Antagonist effects of seglitide (MK 678) at somatostatin receptors in guinea-pig isolated right atria

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Somatostatin (SS) exerts a negative inotropic effect in isolated atria. Here we report that in guinea-pig isolated right atria, seglitide, a potent cyclic hexapeptide somatostatin agonist, behaves as a competitive somatostatin receptor antagonist with pA_2 values against SS_{14} , SS_{25} and SS_{28} , of 6.50 ± 0.40 , 6.24 ± 0.08 and 6.09 ± 0.06 , respectively. Seglitide had little or no effect on the negative inotropic action of carbachol or N^6 -cyclohexyladenosine. Our findings indicate that the receptor-response coupling characteristics of guinea-pig atria are such that in this preparation seglitide has low intrinsic activity and behaves specifically as a somatostatin receptor antagonist.

Keywords: Seglitide; somatostatin; atria; antagonist

Introduction The tetradecapeptide somatostatin, first isolated from ovine hypothalamus (Brazeau *et al.*, 1973), exerts a negative inotropic effect in guinea-pig atria (Quirion *et al.*, 1979). However the pharmacology of somatostatin receptors is not well understood, due to a paucity of good drug tools.

The cyclic hexapeptide somatostatin analogue, seglitide (MK678), has been shown to be a potent somatostatin receptor agonist in various functional studies (Veber *et al.*, 1984) and selectively labels a somatostatin receptor subtype in radioligand binding studies (for review see Bell & Reisine, 1993). In the present study we have investigated the actions of some somatostatin analogues on spontaneously beating guinea-pig isolated right atria and shown that seglitide exhibits negligible agonist activity; rather, it acts as a somatostatin receptor antagonist in this preparation.

Methods Male Dunkin-Hartley guinea-pigs (220–450 g) were killed by cervical dislocation. Hearts were removed and transferred to a petri dish containing Krebs solution of the composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 0.6, KH_2PO_4 1.2, D-glucose 11.1 and $CaCl_2 \cdot 6H_2O$ 2.6.

Spontaneously beating right atria were prepared and mounted in 10 ml organ baths filled with Krebs solution, gassed with 95% O_2 /5% CO_2 maintained at 32°C and connected to a Dynamometer UF1 isometric force transducer by a cotton thread. Isometric contractions were recorded on a Lectromed multitrace 8-channel chart recorder, through a Lectromed MT8P preamplifier.

A resting tension of 1 g was applied before leaving preparations to equilibrate for 1 h, during which time tension was readjusted to 1 g with washes every 15 min. After equilibration, cumulative concentration-effect curves to SS_{14} (1 nM–3 μ M) were performed. Responses were expressed as percentage inhibition of developed tension. Following a 1 h interval with washes every 15 min, this was either repeated, or a cumulative concentration-effect curve to another agonist was constructed. When either antagonism or the influence of peptidase inhibitors was investigated, seglitide or peptidase inhibitors were added to the bath 30 min before the start of the repeat agonist concentration-effect curve. Following two further 15 min washout periods, preparations were exposed to 1 μ M carbachol.

Equi-effective concentration-ratios for all experiments were calculated at the level of 20% inhibition. Antagonism was quantified according to the method of Arunlakshana & Schild (1959). Results are expressed as means $\pm$ s.e.mean of n

observations. Statistical significance was ascertained by use of an unpaired t test, at a value of $P = 0.05$.

Drugs N^6 -cyclohexyladenosine, carbamylcholine chloride (carbachol), somatostatin₁₄ (SS_{14}), somatostatin₂₈ (SS_{28}), somatostatin₂₅ (SS_{25}), phosphoramidon and amastatin were all obtained from Sigma. Seglitide (MK678) (cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe)) was synthesized by Dr J. Kitchen's Chemistry team at Glaxo Group Research, Greenford. All peptides used were acetate salts.

Results *Agonist studies* SS_{14} (1 nM–3 μ M), SS_{25} (0.1 nM–0.3 μ M) and SS_{28} (0.1 nM–0.3 μ M) all decreased the force of developed tension in spontaneously beating guinea-pig right atria, (initial tension 476 ± 15 mg, $n = 92$) with maximum inhibitory effects of $46.6 \pm 2.6\%$, $60.0 \pm 2.0\%$ and $55.8 \pm 7.0\%$ ($n = 4$), respectively at the highest concentration tested (Figure 1). Carbachol (1 μ M) abolished spontaneously developed tension in all experiments. SS_{25} and SS_{28} were both more potent than SS_{14} , with equi-active molar ratios (EMR) of 0.08 ± 0.02 and 0.03 ± 0.01 ($SS_{14} = 1$), respectively ($n = 4$). Seglitide (1 nM–3 μ M) had little agonist activity producing only $9.0 \pm 1.1\%$ ($n = 4$) inhibition of developed force in the absence and a $12.2 \pm 4.8\%$ ($n = 4$) in the presence of a combination of the peptidase inhibitors phosphoramidon (1 μ M) and amastatin (10 μ M). No significant chronotropic changes were seen with any of these peptides.

Antagonist studies Seglitide (1–10 μ M) produced a concentration-dependent rightward shift of the concentration-effect curves to SS_{14} . Pre-incubation of 3 μ M seglitide for periods of 15, 30 and 60 min gave SS_{14} concentration-ratios of 6.4 ± 0.8 , 11.1 ± 6.4 and 7.2 ± 1.8 respectively ($n = 4$). Using a 30 min pre-incubation period, pA_2 values for seglitide against SS_{14} , SS_{25} and SS_{28} were 6.50 ± 0.40 , 6.24 ± 0.08 and 6.09 ± 0.06 , with slopes of the Schild plots of 0.86 ± 0.19 , 0.97 ± 0.12 and 0.89 ± 0.05 , respectively (see Figure 1). The pA_2 values were not statistically different from each other and the slopes of the Schild plots for all three agonists did not differ significantly from unity. In these experiments seglitide itself (10 μ M), produced only a small decrease in force of contraction $4.8 \pm 1.6\%$ ($n = 12$) which was transient and completely reversed within 5 min.

While seglitide (10 μ M), produced a marked displacement of the concentration-effect curves to SS_{14} , it had little or no effect on the negative inotropic action of either carbachol or cyclohexyladenosine, agonist concentration-ratios were 13.2 ± 2.1 , 2.4 ± 0.4 and 0.8 ± 0.2 , respectively. Corresponding control agonist concentration ratios were 1.2 ± 0.3 , 0.9 ± 0.2 and 1.2 ± 0.4 ($n = 4-6$).

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Figure 1 Inhibition of developed tension by (a) SS₁₄, (b) SS₂₅ and (c) SS₂₈ in the absence (●) and presence of seglitide 1 (△), 3 (□) and 10 μM (▽). The pre-incubation time for seglitide was 30 min. Values are means ± s.e.mean from 4 experiments.

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Discussion Somatostatin produced a negative inotropic effect in atria, with SS₂₅ and SS₂₈ being approximately 13 and 30 times more potent than SS₁₄, respectively. However, seglitide which has been used to characterize somatostatin receptors in both functional and radioligand binding studies, behaved primarily as a somatostatin receptor antagonist.

Veber *et al.* (1984) reported that seglitide is a potent inhibitor of insulin, glucagon and growth hormone release in rats, while it has also been shown to inhibit forskolin stimulated adenylate cyclase, voltage activated Ca²⁺ currents and potentiate a delayed rectifier K⁺ current in rat cultured neo-cortical neurones (Raynor *et al.*, 1991). Radioligand binding studies have demonstrated that seglitide shows high affinity for certain somatostatin receptor subtypes (Raynor & Reisine, 1993).

In the present study seglitide antagonized the negative inotropic response to SS₁₄, SS₂₅ and SS₂₈ but had little or no effect on the negative inotropic action of either carbachol or cyclohexyladenosine at 10 μM, indicating that seglitide acts specifically at somatostatin receptors. The antagonism displayed by seglitide was optimal with a 30 min incubation time, since extending this to 60 min incubation did not alter the degree of shift of the SS₁₄ concentration-effect curve produced by 3 μM seglitide.

The Schild plots had slopes that were not significantly different from unity, indicating that the antagonism displayed by seglitide was competitive, such that the pA₂ values provide an estimate of the dissociation constant of seglitide for the somatostatin receptor subtype present in guinea-pig atria. Since the pA₂ values for the three agonists are not significantly different from each other, it seems likely that all three agonists are acting at a common receptor.

The low intrinsic activity of seglitide is unlikely to be explained by the hydrolysis of the peptide by endogenous peptidases, since a mixture of peptidase inhibitors, previously shown to potentiate the effects of SS₁₄ in guinea-pig vas deferens (Feniuk *et al.*, 1993), had no significant effect on the response to seglitide in this preparation. Our findings suggest that seglitide is a low efficacy agonist and that either the receptor density and/or the receptor-response coupling efficiency in guinea-pig atria is low.

In conclusion, while seglitide has previously been shown to be a potent somatostatin receptor agonist, we describe here results which demonstrate that seglitide can behave as a specific antagonist, inhibiting the negative inotropic effect of somatostatin in guinea-pig isolated right atria, with little or no effect on the negative inotropic effects of either carbachol or cyclohexyladenosine.

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