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Effect of somatostatin on ^{45}Ca fluxes in guinea-pig isolated atria

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1 The effect of somatostatin (SS, 10^{-6} M and 5×10^{-6} M) was studied on ^{45}Ca fluxes in guinea-pig isolated atria.

2 SS produced a dose-dependent decrease in ^{45}Ca uptake, this effect being dependent on the stimulation rate and Ca concentration in the bathing media.

3 The decrease in ^{45}Ca uptake was more evident at faster (60 and 180 beats min^{-1}) than at slower frequencies (15 beats min^{-1}) and was less evident in high Ca (5.4 mM).

4 SS had no effect on ^{45}Ca efflux.

5 These results suggest that SS inhibits the slow inward Ca current in guinea-pig atrial fibres.

Introduction

Somatostatin (SS) is a 14 amino acid peptide originally isolated from the bovine hypothalamus and subsequently found also in peripheral nerves (Reichlin, 1983). SS has been found to cause bradycardia and a transient rise in blood pressure (Lundbaek, 1978), reduce cardiac output (Rosenthal *et al.*, 1978) and restore sinus rhythm in patients with paroxysmal supraventricular and junctional tachycardia (Greco *et al.*, 1984). SS also reduced the force of contraction in various atrial heart muscle preparations (Quirion *et al.*, 1979; Diez *et al.*, 1984; 1985).

Recently, Diez *et al.* (1985) demonstrated that this negative inotropic effect appeared at concentrations of SS which inhibited contractility, slow action potentials and ^{45}Ca uptake in guinea-pig atrial muscle fibres. Therefore, these authors concluded that SS may inhibit Ca influx via the slow inward Ca current, I_{Ca}, in atrial muscle fibres. To elucidate further this possibility, the effects of SS on ^{45}Ca uptake and efflux were studied in guinea-pig left atria under different experimental conditions.

Methods

General procedure

Guinea-pigs of either sex weighing 350–400 g were stunned by a sharp blow on the head and their hearts were removed rapidly. The 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 ± 0.5°C. In some experiments the Ca concentration in the bath $[Ca]_o$, was varied from 0.45 to 5.4 mM. The atria were electrically stimulated through bipolar platinum electodes with square-wave pulses (1 ms duration, twice threshold strength) delivered from a programmable stimulator. Under basal conditions, atrial muscle was stimulated at a basal rate of 60 beats min^{-1}. In some experiments the stimulation rate was changed to 15 or 180 beats min^{-1}. Resting tension was adjusted to 1 g and a 30 min equilibration period elapsed before starting the experiments.

^{45}Ca uptake

To determine ^{45}Ca uptake left atria were bisected and one half of the atrium served as control and the other half as the experimental preparation. Following the equilibration period the Ca concentration of the bath, the rate of stimulation or both parameters were modified according to the experimental conditions. After 15 min the experimental half was treated with SS for 10 min. Then both preparations were exposed for 5 min to ^{45}Ca-labelled Tyrode solution (sp. act. 1 μCi ml^{-1}; Radiochemical Centre, Amersham). Afterwards atria were removed, blotted on filter paper, dipped three times for 10 s into Ca-free Tyrode

solution, reblotted and weighed. The atria were placed in scintillation vials and 0.5 ml of Soluene-350 (Packard) added and digested overnight at 50°C. Radioactivity was assayed in a liquid scintillation counter (Intertechnique Model SL-3000) as previously described (Barrigón *et al.*, 1982).

^{45}Ca efflux

To determine ^{45}Ca efflux following the equilibration period left atria were incubated in ^{45}Ca-labelled Tyrode solution (sp. act. 2 μCi ml^{-1}) for 2 h. Thereafter, the preparations were placed every 5 min for the 60 min duration of the washout in succesive tubes containing 2 ml of Tyrode solution. In these experiments, SS (10^{-6} M and 5×10^{-6} M) was added to the bathing solution sequentially during the 15 to 30 min period of the washout. After the efflux period of 60 min, radioactivity lost into the tubes and present at the end of the experiment in the atria was determined as described for the uptake experiments. From the final activity in muscles and from the amount of radioactivity lost from the atria at 5 min intervals the percentage of ^{45}Ca remaining in the atria at each collecting time was obtained. Washout data were expressed as % of radioactivity remaining in the atria after each 5 min interval and were plotted semilogarithmically as desaturation curves. The rate coefficient of the ^{45}Ca efflux, K_{cm}, was calculated as described by Holland *et al.* (1978).

Somatostatin (Serono) was dissolved in distilled water and added to the Tyrode solution to obtain final concentrations of 10^{-6} M and 5×10^{-6} M. Throughout the paper results are expressed as mean ± s.e.mean. The statistical significance of differences from control values was estimated by Student's *t* test. A *P* value of less than 0.05 was considered significant.

Results

Effect of somatostatin on ^{45}Ca uptake at different rates of stimulation

The effect of SS on ^{45}Ca uptake was studied in guinea-pig atrial muscle fibres driven at three different stimulation rates. An increase in the frequency of stimulation from 15 to 60 and 180 beats min^{-1} aug-

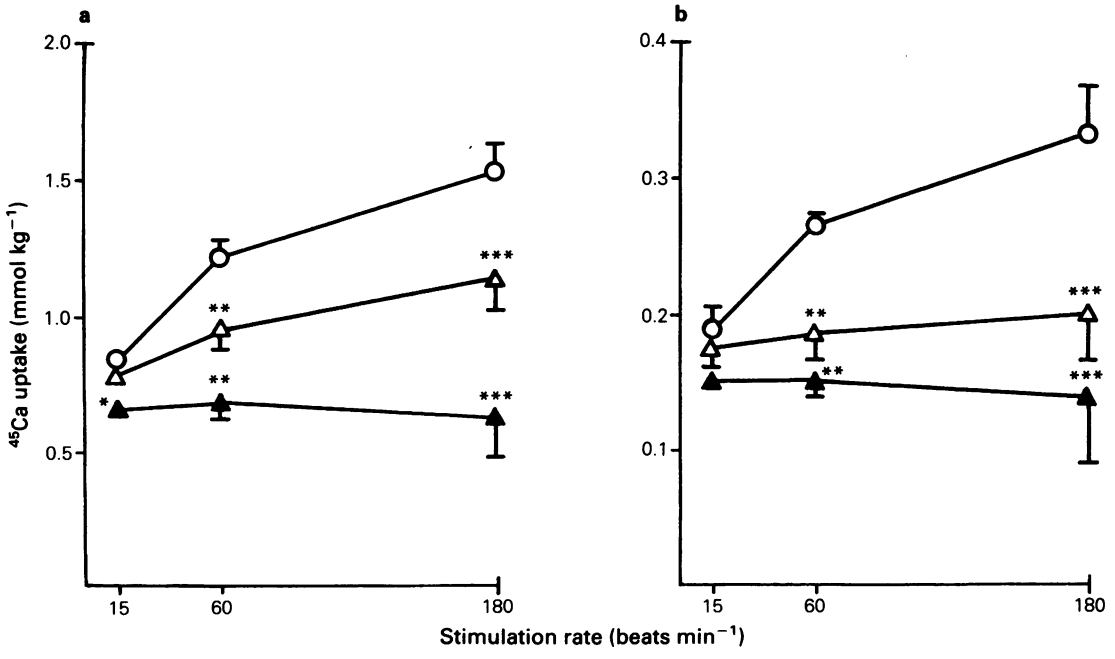

Figure 1 Effect of somatostatin (SS) on ^{45}Ca uptake in left atria after 5 min exposure at different stimulation rates and incubation in Tyrode solution with (a) normal (1.8 mM) or (b) low (0.45 mM) Ca concentration. Ordinate scales: ^{45}Ca uptake in mmol kg^{-1} wet weight. Abscissa scales: stimulation rate (beats min^{-1}). Each point represents the mean of 7–16 experiments; vertical lines show s.e.mean. (○) Control; (△) SS, 1×10^{-6} M; (▲) SS, 5×10^{-6} M. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

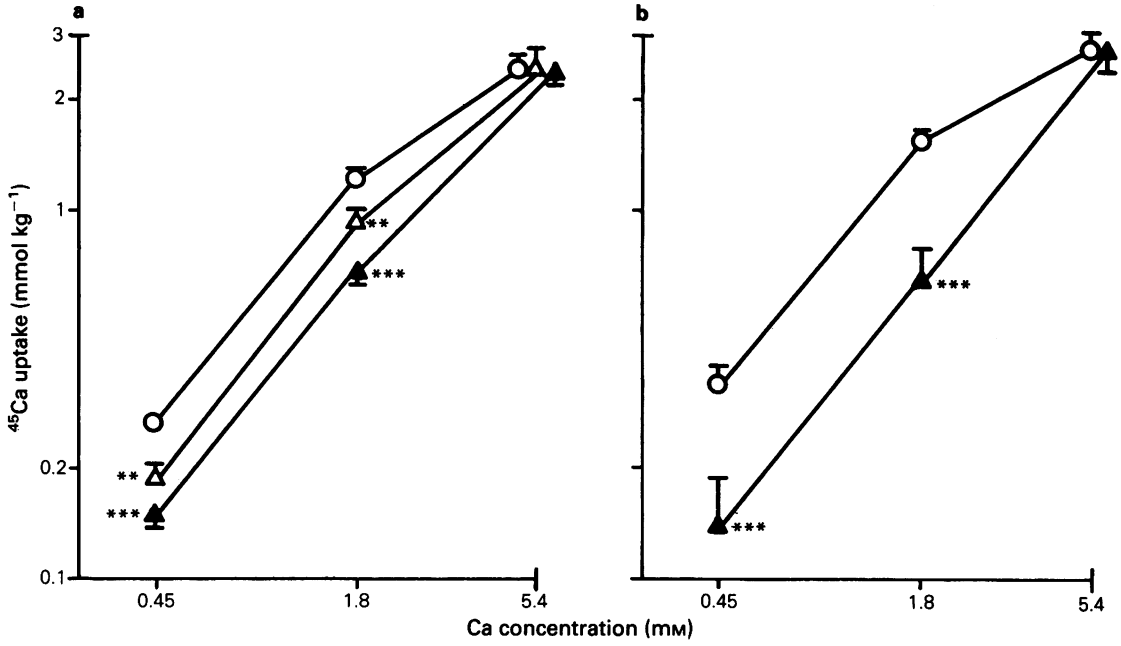

Figure 2 Effect of somatostatin (SS) on ^{45}Ca uptake in left atria after 5 min exposure to different extracellular Ca concentrations. (a) and (b) show data at stimulation rates of 60 and 180 beats min^{-1}, respectively. Ordinate scales: ^{45}Ca uptake in mmol kg^{-1} wet weight. Abscissa scales: Ca concentration in the bathing media. Each point represents the mean of 6–14 experiments; vertical lines show s.e.mean. (○) Control (△) SS, 1×10^{-6} M; (▲) SS, 5×10^{-6} M. $^{**}P < 0.01$, $^{***}P < 0.001$.

mented ^{45}Ca uptake progressively from 0.853 ± 0.021 to 1.224 ± 0.071 and to 1.535 ± 0.101 mmol kg^{-1}, respectively (Figure 1a). SS, 10^{-6} M and 5×10^{-6} M, produced a dose-dependent decrease in ^{45}Ca uptake at all stimulation rates, but this effect was more evident at faster (60 and 180 beats min^{-1}) than at lower frequencies (15 beats min^{-1}). Thus, at 10^{-6} M SS decreased ^{45}Ca uptake by $26.6 \pm 3.2\%$ ($P < 0.01$), $22.8 \pm 2.5\%$ ($P < 0.01$) and $5.9 \pm 1.1\%$ ($P > 0.05$) in atria driven at 180, 60 and 15 beats min^{-1}, respectively. At 5×10^{-6} M, SS decreased ^{45}Ca uptake by $58.0 \pm 7.0\%$ ($P < 0.001$), $43.7 \pm 3.8\%$ ($P < 0.01$) and $22.5 \pm 3.2\%$ ($P < 0.05$), respectively.

In atria incubated in low $[Ca]_o$ (0.45 mM) an increase in stimulation rate also increased the ^{45}Ca uptake (Figure 1b). SS, 10^{-6} M and 5×10^{-6} M, produced a dose-dependent decrease in ^{45}Ca uptake which again was more evident at faster than at slower frequencies of stimulation. Furthermore, in low Ca media the inhibitory effect of 10^{-6} M SS was significantly higher ($P < 0.05$) than in control Tyrode solution in atria driven at 60 ($30.8 \pm 4.6\%$, $P < 0.01$) and 180 beats min^{-1} ($40.1 \pm 5.1\%$, $P < 0.01$). However, the effects of SS, 5×10^{-6} M, were almost equal in both experimental series ($P > 0.05$).

Effects of somatostatin on ^{45}Ca uptake at different $[Ca]_o$

In another group of experiments the influence of Ca concentration in the organ bath, $[Ca]_o$, on the inhibitory effect of SS on ^{45}Ca uptake was examined in atrial muscle fibres driven at a basal rate of 60 beats min^{-1}. As shown in Figure 2a, an increase in $[Ca]_o$ from 0.45 to 5.4 mM produced a dose-dependent increase in ^{45}Ca uptake from 0.267 ± 0.009 to 2.41 ± 0.12 mmol kg^{-1}, respectively. SS, 10^{-6} M, produced a dose-dependent decrease ($P < 0.01$) in ^{45}Ca uptake in atria incubated in normal (1.8 mM) or low (0.45 mM) Ca media. However, SS (10^{-6} M and 5×10^{-6} M) had no significant effects on ^{45}Ca uptake in atria incubated in high Ca media (5.4 mM) (2.41 ± 0.12 as compared to 2.41 ± 0.29 and 2.40 ± 0.29 mmol kg^{-1}, respectively, $P > 0.05$). These results suggest that Ca antagonizes the inhibitory effects of SS on ^{45}Ca uptake in atrial muscle fibres.

The effects of SS, 5×10^{-6} M, on ^{45}Ca uptake were also studied in atrial fibres driven at 180 beats min^{-1} and incubated in low, normal or high Ca media (Figure 2b). Again SS significantly decreased ($P < 0.001$) the ^{45}Ca uptake in atria incubated in low

and normal Ca media, whereas it had no effect ($P > 0.05$) in atria incubated in high Ca media.

Effect of somatostatin on ^{45}Ca efflux

The effect of SS (10^{-6} M and 5×10^{-6} M) on ^{45}Ca efflux was evaluated in left atria after an incubation period of 2 h. In 6 left atria the percentage of ^{45}Ca remaining in the atria after the 60 min of washout was $7.53 \pm 0.91\%$. SS, 10^{-6} M, modified neither the percentage of ^{45}Ca remaining in the atria nor the K_{cm} for ^{45}Ca efflux from the tissue (not shown). Figure 3 shows the effects of SS, 5×10^{-6} M, on ^{45}Ca remaining and K_{cm} when added for 15 min following a 15 min washout in drug-free solution. In this group of 6 left atria the percentage of ^{45}Ca remaining in the atria after 60 min of washout was similar to that obtained in control atria ($7.41 \pm 0.72\%$, $P > 0.05$). Moreover, at this concentration SS had no effect on K_{cm} (see inset in Figure 3). The semilog plot of percentage ^{45}Ca remaining against time did not become linear even after 60 min of washout. Therefore, and to study further the effects of SS on ^{45}Ca efflux, the empirical approach of Reuter & Seitz (1968) was employed. They found that the rate efflux of ^{45}Ca from guinea-pig atria in Ca-free media was a function of the square of the amount of Ca

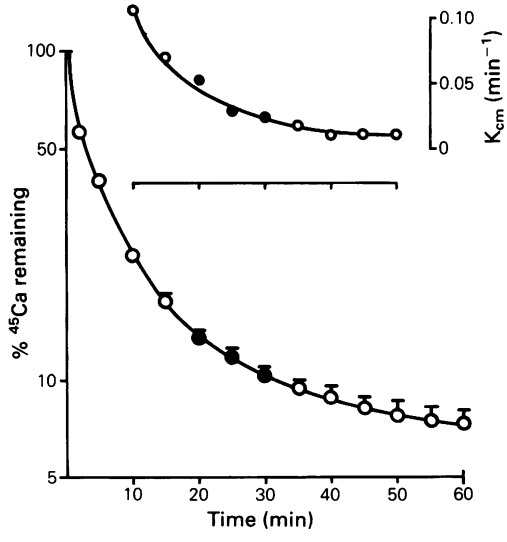

Figure 3 Effect of somatostatin (SS) on ^{45}Ca efflux in left atria after 2 h incubation in radioactive Tyrode solution. Ordinate scale: percentage of ^{45}Ca remaining in the atria. Abscissa scale: time (min) of washout. The inset shows the effect of SS on the rate coefficent of the ^{45}Ca efflux (K_{cm}). Each point represents the mean of 6 experiments; vertical lines show s.e.mean. (○) Control; (●) SS, 5×10^{-6} M.

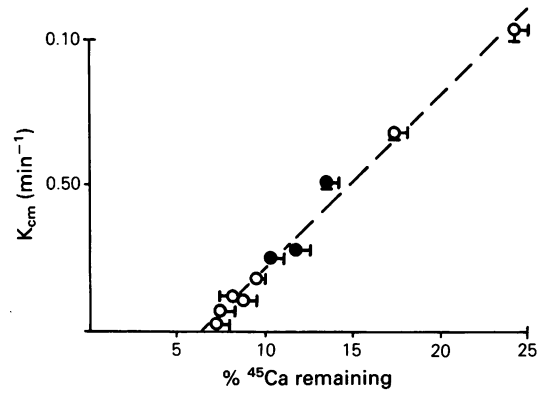

Figure 4 Relationship between the rate coefficient of ^{45}Ca efflux (K_{cm}, min^{-1}) and the percentage of ^{45}Ca remaining in the guinea-pig atria in the absence of (○) and presence of (●) somatostatin, 5×10^{-6} M. $r = 0.9921$; $P < 0.001$.

remaining in the tissue. Such a relationship would produce a straight line when the K_{cm} of the ^{45}Ca efflux is plotted against the percentage of ^{45}Ca remaining in the tissue. As is shown in Figure 4 this relationship was adjusted to a straight line ($r = 0.992$, $P < 0.001$) and this linearity was not modified in the presence of SS, 5×10^{-6} M. This confirms that SS had no effect on ^{45}Ca efflux in guinea-pig isolated atria.

Discussion

The results of this paper demonstrate that in guinea-pig isolated atria, SS inhibited ^{45}Ca uptake without altering ^{45}Ca efflux. This observation confirms previous evidence suggesting that the negative inotropic effect described in this preparation might be attributed to a reversible interference of SS with the Ca diffusion and/or transport into atrial fibres (Quirion *et al.*, 1979; Diez *et al.*, 1984; 1985). The inhibitory effect of SS on ^{45}Ca uptake was markedly dependent on the stimulation rate and $[Ca]_o$ in the bathing media. Thus, the effects of SS were more evident in atrial fibres driven at fast stimulation rates (60 and 180 beats min^{-1}) and in low or normal Ca media. However, the effect of SS was significantly reduced at slow stimulation rates (15 beats min^{-1}) and was counteracted by increasing the $[Ca]_o$ to 5.4 mM.

In guinea-pig isolated atria an increase in stimulation rate has a striking inotropic effect (Koch-Weser & Blinks, 1963) which has been attributed to an increase in Ca entry into atrial fibres accompanying the increased number of action potentials per unit of time (Grossman & Furchgott, 1964b; Edman & Johannsson, 1976). Measurements of ^{45}Ca uptake in guinea-

pig atria have demonstrated that increased frequency of contractions was paralleled by a greater influx of Ca and increased uptake of ^{45}Ca, whereas a decrease in stimulation rate resulted in a decreased intracellular ^{45}Ca content (Grossman & Furchgott, 1964a,b; Langer, 1965; 1973). Furthermore, a slow stimulation rate causes decreased Ca influx per time although the Ca influx per action potential is potentiated (Haacke *et al.*, 1970). Therefore, the inhibitory effect of SS on ^{45}Ca uptake found in atria driven at fast stimulation rates suggests that it may be due to an inhibitory effect on Ca entry via the slow inward Ca current. This possibility is consistent with previous results from this laboratory (Díez *et al.*, 1984; 1985) which demonstrated that in guinea-pig atria and at the same range of concentrations which inhibited ^{45}Ca uptake SS: (a) exerted a negative inotropic effect which was significantly greater at fast than at slow stimulation rates (b) shortened the duration of the plateau phase of the action potential; and (c) inhibited the amplitude and V_{max} of the slow action potentials induced in atrial fibres depolarized to levels where the fast channels are inactivated. Finally, our results are in agreement with the frequency-dependent block of the slow inward Ca current observed with verapamil and D600 (Ehara & Kaufmann, 1978; Pelzer *et al.*, 1982), drugs which exerted no negative inotropic effect at a stimulation rate of 6 beats min^{-1} but exerted strong negative inotropic effects at 60 beats min^{-1} (McCans *et al.*, 1974; Bayer *et al.*, 1975).

The effect of SS was also dependent on the $[Ca]_o$. In guinea-pig atria an increase of $[Ca]_o$ from 0.45 to 5.4 mM produced a positive inotropic effect which was accompanied by an increase in ^{45}Ca uptake (Winegrad & Shanes, 1962; Grossman & Furchgott, 1964a). Furthermore, voltage clamp studies also demonstrated that slow inward Ca current increased as $[Ca]_o$ and contractile force were augmented (Beeler & Reuter, 1970; New & Trautwein, 1972). Therefore, the inhibition of ^{45}Ca uptake observed at low and normal $[Ca]_o$ suggests that SS might inhibit the entry of Ca through the slow channels. Moreover, increasing $[Ca]_o$ to 5.4 mM reversed the negative inotropic effects of SS (Díez *et al.*, 1985) and counteracted its inhibitory effect on ^{45}Ca uptake. Thus Ca behaves as an antagonist of SS on guinea-pig isolated atria. In fact, the inhibitory effects of SS on hormone release are completely reversed by exposure to A23187, a Ca ionophore (Kraicer & Spence, 1981) and the negative inotropic effect of isoprenaline, a drug which increases Ca current in cardiac fibres (Díez *et al.*, 1985).

The physiological role of these results remains to be determined. However, it has been found very recently that SS is present in cardiac nerves in mammals (Day *et al.*, 1985), with higher concentrations being found in the atrioventricular (A–V) node and right atria. Since depolarization in sinoatrial and A–V nodes is due to the activation of I_{Ca} it may be possible that SS plays some role in the regulation of sinus function and A–V conduction. Thus, by blocking slow channel conduction in the A–V node, SS may inhibit nodal re-entrant supraventricular and junctional tachycardias and reduce ventricular rate in atrial flutter and fibrillation (Greco *et al.*, 1984). However, further studies are needed before this hypothesis can be accepted.

References

BARRIGON, S., DE MIGUEL, B., TAMARGO, J. & TEJERINA, T. (1982). The mechanism of the positive inotropic action of ketamine on isolated atria of the rat *Br. J. Pharmac.*, **76,** 85–93.

BAYER, R., HENNEKES, R., KAUFMANN, R. & MANNHOLD, R. (1975). Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. I. Pattern of inotropic effects of the racemic compounds. *Naunyn-Schmiedebergs Arch. Pharmac.*, **290,** 49–68.

BEELER, G. & REUTER, H. (1970), The relation between membrane potential, membrane currents and the activation of concentration in ventricular myocardial fibres. *J. Physiol.*, **207,** 211–229.

DAY, S., GU, J., POLAK, J. & BLOOM, S. (1985), Somatostatin in the human heart and comparison with guinea-pig and rat heart. *Br. heart J.*, **53,** 153–157.

DIEZ, J., VALENZUELA, C., DELGADO, C. & TAMARGO, J. (1984). Efectos de la somatostatina en fibras musculares auriculares de cobaya. *Rev. Esp. Cardiol.*, **37,** Supl. II–33.

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

EDMAN, K. & JOHANNSON, M. (1976). The contractile state of rabbit papillary muscle in relation to stimulation frequency. *J. Physiol.*, **245,** 565–581.

EHARA, T. & KAUFMANN, R. (1978). The voltage- and time-dependent effects of (−)-verapamil on the slow inward current in isolated cat ventricular myocardium. *J. Pharmac. exp. Ther.*, **207,** 49–55.

GRECO, A., GHIRLANDA, G., BARONE, C., BERTOLI, A., CAPUTO, S., UCCIOLI, L. & MANNA, R. (1984). Somatostatin in paroxysmal supraventricular tachycardia. *Br. med. J.*, **288,** 28–29.

GROSSMAN, A. & FURCHGOTT, R. (1964a). The effects of external calcium concentration on the distribution and exchange of calcium in resting and beating guinea-pig auricles. *J. Pharmac exp. Ther.*, **143,** 107–113.

GROSSMAN, A. & FURCHGOTT, R. (1964b). The effect of frequency of stimulation and calcium concentration on ^{45}Ca exchange and contractility on the isolated guinea-pig auricle. *J. Pharmac. exp. Ther.*, **143,** 120–130.

HAACKE, H., LULLMAN, H. & VAN ZWITEN, P. (1970). Calcium metabolism in atrial tissue during frequency potentiation and paired stimulation. *Pflügers Arch.*, **314,** 113–123.

HOLLAND, D., AMSTRONG, McD. & STAINBERG, M. (1978). Calcium fluxes and contractility in isolated guinea-pig atrium: effect of A23187. *Am. J Physiol.*, **235,** C13–C19.

KOCH-WESER, J. & BLINKS, J. (1963). The influence of the interval between beats on myocardial contractility. *Pharmac. Rev.*, **15,** 601–652.

KRAICER, J. & SPENCE, J. (1981). Release of growth hormone from purified somatotrophs: use of high K and the ionophore A23187 to elucidate interrelations among Ca, adenosine 3′, 5′-monophosphate and somatostatin. *Endocrinology*, **108,** 651–657.

LANGER, G. (1965). Calcium exchange in dog ventricular muscle: Relation to frequency of contraction and maintenance of contractility. *Circulation Res.*, **17,** 78–89.

LANGER, G. (1973). Heart: excitation-contraction coupling. *A. Rev. Physiol.*, **35,** 55–86.

LUNDBAECK, K. (1978). Somatostatin: clinical importance and outlook. *Metabolism*, **27,** 1463–1469.

McCANS, J., LINDEMAYER, R., MUNSON, R., EVANS, R. & SCHWARTZ, A. (1974). A dissociation of positive staircase (Bowditch) from ouabain-induced positive inotropism. *Circulation Res.*, **35,** 439–445.

NEW, W. & TRAUTWEIN, W. (1972). The ionic nature of slow inward current and its relation to contraction. *Pflügers Arch.*, **334,** 24–38.

PELZER, P., TRAUTWEIN, W. & McDONALD, T. (1982). Calcium channel block and recovery from block in mammalian ventricular muscle treated with organic channel inhibitors. *Pflügers Arch.*, **394,** 97–105.

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

REICHLIN, S. (1983). Somatostatin. *N. Engl. J. Med.*, **309,** 1495–1500, 1556–1563.

REUTER, H. & SEITZ, N. (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.*, **195,** 451–470.

ROSENTHAL, J., RAPTIS, S., ZOUPAS, C. & JIMENEZ-ESCOBAR, F. (1978). Inhibition of somatostatin of renin, blood pressure and cardiac and stroke index in essential hypertension. *Circulation Res.*, **43,** (suppl. 1), 1361–1363.

WINEGRAD, S. & SHANES, M. (1962). Calcium flux and contractility in guinea-pig atria. *J. gen. Physiol.*, **45,** 371–394.

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