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Evidence that the novel imidazoline compound FT005 is an α_2-adrenoceptor agonist

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1 The aim of this study was to determine whether the hyperglycaemic action of the novel imidazoline compound FT005 could be mediated by activation of α_2-adrenoceptors, using a variety of *in vivo* and *in vitro* methods including radioligand binding.

2 FT005 produced a dose-dependent increase in blood glucose levels of CBA/Ca mice (0.125–25 mg kg^{-1}, i.p.). The time course of this hyperglycaemic effect matched that of adrenaline (1 mg kg^{-1}) more closely than glucagon (1 mg kg^{-1}) or the K_{ATP} channel opener diazoxide (25 mg kg^{-1}). The hyperglycaemic effect of FT005 (1 mg kg^{-1}) was significantly reduced by the α_2-adrenoceptor antagonist rauwolscine (0.5 mg kg^{-1}).

3 FT005 produced a significant reduction in plasma insulin levels of mice 30 min after administration. The hyperglycaemic effect of FT005 (25 mg kg^{-1}), although still present, was significantly less in fasted mice in which insulin levels are lower, suggesting that a reduction of insulin secretion contributes to the action of FT005.

4 When studied in the mouse isolated vas deferens preparation, FT005 produced a complete inhibition of neurogenic contractions, which was blocked by rauwolscine. This is consistent with activation of pre-synaptic α_2-adrenoceptors.

5 In radioligand binding studies FT005 completely displaced the α_2-adrenoceptor antagonist [^{3}H]-RX821002 from mouse whole brain homogenates. The displacement was best described by a two-site model of interaction comprising high and low affinity components.

6 The results indicate that FT005 is an agonist at α_2-adrenoceptors. A reduction in insulin secretion contributes to the hyperglycaemic action of FT005, although an additional mechanism can not be excluded.

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Abbreviations: NIDDM; non-insulin-dependent diabetes mellitus (Type II diabetes)

Introduction

Early evidence that α_2-adrenoceptor agonists inhibit the release of insulin from the pancreas (Nakadate *et al*., 1980; Langer *et al*., 1983) led to the suggestion that α_2-adrenoceptor antagonists might be useful in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). The α_2-adrenoceptor antagonist phentolamine was subsequently shown to improve glucose homeostasis in patients with NIDDM (Broadstone *et al*., 1987). However, when examined in isolated mouse islets, in the absence of adrenoceptor activation, phentolamine, but not rauwolscine, increased insulin secretion, demonstrating a more direct effect on insulin secretion (Schulz & Hasselblatt, 1988). This was later shown to be due to the presence of an imidazoline moiety, absent from rauwolscine, and unrelated to antagonism of α_2-adrenoceptors (Schulz & Hasselblatt, 1989). Imidazoline compounds now represent a novel class of pharmacological agents with the potential for treating type II diabetes, and a number of them increase glucose-stimulated insulin secretion from the pancreatic β-cell (Schulz & Hasselblatt, 1988; Chan & Morgan, 1989; Le Brigand *et al*., 1997).

Around this time the concept of a putative imidazoline receptor was developing, leading to the identification of three subtypes with distinct pharmacology: the I_1 binding site, which appears to be involved in cardiovascular regulation; the I_2 binding site which is involved in monoamine regulation; and a third distinct imidazoline binding site, the I_3 site, which was proposed to exist in the pancreatic β-cells, and is responsible for the insulin secretory effect of imidazolines (for review see Eglen *et al*., 1998). I_1 receptors are not thought to be expressed by β-cells (Brown *et al*., 1993a), whilst I_2 binding sites have been shown to be present (Olmos *et al*., 1994); however, the pharmacology of these receptors is not consistent with the pharmacology of the imidazoline binding site involved in regulating insulin secretion (Morgan *et al*., 1995). Compounds such as efaroxan act at the putative I_3 site in β-cells, where they modulate the open state of the adenosine 5′-triphosphate (ATP)-sensitive potassium channel (K_{ATP}). Efaroxan induces closure of the K_{ATP} channel (Chan *et al*., 1991), leading to membrane depolarization and the opening of voltage-sensitive Ca^{2+}

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channels to initiate insulin exocytosis. Interestingly, efaroxan only stimulates insulin secretion at a stimulatory glucose concentration ($\geqslant$4 mM, Chan *et al*., 1991). Sulphonylurea compounds such as glibenclamide also increase insulin secretion by modulating K_{ATP} channels but, in contrast with efaroxan, increase insulin secretion at non-stimulatory glucose concentrations, and as such are intrinsically hypoglycaemic. Thus, the I_3 binding site may represent a target for new oral antihyperglycaemic agents which lack the risk of hypoglycaemia seen with sulphonylurea compounds.

The exact nature of the I_3 binding site remains unclear. The site displays a degree of stereoselectivity; the (−)-enantiomer of efaroxan reverses the inhibition of insulin secretion caused by diazoxide, while the (+)-enantiomer is an α_2-adrenoceptor antagonist (Chan *et al*., 1993). The binding site is also subject to down-regulation following prolonged agonist exposure (Chan *et al*., 1993). The location of the I_3 binding site on the K_{ATP} channel appears to be the pore-forming Kir6.2 subunit rather than the regulatory SUR subunit. Radioligand binding studies have confirmed that the I_3 site is distinct from the sulphonylurea binding site, since efaroxan failed to displace [^{3}H]-glibenclamide (Brown *et al*., 1993b).

Two compounds have been identified as antagonists at the putative I_3 binding site. Both RX801080 (Brown *et al*., 1993b) and the efaroxan derivative KU14R (Chan *et al*., 1997) prevent the ability of efaroxan to promote glucose-induced insulin secretion whilst having no direct effect on insulin secretion themselves. However, RX801080 has been shown to inhibit glibenclamide-induced insulin secretion, suggesting that the antagonism may also occur at sulphonylurea receptors (Brown *et al*., 1993b). In patch-clamp studies KU14R has been shown to produce a slight reduction in the K_{ATP} channel opening, consistent with the action of agonists such as efaroxan (Chan *et al*., 1998). In contrast, in BRIN-BD11 cells, KU14R and RX801080 have been shown to promote glucose-induced insulin secretion, and neither compound inhibited the ability of efaroxan to increase insulin secretion; KU14R and RX801080 both enhanced the secretion seen in the presence of efaroxan (Ball *et al*., 2000). Consequently, the antagonist action of KU14R and RX801080 at the I_3 site is not clear cut.

We have recently shown that the novel imidazoline compound FT005 (S22954, 4-methyl-2-(4,5-dihydro-1H-imidazol-2-yl) benzo(β)morpholine) increases blood glucose levels in mice *in vivo* (Slough & Taberner, 1999), and have suggested that it might represent a novel I_3 antagonist. Alternatively, given that the imidazoline moiety is present in many α_2-adrenoceptor ligands it is possible that FT005 may be a novel α_2-adrenoceptor agonist (see Figure 1). Here we present results from further experiments designed to establish whether FT005 acts as an α_2-adrenoceptor agonist by testing the ability of known α_2-adrenoceptor antagonists to block the hyperglycaemic effect of FT005, by examining the hypothermic effect of FT005, and by measuring competition binding with [^{3}H]-RX821002 in mouse brain tissue.

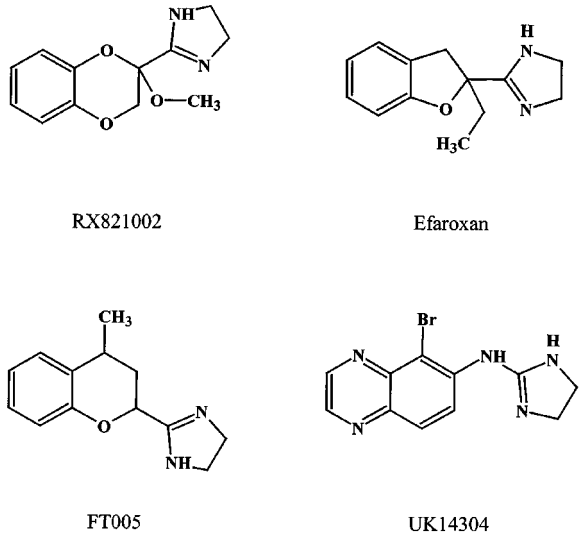

Figure 1 Structures of imidazoline compounds.

Methods

Animals and treatment

Male CBA/Ca mice (26–36 g) aged 16–24 weeks were housed at 19–23°C, relative humidity 40–60%, on a 12 h light/dark cycle of 07:00 h to 19:00 h and were provided with standard rat and mouse pelleted chow (Beekay Standard Rat and Mouse Diet, BK Universal) and water *ad libitum*. All animals were bred in-house at Bristol University. Drugs were administered intra-peritoneally (i.p.) or orally by gavage (p.o.), at a dose volume of 10 ml kg^{-1}; control mice received an equivalent volume of physiological saline or 0.1 M NaOH. Food was withdrawn at the start of each experiment unless stated otherwise. Experiments were begun between 09:00 and 10:00 h.

Drugs

Diazoxide and adrenaline were purchased from Sigma (Poole, U.K.). Glucagon was purchased from Lilly (Basingstoke, U.K.). FT005 (4-methyl-2-(4,5-dihydro-1H-imidazol-2-yl) benzo(β)morpholine) was a gift from Servier (Paris, France). All other reagents were of analytical reagent grade.

Blood glucose measurement

Blood (10 μl) was obtained by venesection of the tail tip under ether anaesthesia. Blood glucose levels were determined using a Boehringer Mannheim Glucochek II, and BM 1-44 glucocheck strips. Blood glucose levels were determined immediately before drug administration (time 0) and at 30 or 60 min intervals thereafter for up to 6 h.

Plasma insulin assays

Blood (200–250 μl) was collected from mice following venesection of the tail, immediately prior to administration of FT005 (1 mg kg^{-1}, i.p.) or saline (time t = 0 min). A second sample was taken at 30 min, by cardiac puncture under ether anaesthesia. A final sample was collected at 60 min from the jugular vein under ether anaesthesia and the mice were killed. Due to the blood volume required for each sample, mean results for different time points were combined from different animals randomized between experiments. The blood was centrifuged for 10 min at 3000 × *g*, at 4°C to

obtain plasma. Plasma insulin was then determined using a radioimmunoassay kit (Amersham, RPA 547).

Isolated vas deferens experiments

Mouse vasa deferentia were rapidly removed and placed in Krebs solution of the following composition (mM): NaCl 118.0, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 0.93, $NaHCO_3$ 25, glucose 11. Magnesium was excluded as more consistent responses are obtained in its absence in mouse tissue (Hughes *et al*., 1975). The vasa deferentia were mounted singly in a 3.5 ml organ bath containing Krebs solution at 37°C, bubbled with 95% O_2 and 5% CO_2, under 0.5 g resting tension, and allowed to equilibrate for 30 min prior to beginning experiments.

The intramural nerves of the tissue were excited using electrical field stimulation; the vas deferens was mounted between two platinum wire electrodes and stimulated every 10 s by a train of three pulses at 80–100 V, 1 ms duration at 10 Hz, using a Grass S88 stimulator. A pulse width of 1 ms was used so as to avoid stimulating the muscle, which can occur at pulse width greater than 5 ms. Contractions of the vas deferens were recorded on a chart recorder (Graphtec Linearcorder). A cumulative concentration response curve was constructed for FT005 (1 nM–100 μM), and for the α_2-adrenoceptor agonist UK14304 (1–30 nM), by adding the relevant drug to the organ bath using a 2 min application period. The effect of the α_2-adrenoceptor antagonist rauwolscine (1–10 μM) on the response to FT005 was also studied; in these experiments the rauwolscine was applied 10 min prior to FT005.

Membrane preparation

Mice were killed by cervical dislocation and whole brains were removed rapidly over ice. Brains were homogenized in 10 volumes of ice-cold sucrose buffer (0.32 M sucrose, 50 mM Tris HCl, pH 7.4 at 4°C) using 10 passes of a Teflon-glass homogenizer at 3000 r.p.m. Homogenates were then centrifuged for 10 min at $1000 \times g$ at 4°C. The resultant supernatants were then centrifuged at $32\,000 \times g$ for 20 min at 4°C. The resultant pellets (P2) were resuspended in assay buffer (50 mM Tris HCl, 1 mM $MgCl_2$, pH 7.4) at 4°C and pooled in pairs before being washed twice in assay buffer by further centrifugation at $32\,000 \times$ g for 20 min at 4°C. Final pellets were stored at −20°C until use.

Saturation binding studies

When used for binding studies pellets were thawed at room temperature and washed twice by resuspension in assay buffer, and centrifugation at $32\,000 \times g$ for 20 min at 4°C.

After washing, pellets were suspended in assay buffer at a concentration of 0.75–1.0 mg ml^{-1}, such that a 400 μl aliquot contained 300–400 μg protein. Binding studies were carried out in a final volume of 500 μl. Aliquots of membrane were incubated for 30 min at room temperature with 10 concentrations of [^{3}H]-RX821002 (0.03–30 nM) in triplicate. Non-specific binding was determined in triplicate at each free ligand concentration using 10 μM rauwolscine. Following incubation, free radioligand was separated from bound radioligand by rapid filtration through Whatman GF/B filter paper (pre-soaked in 0.1% (w v^{-1}) polyethyleneimine in deionized water) using a Brandel M-30 cell harvester; filter papers were then washed twice with 3 ml ice-cold assay buffer. Radioactivity bound to the filter papers was determined by liquid scintillation counting.

Competition binding studies

Washed pellets were suspended in assay buffer at a concentration of 0.75–1.0 mg ml^{-1}. Aliquots of 400 μl were incubated for 30 min at room temperature with 1 nM [^{3}H]-RX821002 and increasing concentrations of competing ligands over the range 0.1 pM–1 mM in triplicate, in a final volume of 0.5 ml. Non-specific binding was determined using 10 μM rauwolscine. Incubation was terminated by rapid filtration through Whatman GF/B filter paper using a Brandel M-30 cell harvester; filters were then washed twice using 3 ml of ice-cold assay buffer. Radioactivity was measured using liquid scintillation counting.

Analysis of binding data

Saturation and competition binding data were analysed by iterative non-linear regression using GraphPAD Prism (Graphpad Software, version 2.00). K_i values for competing drugs were calculated using the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + L/K_D)$. When analysing binding data, the *F*-test was used to determine whether the data were best fit by one- or two-site models.

Statistics

Data are expressed as mean ± s.e.mean. The level of significance of differences between groups was determined using two-tailed Student's *t*-test; a *P* value of less than 0.05 was considered as statistically significant.

Results

Effect of FT005 in vivo

The dose-dependent effect of acute FT005 on the blood glucose level is shown in Figure 2. The maximal blood glucose level (19.13 ± 0.35 mM, $n = 6$) was reached 90 min after a dose of 25 mg kg^{-1}, but a highly significant increase was already seen after 30 min (17.2 mM, $P < 0.001$, relative to saline treated mice $n = 13$). Blood glucose levels remained elevated relative to those in saline treated mice for over 4 h following administration. The minimal effective dose was 0.50 mg kg^{-1}, which significantly increased blood glucose levels for 2 h following administration. A dose of 0.125 mg kg^{-1} produced a slight but not statistically significant increase in blood glucose.

Orally administered FT005 (1 mg kg^{-1}) also produced hyperglycaemia (data not shown), although the peak blood glucose level (12.22 ± 0.63 mM, $n = 6$) was lower than that observed following i.p. administration (14.32 ± 0.52 mM, $n = 6$).

The potency and time course of action of FT005 were compared to those of adrenaline, diazoxide and glucagon (Figure 3). The increase in blood glucose levels following

adrenaline administration (1 mg kg^{-1}) was very similar to that produced by FT005, with a peak (14.18 ± 1.21 mM, $n=5$) observed 60 min after administration, and with both drugs blood glucose had returned to normal levels after 180 min. Glucagon (1 mg kg^{-1}) was found to have a shorter duration of action than FT005, and blood glucose was thus determined at 20 min intervals. The peak blood glucose level (12.33 ± 0.77 mM, $n=4$) was lower than that following FT005, and was observed at 40 min; blood glucose levels had returned to basal levels 80 min after administration. Diazoxide (25 mg kg^{-1}) produced a similar peak blood glucose level (14.76 ± 0.90 mM, $n=9$) to FT005 (1 mg kg^{-1}), but the onset of action was much slower than that of FT005, the peak effect occurring 120 min after administration. Blood glucose levels had returned to basal levels by 240 min.

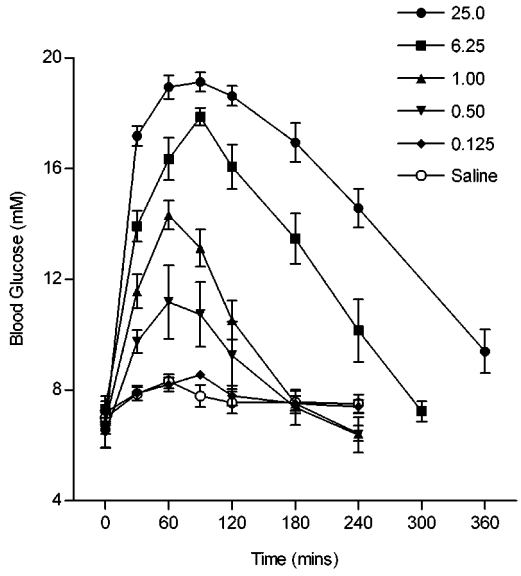

Figure 2 Blood glucose levels in mice following administration of FT005 (0.125–25.0 mg.kg^{-1}, i.p., $n=6$) or saline (i.p., $n=13$) at time = 0 min. Data represent mean ± s.e.mean blood glucose levels in mM. Symbols indicating levels of statistical significance have been omitted for clarity.

Effect of FT005 on plasma insulin

Since the time course study suggested similarities between the action of FT005 and adrenaline we examined the effect of FT005 on plasma insulin levels. Plasma insulin levels were determined immediately prior to administration of FT005 or saline, and 30 and 60 min thereafter (see Figure 4). In saline-treated mice there was a smaller decrease in plasma insulin after 30 min but this was not statistically significant. In FT005 treated animals, plasma insulin was significantly reduced from 1.31 ± 0.20 ng ml^{-1} ($n=14$) at t = 0 min to 0.33 ± 0.07 ng ml^{-1} ($n=11$) 30 min after administration ($P<0.001$). After 60 min plasma insulin was lower, although not significantly different from basal levels. Comparing these results with the blood glucose changes observed in Figure 3, it is apparent that the hyperglycaemia following FT005 persists beyond the transient fall in insulin levels, which may reflect a secondary action of FT 005.

Effect of FT005 in fasted animals

The effect of 25 mg kg^{-1} FT005 on blood glucose following overnight fasting was compared to that seen in fed mice. This higher dose was selected in order to produce a larger response to help distinguish between the responses in fed and fasted animals. Figure 5 shows the rise in blood glucose from the same arbitrary starting point (at t = 0) rather than the absolute blood glucose levels. The basal levels of blood glucose were 6.57 ± 0.65 mM ($n=6$) and 2.62 ± 0.27 mM ($n=6$) in fed and fasted animals respectively. Administration

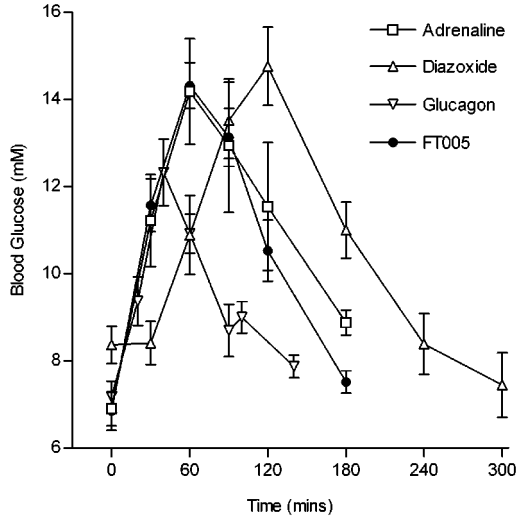

Figure 3 Blood glucose levels in mice following administration of adrenaline (1 mg.kg^{-1}, i.p., $n=5$), diazoxide (25 mg.kg^{-1}, i.p., $n=9$) glucagon (1 mg.kg^{-1}, i.p., $n=4$) and FT005 (1 mg.kg^{-1}, i.p., $n=6$) at time = 0 min. Data represent mean ± s.e mean blood glucose levels in mM. Symbols indicating levels of statistical significance have been omitted for clarity.

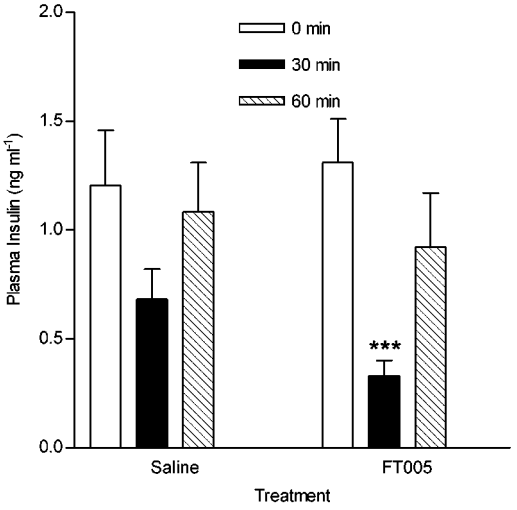

Figure 4 Plasma insulin levels in mice following administration of FT005 (1 mg.kg^{-1}, i.p.) or saline (i.p.). Plasma insulin was determined in blood samples taken prior to, and 30 and 60 min after drug administration. ***Represents a level of statistical significance of $P<0.001$ relative to the plasma insulin concentration at time 0 in the same treatment group.

of FT005 to fasted mice produced a significant increase in blood glucose concentration of 3.90 ± 0.56 mM ($n=6$) after 30 min, and this rose gradually to a maximal increase of 6.9 mM at 270 min before returning to basal levels after 390 min. This hyperglycaemic response to FT005 was less than that observed in fed animals. The mean plasma insulin level in fasted control mice was 0.38 ± 0.09 ng ml^{-1} ($n=8$). Since this level was very similar to the minimum insulin level observed following FT005 and was close to the limit of detection of the assay, we did not measure the plasma insulin levels in fasted mice following FT005.

Antagonism of the hyperglycaemic response by rauwolscine

We examined the possibility that FT005 might be acting as an α_2-adrenoceptor agonist by determining whether the hyperglycaemia could be blocked using a non-imidazoline α_2-adrenoceptor antagonist. The hyperglycaemic effect of FT005 (1 mg kg^{-1}) was significantly reduced but not prevented by a dose of rauwolscine (0.5 mg kg^{-1}) shown to reduce the area under the glucose curve produced by 1 mg kg^{-1} adrenaline from 2203 ± 111 mM.min in saline pre-treated mice to 1676 ± 76 mM.min ($n=4$, $P<0.01$, data not shown). The same dose of rauwolscine also significantly reduced the area under the glucose curve produced by 1 mg kg^{-1} FT005 (Figure 6), from 2391 ± 41 mM.min ($n=8$) to 2104 ± 63 mM.min ($n=6$), and reduced the peak blood glucose level following FT005 from 14.32 ± 0.52 mM to 12.65 ± 0.97 mM.

In saline-treated control mice rauwolscine produced no overall change in blood glucose levels, indicating that its antagonism of the hyperglycaemic effect of FT005 was pharmacological and not physiological. A consistent initial rise in blood glucose was observed 15 min after rauwolscine. This is due in part to the short-term stress of the initial handling and is seen also after saline or sham injection (Slough, 2000). The effect is no longer evident by 30 min (see Figure 6).

Effect of FT005 on body temperature

A dose of FT005 (1 mg kg^{-1} i.p.) which had previously been shown to produce hyperglycaemia was also found to significantly reduce core body temperature (see Figure 7). The peak reduction in body temperature from 38.4 ± 0.2°C to 32.3 ± 0.3°C ($n=8$) was observed 30 min after administration; body temperature returned to normal levels 3 h after administration. No significant changes in body temperature were observed in saline treated mice over the same time period.

Effect of FT005 on isolated vas deferens

The mouse isolated vas deferens was used to examine the possible action of FT005 at pre-synaptic α_2-adrenoceptors, since α_2-adrenoceptor agonists are known to reduce the magnitude of electrically-induced twitches of this tissue as a result of reduced neurotransmitter release. FT005 produced a concentration-dependent reduction in the magnitude of the electrically-induced contraction of the isolated vas deferens. Complete inhibition was seen at approximately 1 μM, with an estimated IC_{50} of 128 ± 50 nM (Figure 8). The α_2-adrenoceptor agonist UK14304 also completely inhibited neurogenic twitches, with an IC_{50} of 2.44 ± 0.41 nM, ($n=4$). In the presence of rauwolscine (1 and 10 μM) the inhibition curve for FT005 showed a parallel shift to the right; the IC_{50} values were 1.04 ± 0.09 μM and 4.52 ± 1.13 μM respectively.

Radioligand binding studies

In saturation binding studies the binding of [^{3}H]-RX821002 to mouse whole brain homogenates was saturable and of high affinity (data not shown). Iterative non-linear regression and

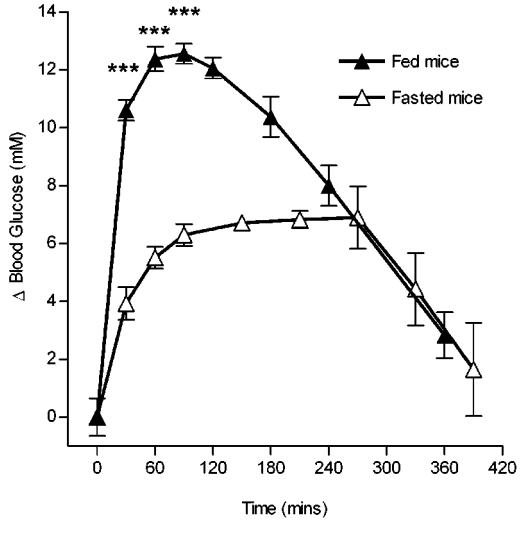

Figure 5 Change in blood glucose concentration following administration of FT005 (25 mg.kg^{-1}, i.p.) to fed ($n=6$) and fasted ($n=6$) mice. Data represent the mean ± s.e. mean increase in blood glucose levels from those observed at time = 0 min, prior to drug administration.

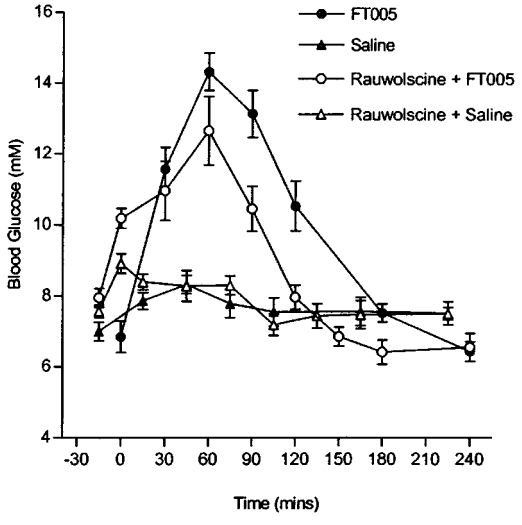

Figure 6 Effect of rauwolscine on the hyperglycaemic response to FT005. Rauwolscine (0.5 mg.kg^{-1}, i.p.) was administered to mice at time = −15 min, followed by FT005 (1 mg.kg^{-1}, i.p., $n=8$) or saline (i.p., $n=6$) at time = 0 min. Control mice received either FT005 (1 mg.kg^{-1}, i.p., $n=6$) or saline ($n=13$) or saline without rauwolscine pretreatment.

Scatchard analysis suggested the binding was to two populations. The higher affinity site had an equilibrium dissociation constant (K_D) of 0.68 ± 0.10 nM, and a B_{max} of 152.9 ± 21.9 fmol mg^{-1} protein (data from four independent experiments). The lower affinity site had an equilibrium dissociation constant (K_D) of 13.5 ± 4.2 nM, and a B_{max} of 86.1 ± 16.8 fmol mg^{-1} protein. Non-specific binding, as determined using 10 μM rauwolscine, was less than 2% for [^{3}H]-RX821002 at 1 nM. Competition binding studies were performed using 1 nM [^{3}H]-RX821002. Both RX821002 and rauwolscine produced concentration-dependent inhibition of specific [^{3}H]-RX821002 binding (Figure 9). Displacement curves were monophasic, with Hill coefficients close to unity; RX821002 and rauwolscine had K_i values of 1.12 ± 0.21 nM and 23.06 ± 2.35 nM respectively. Both competing ligands were capable of completely displacing [^{3}H]-RX821002.

Competition experiments performed using FT005 demonstrated that this novel compound was also capable of completely inhibiting specific [^{3}H]-RX821002 binding to α_2-adrenoceptors in whole mouse brain homogenates (Figure 9). The competition appeared to be biphasic, with the two populations approximately equal in size, and with high and low affinity sites ($K_i = 3.3 \pm 0.6$ nM and 152.6 ± 18.4 nM respectively, $n = 4$). To determine whether FT005 was binding to different affinity states of the receptor, the competition experiment was repeated in the presence of the non-hydrolysable GTP analogue Gpp(NH)P (100 μM). Under these conditions the fraction of the high affinity site was reduced from 46.3 ± 0.02% to 18.6 ± 5.3% (data not shown).

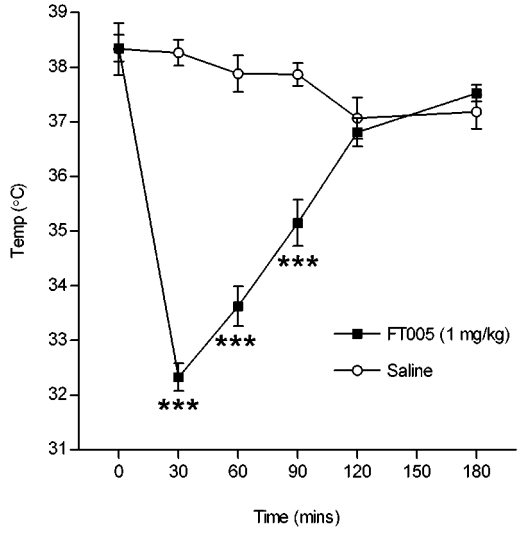

Figure 7 Effect of FT005 on body temperature. FT005 (1 mg.kg^{-1}, i.p., $n = 8$) or saline (i.p., $n = 6$) was administered to mice at time = 0 min. Temperature was determined at 30 and 60 min intervals. Data represent mean ± s.e.mean. ***Represents a level of statistical significance of $P < 0.001$ relative to saline treated mice.

Discussion

Whilst there is growing evidence of the ability of certain imidazoline compounds to increase insulin secretion from pancreatic β-cells *via* interaction with a putative I_3 imidazoline binding site, reports of antagonists have been confused by the recent finding that KU14R and RX801080 enhanced rather than inhibited the ability of efaroxan to promote insulin secretion from BRIN-BD11 cells (Ball *et al*., 2000). It is noteworthy, however, that similar results have yet to be reported from different insulin-secreting cell-lines and isolated β-cells. A specific I_3 antagonist might be expected to elevate blood glucose levels *in vivo* and have a potential therapeutic role in treating the hypoglycaemia occurring acutely as a result of excessive insulin administration, or chronically in cases of islet cell tumour or islet cell hyperplasia. An I_3 selective drug would also have the advantage of avoiding the central cardiovascular effects associated with α_2-adrenoceptor activation. We have previously reported the dramatic increase in blood glucose levels seen with the novel imidazoline

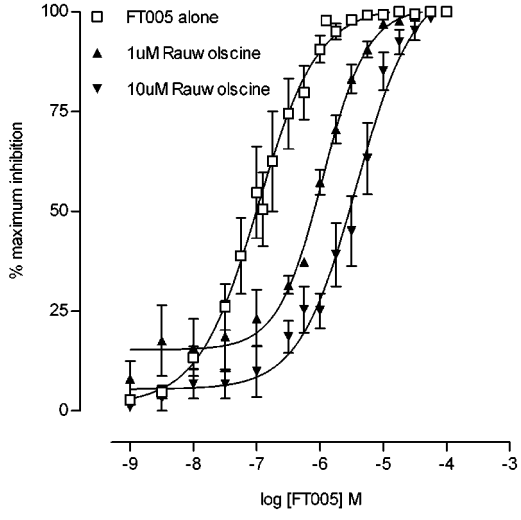

Figure 8 Concentration-dependent inhibition of neurogenic twitches of the mouse isolated vas deferens by FT005. FT005 was applied to the bath alone ($n = 7$) or in the presence of 1 μM or 10 μM rauwolscine ($n = 3$).

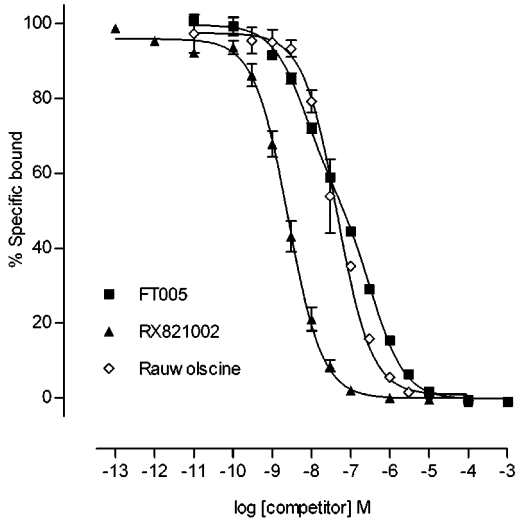

Figure 9 Concentration-dependent inhibition of specific [^{3}H]-RX821002 (1 nM) binding in mouse whole brain homogenates by FT005 ($n = 4$), unlabelled RX821002 ($n = 4$) and rauwolscine ($n = 3$).

compound FT005, which may have been due to an antagonistic action at the novel I_3 site (Slough & Taberner, 1999). The further examination of the properties of FT005 presented here suggest that this hyperglycaemic effect is more likely to be a result of α_2-adrenoceptor stimulation.

FT005 was found to produce a very rapid dose-dependent increase in blood glucose levels, which is the opposite of the effect previously observed *in vivo* with imidazoline compounds such as efaroxan (Berridge *et al*., 1992; Berdeu *et al*., 1994), S21663 (Wang *et al*., 1996) and S22068 (Pele-Tounian *et al*., 1998), where an elevation of plasma insulin, or an improvement of glucose tolerance is observed, presumably as a result of I_3 activation. This suggested the possibility that FT005 may have an antagonistic action at I_3 sites.

A variety of compounds produce hyperglycaemia *in vivo*, including: adrenaline which reduces insulin secretion from the pancreas *via* α_2-adrenoceptor stimulation (Angel & Langer, 1988), and increases glycogenolysis by stimulating hepatic β_2-adrenoceptors (Kuo *et al*., 1977); glucagon, which reduces the effects of insulin secretion and increases glycogenolysis and gluconeogenesis in the liver; and diazoxide, a K_{ATP} channel opener which inhibits pancreatic insulin secretion (Trube *et al*., 1986) and reduces plasma insulin levels (Quast & Cook, 1989; Pratz *et al*., 1991). In the present study, the hyperglycaemic response observed following diazoxide was very different from that observed after FT005, with both the time of onset and the time taken to reach a maximum effect being much slower after diazoxide. Glucagon on the other hand was found to have a much shorter duration of action than the same dose of FT005. Adrenaline was found to be equipotent with FT005 at producing hyperglycaemia at 1 mg kg^{-1}, and had a similar time course of action.

α_2-Adrenoceptor agonists produce hyperglycaemia by inhibiting insulin secretion. When plasma insulin levels were measured following FT005 administration they were indeed found to be significantly reduced, consistent with α_2-adrenoceptor stimulation. In addition, the hyperglycaemic effect of FT005 was significantly reduced by prior administration of the non-imidazoline α_2-adrenoceptor antagonist rauwolscine, suggesting the involvement of α_2-adrenoceptors. Rauwolscine itself was found to have no significant effect on blood glucose levels, suggesting low basal sympathetic tone. In the presence of an irreversible α_2-adrenoceptor antagonist, e.g. phenoxybenzamine, any hyperglycaemic response to FT005 could be attributed to antagonism at I_3 receptors. Studies using isolated islets incubated with phenoxybenzamine have shown that whilst racemic efaroxan retained its ability to evoke a transient increase in insulin output in response to glucose, the α_2-adrenoceptor-mediated action of the (+) enantiomer was blocked (Mourtada *et al*., 1997). However, in the intact animal, *in vivo* interactions with different receptors can not be excluded. Ideally, radioligand binding studies would be performed at I_3 receptors; however, due to the practical difficulties in obtaining sufficient β-cell protein for binding, and the lack of an adequately selective radioligand such studies were not performed.

Since food was withdrawn at the start of all *in vivo* experiments, the hyperglycaemia observed following FT005 administration cannot be due to increased food consumption as a result of an appetite effect. To examine whether the hyperglycaemia could be explained solely by a reduction of plasma insulin levels, FT005 was administered to mice under fasting conditions when insulin secretion is suppressed. Under these conditions FT005 still produced a sustained increase in blood glucose, although the response was attenuated relative to that in fed animals. This suggests that whilst suppression of insulin release contributes to the hyperglycaemic response, it is likely that other mechanisms, such as inhibition of glucose disposal may also play a significant role.

Central post-synaptic α_2-adrenoceptors are involved in regulating body temperature (Bill *et al*., 1989). Activation of α_2-adrenoceptors located in the pre-optic area of the hypothalamus has been demonstrated to induce hypothermia in rats (Quan *et al*., 1992). FT005 produced a rapid hypothermic response which lasted approximately 2 h, providing further evidence of FT005 being an α_2-adrenoceptor agonist. This hypothermia may contribute to the hyperglycaemic response by reducing metabolic rate and hence glucose demand by the tissues.

In the periphery, stimulation of prejunctional α_2-adrenoceptors causes inhibition of neurogenic contractions of isolated vas deferens by reducing neurotransmitter release (Marshall *et al*., 1978). We demonstrated that FT005 was also capable of causing complete inhibition of these contractions. Although there are reports suggesting the presence of imidazoline receptors in the vas deferens (Carratu *et al*., 1992) they remain to be demonstrated conclusively and there are reports questioning their existence in this tissue (Avellar & Markus, 1996). The competitive antagonism of the inhibitory response of FT005 by rauwolscine strongly suggests that the effect is mediated by α_2-adrenoceptors.

To confirm that FT005 is an α_2-adrenoceptor ligand we used radioligand binding to demonstrate the affinity of FT005 for α_2-adrenoceptors. The methoxy analogue of idazoxan, [^{3}H]-RX821002, was used to label α_2-adrenoceptors in mouse whole brain homogenates. Prior binding studies with this compound have shown binding to a single population of high affinity sites in rat whole brain homogenates (Hudson *et al*., 1992; Mallard *et al*., 1992), and demonstrated a low affinity for non-adrenoceptor imidazoline binding sites, since it is completely displaced by (−)-adrenaline. In the present study rauwolscine caused 100% inhibition of specific [^{3}H]-RX821002 binding in competition studies, suggesting that at 1 nM [^{3}H]-RX821002 binds exclusively to α_2-adrenoceptors. Interestingly the saturation data was best fit by a two-site model. This is in contrast with numerous studies performed in rat brain where [^{3}H]-RX821002 binding fits a one-site saturation curve (Hudson *et al*., 1992; Mallard *et al*., 1992; Renouard *et al*., 1994). Species differences between the rat and the mouse could explain the unexpected two-site binding observed with [^{3}H]-RX821002 in the mouse. The most likely explanation for the two-site saturation would appear to be binding to α_2-adrenoceptor subtypes. In a study using a variety of cell lines and tissues [^{3}H]-RX821002 has been shown to bind with approximately equal affinity to the four α_2-adrenoceptor subtypes (O'Rourke *et al*., 1994). In native receptors in one species, however, this might not necessarily be the case.

In competition studies specific [^{3}H]-RX821002 binding was completely displaced by FT005, rauwolscine and unlabelled RX821002. Given that rauwolscine has no affinity for imidazoline binding sites (Lachaud-Pettiti *et al*., 1991) these data support the argument that the two binding sites observed in the saturation binding assay did not include an

imidazoline binding site. Both rauwolscine and unlabelled RX821002 appeared to bind to a single population of binding sites, whilst FT005 competition appeared to be biphasic, with the two populations approximately equal in size. Since FT005 appears to be an α_2-adrenoceptor agonist it is possible that the two different populations could represent high- and low-affinity states of the G-protein coupled α_2-adrenoceptor. In order to determine whether this was indeed the case competition experiments were repeated in the presence of the GTP analogue Gpp(NH)P (100 μM). Under these conditions, the G-protein coupled receptors are more likely to exist in the low affinity state, with the fraction of high affinity binding decreasing. The reduction of the high affinity fraction of the population suggests that FT005 does displace [^{3}H]-RX821002 from two different affinity states of the α_2-adrenoceptor consistent with agonist binding.

The data presented here demonstrate that the hyperglycaemia observed in mice following administration of FT005 can be explained largely in terms of activation of α_2-adrenoceptors, with a fall in insulin secretion responsible in part for the elevation of blood glucose. The interaction of FT005 with α_2-adrenoceptors has been verified in radioligand binding studies. However, reduction of insulin secretion is not solely responsible for the hyperglycaemia; a second mechanism producing hyperglycaemia in fasted mice remains unexplained.

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