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RESEARCH PAPER

α_{2A}-Adrenoceptor antagonism increases insulin secretion and synergistically augments the insulinotropic effect of glibenclamide in mice

V Fagerholm[1], M Scheinin[2,3] and M Haaparanta[1]

[1]*Turku PET Centre/Preclinical Imaging, Turku, Finland;* [2]*Department of Pharmacology, Drug Development and Therapeutics, University of Turku, Turku, Finland and* [3]*Clinical Pharmacology, TYKSLAB, Hospital District of Southwest Finland, Turku, Finland*

Background and purpose: The imidazoline-type α_2-adrenoceptor antagonists (±)-efaroxan and phentolamine increase insulin secretion and reduce blood glucose levels. It is not known whether they act by antagonizing pancreatic β-cell α_2-adrenoceptors or by α_2-adrenoceptor-independent mechanisms. Many imidazolines inhibit the pancreatic β-cell K_{ATP} channel, which is the molecular target of sulphonylurea drugs used in the treatment of type II diabetes. To investigate the mechanisms of action of (±)-efaroxan and phentolamine, α_{2A}-adrenoceptor knockout (α_{2A}-KO) mice were used.
Experimental approach: Effects of (±)-efaroxan, 5 mg kg^{-1}, and phentolamine, 1 mg kg^{-1}, on blood glucose and insulin levels were compared with those of the non-imidazoline α_2-adrenoceptor antagonist [8a*R*,12a*S*,13a*S*]-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6*H*-isoquino[2,1-g][1,6]naphthyridine (RS79948-197), 1 mg kg^{-1}, and the sulphonylurea glibenclamide, in α_{2A}-KO and control (wild type (WT)) mice.
Key results: In fed WT mice, (±)-efaroxan, phentolamine and RS79948-197 reduced blood glucose and increased insulin levels. Fasting abolished these effects. In fed α_{2A}-KO mice, (±)-efaroxan, phentolamine and RS79948-197 did not alter blood glucose or insulin levels, and in fasted α_{2A}-KO mice, blood glucose levels were increased. Glibenclamide, at a dose only moderately efficacious in WT mice (5 mg kg^{-1}), caused severe hyperinsulinaemia and hypoglycaemia in α_{2A}-KO mice. This was mimicked in WT mice by co-administration of RS79948-197 with glibenclamide.
Conclusions and implications: These results suggest that (±)-efaroxan and phentolamine increase insulin secretion by inhibition of β-cell α_{2A}-adrenoceptors, and demonstrate a critical role for α_{2A}-adrenoceptors in limiting sulphonylurea-induced hyperinsulinaemia and hypoglycaemia.
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Keywords: α_{2A}-adrenoceptor knockout; efaroxan; glibenclamide; glucose; hypoglycaemia; insulin; mouse; pharmacology; phentolamine; RS 79948-197

Abbreviations: α_{2A}-KO, α_{2A}-adrenoceptor knockout; WT, wild type

Introduction

Stimulatory and inhibitory neurotransmitters and hormones modify the responses of pancreatic β-cells to metabolized nutrients. Noradrenaline, released by sympathetic efferent nerves innervating the pancreatic islets, and adrenaline, secreted into the blood by the chromaffin cells of the adrenal medulla, inhibit insulin secretion by activating α_2-adrenoceptors on pancreatic β-cells (Metz *et al*., 1978; Nakaki *et al*., 1980; Filipponi *et al*., 1986; Angel and Langer, 1988). Increased insulin secretion and improved glucose tolerance following α_2-adrenoceptor antagonist administration in healthy animal and human subjects and in subjects with type II diabetes have been demonstrated in most (Linde and Deckert, 1973; Robertson and Porte, 1973; Ahrén *et al*., 1984; Hsu *et al*., 1987; Kawazu *et al*., 1987; Langer, 1987; Ortiz-Alonso *et al*., 1991; Berridge *et al*., 1992; Berlin *et al*., 1994; Angel *et al*., 1996; Abdel-Zaher *et al*., 2001), but not all studies (Östenson *et al*., 1988; John *et al*., 1990; Karhuvaara *et al*., 1990; Hiyoshi *et al*., 1995; Natali *et al*., 1998).

Initially, the insulinotropic effect of α_2-adrenoceptor antagonists was suggested to be mediated by inhibition of tonically activated α_2-adrenoceptors on pancreatic β-cells (Robertson and Porte, 1973; Robertson *et al*., 1976). However, most investigated α_2-adrenoceptor antagonists were imidazolines, and from *in vitro* experiments on isolated pancreatic islets and insulin-secreting cell lines, it is evident that imidazoline-type compounds are capable of stimulating insulin secretion independently of α_2-adrenoceptors

Correspondence: Dr V Fagerholm, Turku PET Centre/Preclinical Imaging, MediCity, Tykistökatu 6A, FI-20520 Turku, Finland.
E-mail: veronica.fagerholm@abo.fi
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(Schulz and Hasselblatt, 1988; Smith and Furman, 1988; Schulz and Hasselblatt, 1989a, b; Jonas *et al*., 1992; Hirose *et al*., 1997; Mourtada *et al*., 1997). Many imidazoline-type compounds inhibit K_{ATP} channels, resulting in β-cell depolarization followed by Ca^{2+} influx, which triggers the exocytosis of insulin granules. Moreover, several lines of evidence suggest additional, K_{ATP} and Ca^{2+} channel-independent, mechanisms in the insulinotropic effect of imidazolines (Efanov *et al*., 2001; Morgan and Chan, 2001). *In vivo*, it has been difficult to pinpoint effects specifically attributable to imidazoline-binding sites, as most insulinotropic imidazolines exhibit at least some degree of α_2-adrenoceptor antagonism. Pancreatic imidazoline-binding sites have, nevertheless, been proposed to represent putative new drug targets for the treatment of type II diabetes (reviewed by Morgan and Chan, 2001).

Of the three mammalian α_2-adrenoceptor subtypes, α_{2A}, α_{2B} and α_{2C}, both α_{2A}- and α_{2C}-adrenoceptors have been implicated in inhibition of insulin secretion in mouse islets *in vitro* (Peterhoff *et al*., 2003). We have previously reported that α_{2A}-adrenoceptor-deficient (knockout) (α_{2A}-KO) mice exhibit increased plasma insulin levels, reduced blood glucose levels and improved glucose tolerance (Savontaus *et al*., 2008). These findings support the idea of α_{2A}-adrenoceptor-mediated tonic inhibition of insulin secretion. The potent subtype non-selective α_2-adrenoceptor agonist dexmedetomidine increased blood glucose levels and decreased insulin levels in control mice, but was without effect in α_{2A}-KO mice, demonstrating that only the α_{2A}-adrenoceptor subtype contributes significantly to blood glucose control *in vivo* (Fagerholm *et al*., 2004). Therefore, in the present study, α_{2A}-KO mice and their wild-type (WT) controls were used to investigate putative α_2-adrenoceptor-independent effects of the imidazoline α_2-adrenoceptor antagonists (±)-efaroxan and phentolamine, the non-imidazoline α_2-adrenoceptor antagonist RS79948-197 ([8a*R*,12a*S*,13a*S*]-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6*H*-isoquino[2,1-g][1,6]naphthyridine) and the K_{ATP} channel inhibitor glibenclamide, on blood glucose and insulin levels. RS79948-197 exhibits high α_2-adrenoceptor specificity, potency and affinity, including high affinity also for the rodent α_{2A}-adrenoceptor, as compared with the other commonly employed non-imidazoline α_2-adrenoceptor antagonists, yohimbine and rauwolscine (Milligan *et al*., 1997; Uhlén *et al*., 1998). The structural formulae of (±)-efaroxan, phentolamine and RS79948-197 are shown in Figure 1.

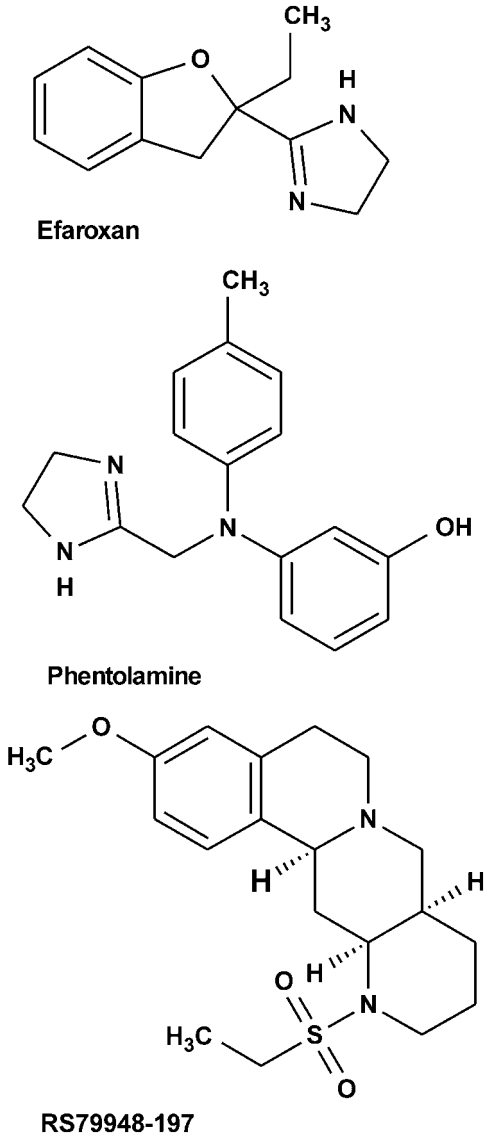

Figure 1 Chemical structures of the α_2-adrenoceptor antagonists studied.

The results of the study suggest that the insulinotropic actions of the investigated imidazoline compounds are due to antagonism of α_{2A}-adrenoceptors. α_{2A}-Adrenoceptors were found to be necessary for the counter-regulatory response to glibenclamide-induced hyperinsulinaemia and hypoglycaemia.

Methods

Animals and experimental design

Animal care and handling complied with the ethical guidelines of the International Council for Laboratory Animal Science. All experiments were performed on conscious mice. The experimental procedures were approved by the laboratory animal welfare committee of the University of Turku, Finland.

Adult (2–6 months old) age-matched male α_{2A}-KO mice (Altman *et al*., 1999) on a C57BL/6J genetic background and C57BL/6J control mice were used. The mice were maintained on a 12 h:12 h light–dark cycle (lights on at 0600 hours) and were provided standard pelleted mouse chow (Dietex International, Special Diet Services, Witham, UK) and tap water *ad libitum*.

A total number of 26 mice were used. The experiments were performed over a period of 15 weeks. For each mouse, the time interval between test compound administrations was at least 72 h. The experiments were performed on the same cohort of mice, unless stated otherwise. Experiments started at 0800 hours. Fed mice had access to food until the start of the experiment, at which food was removed to prevent possible effects of the test compounds on feeding behaviour. When fasted mice were used, food was removed at

0600 hours. Water was available at all times. Test compounds were administered intraperitoneally at a dose volume of 10 mL kg^{-1}. Blood samples for glucose and insulin determinations were obtained from the tail by venepuncture.

Effects of (±)-efaroxan, phentolamine, RS79948-197 and glibenclamide on blood glucose and insulin levels in fed and fasted α_{2A}-KO and WT mice

Ten mice of each genotype were used. The mice were divided into two groups with approximately the same number of mice of each genotype, so that no more than 10 mice were assessed on each experimental day. Blood glucose was measured immediately before test compound injection, and the effects of test compounds on blood glucose levels were followed for up to 4 h. At 1 h after test compound administration, blood was sampled for determination of plasma insulin levels.

Each mouse was tested with (±)-efaroxan (5 mg kg^{-1}; 20 μmol kg^{-1}), phentolamine (1 mg kg^{-1}; 3 μmol kg^{-1}) and RS79948-197 (1 mg kg^{-1}; 2 μmol kg^{-1}), once in the fasting state and once in the fed state. Effects of glibenclamide were determined in fasted mice only. α_{2A}-KO mice were administered a 5 mg kg^{-1}; 10 μmol kg^{-1} dose of glibenclamide. WT mice were administered 5 and 20 mg kg^{-1} doses of glibenclamide, and in addition, a combination of glibenclamide, 5 mg kg^{-1}, and RS79948-197, 1 mg kg^{-1}. Saline experiments were performed twice in both fed and fasted mice; once at the beginning, and once towards the end of each experimental series. As similar results were obtained in the two saline experiments, mean blood glucose and insulin values were calculated for each mouse, and used for the statistical comparisons of saline and drug effects.

During the course of these experiments, one α_{2A}-KO mouse died from hypoglycaemia following administration of 5 mg kg^{-1} glibenclamide, one WT mouse was injured during the experimental procedures and therefore excluded, and four α_{2A}-KO mice died in their home cages from causes apparently unrelated to experimentation. Towards the end of the study, the mice approached 6 months of age. We have noted increased mortality in the α_{2A}-KO mice, as compared with WT mice, with advancing age. The mice die suddenly, without prior externally visible signs of disease, possibly as a result of cardiac failure associated with chronically elevated sympathetic tone (see Brede *et al*., 2002).

Effects of (±)-propranolol and methyl atropine on blood glucose and plasma insulin levels in (±)-efaroxan-administered fed WT and α_{2A}-KO mice

Because of the deaths of some of the α_{2A}-KO mice, as detailed above, the α_{2A}-KO cohort was complemented with six new mice for the (±)-propranolol and methyl atropine experiments. (±)-Efaroxan (5 mg kg^{-1}) was administered, and 30 min later, saline, (±)-propranolol (5 mg kg^{-1}; 17 μmol kg^{-1}) or methyl atropine (10 mg kg^{-1}; 27 μmol kg^{-1}) was injected. After an additional 30 min, blood was sampled for glucose and insulin determinations. The effects of (±)-propranolol and methyl atropine on blood glucose and insulin levels were compared with the effects of saline.

Blood glucose measurement and plasma immunoreactive insulin assay

Blood glucose was measured using an Accu-Chek Aviva glucometer and plasma-calibrated Accu-Chek Aviva test strips (Roche Diagnostics, Mannheim, Germany). Plasma insulin levels were determined using a mouse insulin ELISA kit (Mercodia, Uppsala, Sweden).

Data analysis and statistical procedures

Results are reported as mean ± s.e.mean for the indicated number of observations. Statistical tests were performed with GraphPad Prism, version 4.01 (GraphPad Software Inc., San Diego, CA, USA). The effects of test compounds were evaluated using Student's two-tailed *t*-test or Wilcoxon signed-rank test when comparing two groups. For comparisons of more than two groups, one-way ANOVA was used when assessing a single parameter (drug effect), and two-way ANOVA when assessing two parameters (drug and time effects). When ANOVA indicated statistically significant differences, Bonferroni post-tests were performed. The level of statistical significance was set at $P<0.05$.

Drugs

Glibenclamide, (±)-efaroxan and RS79948-197 were purchased from Tocris Bioscience (Bristol, UK). Phentolamine, (±)-propranolol and atropine methyl nitrate (methyl atropine) were purchased from Sigma-Aldrich (St Louis, MO, USA). (±)-Efaroxan, phentolamine and RS79948-197 were prepared as 10 mM stock solutions in H_2O. Glibenclamide was prepared as a 100 mM stock solution in dimethylsulphoxide. The stock solutions were stored at −20 °C, and diluted in physiological saline on the day of the experiment. The maximum dose of dimethylsulphoxide injected was 0.4 mL kg^{-1}. (±)-Propranolol and methyl atropine were dissolved in physiological saline on the day of the experiment. The drug and molecular target nomenclature conforms with the BJP's Guide to Receptors and Channels (Alexander *et al*., 2008).

Results

Effects of (±)-efaroxan, phentolamine and RS79948-197 on blood glucose and plasma insulin levels in fed and fasted WT and α_{2A}-KO mice

Basal blood glucose levels were lower in fed α_{2A}-KO mice (6.5 ± 0.3 mM, $n=7$) than in fed WT mice (9.6 ± 0.4 mM, $n=9$, $P<0.0001$, Student's *t*-test) and also lower in fasted α_{2A}-KO mice (5.8 ± 0.1 mM, $n=10$) than in fasted WT mice (7.7 ± 0.3 mM, $n=10$, $P<0.0001$, Student's *t*-test). Basal insulin levels were higher in fed α_{2A}-KO mice (1.7 ± 0.3 μg L^{-1}, $n=7$) than in fed WT mice (0.5 ± 0.04 μg L^{-1}, $n=9$, $P=0.0002$, Student's *t*-test) as well as in fasted α_{2A}-KO mice (1.3 ± 0.1 μg L^{-1}, $n=10$) compared with fasted WT mice (0.4 ± 0.1 μg L^{-1}, $n=10$, $P<0.0001$, Student's *t*-test). These results are in line with our previous findings (Fagerholm *et al*., 2004; Savontaus *et al*., 2008).

WT and α_{2A}-KO mice received (±)-efaroxan (5 mg kg^{-1}), phentolamine (1 mg kg^{-1}) and RS79948-197 (1 mg kg^{-1}) in both the fed and the fasted states. The experimental protocol and the dose of (±)-efaroxan were chosen based on a study in fed mice by Mayer and Taberner (2002), in which the maximal hypoglycaemic response to (±)-efaroxan was observed at 5 mg kg^{-1}, as compared with 1 and 10 mg kg^{-1}. Phentolamine and RS79948-197 were used at doses reported to antagonize α_2-adrenoceptors in rodents *in vivo* (Medgett and Rand, 1983; Zhu *et al*., 1999).

In WT mice that were fully fed at the start of the experiment, blood glucose levels were reduced and plasma insulin levels were increased by both the imidazoline α_2-adrenoceptor antagonists (±)-efaroxan and phentolamine, and by the non-imidazoline α_2-adrenoceptor antagonist RS79948-197. In fed α_{2A}-KO mice, none of the compounds significantly affected blood glucose or insulin levels. These results are presented in Figure 2. However, in 2-h fasted WT mice (Figure 3), (±)-efaroxan and RS79948-197 reduced blood glucose levels 60 min after drug administration, but not at later time points. Phentolamine increased blood glucose levels at 4 h after administration. Plasma insulin levels in fasted WT mice, measured 60 min after drug administration, were significantly increased by (±)-efaroxan; the other compounds were without statistically significant effects. In fasted α_{2A}-KO mice, there were no effects on blood glucose or insulin levels at the 60 min time point following drug administration. However, all drugs raised blood glucose levels at later time points.

Effects of (±)-propranolol and methyl atropine on blood glucose and plasma insulin levels in (±)-efaroxan-administered fed WT and α_{2A}-KO mice

The possible contributions of β-adrenoceptors and muscarinic ACh receptors to the reduction in blood glucose and increase in plasma insulin levels induced by (±)-efaroxan (5 mg kg^{-1}) were investigated using the β-adrenoceptor antagonist (±)-propranolol (5 mg kg^{-1}) and the peripherally acting muscarinic ACh receptor antagonist methyl atropine (10 mg kg^{-1}). (±)-Propranolol and methyl atropine altered neither blood glucose nor insulin levels in either genotype (Figure 4).

Effects of glibenclamide on blood glucose and plasma insulin levels in WT and α_{2A}-KO mice

K_{ATP} channel inhibition may be at least partly responsible for the insulinotropic effects of (±)-efaroxan and phentolamine. To assess putative genotype differences in the responses to K_{ATP} channel inhibition *in vivo*, a standard sulphonylurea, glibenclamide, was administered to 2-h fasted WT and α_{2A}-KO mice. The results are presented in Figure 5. In WT mice, a moderate and transient dose-dependent reduction of blood glucose was evident following 5 and 20 mg kg^{-1} glibenclamide. Plasma insulin levels in WT mice 60 min after drug administration were slightly, but significantly, increased by the higher dose of glibenclamide. In contrast to the WT mice, the α_{2A}-KO mice were unable to defy hypoglycaemia. Following 5 mg kg^{-1} glibenclamide, their blood glucose decreased to fatally low levels within 1 h, at which time the experiment was discontinued and the mice were rescued using repeated intraperitoneal injections of 5% (w/v) glucose solution. The fall in blood glucose was accompanied by a rise in plasma insulin from 1.27 μg L^{-1} to more than 10 μg L^{-1}. To verify the role of α_2-adrenoceptors in hypoglycaemic counter-regulation, glibenclamide (5 mg kg^{-1}) and RS79948-197 (1 mg kg^{-1}) were co-administered to WT mice. Combined K_{ATP} channel and α_2-adrenoceptor inhibition in WT mice resulted in similarly reduced blood glucose levels and increased plasma insulin levels as seen in α_{2A}-KO mice administered with glibenclamide only, although in contrast to the α_{2A}-KO mice, only some of the WT mice required glucose injections to restore euglycaemia.

Discussion and conclusions

The imidazoline α_2-adrenoceptor antagonists (±)-efaroxan and phentolamine, and also the non-imidazoline α_2-adrenoceptor antagonist RS79948-197, reduced blood glucose levels and increased insulin levels in fed WT mice, clearly demonstrating that an imidazoline moiety was not necessary for this function. The (±)-efaroxan-induced reduction in blood glucose in WT mice was more prominent than the reductions induced by RS79948-197, and especially by phentolamine. These differences in drug efficacy could be related to dose or bioavailability, or to nonspecific interactions with receptors that influenced the effects of α_{2A}-adrenoceptor antagonism. None of the compounds reduced blood glucose or increased insulin levels in α_{2A}-KO mice. Hence the results did not support the hypothesis that imidazoline compounds augment insulin secretion and regulate blood glucose homoeostasis independently of α_2-adrenoceptors.

A significant effect of the nutritional state of the mice was, however, observed. In 2-h fasted WT mice, a reduction in blood glucose levels by (±)-efaroxan and RS79948-197, and an increase in plasma insulin levels by (±)-efaroxan, was still seen 60 min after drug administration, that is, 3 h after food removal. At later time points of the fasting period, blood glucose levels were no longer decreased by any of the compounds, and phentolamine actually increased blood glucose levels at 240 min after drug administration. In 2-h fasted α_{2A}-KO mice, all compounds increased blood glucose levels from 120 min and onwards after drug administration, or rather, appeared to offset a more prominent fasting-induced decrease in blood glucose in this genotype. α_{2A}-KO mice exhibit reduced blood glucose levels, increased insulin and glucagon levels, and increased sympathetic tone (Altman *et al*., 1999; Savontaus *et al*., 2008). As fasting abolished the insulinotropic effects of (±)-efaroxan, phentolamine and RS79948-197 in fed WT mice, a role for altered energy homoeostasis or autonomic function in the abolished insulinotropic capacity of these drugs also in fed α_{2A}-KO mice cannot be excluded.

In the fasting state, factors such as low blood glucose are probably more important for limiting insulin secretion than sympathetic tone. It may be that the rise in blood glucose in

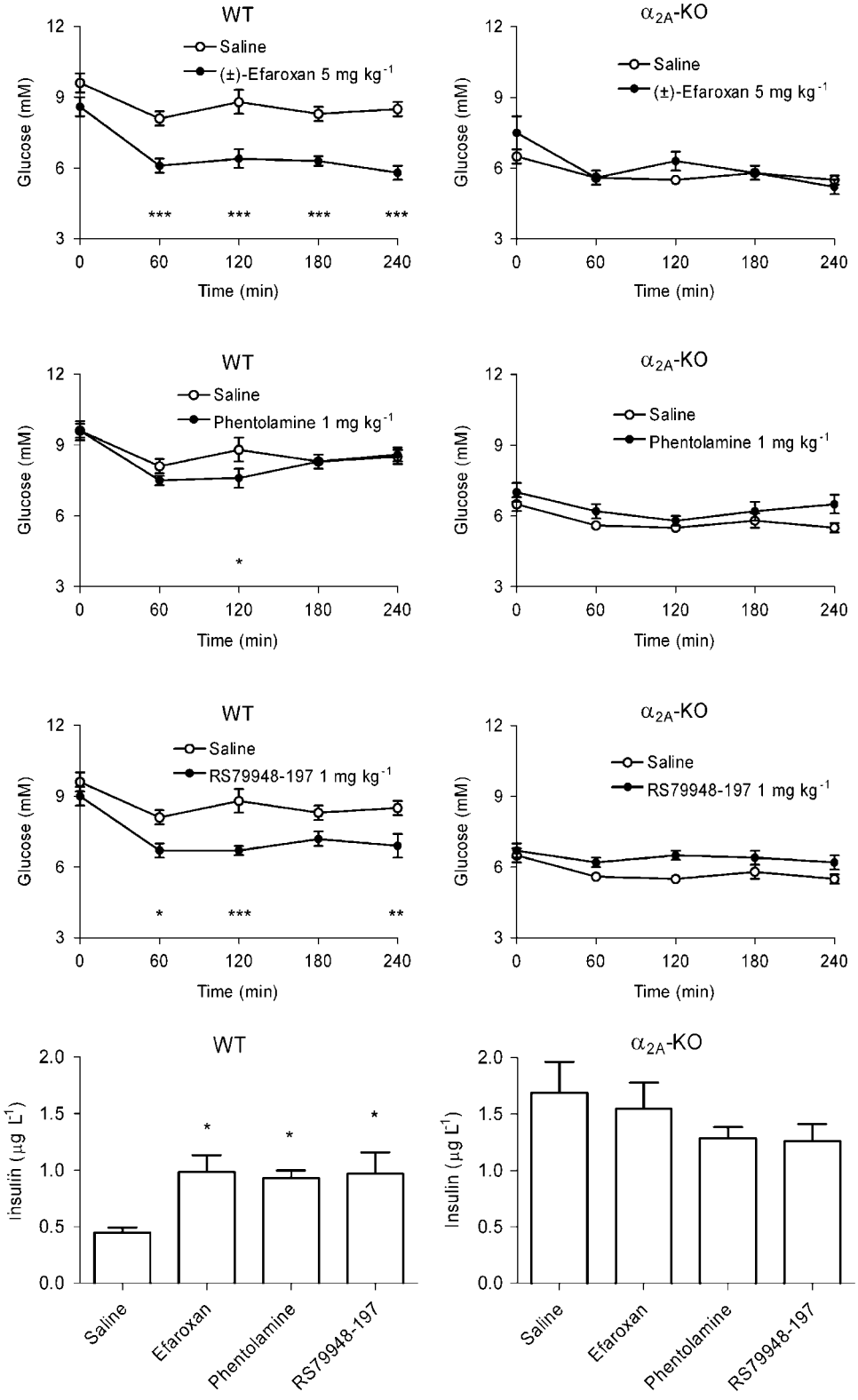

Figure 2 Effects of the imidazoline α_2-adrenoceptor antagonists (±)-efaroxan and phentolamine, and the non-imidazoline α_2-adrenoceptor antagonist RS79948-197 in fed WT ($n=8$–9) and α_{2A}-KO ($n=6$–7) mice. Blood glucose levels were measured immediately before drug administration at time zero, and thereafter at the indicated time points. Insulin levels were measured at 60 min after drug administration. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with saline; two-way ANOVA (glucose results) or one-way ANOVA (insulin results).

fasted mice, induced by all of the investigated α_2-adrenoceptor antagonists in α_{2A}-KO mice, and by phentolamine at the latest time point in WT mice, resulted from increased hepatic glucose production secondary to increased sympathetic or adrenal outflow. It has been demonstrated in mice that both α_{2A}- and α_{2C}-adrenoceptors tonically inhibit

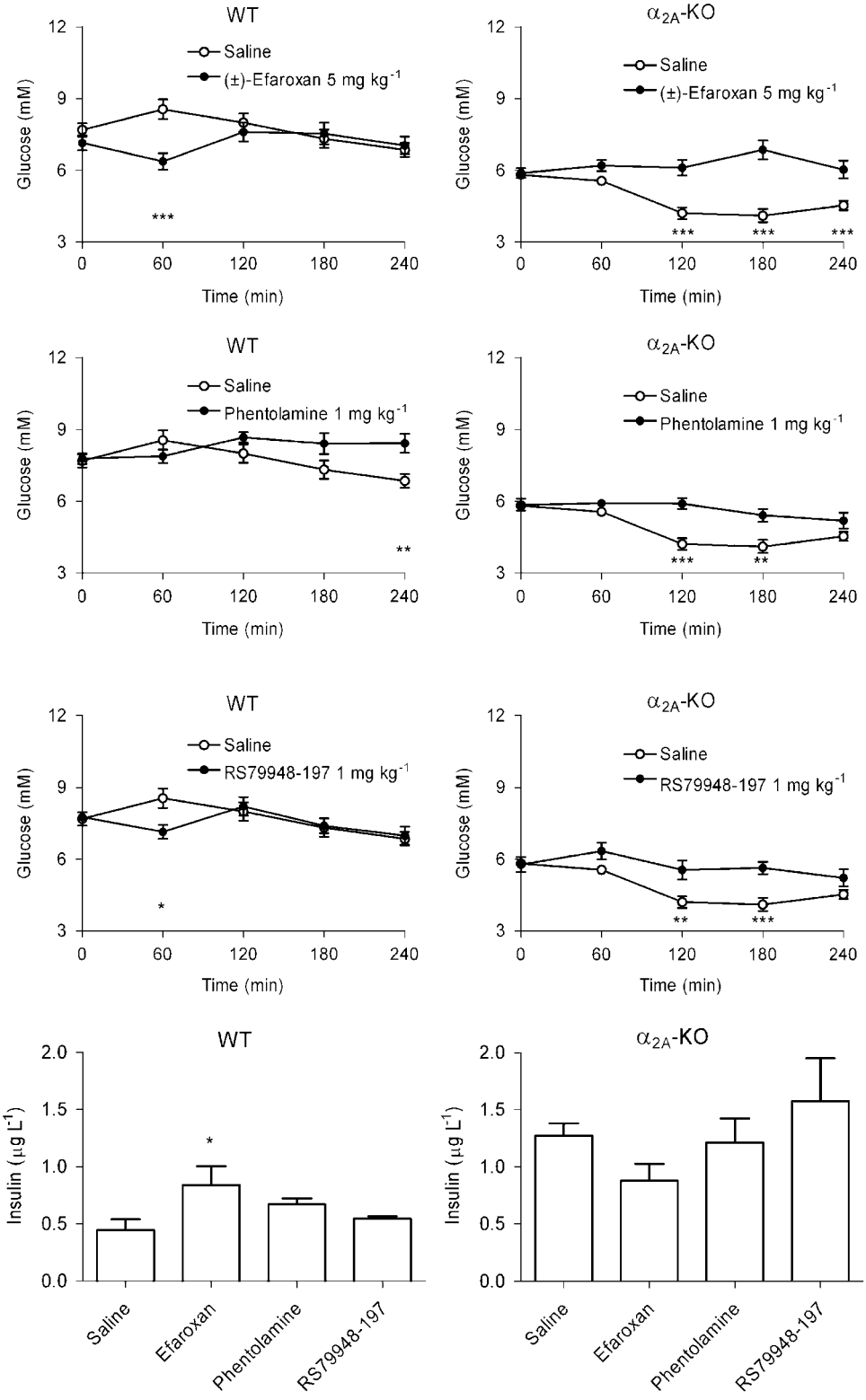

Figure 3 Effects of the imidazoline α_2-adrenoceptor antagonists (±)-efaroxan and phentolamine, and the non-imidazoline α_2-adrenoceptor antagonist RS79948-197 in 2-h fasted WT ($n=8$–10) and α_{2A}-KO ($n=9$–10) mice. Blood glucose levels were measured immediately before drug administration at time zero, and thereafter at the indicated time points. Insulin levels were measured at 60 min after drug administration. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with saline; two-way ANOVA (glucose results) or one-way ANOVA (insulin results).

noradrenaline release from sympathetic nerves, and that the α_{2C}-adrenoceptor tonically inhibits the secretion of adrenaline from the adrenal medulla (Hein *et al.*, 1999; Moura *et al.*, 2006). (±)-Efaroxan, phentolamine and RS79948-197 could therefore increase noradrenaline and adrenaline release through inhibition of α_{2A}- and α_{2C}-adrenoceptors in

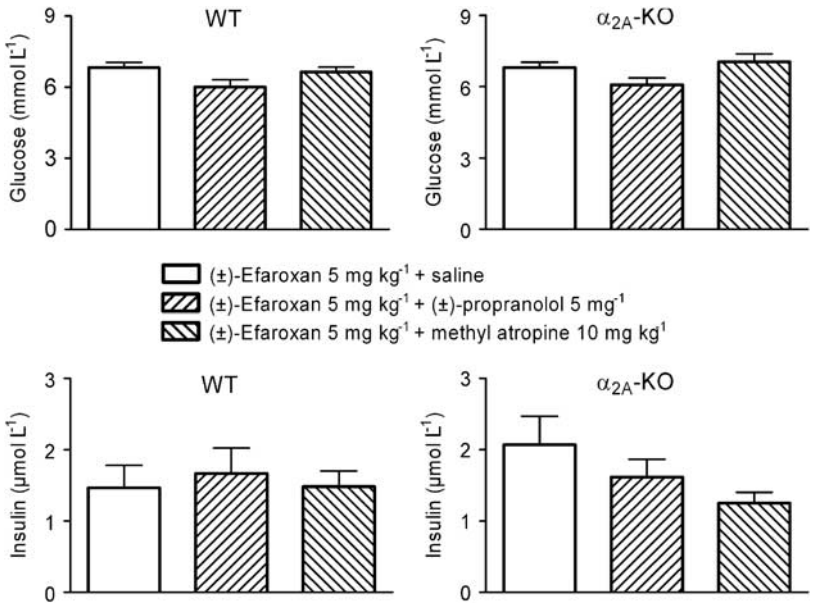

Figure 4 (±)-Efaroxan was injected, followed by injections of either saline, the β-adrenoceptor antagonist (±)-propranolol or the muscarinic ACh receptor antagonist methyl atropine 30 min later. Blood glucose and insulin levels were measured after another 30 min. (±)-Propranolol or methyl atropine did not significantly modify the effects of (±)-efaroxan on blood glucose and insulin levels in fed WT and α_{2A}-KO mice. $n = 9$–10 for both genotypes (one-way ANOVA).

WT mice, and through inhibition of α_{2C}-adrenoceptors in α_{2A}-KO mice.

Enhanced sympathetic activity, especially during concomitant lack of postjunctional inhibitory α_{2A}-adrenoceptors on pancreatic β-cells, may increase insulin secretion through activation of pancreatic β-cell β-adrenoceptors (Hermann and Deckert, 1977; Lacey *et al*., 1991). Also, disinhibition of α_2-adrenoceptors on postganglionic parasympathetic nerve terminals (Blandizzi *et al*., 1995; Scheibner *et al*., 2002) could enhance insulin secretion through activation of muscarinic ACh M_3 receptors on β-cells (Verspohl *et al*., 1990; Duttaroy *et al*., 2004). Pretreatment with (±)-propranolol or methyl atropine did, however, not diminish the rise in insulin and decrease in blood glucose in response to (±)-efaroxan in fed WT mice. This implies that postjunctional α_{2A}-adrenoceptors on pancreatic β-cells are responsible for the insulinotropic action of α_2-adrenoceptor antagonists.

As (±)-efaroxan, phentolamine and RS79948-197 stimulated insulin secretion and reduced blood glucose levels by relieving tonic inhibition of α_{2A}-adrenoceptors on β-cells, clinically beneficial effects of α_2-adrenoceptor antagonists on glycaemia are likely to be detectable only under conditions of high sympathetic activity. Differences in sympathetic activity or nutritional state may explain some of the inconsistencies between previous studies assessing α_2-adrenoceptor antagonist effects. In addition, the common use of rauwolscine as a non-imidazoline α_2-adrenoceptor antagonist may in some cases have resulted in an underestimation of the role of α_2-adrenoceptor antagonism in mice and rats, in which the affinity of rauwolscine for the α_{2A}-adrenoceptor subtype is approximately 10-fold lower than for the human α_{2A}-adrenoceptor subtype (Uhlén *et al*., 1998).

K_{ATP} channel activity is a major determinant of the pancreatic β-cell resting potential. (±)-Efaroxan and phentolamine inhibit K_{ATP} channels *in vitro*, although probably only at relatively high (micromolar) concentrations (Plant and Henquin, 1990; Chan *et al*., 1991; Rustenbeck *et al*., 1999; Bleck *et al*., 2005). The finding that the K_{ATP} channel inhibitor glibenclamide was more potent in α_{2A}-KO than in WT mice demonstrated that the β-cells of α_{2A}-KO mice efficiently responded to K_{ATP} channel inhibition. It is therefore unlikely that the abolished insulinotropic and hypoglycaemic effects of (±)-efaroxan and phentolamine in fed α_{2A}-KO mice were due to altered K_{ATP} channel function, and there is thus no evidence to suggest that (±)-efaroxan and phentolamine mediated their actions through K_{ATP} channel inhibition.

Although (±)-efaroxan has previously been shown to potentiate the insulinotropic effect of glibenclamide in intact rats and in isolated rat pancreatic islets (Berridge *et al*., 1992; Mourtada *et al*., 1997; Abdel-Zaher *et al*., 2001), the pivotal role of α_{2A}-adrenoceptors in limiting hypoglycaemia following glibenclamide administration was an unexpected finding. The severe hypoglycaemia resulting from combined administration of glibenclamide and RS79948-197 in WT mice, and from administration of glibenclamide alone in α_{2A}-KO mice, would be expected to initiate a maximal counter-regulatory response, engaging both branches of the autonomic nervous system. Adrenergic mechanisms have been shown to be important for increasing glucose production and glucagon secretion during hypoglycaemia (Connolly *et al*., 1996). Increased hepatic glucose production has, however, been attributed to α_1- and β-adrenoceptor activation (Chu *et al*., 2000). Moreover, experimental studies in humans have suggested that adrenergic counter-regulatory mechanisms become critical only when glucagon secretion is impaired (Rizza *et al*., 1979; Bolli *et al*., 1982; Tse *et al*., 1983; Cryer *et al*., 1984; Boyle *et al*., 1989). With the current experimental design, determinations of plasma glucagon levels were not feasible due to the

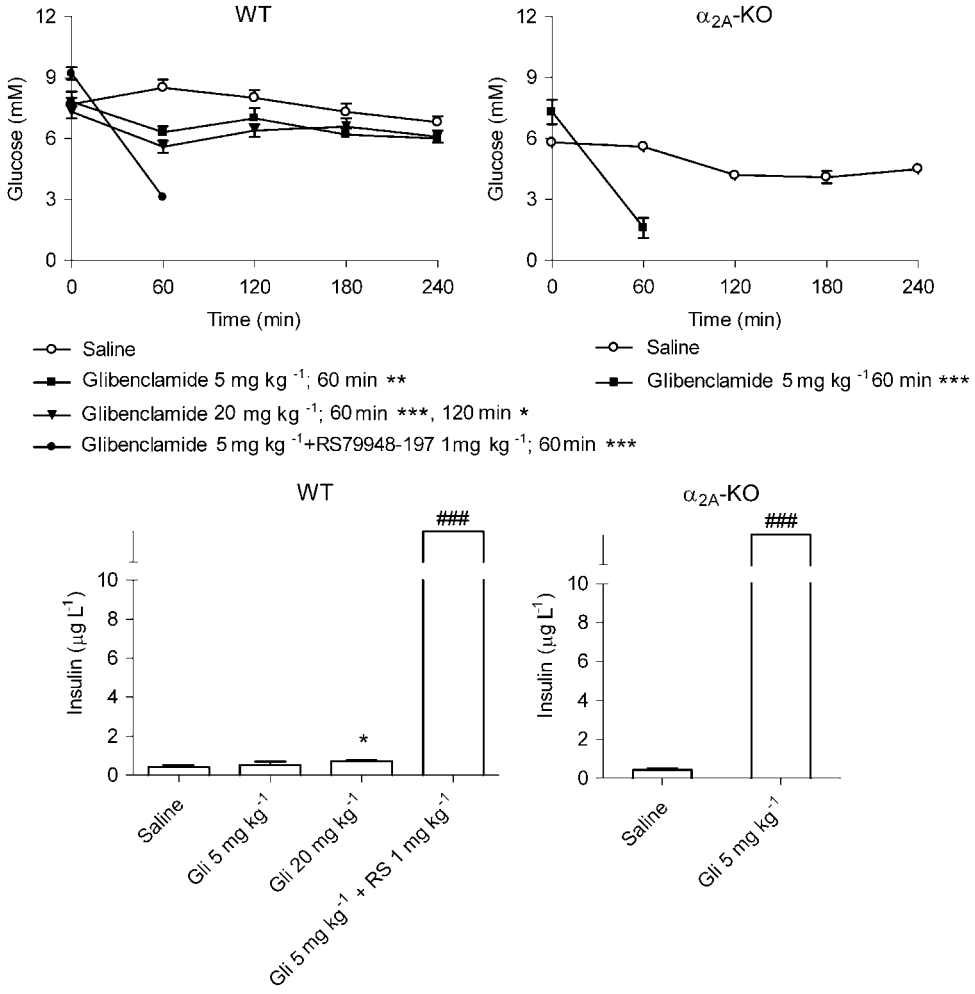

Figure 5 Effects of the sulphonylurea glibenclamide, and glibenclamide co-administered with the non-imidazoline α_2-adrenoceptor antagonist RS79948-197 in 2-h fasted WT mice ($n = 9$–10), and of glibenclamide alone in 2-h fasted α_{2A}-KO mice ($n = 4$–10). Blood glucose levels were measured immediately before drug administration at time zero, and thereafter at the indicated time points. Insulin levels were measured at 60 min after drug administration. In WT mice co-administered with RS79948-197 (RS) and glibenclamide (Gli), and in α_{2A}-KO mice administered with glibenclamide only, all insulin values exceeded 10 μg L^{-1}, which was the highest value of the insulin standard curve of the employed ELISA. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with saline; Student's *t*-test or one-way ANOVA. $^{###}P = 0.001$ compared with saline; Wilcoxon signed-rank test.

large blood volumes required. However, basal fasting glucagon levels were actually elevated in α_{2A}-KO mice (Savontaus *et al.*, 2008), suggesting that lack of α_{2A}-adrenoceptor signalling does not impair pancreatic α-cell function. The α_{2A}-KO mice also spontaneously recovered from hypoglycaemia induced by 1 IU kg^{-1} insulin during an intraperitoneal insulin tolerance test (Savontaus *et al.*, 2008). The present results therefore suggest that in the absence of inhibitory β-cell α_2-adrenoceptor signalling, severe hypoglycaemia occurred as a result of massive glibenclamide-induced secretion of insulin. The potential risk of severe hypoglycaemia during combined α_2-adrenoceptor antagonist and sulphonylurea administration would deserve further investigation, as some antidepressants (Garcia-Sevilla *et al.*, 1981; de Boer, 1996) and atypical antipsychotics (Lindström, 2000; Kalkman and Loetscher, 2003) are potent α_2-adrenoceptor antagonists, and the α_2-adrenoceptor antagonist atipamezole is widely used in veterinary medicine.

In conclusion, the α_{2A}-adrenoceptor, and not imidazoline-binding sites, mediates the insulinotropic effects of (±)-efaroxan and phentolamine *in vivo*. In addition, the α_{2A}-adrenoceptor counteracts sulphonylurea-induced insulin secretion by as yet uncharacterized molecular mechanisms.

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Conflict of interest

The authors state no conflict of interest.

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