ELSEVIER

European Journal of Pharmacology 454 (2002) 95–102

www.elsevier.com/locate/ejphar

# Effects of the imidazoline ligands efaroxan and KU14R on blood glucose homeostasis in the mouse

Gaëll Mayer, Peter V. Taberner*

*Department of Pharmacology, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK*

Received 16 August 2002; received in revised form 18 September 2002; accepted 20 September 2002

**Abstract**

The putative imidazoline $I_3$ receptor antagonist 2-(2-ethyl-2,3-dihydrobenzo[*b*]furan-2-yl)-1*H*-imidazole (KU14R) has been shown to block the effects of the atypical $I_3$ agonist efaroxan at the level of the ATP-sensitive $K^+$ ($K_{ATP}$) channel in isolated pancreatic islet β cells, but its effects in vivo are not known. We have therefore investigated the effects of KU14R on blood glucose and insulin level in vivo. When KU14R was administered before or after a hypoglycaemic dose of efaroxan, the fall in blood glucose was at least additive. When the antihyperglycaemic imidazoline ligand S22068 was administered after a dose of KU14R, it did not alter the hypoglycaemic response. In the mouse isolated vas deferens preparation, neither rauwolscine (at concentrations which competitively antagonised the inhibitory response to 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK14304)) nor KU14R affected inhibition produced by S22068. At $10^{-4}$ M, KU14R had weak $\alpha_2$-adrenoceptor antagonist activity. We conclude that KU14R does not act as an antagonist of either efaroxan or S22068 at an imidazoline site in vivo.


*Keywords:* KU14R; Efaroxan; Imidazoline; $\alpha_2$-Adrenoceptor; Insulin; Blood glucose

## 1. Introduction

A number of imidazoline-containing compounds have been shown previously to induce insulin release from isolated pancreatic islets (Chan et al., 1993; Wang et al., 1996) and, more importantly, to improve glucose tolerance in both rats (Berdeu et al., 1997; Pele-Tounian et al., 1999) and mice (Williams et al., 2000). The putative imidazoline receptor site responsible, present on the pancreatic β-cell, has been termed the atypical imidazoline or $I_3$ site (Eglen et al., 1998) since it shows pharmacological properties which distinguish it from the previously identified $I_1$ and $I_2$ sites found in other tissues (Brown et al., 1993; Chan et al., 1994). Electrophysiological studies have shown that the insulin-secreting property of the proto-typical $I_3$ ligand efaroxan is mediated by closure of the ATP-sensitive $K^+$ ($K_{ATP}$) channel (Chan et al., 1998). The $K_{ATP}$ channel has been cloned and shown to consist of two subunits: a sulphonylurea receptor (SUR) and a Kir6.2 subunit. The transmembrane protein Kir 6.2, to which imidazoline containing ligands such as phentolamine bind (Proks and Ashcroft, 1997) is thought to be the pore-forming subunit of the $K_{ATP}$ channel; the nucleotide binding protein, which acts as a regulator of ion flow, is sensitive to sulphonylurea drugs (Aguilar-Bryan et al., 1995). It has been established that the imidazoline $I_3$ site and the sulphonylurea receptor (SUR) are not identical, since imidazoline ligands do not displace binding from the SUR sites (Morgan et al., 1999).

Investigation of the functional activity of putative imidazoline receptors has been hampered by the lack of established antagonists to block the action of agonists. However, it has been demonstrated that 2-(2-ethyl-2,3-dihydrobenzo[*b*]furan-2-yl)-1*H*-imidazole (KU14R) can inhibit the secretory action of sulphonylureas, even though they have physically different binding sites (Morgan et al., 1996). KU14R is a close structural analogue of efaroxan, possessing an imidazole ring in place of the imidazoline ring in efaroxan. KU14R has since been shown to behave as an

* Corresponding author. Tel.: +44-117-928-7637; fax: +44-117-925-0168.

*E-mail address:* peter.v.taberner@bris.ac.uk (P.V. Taberner).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02473-1

antagonist of efaroxan at $I_3$ binding sites in rat isolated β cells both in terms of insulin release and $K_{ATP}$ channel currents, although conserving some weak $\alpha_2$-adrenoceptor activity (Chan et al., 1998). At high concentrations, KU14R directly closed $K_{ATP}$ channels when added in the absence of efaroxan but did not stimulate insulin secretion. It has therefore been suggested that KU14R may be acting as a weak partial agonist of $I_3$ binding sites (Morgan et al., 1999). More recently, KU14R has been shown to stimulate insulin release in BRIN-BD11 cells and enhance insulin release in the presence of efaroxan, suggesting direct agonist activity (Ball et al., 2000). The action of KU14R at $I_3$ sites is therefore not unequivocal.

Although a number of independent groups have investigated the effects of novel imidazoline compounds on glucose homeostasis and insulin secretion in the intact rat or mouse in order to discover useful alternatives to the sulphonylureas as insulin secretagogues (Wang et al., 1996; Williams et al., 2000), there have been no studies to date on the effect of KU14R on blood glucose levels or pancreatic function in vivo. We have therefore examined the effects of KU14R and its interaction with efaroxan in vivo in the mouse, in terms of glucose homeostasis and circulating insulin levels.

Since many imidazolines also have affinity for $\alpha_2$-adrenoceptors, and activation of pancreatic β cell $\alpha_2$-adrenoceptors can inhibit insulin secretion (Langer et al., 1983), it is important to eliminate any $\alpha_2$-adrenoceptor component in the responses to efaroxan and KU14R; indeed, efaroxan itself has marked $\alpha_2$-adrenoceptor antagonist activity, which was originally believed to be responsible for its action on $K_{ATP}$ channels (Chan et al., 1991). For this reason, we have used the mouse isolated vas deferens preparation, which possesses both functional imidazoline sites and presynaptic $\alpha_2$-adrenoceptors (Slough et al., 2000), in order to examine the effects of standard $\alpha_2$-adrenoceptor agonists and antagonists on the responses to KU14R and the $I_3$ imidazoline ligand S22068. The aim of these studies was to further characterise the properties of KU14R in order to establish the possible role of atypical $I_3$ imidazoline sites in glucose homeostasis. A preliminary account of some of these findings has already been presented in abstract form (Mayer and Taberner, 2001).

## 2. Methods

### 2.1. Animals

Male CBA/Ca mice from a colony bred at Bristol University Medical School were used at age 16 weeks or older when they weighed 30–34 g. The animals were kept at 20–22 °C on a 12-h light/12-h dark cycle under conditions of controlled humidity. Mice were fed with standard B and K Universal mouse diet ad libitum and tap water was always available.

### 2.2. Drugs and reagents

KU14R, efaroxan and 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK14304) were obtained from Tocris Cookson, Avonmouth, Bristol, UK. Rauwolscine hydrochloride was purchased from Sigma, Dorset, UK, and S22068 was obtained from I.R.I.S., Courbevoie, Paris, France. In the initial dose finding experiments, KU14R was dissolved in 20% (v/v) DMSO in order to ensure complete solubility at the higher doses. Subsequently, the standard dose of KU14R employed (16 mg $kg^{-1}$ body weight) was made up in physiological (0.9% w/v) saline to which a small drop of Tween 80 was added. Efaroxan was dissolved in physiological saline. For the in vivo experiments, KU14R, efaroxan, and either Tween/saline or saline as control were injected intraperitoneally (i.p.) in a dose volume of 10 ml $kg^{-1}$. In all the in vivo experiments, mice were fully fed but had no access to food during the duration of the experiment.

### 2.3. Blood glucose levels

Each experiment started between 09:00 and 10:00 h. Blood glucose concentrations were determined in fed animals using a Boehringer Glucocheck 90 and BM 1-44 glucocheck strips. The animal was lightly anaesthetised by diethyl ether (BDH) inhalation and a blood sample of approximately 10 μl obtained from the tail by distal venesection. Drugs or saline were given immediately following collection of an initial blood sample (0 min), subsequent blood samples were taken at 30, 60, 90, 120, 180, 240, 300 and 360 min for analysis.

### 2.4. Plasma insulin levels

Blood samples were taken immediately prior to the drug or Tween/saline vehicle administration and subsequently at 60, 120, 180 and 240 min. The blood samples (30–100 μl) were collected into heparinized capillary tubes and centrifuged. The plasma was extracted and stored at −20 °C prior to assay. No more than two samples were obtained from each mouse prior to the terminal sample and the time points were randomised between experimental sessions. Plasma insulin levels were measured using a rat insulin radioimmunoassay kit (SRI-13K, Linco Research, St. Charles, MO, USA) with centrifugation to separate the free and antibody-bound insulin. Although the kit has been marketed for rat insulin, it has been found to work equally well for mouse plasma samples (Williams et al., 2000).

### 2.5. In vitro vas deferens experiments

CBA/Ca mice were killed by cervical dislocation. The two vasa deferentia were rapidly removed and placed immediately in Krebs solution prepared as follows (compo-

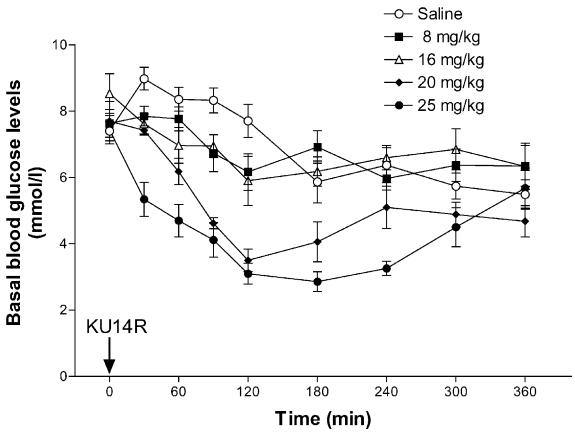


Fig. 1. Time course of the effect of KU14R (8–25 mg $kg^{-1}$ i.p.) on blood glucose levels. Basal blood glucose was measured at $t=0$ min and the KU14R administered immediately afterwards. Each point is the mean ± S.E.M. of 15 (saline) or 5–6 (KU14R) mice. Individual statistical differences have been omitted for clarity. Two-way ANOVA revealed a statistically significant difference in effect of KU14R at 20 and 25 mg $kg^{-1}$ compared to saline with $F(1,162)=43.51$, $F(1,162)=90.28$ ($P<0.0001$), respectively. There was a significant effect of time following every dose.

sition in mM): NaCl 118.0, KCl 4.75, $CaCl_2$ 2.54, $KH_2PO_4$ 0.93, $NaHCO_3$ 25, glucose 11. It has been found that more consistent responses are obtained in the absence of magnesium (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 0.3 g resting tension. Prior to experiment, the tissue was left to equilibrate for 30 min. 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 to avoid stimulating the muscle, which can occur at pulse widths greater than 5 ms. Contractions were recorded on a chart recorder (Graphtec Linearcorder).

The imidazoline agonist S22068 was applied every 2 min and the $\alpha_2$-adrenoceptor agonist UK14304 every 3 min to produce cumulative concentration–response curves. The effects of rauwolscine (1 and 10 μM) and KU14R (1, 10 and 100 μM) on the response to S22068 and UK14304 were investigated by applying the antagonist before the agonist and leaving the tissue to equilibrate for 10 min before commencing addition of the agonist.

### 2.6. Statistical methods

All data are given as means ± S.E.M. For single comparisons between groups, a two-tailed unpaired Student's *t*-test was used. For the multiple comparisons of treatment and time course, we used analysis of variance (ANOVA) (Instat, Graphpad Software, San Diego, USA).

## 3. Results

### 3.1. KU14R and efaroxan on blood glucose and plasma insulin levels

In a preliminary experiment to determine the appropriate dose of KU14R, groups of five to six mice were injected i.p. with doses of between 4 and 25 mg $kg^{-1}$, and their blood glucose measured at time intervals between 0 and 360 min post-injection. At 4 mg $kg^{-1}$, KU14R was without effect, but increasing doses produced increasing hypoglycaemic responses (see Fig. 1). The maximum response, observed as the fall in blood glucose levels between 120 and 180 min, occurred at doses of 20–25 mg $kg^{-1}$. The median effective dose was 16 mg $kg^{-1}$, which was used in the subsequent experiments with efaroxan in order that either synergistic or antagonistic effects could be observed.

Firstly, in order to determine whether the fall in blood glucose levels following KU14R was related to a stimulation of insulin release, we conducted an experiment in which the blood glucose levels and plasma insulin levels were measured in parallel following a dose of 20 mg $kg^{-1}$ KU14R compared to saline/Tween-treated controls (see Fig. 2). It can be seen that there was a significant rise in plasma insulin after KU14R which coincided with the fall in blood glucose levels at 120 min. Interestingly, the blood glucose level falls before an increase in insulin concentration is seen, i.e. 60 min, but by 120 min, insulin increases and basal blood glucose levels still decreased.

In order to determine the appropriate dose of efaroxan, groups of five to six mice were injected i.p. with doses of between 1, 5 and 10 mg $kg^{-1}$, and their blood glucose

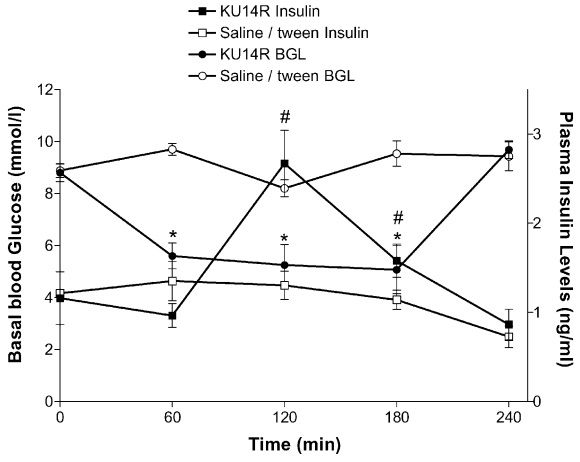


Fig. 2. Effect of KU14R (20 mg $kg^{-1}$) on blood glucose and plasma insulin levels. Results are shown as means ± S.E.M. of *n*. For blood glucose levels data: $n=3–25$ (saline), $n=6–25$ (KU14R); for insulin data: $n=3–10$ (saline), $n=6–8$ (KU14R). Basal insulin levels and blood glucose levels were measured at $t=0$ min and KU14R administered immediately afterwards. For blood glucose levels data: $*P<0.05$; for insulin data: $\#P<0.05$.

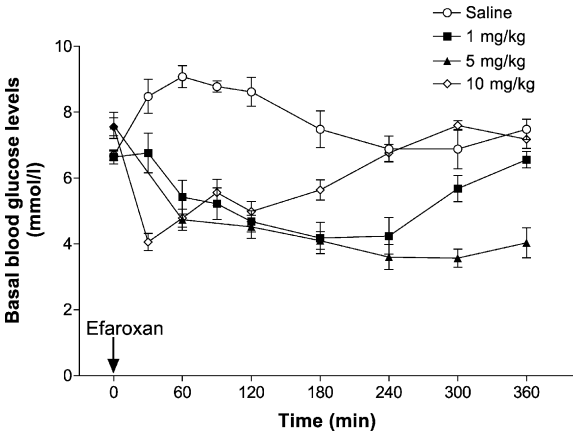


Fig. 3. Time course of the effect of efaroxan (1–10 mg $kg^{-1}$ i.p.) on blood glucose levels. Basal blood glucose was measured at $t=0$ min and the efaroxan administered immediately afterwards. Each point is the mean ± S.E.M. of six (saline) or five (efaroxan) mice. Individual statistical differences have been omitted for clarity. Two-way ANOVA a revealed statistically significant difference in effect of efaroxan at 1, 5 and 10 mg $kg^{-1}$ compared to saline with $F(1,77)=136.88$, $F(1,59)=209.65$ and $F(1,77)=114.59$ ($P<0.0001$), respectively. There was a significant effect of time following every dose.

measured at time intervals between 0 and 360 min post-injection. All three doses of efaroxan produced significant hypoglycaemic responses (see Fig. 3). The maximum and more constant response occurred at a dose of 5 mg $kg^{-1}$, which was used in the subsequent experiments. The greater hypoglycemic effect of efaroxan at 10 mg/kg could provoke a physiological response, which leads to a quicker recovery to normal blood glucose levels. The effects of efaroxan on blood glucose and insulin levels were conducted using efaroxan at a dose of 5 mg $kg^{-1}$; the results are shown in Fig. 4. In contrast to KU14R, there was a smaller but still significant fall ($P<0.005$) in blood glucose levels by 60 min but no change in the plasma insulin level at any time

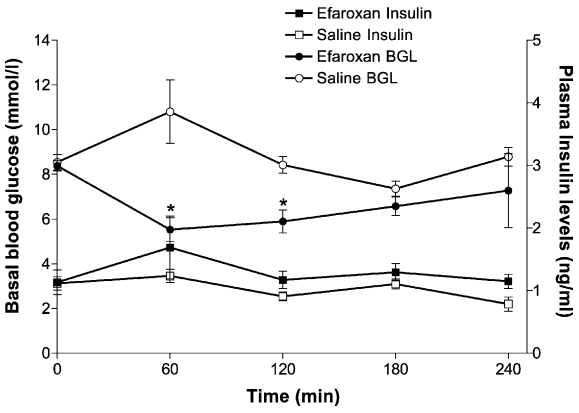


Fig. 4. Effect of efaroxan (5 mg $kg^{-1}$) on blood glucose and plasma insulin levels. Results are shown as means ± S.E.M. of $n$. For blood glucose levels: $n=5–25$ (saline), $n=4–25$ (efaroxan); for insulin data: $n=6–9$ (saline), $n=4–10$ (efaroxan). Basal insulin levels and blood glucose levels were measured at $t=0$ min and efaroxan administered immediately afterwards. For blood glucose levels data: $^*P<0.05$.

point. Therefore, the origin of the hypoglycaemia seen with 5 mg/kg of efaroxan could be explained by an increase of glucose uptake by tissues.

### 3.2. Interaction between KU14R, efaroxan and S22068 in vivo

In order to examine the possible interaction between KU14R and efaroxan, two further experiments were conducted in which the KU14R was given either 60 min prior to efaroxan (Fig. 5A) or 60 min after efaroxan (Fig. 5B). When KU14R preceded efaroxan, the fall in blood glucose levels was significantly greater and more prolonged ($P<0.001$) than after efaroxan alone (see Fig. 5A). The blood glucose levels were still significantly reduced at 360 min following the combination treatment. The pre-injection value for

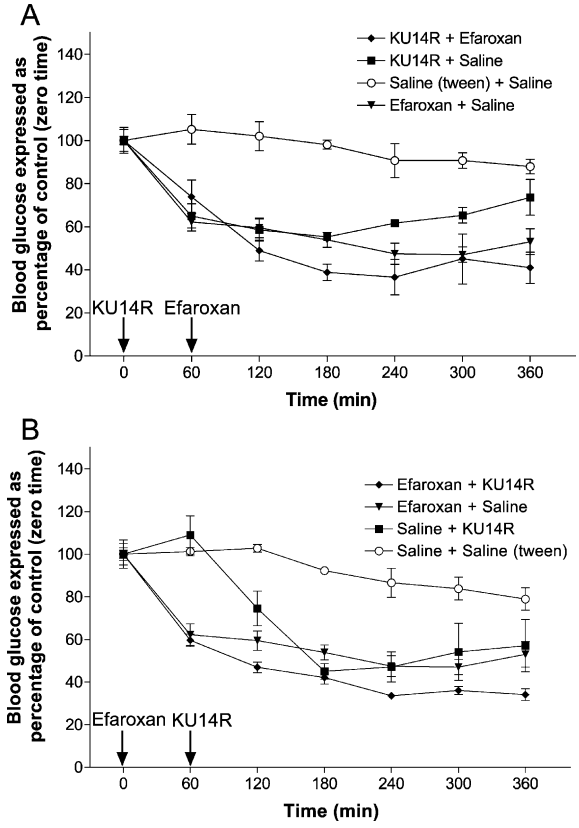


Fig. 5. Interaction between KU14R and efaroxan on blood glucose level. KU14R (16 mg $kg^{-1}$) administered 60 min prior to efaroxan (5 mg $kg^{-1}$) (A) or 60 min following efaroxan (B). Each point is the mean ± S.E.M. of $n=4$ (KU14R + efaroxan, KU14R + saline), $n=6$ (efaroxan + KU14R, efaroxan + saline) or $n=3$ (vehicle). (A) Effects of KU14R and KU14R + efaroxan significantly different from saline with $F(1,35)=105.65$ and $F(1,35)=129.02$ ($P<0.001$), respectively (two-way ANOVA). Effects of KU14R + efaroxan significantly different from KU14R alone with $F(1,42)=17.89$ ($P<0.001$). (B) Effects of efaroxan, KU14R and efaroxan + KU14R significantly different from saline with $F(1,49)=127.72$, $F(1,35)=28.02$ and $F(1,49)=579.39$ ($P<0.0001$), respectively (two-way ANOVA). Effects of efaroxan + KU14R significantly different from KU14R alone and efaroxan alone with $F(1,56)=41.21$ and $F(1,70)=24.33$ ($P<0.001$), respectively.

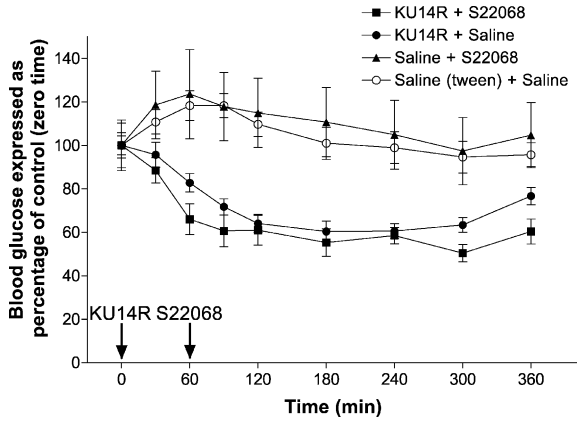


Fig. 6. Effect of S22068 (24 mg $kg^{-1}$) administered 60 min after KU14R (16 mg $kg^{-1}$) on basal blood glucose levels. Each point is the mean ± S.E.M. of $n=8$ (KU14R + saline), $n=6$ (KU14R + S22068), $n=4$ (S22068 + saline) and $n=7$ (saline Tween + saline). Effects of KU14R and KU14R + S22068 significantly different from saline (Tween) with $F(1,72)=76.25$ and $F(1,99)=157.12$ ($P<0.0001$), respectively (two-way ANOVA). There was a significant effect of time following KU14R and KU14R + S22068 with $F(8,72)=3.47$ and $F(8,99)=4.67$ ($P<0.0001$), respectively. Two-way ANOVA revealed no statistically difference in the effect of S22068 compared to saline (Tween).

KU14R + efaroxan was 6.70 ± 0.122 mmol/l and the maximal hypoglycemic level was reached at 180 min with 2.60 ± 0.25 mmol/l (see Fig. 5A).

When efaroxan was given first, a fall in blood glucose levels was observed by 60 min as before, but subsequently, the fall in blood glucose levels was significantly greater than that following either drug alone (see Fig. 5B). The pre-injection value for efaroxan + KU14R was 7.08 ± 0.20 mmol/l and the maximal hypoglycemic level was reached at 180 min with 2.98 ± 0.22 mmol/l (see Fig. 5B). Neither of these experiments demonstrated any antagonism between efaroxan and KU14R, but rather a synergistic or at least additive effect on the blood glucose levels.

Thereafter, the possibility of an antagonistic effect of KU14R on S22068 was investigated. When KU14R and S22068 were administered together, there was no change in the hypoglycaemic profile of KU14R (Fig. 6). Therefore, this experiment did not demonstrate any antagonistic effect of KU14R on S22068.

### 3.3. Effects of KU14R, efaroxan and S22068 in vitro

In order to distinguish between the specific effects of KU14R at the atypical imidazoline site and $\alpha_2$-adrenoceptor, the effects of KU14R were examined in the isolated vas deferens. Concentration–response curves to an authentic $\alpha_2$-adrenoceptor agonist (UK 14304) in the absence and presence of an antagonist (rauwolscine) are shown in Fig. 7A. UK14304 was capable of complete inhibition of the twitch and had an $IC_{50}$ of $8.37 \times 10^{-9}$ M ($n=4$). It can be

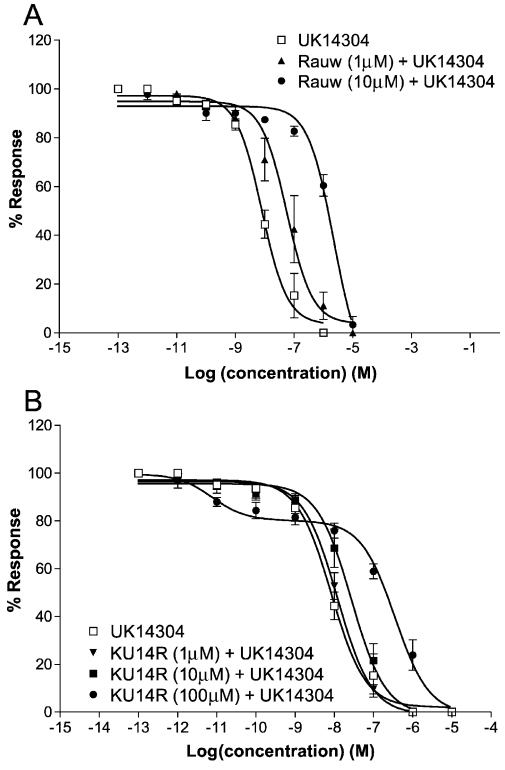


Fig. 7. Cumulative concentration–response curves for the inhibition of electrically induced twitches in vas deferens by the $\alpha_2$-adrenoceptor agonist UK14304 and the antagonism of UK 14304 by rauwolscine (A) or KU14R (B). Each point is the mean ± S.E.M. of $n=4-5$.

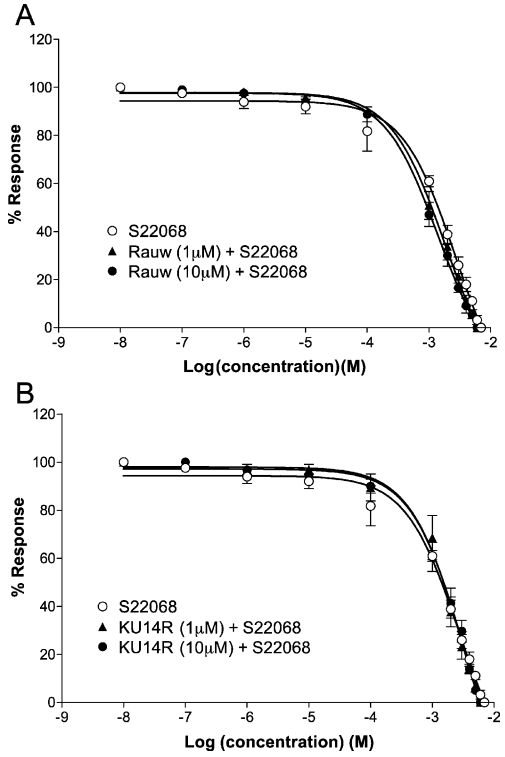


Fig. 8. Cumulative concentration–response curves for the inhibition of electrically induced twitch in vas deferens by the imidazoline agonist S22068 and the response to S22068 in the presence of rauwolscine (A) or KU14R (B). Each point is the mean ± S.E.M. of $n=4$.

seen that rauwolscine produced a concentration-dependent parallel shift to the right of the inhibitory response to UK 14304, thus confirming competitive antagonism. With $10^{-6}$ M rauwolscine, the $IC_{50}$ was shifted from $8.37 \times 10^{-9}$ to $5.72 \times 10^{-8}$ M; the apparent $K_i$ for rauwolscine was $1.0 \times 10^{-7}$ M The effect of KU14R on the UK 14304 response is shown in Fig. 7B. No effect was observed at $10^{-6}$ M KU14R, but inhibition occurred with $10^{-5}$ M ($K_i = 4.44 \times 10^{-6}$ M) and $10^{-4}$ M ($K_i = 4.14 \times 10^{-6}$ M). At the highest concentration of KU14R ($10^{-4}$ M), there was evidence of some direct inhibition (maximally 15%) of the twitch response when subeffective concentrations of UK 14034 were present (see Fig. 7B). A comparative line fit ($F$-test) indicated that a two-site model gave the best fit for KU14R at this concentration ($P < 0.001$), suggesting a separate low affinity inhibitory site.

The cumulative concentration–response of the isolated vas deferens to the imidazoline S22068 is shown in Fig. 8A. S22068 was capable of producing complete inhibition and the $IC_{50}$ was $2.77 \times 10^{-3}$ M ($n = 4$). In this case, rauwolscine produced no shift of the inhibition curve, indicating that S22068 was not acting via an $\alpha_2$-adrenoceptor. In the presence of $10^{-6}$ or $10^{-5}$ M KU14R, there was again no shift of the S22068 response curve (Fig. 8B), indicating that KU14R does not compete for the same functional site as S22068.

## 4. Discussion

KU14R has been proposed as a novel imidazoline $I_3$ receptor antagonist on the basis of evidence from in vitro studies which have relied largely upon the use of efaroxan as the selective agonist. The acute hypoglycemia and increase in insulin levels produced by KU14R that we find in vivo are in contradiction with the results from the in vitro secretory studies on rat islets (Chan et al., 1997a,b, 1998) and patch-clamp studies of cultured rat β-cells (Chan et al., 1998), which demonstrated antagonism by KU14R of the $K_{ATP}$ channel-closing action of efaroxan. However, our results are in agreement with the more recent findings of Ball et al. (2000), who demonstrated a stimulation of insulin secretion by KU14R in the cloned rat pancreatic β-cell line BRIN-BD11. The imidazoline ring of efaroxan has been replaced in KU14R by an imidazole ring whilst retaining the stereogenic centre (Morgan et al., 1996; Chan et al., 1998). Consequently, both (+)- and (−)-enantiomers will be present in the racemate. Racemic efaroxan is known to promote insulin secretion by inhibition of $K_{ATP}$ channels in isolated pancreatic islets (Chan et al., 1991), although it was subsequently demonstrated that the (−)-enantiomer was responsible for the stimulation of insulin secretion (presumably through $I_3$ binding sites) whereas the (+)-enantiomer possessed the $\alpha_2$-adrenoceptor activity (Chan et al., 1993). It is therefore possible that the enantiomers of KU14R also have different receptor selectivity. The separate enantiomers of KU14R are not available commercially, although they would make it possible to confirm their receptor selectivity.

Efaroxan at the doses used here significantly decreased the basal blood glucose of fed mice (Fig. 3) without any significant increase in insulin levels (Fig. 4). This is in contrast to the findings of Berridge et al. (1992), who reported that the same dose of efaroxan (5 mg $kg^{-1}$) in fed rats produced an increase in plasma insulin although without affecting the blood glucose level. This discrepancy may reflect differences either in the species sensitivity to efaroxan or its relative bioavailability. In the mouse, it appears that KU14R and efaroxan act in the same qualitative manner, possibly through binding to more than one site.

The effect of a combination of KU14R and efaroxan on basal blood glucose levels was investigated since a partial agonist may appear to be an antagonist when given in combination with a full agonist. KU14R and efaroxan together produced a more profound hypoglycaemia than that produced by efaroxan or KU14R alone. This additive effect was not dependent upon the order of injection of the drugs, confirming that the two drugs are not acting antagonistically in their effects on blood glucose levels and precluding a partial agonist action of KU14R. The overall response could therefore be a combination of a direct action on $K_{ATP}$ channels through the $I_3$ binding site and/or an antagonist action at the $\alpha_2$-adrenoceptor through which noradrenaline inhibits insulin secretion (Nakaki et al., 1980). More recently, Chan et al. (2001) have raised the possibility of another mechanism involved in the control of insulin secretion which is independent of the membrane potential, and have proposed that efaroxan and KU14R could potentiate insulin secretion by interacting with components of the exocytotic pathway.

Radioligand binding studies with KU14R and efaroxan in rat brain have revealed that efaroxan has high affinity for the $\alpha_2$-adrenoceptor ($K_i = 3.77 \pm 0.11$ nM) compared to KU14R ($K_i = 349 \pm 68$ nM), and that both ligands have negligible affinity for $I_2$ sites ($K_i$>10,000 nM) (Tyacke et al., 1997). There have been no radioligand binding studies on the islet $I_3$ site due to the very limited amount of tissue available. Imidazoline ligands with low affinity for $\alpha_2$-adrenoceptors are known to enhance insulin release in isolated islets in the presence of $\alpha_2$-adrenoceptor antagonists (Schulz and Hasselblatt, 1989), and a number of imidazoline ligands which are devoid of $\alpha_2$-adrenoceptor activity can lower plasma glucose in vivo (Berdeu et al., 1997). The imidazoline S22068 has antihyperglycaemic effects in both the rat (Pele-Tounian et al., 1998, 1999) and the mouse (Williams et al., 1999, 2000). In the mildly diabetic rat, S22068 acts by promoting insulin secretion (Le Brigand et al., 1999). We have shown here that the hypoglycemic profile following KU14R is unaltered by the addition of an anti-hyperglycemic dose of S22068 (see Fig. 6), suggesting that the two compounds are not competing at the same site either positively or antagonistically.

Although the imidazolines clonidine and rilmenidine inhibit the contractile response in the vas deferens through a presynaptic $\alpha_2$-adrenoceptor (Avellar and Markus, 1996), presynaptic non-adrenergic imidazoline receptors have been identified in a number of peripheral tissues (Göthert et al., 1999), and we have since demonstrated that S22068 inhibits the vas deferens twitch response through a non-$\alpha_2$-adrenoceptor imidazoline preferring site which also regulates noradrenaline release (Slough et al., 2000). Neither rauwolscine nor KU14R was able to block the inhibitory effects of S22068 and, whilst this is not sufficient evidence to argue that S22068 and KU14R are acting at different sites, it does confirm that the inhibitory effects of S22068 are not mediated via activation of an $\alpha_2$-adrenoceptor. On the other hand, KU14R at the highest concentration ($10^{-4}$ M) did show evidence of competitive inhibition of the response to UK14304. This suggests that KU14R may have low affinity for the presynaptic $\alpha_2$-adrenoceptor and confirms previous reports of the weak $\alpha_2$-adrenoceptor activity of KU14R (Chan et al., 1998; Morgan et al., 1999).

In conclusion, our data indicate that KU14R is not a functional antagonist of efaroxan in vivo, suggesting that these drugs may act at different sites to reduce blood glucose. Racemic KU14R, like efaroxan, may have multiple actions associated with the separate enantiomers, although the possibility that KU14R is acting as a prodrug cannot be ruled out. Thus, there is still a need for a selective imidazoline $I_3$ receptor antagonist to characterise these sites and define their functional role.

## Acknowledgements

We are grateful to Tocris Cookson, Avonmouth, Bristol, UK, for supplying KU14R and I.R.I.S., Courbevoie, France, for S22068. The whole animal experiments described here were conducted within the conditions of a Project Licence under the terms set out in the Animals (Scientific Procedures) Act 1986. G. Mayer is supported by a University of Bristol Scholarship.

## References

Aguilar-Bryan, L., Nichols, C.G., Wechsler, S.W., Clement IV, J.P., Boyd III, A.E., González, G., Herrera-Sosa, H., Nguy, K., Bryan, J., Nelson, D.A., 1995. Cloning of the β cell high-affinity sulphonlyurea receptor: a regulator of insulin secretion. Nature 268, 423–426.

Avellar, M.C.W., Markus, R.P., 1996. Are imidazoline receptors involved in sympathetic neurotransmission in rat vas deferens. Gen. Pharmacol. 27, 1273–1278.

Ball, B.J., Flatt, P.R., McClenaghan, N.H., 2000. Stimulation of insulin secretion in clonal BRIN-BD11 cells by the imidazoline derivatives KU14R and RX801080. Pharmacol. Res. 42, 575–579.

Berdeu, D., Puech, R., Ribes, G., Loubatrieres-Mariani, M.M., Bertrand, G., 1997. Antazoline increases insulin secretion and improves glucose tolerance in rats and dogs. Eur. J. Pharmacol. 324, 233–239.

Berridge, T.L., Doxey, J.C., Roach, A.G., 1992. Comparison of the effects of efaroxan and glibenclamide on plasma glucose and insulin levels in rats. Eur. J. Pharmacol. 213, 213–218.

Brown, C.A., Loweth, A.C., Smith, S.A., Morgan, N.G., 1993. Stimulation by imidazoline compounds is not due to interaction with non-adrenoceptor idazoxan binding sites. Br. J. Pharmacol. 108, 312–317.

Chan, S.L.F., Dunne, M.J., Stillings, M.R., Morgan, N.G., 1991. The $\alpha_2$-adrenoceptor antagonist efaroxan modulates $K^+_{ATP}$ channels in insulin secreting cells. Eur. J. Pharmacol. 204, 41–48.

Chan, S.L.F., Brown, C.A., Morgan, N.G., 1993. Stimulation of insulin secretion by the imidazoline $\alpha_2$-adrenoceptor antagonist efaroxan is mediated by a novel, stereoselective, binding site. Eur. J. Pharmacol. 230, 375–378.

Chan, S.F., Brown, C.A., Scarpello, K.E., Morgan, N.G., 1994. The imidazoline site involved in the control of insulin secretion: characteristics that distinguish it from $I_1$- and $I_2$-sites. Br. J. Pharmacol. 112, 1065–1070.

Chan, S.L.F., Pallett, A.L., Clews, J., Ramsden, C.A., Morgan, N.G., 1997a. Evidence that the ability of imidazoline compounds to stimulate insulin secretion is not due to interaction with σ receptors. Eur. J. Pharmacol. 323, 241–244.

Chan, S.F., Pallett, A.L., Morgan, N.G., 1997b. Clotrimazole and efaroxan stimulate insulin secretion by different mechanisms in rat pancreatic islets. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 763–768.

Chan, S.L.F., Pallett, A.L., Clews, J., Ramsden, C.A., Chapman, J.C., Kane, C., Dunne, M.J., Morgan, N.G., 1998. Characterisation of new efaroxan derivatives for use in purification of imidazoline-binding sites. Eur. J. Pharmacol. 355, 67–76.

Chan, S.L.F., Mourtada, M., Morgan, N.G., 2001. Characterization of $K_{ATP}$ channel-independent pathway involved in potentiation of insulin secretion by efaroxan. Diabetes 50, 340–347.

Eglen, R.M., Hudson, A.L., Kendall, D.A., Nutt, D.J., Morgan, N.G., Wilson, V.G., Dillon, M.P., 1998. 'Seeing through a glass darkly': casting light on imidazoline 'I' sites. TIPS 19, 381–390.

Göthert, M., Brüss, M., Bönish, H., Molderings, G.J., 1999. Presynaptic imidazoline receptors, new development in characterization and classification. Ann. N.Y. Acad. Sci. 881, 171–184.

Hughes, J., Kosterlitz, H.W., Leslies, F.M., 1975. Effect of morphine on adrenergic transmission in mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. Br. J. Pharmacol. 53, 371–381.

Langer, J., Panten, U., Zielmann, S., 1983. Effects of α-adrenoceptor antagonists on clonidine-induced inhibition of insulin secretion by isolated pancreatic islets. Br. J. Pharmacol. 79, 415–420.

Le Brigand, L., Virsolvy, A., Manechez, D., Godfroid, J.J., Guardiola-Lemaitre, B., Gribble, F.M., Ashcroft, F.M., Bataille, D., 1999. In vitro mechanism of action on insulin release of S-22068, a new putative antidiabetic compound. Br. J. Pharmacol. 128, 1021–1026.

Nakaki, T., Nakadate, T., Kato, R., 1980. $\alpha_2$-Adrenoceptors modulating insulin release from isolated pancreatic islets. Naunyn-Schmiedeberg's Arch. Pharmacol. 313, 151–153.

Mayer, G., Taberner, P.V., 2001. Stimulation of insulin secretion in vivo by the imidazoline analogue KU14R. Br. J. Pharmacol. 134, 51.

Morgan, N.G., Pallett, A.L., Mourtada, M., Chan, S.L.F., Clews, J., Ramsden, C.A., 1996. Synthesis and characterisation of KU14R a novel antagonist of the pancreatic islet imidazoline binding site. Diabetologia 39, 456.

Morgan, N.G., Chan, S.L.F., Mourtada, M., Monks, L.K., Ramsden, C.A., 1999. Imidazolines and pancreatic hormone secretion. Ann. N.Y. Acad. Sci. 881, 217–228.

Pele-Tounian, A., Wang, X., Rondu, F., Lamouri, A., Touboul, E., Marc, S., Dokhan, R., Pfeiffer, B., Manechez, D., Renard, P., Guardiola-Lemaitre, B., Godfroid, J.J., Penicaud, L., Ktorza, A., 1998. Potent antihyperglycaemic property of a new imidazoline derivative S-22068 (PMS 847) in a rat model of NIDDM. Br. J. Pharmacol. 124, 1591–1596.

Pele-Tounian, A., Chan, S.L.F., Rondu, F., Le Bihan, G., Giroix, M.-H., Lamouri, A., Touboul, E., Pfeiffer, B., Manechez, D., Renard, P., Guardiola-Lemaitre, B., Godfroid, J.-J., Penicaud, L., Morgan, N.G., Ktorza, A., 1999. Effect of the new imidazoline derivative S-22068 (PMS 847)

on insulin secretion in vitro and glucose turnover in vivo in rats. Eur. J. Pharmacol. 377, 81–87.

Proks, P., Ashcroft, F.M., 1997. Phentolamine block of $K_{ATP}$ channels is mediated by Kir6.2. Proc. Natl. Acad. Sci. U. S. A. 94, 11716–11720.

Schulz, A., Hasselblatt, A., 1989. Dual action of clonidine on insulin release: suppression, but stimulation when α-adrenoceptors are blocked. Naunyn-Schmiedeberg's Arch. Pharmacol. 340, 712–714.

Slough, S., Watkins, J., Taberner, P.V., 2000. Evidence for a functional presynaptic imidazoline receptor in the mouse isolated vas deferens. Br. J. Pharmacol. 131, 60.

Tyacke, R.J., Nutt, D.J., Chan, S.L.F., Ramsden, C.A., Morgan, N.G., Hudson, A.L., 1997. Affinities of efaroxan derivatives for $\alpha_2$-adrenoceptors and $I_2$-binding sites in rat brain membranes. Br. J. Pharmacol. 122, 66.

Wang, X., Rondu, F., Lamouri, A., Dokhan, R., Marc, S., Touboul, E., Pfeiffer, B., Manechez, D., Renard, P., Guardiola-Lemaitre, B., Godfroid, J., Ktorza, A., Penicaud, L., 1996. Effect of S-21663 (PMS 812), an imidazoline derivative, on glucose tolerance and insulin secretion in a rat model of type II diabetes. J. Pharmacol. Exp. Ther. 278, 82–89.

Williams, C.A., Shih, M.-F., Taberner, P.V., 1999. A simple acute in vivo comparative test for sensitivity to insulin in the mouse. Horm. Metab. Res. 31, 580–582.

Williams, C.A., Shih, M.-F., Taberner, P.V., 2000. Effect of acute and subchronic administration of the imidazoline compound S 22068 on in vivo glucose and insulin responses in normal lean CBA/Ca mice. Gen. Pharmacol. 34, 183–191.