of single test contractions elicited after a period of rest following frequent stimulation. The steady-state amplitude attained during conditioning stimulation was taken as 100%. Ordinate scale: force of test contractions (% of control values). Abscissa scale: resting interval (s). Each point represents the mean of 8 experiments; vertical bars show s.e.mean. (●) Control; (○) SS, 1×10^{-7} M; (△) SS, 1×10^{-6} M; (▲) SS, 5×10^{-6} M. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

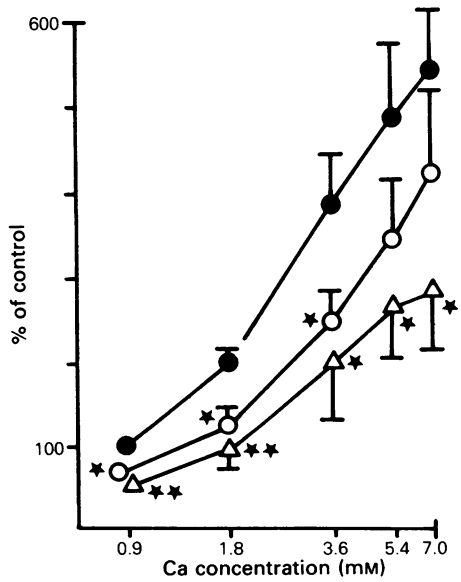

Figure 4 Effect of somatostatin (SS) on the positive inotropic effect of increases in Ca concentration in guinea-pig left atria. Ordinate scale: contractile force (% of control values). Abscissa scale: concentration (mM) of calcium in Tyrode solution. Each point represents the mean of 8 experiments; vertical bars show s.e.mean. (●) Control; (○) SS, 1×10^{-7} M; (△) SS, 1×10^{-6} M. $*P < 0.05$; $**P < 0.01$.

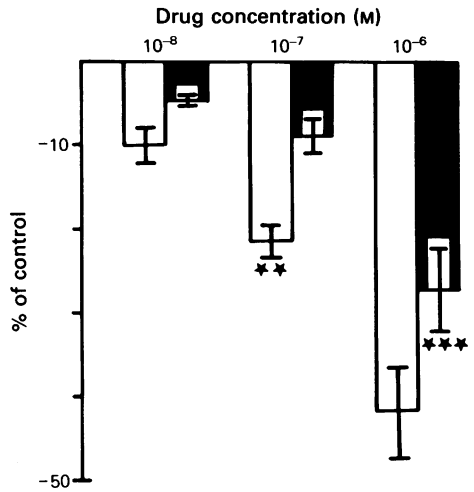

Figure 5 Effect of somatostatin, 1×10^{-8} M $- 1 \times 10^{-6}$ M on contractile force of electrically driven left atria incubated in normal Tyrode solution (open columns) or in 70% Na Tyrode solution (solid columns) expressed as percentage of control contractile force. Values are the mean of experiments; vertical bars shows s.e.mean. Significantly different from normal Tyrode solution: $^{**}P<0.01$; $^{***}P<0.001$.

significant differences were found when compared with the control curve.

Effect of variations of the extracellular concentration of Na and Ca on the negative inotropic effect of somatostatin

The influence of SS on the positive inotropic effect of increasing extracellular Ca concentrations was evaluated in a paired basis in 8 left atria. A stepwise increase in the extracellular Ca concentration, $[Ca]_o$, from 0.9 to 7.0 mM increased dose-dependently atrial contractile force. As is shown in Figure 4, SS (1×10^{-7} M and 1×10^{-6} M) shifted the dose-response curve of Ca downward, this effect being more significant at $[Ca]_o$ below 3.6 mM, i.e. increasing the $[Ca]_o$ partially or completely reversed the negative inotropic effect of SS. In another group of experiments the negative inotropic effect of SS was studied on a paired basis in 10 left atria equilibrated for 30 min in Tyrode solution with Na concentration reduced to 70% (Figure 5). In 70% Na solution, the peak contractile force was significantly increased over values obtained in atria incubated in normal Tyrode solution (704.6 ± 52.5 mg as compared to 512.3 ± 68.3 mg, $P<0.05$). Moreover, in 70% Na solution the negative inotropic effect of SS, 1×10^{-7} M and 1×10^{-6} M, was significantly reduced when compared with the effect observed in atria in normal Tyrode solution ($9.0 \pm 2.0\%$ and $27.5 \pm 4.8\%$ as compared to $21.5 \pm 2.4\%$ and $42.1 \pm 5.5\%$, respectively, $P<0.01$).

Effect of somatostatin on transmembrane potentials

The effects of SS, 1×10^{-9} M-1×10^{-6} M, were studied on transmembrane action potential (AP) characteristics of 12 atria. Results are summarized in Table 1. SS did not significantly affect at any of the concentrations tested, the amplitude and V_{max} of the AP or the resting membrane potential. However, at 1×10^{-9} M SS shortened the amplitude and duration of the plateau phase and significantly shortened the duration of the AP measured at the 50% level of repolarization. At higher concentrations this effect was accompanied by an increase in the slope of phase 3 of repolarization, which explains the progressive shortening of the duration of the AP measured at both 50% and 90% level of repolarization. The shortening of the AP was accompanied by a parallel shortening in the duration of the ERP which from the control values of 113.9 ± 6.2 ms ($n = 12$) decreased to 81.0 ± 4.0 ms ($P<0.001$) after perfusion with SS, 5×10^{-6} M. Pretreatment with atropine (1×10^{-6} M) did not modify the shortening of the APD_{50} ($38.7 \pm 4.8\%$ as compared to $32.6 \pm 6.8\%$, $P>0.05$) and APD_{90} values ($32.7 \pm 4.2\%$ as compared to 33.2 ± 8.1, $P>0.05$).

Table 1 Electrophysiological effects of somatostatin on transmembrane potentials in guinea-pig atrial fibres

Concentration (M)	*Resting potential* (mV)	*Amplitude* (mV)	V_{max} (Vs^{-1})	APD_{50} (ms)	APD_{90} (ms)	*ERP* (ms)
0	85.2±0.7	107.9±1.8	189.0±8.9	56.2±2.7	117.5±5.6	113.9±6.2
10^{-9}	85.9±0.9	107.6±1.9	186.7±7.6	46.9±3.1*	107.9±5.7	106.0±4.1
10^{-8}	85.7±0.9	109.0±2.4	190.8±8.3	45.5±3.0**	96.2±5.5**	99.9±4.2*
10^{-7}	85.9±1.0	107.3±2.1	192.5±8.5	41.0±3.2**	84.6±2.5***	89.9±4.9***
10^{-6}	86.6±1.2	109.1±2.0	189.5±7.4	34.5±5.5***	79.0±4.8***	81.0±4.0***

Mean values ± s.e.mean, $n = 13$.
$^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

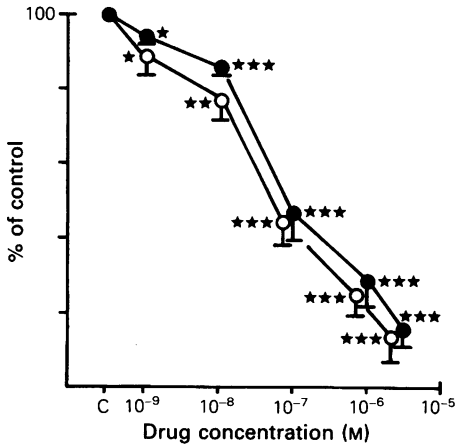

Figure 6 Effect of somatostatin (SS) on the slow contractions induced by isoprenaline (○, 1×10^{-6} M) or caffeine (●, 5×10^{-3} M) on K (27 mM)-depolarized atria. Ordinate scale: % of control values. Abscissa scale: drug concentration (M). Each point represents the mean of 8 experiments; vertical bars show s.e.mean. $*P<0.05$; $**P<0.01$; $***P<0.001$.

Effect of somatostatin on slow contractions and slow action potentials

In order to ascertain the effects of SS on the slow inward current, the fast inward Na current was voltage-inactivated by a partial depolarization (to about −46 mV) by perfusion with Tyrode solution containing 27 mM K. Atria so treated became inexcitable despite intense electrical stimulation. Isoprenaline (1×10^{-6} M) and caffeine (5×10^{-3} M) were then used to restore electromechanical activity, i.e. slow APs and contractions, in atria stimulated at a basal rate of 0.12 Hz. Peak contractile force of slow contractions induced by isoprenaline and caffeine averaged 550.6 ± 60.6 mg ($n = 8$) and 585.8 ± 50.3 mg ($n = 8$) respectively. As is shown in Figure 6, SS (1×10^{-9} M–5×10^{-6} M) produced a dose-dependent decrease in the amplitude of the slow contractions induced by isoprenaline or caffeine. Furthermore, Table 2 shows the effects of SS on the slow APs induced by caffeine. SS, 1×10^{-8} M–1×10^{-7} M, produced a dose-dependent decrease in amplitude and V_{max} and a shortening of the APD_{50} and APD_{90} values, without altering the resting membrane potential. However, at 1×10^{-6} M, the effects of SS on phase 0 characteristics and APD were accompanied by a slight depolarization of the resting membrane potential. Similar effects were observed in another 4 atria where the slow APs were induced by isoprenaline.

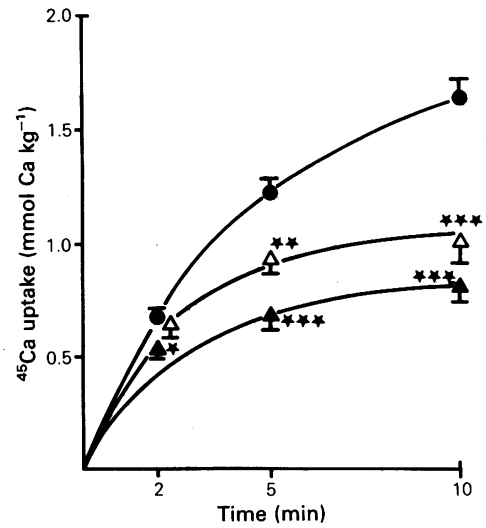

Figure 7 Effect of somatostatin (SS) on ^{45}Ca uptake in electrically driven left atria. Ordinate scale: ^{45}Ca uptake in mmol kg^{-1} wet weight. Abscissa scale: time (min) in radioactive solution. Each point represents the mean of 7 experiments; vertical lines represent s.e.mean. (●) Control; (△) SS, 1×10^{-6} M; (▲) SS, 5×10^{-6} M. $*P<0.05$; $**P<0.01$; $***P<0.001$.

Table 2 Electrophysiological effects of somatostatin on slow action potentials induced by caffeine in guinea-pig atrial fibres perfused with high K (27 mM) Tyrode solution

Concentration (M)	*Resting potential* (mV)	*Amplitude* (mV)	V_{max} (Vs^{-1})	APD_{50} (ms)	APD_{90} (ms)
0	47.4 ± 1.0	70.5 ± 2.4	10.3 ± 0.5	66.8 ± 10.3	89.9 ± 9.2
10^{-8}	47.0 ± 0.9	67.1 ± 2.2*	8.9 ± 0.3*	49.4 ± 6.7*	67.4 ± 7.4*
10^{-7}	46.7 ± 0.8	64.4 ± 2.9**	7.2 ± 0.3**	47.4 ± 6.8**	63.1 ± 9.1**
10^{-6}	45.7 ± 1.8*	58.8 ± 4.1**	5.8 ± 0.9**	42.8 ± 9.3**	58.5 ± 9.5**

Values are mean ± s.e.mean, $n = 9$.
$*P < 0.05$; $**P < 0.01$.

Effect on ^{45}Ca uptake

The effects of SS on ^{45}Ca uptake were studied in paired atria driven at a basal rate of 1 Hz. As is shown in Figure 7, at 1×10^{-6} M SS had no effect on ^{45}Ca uptake after 2 min exposure in labelled Tyrode solution but significantly decreased ^{45}Ca uptake after 5 ($P < 0.01$) and 10 min ($P < 0.001$). At 5×10^{-6} M, SS significantly decreased ^{45}Ca uptake at all time intervals tested.

Discussion

The present experiments demonstrated that in guinea-pig atrial fibres SS produced a dose-dependent decrease in peak contractile force similar to that previously observed in guinea-pig atria (Quirion *et al.*, 1979). This negative inotropic effect was accompanied by parallel changes in the df/dt_{max}, but no changes in the time to peak tension and time for total contraction were observed. Moreover, SS also produced a slight but significant decrease in atrial rate and prolonged the SNRT. This result is opposite to that published by Quirion *et al.* (1979) who found that SS did not modify the rate of spontaneous contractions in guinea-pig right atria. This discrepancy may be attributed to the small number of experiments performed or to the fact that the maximum concentration that they tested was 1×10^{-6} M. Moreover, they did not measure the SNRT, which is a more sensitive index of the sinus function and that may be of value in unmasking occult sinus node disfunction (Narula, 1975).

The results of this paper are clearly consistent with the hypothesis that the negative inotropic effect of SS in guinea-pig atria may represent a decrease in transmembrane Ca influx into atrial fibres. The evidence for this is 5 fold: (1) In our experiments the negative inotropic effect of SS occurred simultaneously with a decrease in amplitude and duration of the plateau phase which accelerated the time course of repolarization and shortened the APD_{50}. Because the activation of the slow inward Ca current is of prime importance for the genesis of the plateau phase of the AP (Carmeliet & Vereecke, 1979), it is reasonable to assume that because SS shortened the plateau it may reduce Ca influx and peak contractile force in atrial muscle fibres.

(2) SS at the same range of concentrations which exerted a dose-dependent negative inotropic effect also decreased the amplitude and V_{max} of the slow APs as well as the amplitude of the slow contractions induced by isoprenaline and caffeine in K-depolarized atrial fibres. Slow APs and contractions induced in K-depolarized fibres by isoprenaline and caffeine are an indirect measure of the magnitude of the slow inward Ca current (Pappano, 1970). Therefore, the depressant effect of SS on the slow responses suggests that its negative inotropic effect was due, at least partly, to a decrease in Ca influx via the slow inward Ca current. But the depression of the slow responses by SS would also be attributed to an increase in gK because SS shortened the APD at a time and dose that caused a decrease in amplitude and V_{max} of the slow APs. Furthermore, in fibres perfused with Tyrode solution, depression of the contraction was also associated with a significant shortening of the APD_{90}, which suggests that SS may increase the outward K current responsible for the final phase of repolarization (Carmeliet & Vereecke, 1979). In fact, SS increases K efflux in pancreatic islets (Pace & Tarvin, 1981).

(3) The negative inotropic effect of SS was more pronounced at fast frequencies, which led to a reversal of the ascending branch of the frequency-force relationship. Similar results have been described with various Ca antagonists (Bayer *et al.*, 1975; Tejerina *et al.*, 1984) and can be explained by a use-dependent block of the slow channels (McDonald *et al.*, 1980), that is, block occurs only during excitation, so that there is an accumulation of the blocked channels at high frequencies of stimulation.

(4) The effects of SS were reduced when the Ca concentration was increased or Na was reduced, that is, in situations when the Ca influx into atrial cells was increased (Langer, 1973). These results indicate that Ca antagonized the effects of SS in guinea-pig atria. The antagonistic effect of an increase in $[Ca]_o$ on the action of SS has been documented in pancreatic islets (Curry & Bennet, 1974; Oliver, 1976), guinea-pig atria (Quirion *et al.*, 1979), pig gastric antrum (Bolman *et al.*, 1978) and purified somatotrophs (Kraicer & Spence, 1981), which suggests that SS produced some of its effects by reducing Ca influx into the cells. Moreover, the negative inotropic effect of SS was reversed by isoprenaline, a drug that increases the slow inward Ca current in atrial fibres (Reuter, 1974), or by increasing the $[Ca]_o$. In fact, the inhibitory effects of SS on hormone release are also partially or completely reversed by exposure to the Ca ionophore A23187 (Kraicer & Spence, 1981; Pace & Tarvin, 1981). Conversely, SS is able to reduce Ca uptake induced by high K or the Ca ionophore A23187 (Schofield & Bicknell, 1978; Kraicer & Spence, 1981).

(5) Finally, SS decreased dose-dependently the ^{45}Ca uptake in electrically driven atria, which seems to confirm that a decrease in Ca influx is involved in the negative inotropic effect.

The exact mechanism by which SS decreases transmembrane Ca influx into atrial fibres has not been yet determined. Because of its physico-chemical properties and the rapid onset of the effects of SS and the rapid recovery after washing, however, the site of action of SS appears to be primarily on the outer surface of the sarcolema. Moreover, SS did not alter the restitution kinetics of Ca availability from in-

tracellular stores as revealed by the amplitude-interval relationship of single test contractions (Bayer *et al.*, 1975). Since the contraction elicited by the test stimuli after resumption of stimulation is mainly determined by the Ca stored in the sarcoplasmic reticulum (Bass, 1976), the negative inotropic effect of SS does not seem to be associated with a decrease in the availability of Ca from intracellular stores.

The clinical implications of these results remain to be determined. The range of concentration tested, 1×10^{-9} M–5×10^{-6} M (1.64–8190 ng ml^{-1}), are higher than the plasma SS concentration found in peripheral blood in normal subjects (80 to 675 pg ml^{-1}; Reichlin, 1983). However, in patients with somatostatinoma plasma levels of 3000 to 25000 ng ml^{-1} have been reported (Reichlin, 1979; Pipeleers *et al.*, 1979) and it has been reported that some pancreatic tumours may secrete small amounts of SS as an ectopic hormone (Reichlin, 1983). In these groups of patients it should be possible to observe some of the cardiac effects reported in this paper. Because SS did not alter the amplitude and V_{max} of the fast APs it is unlikely that it modifies excitability and conduction velocity in atrial muscle fibres. However, in cells with largely inactivated I_{Na}, as in the cells of sinus and atrioventricular nodes (Carmeliet & Vereecke, 1979), excitation is mainly based on the slow inward Ca current. Thus the inhibitory effect of SS on this current can cause bradycardia on the sinus node and retard the A-V conduction. The slowing of A-V conduction by SS could interrupt a re-entrant tachycardia which uses the A-V node as part of its circuit. In fact, recent evidence has demonstrated (Greco *et al.*, 1984) that in 5 out 6 patients with paroxysmal supraventricular and junctional tachycardia the sinus rhythm was restored after administration of 175–200 μg SS. The effect was preceded by a variable but transient atrioventricular dissociation which suggests that the effect of SS on supraventricular tachycardia is probably caused by a depressant effect on the atrioventricular node, possibly related to a Ca antagonistic effect.

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