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Suppressive effects of somatostatin in dog Purkinje fibres

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1 The effects of somatostatin (SS, 1 nM–3 μM) on the electrical and mechanical activities of isolated Purkinje fibres of the dog were studied.

2 In most Purkinje fibres driven electrically in normal $[K]_o$ Tyrode solution, SS decreased the force of contraction slightly and had very little effect on the fast response action potential. However, in sensitive fibres SS induced a moderate reduction of action potential duration and contractile force in normal $[K]_o$ and depressed the slow response action potentials in high $[K]_o$.

3 In spontaneously beating Purkinje fibres, SS decreased the regular rhythms slightly but abolished bursts of fast rhythms at a concentration as low as 1 nM.

4 When the fibres were depolarized in the presence of 0.2 mM barium or in Na-free solution, SS suppressed the Ca-dependent slow response action potentials.

5 These findings suggest that SS may suppress abnormal automatic activity of dog Purkinje fibres through a reduction of transmembrane Ca influx or a modulation of intracellular calcium.

Introduction

The hypothalamic growth hormone release-inhibiting factor, somatostatin (SS), is also distributed in peripheral tissues such as alimentary tract, pancreas and cholinergic postaganglionic neurones of the cardiac vagus (Campbell *et al.*, 1982). SS also acts as a negative inotropic agent in isolated atria of guinea-pig (Quirion *et al.*, 1979; Diez *et al.*, 1985) and toad (Campbell *et al.*, 1982) but not in atria from rat and rabbit hearts. In a clinical trial, intravenous SS has been shown to restore sinus rhythm in 5 out of 6 patients with paroxysmal supraventricular and junctional tachycardia (Greco *et al.*, 1984). A recent electrophysiological study in human isolated atrial fibres revealed that the effects of SS are highly dependent on the diastolic potential of atrial fibres and that SS may suppress the spontaneous low amplitude action potentials and the triggered activity through a reduction of cellular Ca (Hou *et al.*, 1987). To the best of our knowledge, however, no report exists concerning the electrophysiological action of SS in ventricular tissues. We have therefore performed experiments to see whether SS could also induce antiarrhythmic effects in mammalian ventricular tissues and to explore the underlying mechanism for these actions.

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Methods

Mongrel dogs of either sex weighing 5–10 kg were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.p.) and the heart was quickly excised. Strands of Purkinje fibres with a diameter of 1 mm or less were removed from ventricles and placed in a tissue bath perfused with oxygenated (97% O_2 and 3% CO_2) Tyrode solution at 37°C. The composition of normal Tyrode solution was as follows (mM): NaCl 137, KCl 4, $NaHCO_3$ 11.9, NaH_2PO_4 0.5, $MgCl_2$ 0.5, $CaCl_2$ 2.7 and dextrose 5.5. In the high $[K]_o$ solution, $[K]_o$ was increased to 27 mM and 5.5×10^{-7} M adrenaline was added. In the Na-free solution, $[Na]_o$ was substituted by tetraethylammonium (see Lin & Vassalle, 1979). The preparations were driven at 60 min^{-1} with suprathreshold electrical stimuli of 2 ms duration provided by a Grass S88 stimulator. One end of the preparation was fixed and the other end was connected to a Grass FTO3C force-displacement transducer. Transmembrane potentials were recorded by means of two glass microelectrodes filled with 3 M KCl and connected to a WPI 707 microprobe system. Both electrical and mechanical events were displayed simultaneously on a Tektronix 5223 storage oscilloscope and a Grass polygraph. The tracings were

recorded occasionally on film with a Tektronix C5C camera.

Diltiazem was obtained from Tanabe Seiyaku Company. SS and other chemicals were obtained from Sigma Chemicals. Values are expressed as means ± s.e. Statistical analysis was performed with Student's t test and a P value smaller than 0.05 was regarded as significant.

Results

Somatostatin effects in normal and high $[K]_o$

Figure 1 shows the differential effects of somatostatin on the action potential and contraction of Purkinje fibres driven electrically at 60 min^{-1} in normal $[K]_o$ Tyrode solution. Recordings in the left panel of Figure 1a are an example of a reduction of action potential duration at 50% repolarization (−20 ms) and a decrease in the contractile force (−40%) induced by a 4 min exposure to 1 nM SS. In most preparations, however, even high concentrations (0.1–1 μM) of SS induced only a small reduction of contractile force as illustrated in a preparation shown in Figure 1b. The maximal change in force of contraction induced by SS (from 1 nM to 1 μM) in 15 preparations are summarized in Figure 1c. The changes in the upstroke velocity ($\dot{V}_{max}$) and the amplitude of action potential were small (Figure 2a) except in one preparation with a depressed fast response action potential. The $\dot{V}_{max}$ was reduced from 88 to 63 Vs^{-1} after 5 min exposure to 1 nM SS as illustrated in Figure 2b. Results of the electrophysiological effects of 1 nM and 1 μM SS in 11 normal preparations are summarized in Table 1.

Effects of SS were also studied in Purkinje fibres depolarized in 27 mM $[K]_o$ in the presence of 0.55 μM adrenaline. In preparations resistant to the effect of SS in normal Tyrode solution, the changes in the slow response and force induced by SS were also small (Figure 1b). In 8 preparations, 1 nM and 1 μM SS decreased the amplitude of slow responses only slightly by 2.1 ± 0.7 mV and 2.3 ± 0.7 mV ($P < 0.02$), respectively. The force of contraction was reduced markedly nevertheless by −31 ± 11% ($P < 0.05$) and by −46 ± 10% ($P < 0.01$), respectively. When Purkinje fibres sensitive to the suppressive effects of SS in normal $[K]_o$ were depolarized in high $[K]_o$, SS decreased significantly the upstroke velocity and the amplitude of slow response action potential as well as the force of contraction. Eventually the fibres could become inexcitable and dropped beats occurred (right panel in Figure 1a). The greater depression of slow responses in high $[K]_o$ indicates a larger reduction of the transmembrane Ca influx (Sperelakis & Schneider, 1976) during SS exposure in the sensitive preparation.

Effects of somatostatin on normal and abnormal automaticity

In 7 experiments, the Purkinje fibres were not driven and 6 of them were discharged regularly at a rate of 24 ± 3.7 beats min^{-1} with a mean maximum diastolic potential of −78.9 ± 2.4 mV in normal Tyrode solution. SS at a low concentration (1 nM) did not change significantly the rate of spontaneous activity but at a high concentration (1 μM) reduced the rate to 17.3 ± 4.7 (−6.7 ± 1.4 beats min^{-1}, $P < 0.01$). In one spontaneously active preparation with a maximal diastolic potential around −80 mV, the rate of firing was not regular and bursts of fast rhythms (minimum cycle length 1.2 s) intermingled with slow rhythms (maximum cycle length 31 s). Bursts of fast rhythms occurred every 4–5 min and each burst lasted about 1 min. The fast rhythms were preceded by oscillations in diastolic potential and by a gradual acceleration of the rate of firing from slow to fast. SS in a concentration as low as 1 nM abolished the fast rhythms and suppressed further the slow rhythms.

In 6 experiments, Purkinje fibres were partially depolarized in Tyrode solution containing 0.2 mM $BaCl_2$ which is known to reduce membrane K conductance (DiFrancesco, 1981) and induce abnormal automaticity in Purkinje fibres (Dangman & Hoffman, 1980). As shown in Figure 3a, exposure to Ba caused a reduction of the diastolic potential and decreased the upstroke velocity and the amplitude of action potential. Eventually, spontaneous slow responses developed at a depolarized level. SS at a low concentration (1 nM) reduced the rate of discharges from 55 to 48 beats min^{-1}. At a high concentration (3 μM), the spontaneous activity was abolished completely (second panel in Figure 4b). This suppressive effect could be antagonized by increasing $[Ca]_o$ from 2.7 to 5.4 mM (third panel) and mimicked by diltiazem (1 μM, fourth panel). Similar results were obtained in another preparation. In the remaining 4 preparations, however, no significant effects were induced by SS (1 nM–1 μM).

Effects of somatostatin on slow responses developed in Na-free TEA solution

When all sodium in Tyrode solution was substituted with tetraethylammonium, Purkinje fibres depolarized and developed spontaneous low amplitude action potentials (Lin & Vassalle, 1979). As shown in Figure 4, low amplitude action potentials with a spontaneous firing rate of 19 min^{-1} were induced after exposure to Na-free solution for 10 min. SS abolished the spontaneous activity within 2 min. This effect is reversible after washout of SS. Diltiazem (0.1 μM) decreased the spontaneous rate (from 19 to 8 min^{-1}) transiently and abolished the spontaneous

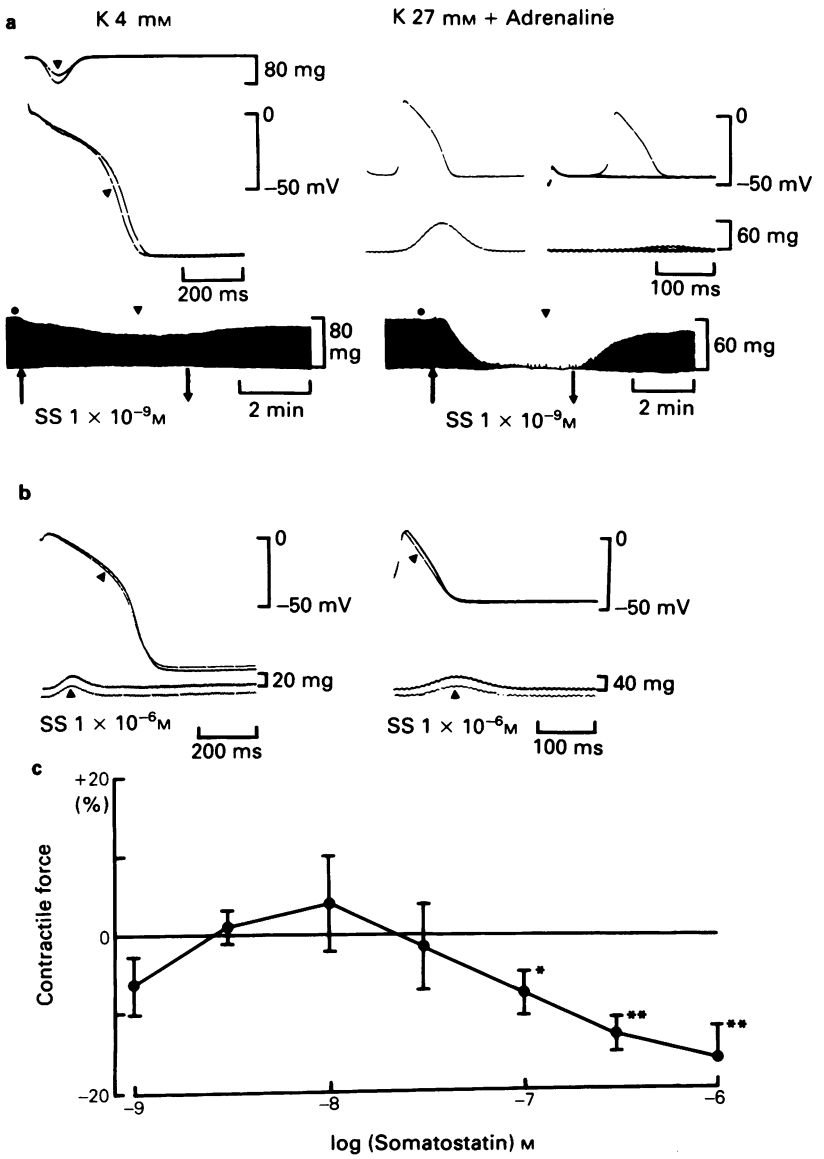

Figure 1 Effect of somatostatin (SS) on electromechanical activity of dog Purkinje fibres. In (a) the bottom traces show the recordings of contractile force in slow speed in normal $[K]_o$ (4 mM, left panel) and in 27 mM $[K]_o$ Tyrode solution plus 0.55 μM adrenaline (right panel). Upward and downward arrows indicate the start and end of SS (1 nM) exposure. The fast speed recordings of twitch (upper traces) and action potentials (middle traces) before and during SS exposure (triangle) were superimposed in the left panel. In the right panel, the dot and triangle on top of slow speed force recording indicate the time at which the fast speed action potential (upper traces) and twitch (middle traces) were taken before (dot) and during SS exposure (triangle). In (b), another preparation was perfused in the absence and presence (triangles) of 1 μM SS in normal (left panel) and high $[K]_o$ (right panel) solution. In (c) the inotropic effect of SS in Purkinje fibres obtained from 15 dogs are shown. The ordinate scale gives the mean and s.e. of change in contractile force in %. The abscissa scale shows the logarithmic concentrations of SS. $n = 15$ in 1 nM and 1 μM SS; $n = 6$ in other concentrations. $^*P < 0.05$, $^{**}P < 0.01$.

Table 1 Effects of somatostatin (SS) on action potential characteristics of dog Purkinje fibres in normal Tyrode solution (mean ± s.e).

	$\dot{V}_{max}$ (Vs^{-1})	*APA* (mV)	APA_{50} (ms)	APD_{90} (ms)
Control	324.3 ± 44.2	125.2 ± 1.0	258.2 ± 10.4	384.4 ± 8.0
SS (10^{-9} M)	298.0 ± 44.3	122.2 ± 1.3	239.2 ± 11.0	358.5 ± 8.0
Difference	−26.3 ± 16.8	−3.0 ± 1.3*	−19.0 ± 5.7**	−25.9 ± 7.5**
SS (10^{-6} M)	308.3 ± 48.3	122.6 ± 1.7	243.6 ± 10.0	362.4 ± 9.5
Difference	−16.0 ± 22.2	−2.6 ± 0.9*	−14.6 ± 10.6	−22.0 ± 11.5
n	6	11	11	11

$\dot{V}_{max}$, maximum upstroke velocity of depolarization; APA, action potential amplitude; APD_{50}, APD_{90}, action potential duration at 50% and 90% repolarization, respectively; *n* = number of preparations. In comparison with control, $P <$ *0.05; **0.01 (Student's paired *t* test).

activity completely at a higher concentration (0.3 μM). In 3 experiments, the maximum diastolic potentials were reduced from −82.3 ± 2.6 mV in normal Tyrode solution to −40.7 ± 3.5 mV in Na-free solution and spontaneous low amplitude action potentials appeared at a rate of 30 ± 6 beats min^{-1}. Exposure to 1 μM SS abolished spontaneous activity in all 3 preparations in 147 ± 48 s.

Discussion

The present study shows that there is differential sensitivity of dog Purkinje fibres to the actions of SS. In most preparations, SS (1 nM–1 μM) induces very little change in action potential characteristics. In sensitive fibres, however, SS at a concentration as low as 1 nM induces a moderate reduction of action

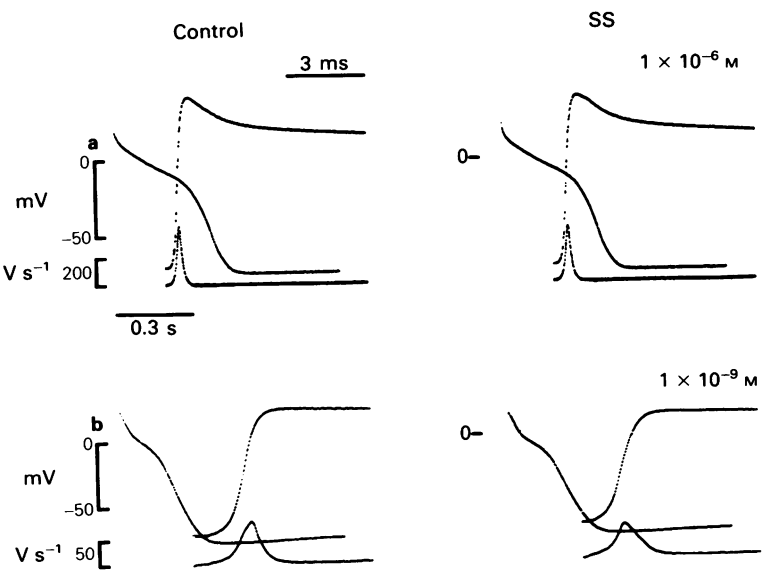

Figure 2 Effect of somatostatin (SS) on rate of upstroke ($\dot{V}_{max}$) of phase 0 depolarization in Purkinje fibres with normal (a) and depressed (b) fast response action potentials. In each panel, upper traces show the action potential at slow speed and the phase 0 depolarization at fast speed whereas the lower trace shows the $\dot{V}_{max}$ at fast speed. The preparations were driven at 60 min^{-1} in normal Tyrode solution before (Control, left panels) and after exposure to SS (1 μM in a and 1 nM in b, right panels) for 5 min.

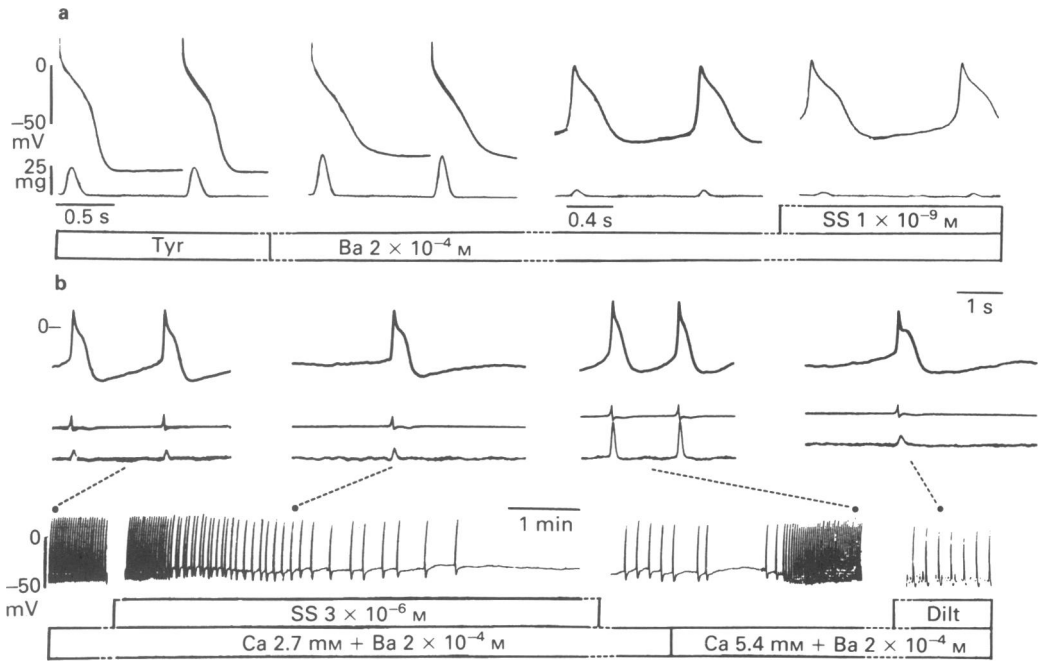

Figure 3 Effects of somatostatin (SS) on spontaneous slow response action potentials in Purkinje fibres depolarized with barium chloride (Ba 0.2 mM). In (a), the first and second panels show action potentials (upper traces) and twitches (lower traces) of the Purkinje fibre preparation driven at 60 min^{-1} in the absence (Tyr) and presence of 0.2 mM Ba. The third and fourth panels show the spontaneous activity before and during SS (1 nM) exposure. In (b), bottom traces show the slow speed chart recordings of action potentials before (first panel), during SS exposure (3 μM, second panel) and after washout. During the recovery, $[Ca]_o$ was increased from 2.7 to 5.4 mM (third panel) and then finally 1 μM diltiazem was added (fourth panel). Dots and dashed lines above chart recordings indicate the time at which the fast-speed oscilloscopic traces of action potentials, their first derivatives and twitches shown at the top were recorded.

potential duration and contractile force in normal $[K]_o$ and depresses the slow response action potential in high $[K]_o$ SS also suppresses calcium-dependent spontaneous small amplitude action potentials in Ba-depolarized fibres or in fibres perfused with Na-free solution. The results suggest that SS should have no major undesirable effects on electromechanical activity of normal Purkinje fibres but might suppress abnormal automaticity in depolarized fibres through a reduction of transmembrane Ca influx.

It has been shown that somatostatin prevented growth hormone release in perifused somatotrophs by blocking the action of cyclic AMP and by decreasing Ca influx into the cell (Kraicer & Chow, 1982). Somatostatin also inhibited insulin and glucagon secretion in pancreatic islets and this effect was readily reversed by calcium ionophore A23187 (Fujimoto & Ensinck, 1976) or high calcium (Taminato *et al.*, 1975). In guinea-pig atria the negative inotropic effect of SS was associated with a decrease of the plateau of fast action potential and the amplitude and rate of rise of the slow action potential (Diez *et al.*, 1985). SS also decreased ^{45}Ca uptake in atrial tissues (Diez & Tamargo, 1987). In human isolated atrial fibres obtained at cardiac surgery, SS decreased dose-dependently the contractile force (Hou *et al.*, 1987) and inhibited the noradrenaline-induced positive inotropic effect (Franco-Cereceda *et al.*, 1986). The inhibition was atropine-resistant and could be reversed by increasing $[Ca]_o$. All these results suggest that the suppressive effects of SS in atrial fibres are related to a reduction of Ca influx across sarcolemma.

Very little is known about the effect of SS on ventricular tissues. In toad ventricle, SS in concentrations up to 6 μM had no effect on the force of beat (Campbell *et al.*, 1982). In patients with high renin hypertension, however, SS (when administered with furosemide) decreased the cardiac and stroke index (Rosenthal *et al.*, 1978). The present study shows that in isolated dog Purkinje fibres, SS in concentrations up to 1 μM changed the high amplitude action potentials very little but suppressed dose-dependently the force

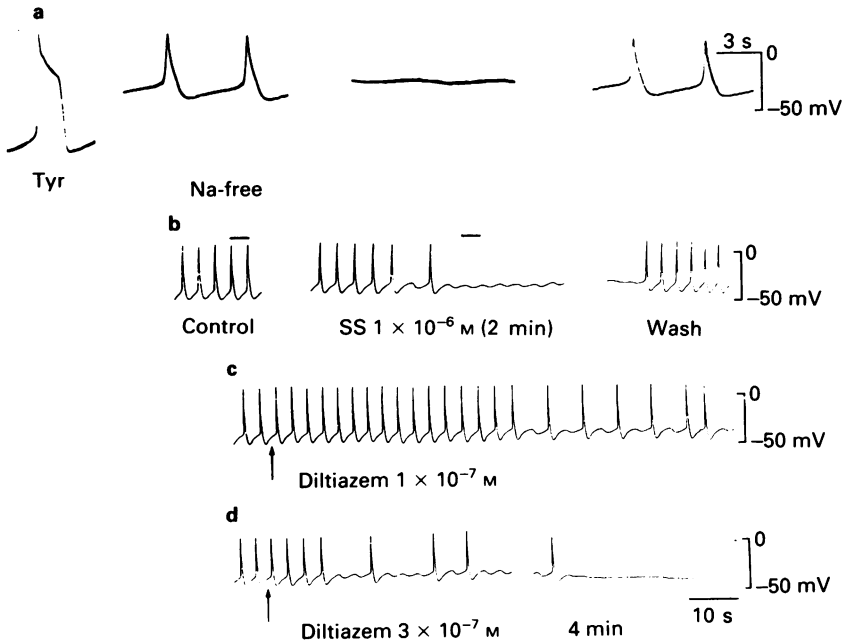

Figure 4 Effects of somatostatin (SS) on spontaneous action potentials in Purkinje fibres perfused in Na-free solution. In (a) oscilloscope traces of action potentials in normal Tyrode solution (Tyr) and in a solution in which all Na had been replaced by tetraethylammonium (Na-free) are shown. In (b) panels show the chart recordings of slow response action potentials before (Control), during SS exposure (1 μM, 2 min) and after washout (Wash) of SS in Na-free solution. Bars above chart recordings indicate the time at which the faster speed recordings in (a) were taken. In (c) and (d), diltiazem (0.1 and 0.3 μM, respectively) was added to the Na-free solution at arrows. In (c) 0.1 μM diltiazem was added to the Na-free solution at arrow. After washout, 0.3 μM diltiazem was added again in (d). The break in trace was 3 min.

of contraction. When the fibres were partially depolarized in high $[K]_o$ solution in the presence of adrenaline, SS could induce a decrease in the upstroke velocity and the amplitude of slow response, indicating a reduction of transmembrane Ca influx (Sperelakis & Schneider, 1976). This conclusion is further supported by the findings that SS suppressed the abnormal automaticity in Ba-depolarized fibres (Figure 3, also see Dangman & Hoffman, 1980) and the Ca-dependent slow responses in Na-free solution (Figure 4, see Lin & Vassalle, 1979). The latter findings also provide evidence that SS still suppressed slow action potentials in the absence of adrenaline.

However, the present results do not exclude other mechanisms for the cardiac effects of SS. It has been shown that SS inhibits the release of growth hormone from purified somatotrophs by blocking the action of cyclic AMP as well as by decreasing Ca influx into the cells (Kraicer & Chow, 1982). Thus SS could act through its cyclic AMP lowering effect which would compromise the function of the calcium channel at sarcolemma and the regulation of intracellular calcium by the sarcoplasmic reticulum. In addition, the suppression of $\dot{V}_{max}$ in depressed fast response action potential (Figure 2b) and the abolition of bursts of fast rhythms in spontaneously active Purkinje fibres with high level of MDP (−80 mV) also indicate an inhibitory action on sodium influx. However, the amplitude and the $\dot{V}_{max}$ of phase 0 depolarization of most Purkinje fibres with fast response are only barely affected by SS (Table 1) suggesting a minimal effect on the fast Na inward current in normal ventricular conducting tissues.

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References

CAMPBELL, G., GIBBINS, I.L., MORRIS, J.L., FURNESS, J.B., COSTA, M., OLIVER, J.R., BEARDSLEY, A.M. & MURPHY, R. (1982). Somatostatin is contained in and released from cholinergic nerves in the heart of the toad *Bufo marinus*. *Neuroscience*, **7**, 2013–2023.

DANGMAN, K.H. & HOFFMAN, B.F. (1980). Effects of nifedipine on electrical activity of cardiac cells. *Am. J. Cardiol.*, **46**, 1059–1067.

DIEZ, J. & TAMARGO, J. (1987). Effect of somatostatin on ^{45}Ca fluxes in guinea-pig isolated atria. *Br. J. Pharmacol.*, **90**, 309–314.

DIEZ, J., TAMARGO, J. & VALENZUELA, C. (1985). Negative inotropic effect of somatostatin in guinea-pig atrial fibres. *Br. J. Pharmacol.*, **86**, 547–555.

DIFRANCESCO, D. (1981). A new interpretation of the pace-maker current in calf Purkinje fibres. *J. Physiol.*, **314**, 359–376.

FRANCO-CERECEDA, A., LUNDBERG, J.M. & HOKFELT, T. (1986). Somatostatin: an inhibitory parasympathetic transmitter in the human heart? *Eur. J. Pharmacol.*, **132**, 101–102.

FUJIMOTO, W.Y. & ENSINCK, J.W. (1976). Somatostatin inhibition of insulin and glucagon secretion in rat islet culture: reversed by ionophore A23187. *Endocrinology*, **98**, 259–262.

GRECO, A.V., GHIRLANDA, G., BARONE, C., BERTOLI, A., CAPUTO, S., UCCIOLI, L. & MANNA, R. (1984). Somatostatin in paroxysmal supraventricular and junctional tachycardia. *Br. Med. J.*, **288**, 28.

HOU, Z.Y., LIN, C.I., CHIU, T.H., CHIANG, B.N., CHENG, K.K. & HO, L.T. (1987). Somatostatin effects in isolated human atrial fibres. *J. Molec. Cell. Cardiol.*, **19**, 177–185.

KRAICER, J. & CHOW, A.E.H. (1982). Release of growth hormone from purified somatotrophs: use of perifusion system to elucidate interrelations among Ca^{++}, adenosine 3',5'-monophosphate, and somatostatin. *Endocrinology*, **111**, 1173–1180.

LIN, C.I. & VASSALLE, M. (1979). Sodium lack prevents strophanthidin toxicity in Purkinje fibers. *Cardiology*, **64**, 110–121.

QUIRION, R., REGOLI, D., RIOUX, F. & ST-PIERRE, S. (1979). An analysis of the negative inotropic action of somatostatin. *Br. J. Pharmacol.*, **66**, 251–257.

ROSENTHAL, J., RAPTIS, S., ZOUPAS, C. & ESCOBAR-JIMENEZ, F. (1978). Inhibition by somatostatin of renin, blood pressure, and cardiac and stroke index in essential hypertension. *Circulation Res.*, **43**, (suppl I), 69–76.

SPERELAKIS, N. & SCHNEIDER, J.A. (1976). A metabolic control mechanism for calcium ion influx that may protect the ventricular myocardial cell. *Am. J. Cardiol.*, **37**, 1079–1085.

TAMINATO, T., SEINO, Y. & IMURA, H. (1975). Interaction of somatostatin and calcium in regulating insulin release from isolated pancreatic islets of rats. *Biochem. Biophys. Res. Commun.*, **66**, 928–934.

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