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Novel I₁-Imidazoline Agonist S43126 Augment Insulin Secretion in Min6 Cells

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Abstract

The I₁-imidazoline receptor is a novel drug target for hypertension and insulin resistance which are major disorders associated with Type II diabetes. In the present study, we examined the effects of a novel imidazoline agonist S43126 on calcium fluxes and insulin secretion from Min6 β-cells. We also examined the effects of S43126 on the induction of IRAS, and phosphorylation of components in the I₁-imidazoline signaling pathways, namely ERK and PKB. Min6 β-cells were treated with varying doses of S43126 [10⁻⁸M to 10⁻⁵M] for various time (5–60mins). S43126 at higher dose [10⁻⁵M] stimulated insulin secretion under elevated glucose concentration compared to basal. In addition, insulin secretion and Ca²⁺ influx mediated by S43126 [10⁻⁵M] were decreased following co-treatment with efaroxan (I₁-antagonist) and nifedipine (L-type voltage-gated Ca²⁺-channel blocker) at various times (5–60mins). Furthermore, S43126 at [10⁻⁵M] increased Ca²⁺ oscillation, [Ca²⁺] and ⁴⁵Ca²⁺ uptake in a time and dose-dependent manner. Moreover, Western blot analysis of treated samples showed that S43126 caused an increased protein expression of IRAS as well as phosphorylation of both ERK1/2 and PKB in a concentration-dependent manner. We conclude that S43126 exerts its insulinotropic effect in a glucose dependent manner by a mechanism involving L-type calcium channels and imidazoline I₁-receptors.

Introduction

Insulin resistance and hypertension are commonly associated with metabolic syndrome, which affects over 75 million Americans, and type 2 diabetes which affects over 18 million Americans [1]. Pharmacologic treatment of many type 2 diabetic patients requires separate agents for treating hyperglycemia, and hypertension. This results in patients having to take multiple medications, which negatively impact patient compliance and increases the risk for drug interaction. In response to this growing health care problem, compounds that have the

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ability to counter both hyperglycemia and hypertension would positively impact compliance and be an asset to patients.

Pharmacologic criteria have defined three main types of imidazoline receptors: the I₁ subtype is labeled by [³H] clonidine and the I₂ subtype is labeled by [³H] idazoxan [2,3]. A third pharmacologically distinct entity, the I₃ subtype, is found in the pancreas and is involved in regulation of insulin secretion [4]. Functionally, I₂-imidazoline sites seem to play a role in depression as the density of I₂-sites were altered in suicide/depressive patients and the I₂-selective compound 2-(2-benzofuranyl)-2-imidazoline (2-BFI) demonstrated antidepressant-like effects in mice according to the tail suspension test and the forced swim test [5]. The I₂-site is also an emerging drug target for pain treatment [6] and I₂-agonists have been shown to enhance the antinociceptive effects of opioids [7]. There is an emerging role for I₂-agonists in the regulation of glucose homeostasis. Cerebral injections of agmatine reduced plasma glucose levels in streptozotocin-induced diabetic (STZ-diabetic) rats by a mechanism not involving insulin secretion but activation of I₂-imidazoline receptors [8]. It was subsequently shown that peripheral administration of agmatine caused activation of I₂-receptors in the adrenal medulla to enhance secretion of β-endorphins, leading to activation of μ-opioid receptors, and lower glucose levels [9]. Additionally it was shown that in rats where insulin resistance was induced by a high fructose diet, agmatine (1mg/kg) ameliorated the insulin resistance by a mechanism involving I₂-imidazoline receptors [10].

Imidazoline compounds, which are agonists at the I₁-imidazoline receptor (I₁R) present in the rostral ventrolateral medulla (RVLM) region of brain [11,12] act centrally to lower blood pressure. Clinical and basic findings also indicate a role for I₁-imidazoline agonists in the treatment of insulin resistance and diabetics with hypertension [13,14].

Several studies have shown that compounds containing the imidazoline moiety are potent stimulators of insulin secretion from pancreatic β-cells [15–19]. The mechanisms by which imidazoline compounds promote insulin secretion have not been fully elucidated. Classical imidazoline compounds mimic the actions of sulfonylurea drugs and interact directly with the pore-forming component (Kir6.2) of the ATP-sensitive potassium (K_{ATP}) channel to promote channel closure, membrane depolarization, Ca²⁺ influx and insulin secretion [15,17,20,21]. These agents also have a direct effect on exocytosis. Other imidazoline compounds have been shown to have no effect on the K_{ATP} channel, but exert their insulinotropic effects only if glucose concentration is elevated [18]. Some agents show a dependence on protein kinase A and C to exert their insulinotropic effects [18]

We have previously shown that S43126 (pK_i I₁=7.46, pK_i I₂=8.28, pK_i α₁<5 and pK_iα₂<5) a novel imidazoline compound with close binding affinities for both I₁ and I₂ imidazoline binding sites [22], lowers blood pressure when injected into the RVLM of spontaneously hypertensive rats. This compound does not contract rat tail arterial strips suggesting that it is inactive at alpha adrenergic receptors [23]. In this study we describe the effects of S43126 on calcium fluxes, insulin secretion and glucose uptake. Imidazoline compounds may prove useful in treating diabetics with hypertension

Materials and Methods

Antibodies and reagents

Primary antibodies used were IRAS, β -actin, p44/42 MAP kinase, phospho-p44/42 MAP kinase (Thr-202/Tyr-204), Akt, phospho-Akt (Ser473) antibody diluted 1:1000, which were detected using a secondary antibody (HRP linked anti-rabbit IgG), diluted 1:2000 and enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech). Treated cells were lysed and aliquots were subjected to western blotting using appropriate antibodies.

Cell culture and drug treatment

Min6 β -cells were cultured in DMEM (Cellgro) supplemented with 15% FBS, 5ml penicillin/streptomycin solution (Sigma), 1 μ L β -mercaptoethanol (Sigma) and maintained in the presence of 5% CO₂ at 37°C. Min6 β -cells were treated with varying doses of S43126 [10^{-5} M– 10^{-8} M] for different times, in the presence or absence of I₁-imidazoline receptor blocker efaroxan [100 μ M] or L-type calcium channel blocker nifedipine [10 μ M].

Single-cell microfluorimetry

Min6 β -cells were seeded on 25 mm glass coverslips in 35 mm plastic dishes. Twenty-four hours before imaging, culture medium was replaced with serum free medium. On the day of the experiment, culture medium was removed; cells were washed twice with Krebs-Ringer bicarbonate (KRB) buffer, pH 7.5, and containing 2.8 mM glucose. Washed cells were incubated with KRB/glucose for 2 hours at 37°C, 5% CO₂. In preparation for imaging, cells grown on the coverslip were loaded with 5 μ l of Ca²⁺ Fluorophore, Fura2/AM for 45 mins at room temperature. The coverslips were rinsed in KRB buffers and placed in a 300 μ l steel chamber attached to the stage of a Nikon inverted microscope (Nikon Instruments, Tokyo, Japan). A low pressure, rapid super fusion system (3 ml/min) was used to change solutions (i.e. S43126, glucose and KRB buffers) in the bath chamber. Ratios of 510 nm emission at 340 nm vs. 380 nm excitation wavelengths were acquired every second by photometric Cool Snap 12-bit digital camera. To facilitate comparison between experiments, data are expressed as 340/380-nm ratios normalized to the baseline ratio [24].

⁴⁵Ca²⁺ influx

⁴⁵Ca influx was determined by a modification of the methods of Edwards et al. and Brigand et al. [25,26]. Min6 β -cell in 6-well plates were treated as above but on the day of the experiment, cells were preincubated for 10 min at 37°C, 5% CO₂ in KRB. The preincubation solution was then replaced with KRB containing 3 uCi/ml ⁴⁵CaCl₂, in the presence or absence of S43126 [10^{-5} M], efaroxan [100 μ M] or nifedipine [10 μ M] for various times (0–30 mins). Treated cells were quickly washed five times with ice-cold saline and then solubilized in 1 ml KRB containing 0.1% Triton for 4 hours at room temperature. Aliquots (200 μ l) of the solution were then assayed for ⁴⁵Ca²⁺ content after the addition of 5 ml liquid scintillation medium (Perkin Elmer). Protein concentrations were determined using the BioRad DC Protein assay reagents (BioRad, Hercules, CA).

Insulin secretion

Monolayers of Min6 β -cells were seeded 3 days before each series of studies in 24-well plates at a density of 500,000 cells per well. 12 hours before each experiment, the culture medium was replaced with serum free medium. On the day of the experiment, the cells were washed twice with KRB buffer, pH 7.5, containing 0.1% BSA (KRB-BSA). Cells were then preincubated for 2 hrs in KRB-BSA containing 2.8 mM glucose at 37°C, 5% CO₂ followed by incubated for 2 hours in KRB-BSA containing various concentration of effectors (S43126, efaroxan and nifedipine) and glucose (2.8mM or 16.7mM) [13]. After incubation, the medium was collected, centrifuged at 600 X g for 10 mins and stored at -20°C. Insulin release was measured using Mercodia ultrasensitive mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden). Protein concentrations were determined using the BioRad DC Protein assay reagents (BioRad, Hercules, CA).

Statistical analysis

Data was analyzed using two-way analysis of variance (ANOVA) or Tukey's test for pairwise multiple comparisons to identify significant differences ($P > 0.05$) between individual samples. Percent blockade was calculated using the formula % blockade = $(S - [S+N]) / (S - \text{baseline}) * 100$ (Figure 3 and 4).

Results

Effects of S43126 on insulin secretion

We examined the ability of S43126 [10^{-7}M – 10^{-5}M] to induce insulin secretion directly from Min6 β -cells at various times (5–60mins) under conditions of basal glucose (2.8 mM) and high glucose (16.7 mM). S43126, a novel I₁-imidazoline agonist (Figure 1), induced a dose-dependent release of insulin at 5 mins, 10 mins and 30 mins under conditions of basal glucose (Figure 2A), and under high glucose (Figure 2B). At 5 mins and 10 mins, the amount of insulin release caused by a particular dose of S43126 was greater under conditions of high glucose than at basal glucose. Under conditions of basal glucose, the maximal release of insulin was 2.3 fold, seen at 5 mins of treatment with 10^{-5}M S43126. Under conditions of high glucose, the maximal release of insulin was 3.1 fold, seen at 5 mins of treatment with 10^{-5}M S43126. Relative insulin secretion decreased with time following stimulation under basal and high glucose.

In order to evaluate the role of I₁-imidazoline receptor and L-type calcium channel in the insulinotropic effect of S43126, we treated Min 6 cells with S43126 [10^{-5}M] in the presence or absence of either efaroxan [100 μM] (I₁-antagonist) or nifedipine [10 μM] (L-type calcium channel blocker) for various times (5–60mins). We used S43126 [10^{-5}M] as this dose produced the maximum release of insulin in our previous study. Under conditions of basal glucose, efaroxan caused 92%, 50%, 166%, and 85% inhibition of insulin release by S43126 at 5 mins, 10 mins, 30 mins, and 60 mins respectively (Figure 3A). Efaroxan by itself caused release of insulin under both basal and high glucose conditions. Under conditions of high glucose, efaroxan caused a greater than 89%, 100%, 93%, 100% inhibition of insulin release by S43126 at 5 mins, 10 mins, 30 mins and 60 mins respectively (Figure 3B).

Co-treatment of Min 6 cells with nifedipine [10 μ M], caused an approximately 73%, 63%, 91% and >80% reduction in insulin release at 5mins, 10 mins, 30 mins and 60 mins respectively under basal glucose conditions (Figure 4A). Inhibition of insulin release by nifedipine was more dramatic under high glucose conditions. Nifedipine caused a greater than 90% reduction in insulin release at all time points (Figure 4B).

Effects of S43126 on [Ca²⁺]_i

When Min6 β -cells were incubated with S43126 [10⁻⁸M – 10⁻⁵M] in the presence of 2.8mM glucose, S43126 caused a dose-dependent increase in [Ca²⁺]_i which was first observed at [10⁻⁶M] of S43126 (Figure 5A). The amplitude of the calcium oscillations evoked by S43126 at 2.8mM glucose were less than those produced by 16.7mM glucose, but greater than control, at higher doses. The increase in [Ca²⁺]_i caused by S43126 [10⁻⁵M] and 2.8mM glucose were similar at 1 min, however S43126 [10⁻⁵M] caused a gradual increase in [Ca²⁺]_i up to 5min followed by a slight decline. Under conditions of basal glucose, [Ca²⁺]_i gradually declined with time. The temporal response of [Ca²⁺]_i to high glucose, mimic that of S43126 [10⁻⁵M] but with higher levels of [Ca²⁺]_i. (Figure 5B).

Effects of S43126 on ⁴⁵Ca²⁺ uptake

Min 6 cells were also incubated with ⁴⁵Ca²⁺ alone or in combination with S43126 [10⁻⁵M] at various times (0–60 mins). There was a time-dependent increase in ⁴⁵Ca²⁺ influx up to 5 mins, followed by a decline at 10 mins and then a plateau between 30–60 mins (Figure 6A). The maximum influx of greater than 3 fold was seen at 5 mins.

Since S43126 [10⁻⁵M] provoked marked ⁴⁵Ca²⁺ influx at 5 mins, we co-treated cells with S43126 [10⁻⁵M] in the presence or absence of either efaroxan (10 μ M) or nifedipine (10 μ M) with the aim of determining whether I₁-imidazoline receptor or L-type calcium channels mediated the observed ⁴⁵Ca²⁺ influx into Min 6 cells. S43126 increased ⁴⁵Ca²⁺ influx by 4 fold and this increase was blocked by efaroxan (Figure 6B). In addition, nifedipine, a blocker of L-type calcium channels, reduced ⁴⁵Ca²⁺ influx by S43126 to near basal levels (Figure 6B).

Effects of S43126 on ERK1/2, PKB phosphorylation and IRAS protein expression

S43126 [10⁻⁷M–10⁻⁵M] induce IRAS protein expression in a dose-dependent manner (Figure 7A). S43126 [10⁻⁷M–10⁻⁵M] also cause a 3-fold increase in ERK1/2 phosphorylation (Figure 7B) and a 2-fold increase in PKB phosphorylation (Figure 7C). ERK1/2 and PKB are components of both the insulin and imidazoline receptor signaling pathways.

Discussion

Compounds that have the ability to treat both hypertension and insulin resistance would greatly improve compliance among patients. The novel imidazoline compound S43126 has been shown previously to lower blood pressure in rats following injection into the RVLM, but did not contract rat tail artery [23]. We therefore wanted to determine whether S43126 also had the ability to affect glucose homeostasis. Treatment of Min6 β -cells with S43126

caused a dose-dependent increase in insulin secretion at 5 mins, 10 mins and 30 mins under conditions of basal glucose (Figure 2A), and under high glucose (Figure 2B). This increase in insulin secretion was modest, but glucose-dependent, with S43126 causing a greater insulin release at higher concentrations of glucose at 5 mins and 10 mins. This is in contrast to sulfonylurea drugs which demonstrate a strong insulinotropic effect at basal glucose concentrations. Agents that stimulate robust insulin secretion at basal levels of glucose are more likely to cause hypoglycemia in patients [27]. A more desirable therapeutic outcome would be achieved by agents such as S43126 that augment glucose-induced insulin secretion.

The effect of imidazoline compounds on glucose homeostasis is mediated by all three subtypes of imidazoline receptors. I₁-imidazoline and I₃-imidazoline agonists mediate insulin release, while I₂-imidazoline agonists mediate insulin sensitization. It was shown previously that cerebral injections of agmatine caused a decrease in plasma glucose in STZ-diabetic rats, but plasma levels of glucose and insulin were not affected in normal rats [8]. This suggested that agmatine was acting through a non-insulin dependent mechanism, and did not cause insulin release in normal rats. The effects of agmatine on glucose levels were attenuated in a dose-dependent manner with the I₂-imidazoline receptor blocker BU-224. It was subsequently shown that peripheral injections of agmatine into STZ-diabetic rats also lowered plasma glucose in a dose-dependent manner [9]. The proposed mechanism for the peripheral glucose lowering effects of agmatine involved secretion of β -endorphins from the adrenal medulla, secondary to activation of adrenal I₂-imidazoline receptors. β -endorphins are known to decrease the gene expression of phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in gluconeogenesis [9]. Increased *GLUT4* gene expression was also involved in the glucose lowering effects of agmatine [9].

The novel compound S4321 has affinity at I₂-imidazoline receptors, however it is unknown whether this agent acts as an agonist or antagonist at the I₂-imidazoline receptors [22]. It is unlikely that the insulin release caused by S43126 was due to an action at I₂-imidazoline receptors, since as stated above, agmatine which also has effects at I₂-imidazoline receptors does not cause insulin release in normal rats. In addition, I₂-ligands such as cirazoline and idazoxan were shown to cause insulin release from RIN-5AH insulinoma cell line, but not by their actions at I₂-imidazoline receptors, since irreversible blockade of the I₂-receptor by clorgyline did not attenuate the effects of these compounds on insulin secretion. The proposed mechanism for insulin release by these ligands was an action on K_{ATP} channels [28].

Imidazoline compounds are not uniform in the mechanism by which they stimulate insulin release from pancreatic beta cells. Some imidazolines such as efaroxan [29,30], phentolamine [31] and RX871024 [32–33] stimulate insulin secretion both *in vivo* and *in vitro* by binding to K_{ATP} channels [29–34]. The pore forming subunit of the K_{ATP} channel, Kir6.2 contains an imidazoline binding site [35] to which certain imidazolines bind, leading to membrane depolarization, activation of voltage-dependent calcium channels, and subsequent calcium influx, and insulin secretion. Some imidazolines such as BL11282 stimulate insulin secretion only at elevated levels of glucose and does not block the K_{ATP} channel [18]. This property suggests that the risk of hypoglycemia would be very low in

patients treated with BL11282. However, we are unaware of any published data suggesting that BL11282 lowers blood pressure. Thus BL11282 may not possess the utility that S43126 has in impacting both hypertension and insulin resistance.

S-21663 is an imidazoline compound that is a non glucose-dependent insulin secretagogue, which does not readily induce hypoglycemia [15]. The authors explained the discrepancy between the ability of S-21663 to stimulate insulin secretion at all levels of glucose and the lack of hypoglycemia seen *in vivo* by the existence of some compensatory mode of action by this compound *in vivo*, but not seen *in vitro*. S-21663 acts in a manner that is dependent on calcium entry via L-type calcium channels that are activated by closure of non K_{ATP} potassium channels [15]. Our novel compound S43126 also caused an increase in intracellular calcium via influx through nifedipine sensitive calcium channels. Unlike other imidazolines, we showed that the insulinotropic effect of S43126 could be blocked by efaroxan, an I_1 -imidazoline antagonist. In addition to its antagonistic effect at I_1 -imidazoline sites, efaroxan can also interact with K_{ATP} channels or the I_3 -imidazoline receptor to cause insulin release. We observed that efaroxan did cause release of insulin presumably through its action at K_{ATP} channels or the I_3 -imidazoline receptor. Combination of S43126 and efaroxan resulted in inhibition of insulin release; this suggested that at 100 μ M, efaroxan acted as an effective blocker of the I_1 -receptor. The degree of antagonism caused by efaroxan was complicated by its ability to release insulin and a purer antagonist would prove more useful. The functional effects of S43126 at the various subtypes of imidazoline receptors need to be further studied, using inhibitors without intrinsic effects at these receptors.

S43126 also caused an increased induction of IRAS protein, and an increased phosphorylation of ERK and PKB which are components of the I_1 -imidazoline signaling pathways. In one study, PKB function in beta cells was disrupted by expression of a kinase-dead dominant negative form of PKB α (rip-*kdpkb*) and defective insulin secretion was observed, but no reduction in islet size [36]. This suggests that at least PKB α may play a role in insulin secretion. There is some controversy surrounding the exact role of different isoforms of PKB in the pancreas. Recent work by Buzzi et al. [37] showed that only PKB α but not PKB γ or PKB β is activated downstream of IRS2 in beta cells. Overexpression of PKB α by adenovirus caused an increased proliferation of beta cells, while overexpression of PKB γ and PKB β were ineffective. In another study, it was shown that PKB α -deficient mice can show enhanced glucose tolerance in addition to improved beta cell function and higher insulin sensitivity in adipocytes. Further studies will be needed to determine the importance of increased phosphorylation of PKB by S43126 on insulin sensitivity, and any possible involvement of I_2 -imidazoline receptors. These studies will need to consider the specific isoforms of PKB, which, as the recent studies showed, may even have opposing effects [37].

In conclusion, S43126 is a novel compound that activated I_1 -imidazoline receptors but not α -adrenoceptors [23,38]. We have shown that S43126 induced insulin secretion in a dose and time dependent manner. Because S43126 increased insulin release at higher glucose levels compared to basal condition, the risk of hypoglycemia associated with the use of S43126 is diminished [9]. Thus, the present study showed that S43126 activated at least two distinct mechanisms to promote insulin release. One of these may involve binding to

imidazoline I₁-receptors, while a second arises from calcium influx due to L-type Ca²⁺ channels. More importantly, S41326 activated components of the I₁-imidazoline signaling pathways, namely IRAS, ERK and PKB in Min6 β-cells have the potential to act as an insulin sensitizer. Imidazoline agonists should be developed as an innovative pharmacological approach for the management of Type II diabetes.

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References

1. Hansen BC. The Metabolic Syndrome X. *Ann NY Acad Sci.* 1999; 892:1–24. [PubMed: 10842649]
2. Bousquet P, Feldman J, Schwartz J. Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. *J Pharmacol Exp Ther.* 1984; 230:232–236. [PubMed: 6146707]
3. Bousquet P. Imidazoline receptors: from basic concepts to recent developments. *J Cardiovasc Pharmacol.* 1995; 26:S1–S6. [PubMed: 8642798]
4. Brown CA, Chan SL, Stillings MR, Smith SA, Morgan NG. Antagonism of the stimulatory effects of efaroxan and glibenclamide in rat pancreatic islets by the imidazoline, RX801080. *Br J Pharmacol.* 1993; 110:1017–1022. [PubMed: 7905338]
5. Tonello R, Villarinho JG, da Silva Sant’anna G, Tamiozzo L, Machado P, et al. The potential antidepressant-like effect of imidazoline I₂ ligand 2-BFI in mice. *Prog Neuropsychopharmacol Biol Psychiatry.* 2012; 37:15–27. [PubMed: 22122878]
6. Li JX, Zhang Y. Emerging drug targets for pain treatment. *Eur J Pharmacol.* 2012; 681:1–5. [PubMed: 22314220]
7. Thorn DA, Zhang Y, Peng BW, Winter JC, Li JX. Effects of imidazoline I₂ receptor ligands on morphine- and tramadol-induced antinociception in rats. *Eur J Pharmacol.* 2011; 670:435–440. [PubMed: 21970802]
8. Jou SB, Liu IM, Cheng JT. Activation of imidazoline receptor by agmatine to lower plasma glucose in streptozotocin-induced diabetic rats. *Neurosci Lett.* 2004; 358:111–114. [PubMed: 15026161]
9. Hwang SL, Liu IM, Tzeng TF, Cheng JT. Activation of imidazoline receptors in adrenal gland to lower plasma glucose in streptozotocin-induced diabetic rats. *Diabetologia.* 2005; 48:767–775. [PubMed: 15756537]
10. Ko WC, Liu IM, Chung HH, Cheng JT. Activation of I(2)-imidazoline receptors may ameliorate insulin resistance in fructose-rich chow-fed rats. *Neurosci Lett.* 2008; 448:90–93. [PubMed: 18926881]
11. Ernsberger P, Westbrooks KL, Christen MO, Schäfer SG. A second generation of centrally acting antihypertensive agents act on putative I₁-imidazoline receptors. *J Cardiovasc Pharmacol.* 1992; 20:S1–S10.
12. Ernsberger P, Giuliano R, Willette RN, Reiss DJ. Role of imidazole receptors in the vasodepressor response to clonidine analogs in the rostral ventrolateral medulla. *J Pharmacol Exp Ther.* 1990; 253:408–418. [PubMed: 2158551]
13. De Luca N, Izzo R, Fontana D, Iovino G, Argenziano L, et al. Haemodynamic and metabolic effects of rilmenidine in hypertensive patients with metabolic syndrome X. A double-blind parallel study versus amlodipine. *J Hypertens.* 2000; 18:1515–1522. [PubMed: 11057441]
14. Hansson L. Therapy of hypertension and metabolic syndrome: today’s standard and tomorrow’s perspectives. *Blood Press Suppl.* 1998; 3:20–22. [PubMed: 10321450]
15. Brigand LL, Virsolvy A, Peyrollier K, Manechez D, Godfroid JJ, et al. Stimulation of insulin Release from the Min6 Cell line by new imidazoline compound, S-21663: evidence for the existence of a novel imidazoline site in β-cells. *Br J Pharmacol.* 1997; 122:786–791. [PubMed: 9375978]

16. Mourtada M, Smith SA, Morgan NG. Effector systems involved in the insulin secretory responses to efaroxan and RX871024 in rat islets of Langerhans. *Eur J Pharmacol.* 1998; 350:251–258. [PubMed: 9696415]
17. Brigand LL, Virsolvy A, Manechez D, Godfroid JJ, Lematre BG, et al. In vitro mechanism of action on insulin release of S-22068, a new putative antidiabetic compound. *Br J Pharmacol.* 1999; 128:1021–1026. [PubMed: 10556939]
18. Efanov AM, Zaitsev SV, Mest HJ, Raap A, Appelskog IB, et al. The Novel Imidazoline Compound BL11282 Potentiates Glucose-Induce Insulin Secretion in Pancreatic β -Cells in the absence of Modulation of K_{ATP} Channel Activity. *Diabetes.* 2001; 50:797–802. [PubMed: 11289044]
19. Olsen HL, Norby PL, Hoy M, Spee P, Thams P, et al. Imidazoline NNC77-0074 stimulates Ca^{2+} -evoked exocytosis in INS-1E cells by a phospholipase A_2 -dependent mechanism. *Biochem Biophys Res Commun.* 2003; 303:1148–1151. [PubMed: 12684056]
20. Proks P, Ashcroft FM. Phentolamine block of K_{ATP} channels is mediated by Kir6.2. *Proc Natl Acad Sci USA.* 1997; 94:11716–11720. [PubMed: 9326676]
21. Monks LK, Cosgrove KE, Dunne MJ, Ramsden CA, Morgan NG, et al. Affinity isolation of imidazoline binding protein from rat brain using 5-amino-efaroxan as ligand. *FEBS Lett.* 1999; 447:61–64. [PubMed: 10218583]
22. Anastassiadou M, Danoun S, Crane L, Baziard-Mouysset G, Payard M, et al. Synthesis and pharmacological evaluation of imidazoline sites I1 and I2 selective ligands. *Bioorg Med Chem.* 2001; 9:585–592. [PubMed: 11310592]
23. Edwards LP, Crane L, Baziard-Mouysset G, Brown-Bryan T. Imidazolines as single agent therapy for diabetics with hypertension. *FASEB J (Meeting Abstract Supplement).* 2010:597.3.
24. Schmid GM, Meda P, Caille D, Wargent E, O'Dowd J, et al. Inhibition of Insulin Secretion by Betagranin, an N-terminal Chromogranin A Fragment. *J Biol Chem.* 2007; 282:12717–12724. [PubMed: 17289672]
25. Edwards L, Hessinger DA. Portuguese Man-of-war (*Physalia physalis*) venom induces calcium influx into cells by permeabilizing plasma membranes. *Toxicol.* 2000; 38:1015–1028. [PubMed: 10708794]
26. Brigand LL, Virsolvy A, Peyrollier K, Manechez D, Godfroid JJ, et al. Stimulation of insulin Release from the Min6 Cell line by new imidazoline compound, S-21663: evidence for the exitance of a novel imidazoline site in beta cells. *Br J Pharmacol.* 1997; 122:786–791. [PubMed: 9375978]
27. Pfeiffer EF, Thum C, Raptis S, Beischer W, Ziegler R. Hypoglycemia in diabetics. *Horm Metab Res.* 1976; 6:112–126. [PubMed: 179924]
28. Olmos G, Kulkarni RN, Haque M, MacDermot J. Imidazolines stimulate release of insulin from RIN-5AH cells independently from imidazoline I1 and I2 receptors. *Eur J Pharmacol.* 1994; 262:41–48. [PubMed: 7813577]
29. Chan SL, Morgan NG. Stimulation of insulin secretion by efaroxan may involve interaction with potassium channels. *Eur J Pharmacol.* 1990; 176:97–101. [PubMed: 2178947]
30. Chapman JC, McClenaghan NH, Cosgrove KE, Hashmi MN, Shepherd RM, et al. ATP-sensitive potassium channels and efaroxan-induced insulin release in the electrofusion derived BRIN-BD11 β -cell line. *Diabetes.* 1999; 48:2349–2357. [PubMed: 10580423]
31. Schulz A, Hasselblatt A. Phentolamine, a deceptive tool to investigate sympathetic nervous control of insulin release. *Naunyn Schmiedebergs Arch Pharmacol.* 1988; 337:637–643. [PubMed: 3063970]
32. Mourtada M, Chan SLF, Smith SA, Morgan NG. Multiple effector pathways regulate the insulin secretory response to the imidazoline RX871024 in isolated rat pancreatic islets. *Br J Pharmacol.* 1999; 127:1279–1287. [PubMed: 10455276]
33. Efanov AM, Zaitsev SV, Efanova IB, Zhu S, Ostenson CG, et al. Signaling and sites of interaction for RX871024 and sulfonylurea in the stimulation of insulin release. *Am J Physiol.* 1998; 274:E751–757. [PubMed: 9575838]
34. Chan SLF, Dunne MJ, Stillings MR, Morgan NG. The α_2 -adrenoceptor antagonist efaroxan modulates K_{ATP} channels in insulin secreting cells. *Eur J Pharmacol.* 1991; 204:41–48. [PubMed: 1687123]

35. Proks P, Ashcroft FM. Phentolamine block of KATP channels is mediated by Kir6.2. *Proc Natl Acad Sci USA*. 1997; 94:11716–11720. [PubMed: 9326676]
36. Bernal-Mizrachi E, Fatrai S, Johnson JD, Ohsugi M, Otani K, et al. Defective insulin secretion and increased susceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet beta cells. *J Clin Invest*. 2004; 114:928–936. [PubMed: 15467831]
37. Buzzi F, Xu L, Zuellig RA, Boller SB, Spinass GA, et al. Differential effects of protein kinase B/Akt isoforms on glucose homeostasis and islet mass. *Mol Cell Biol*. 2010; 30:601–612. [PubMed: 19933838]
38. Tesfai J, Crane L, Mouysset GB, Kennedy W, Edwards LP. Novel I1-Imidazoline S43126 Enhance Insulin Action in PC12 Cells. *Pharmacol Rep*. 2011; 63:1442–1449. [PubMed: 22358092]

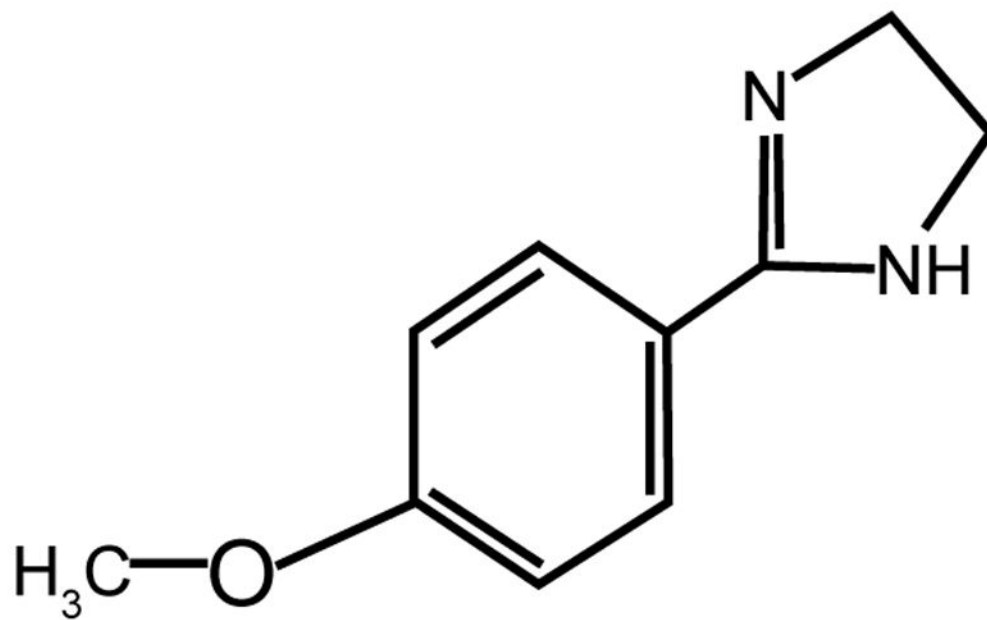


Figure 1.
Chemical Structure of the Novel Imidazoline Compound, S43126.

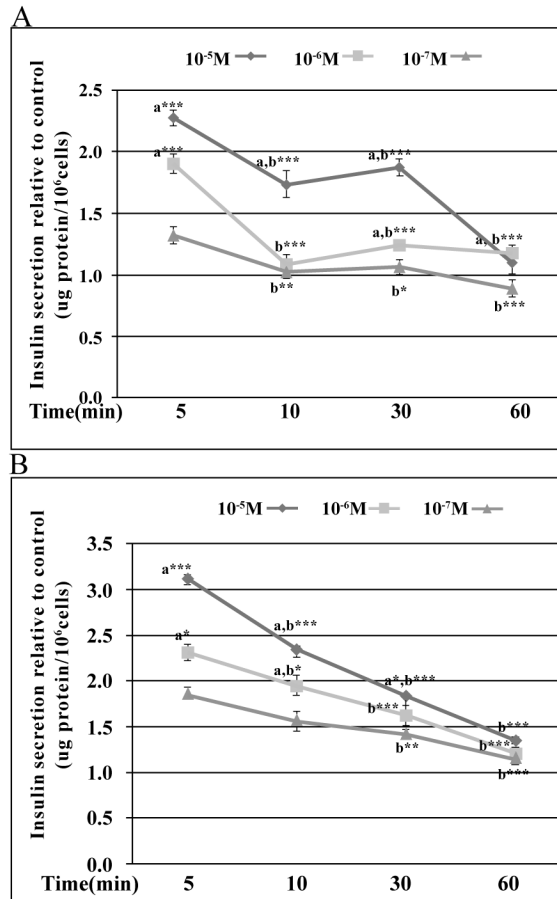


Figure 2. Stimulatory effects of S43126 on insulin release from Min6 cells. S43126 [10^{-5} M] at 5mins induced the highest insulin release (2.3 fold) and 3.1 fold under conditions of (A) low glucose (2.8 mM) and (B) high glucose (16.8 mM) respectively, relative to control. (A) Data are presented as mean percent change \pm standard error from three separate experiments run in duplicate. ($a = ***p < 0.001$ same time point, compared to [10^{-7} M] and $b = ***p < 0.001$ same dose, different time compared to 5mins, ANOVA) (B) Data are presented as mean percent change \pm standard error from three separate experiments run in duplicate. ($a = **p < 0.01$, $***p < 0.001$ same time point, compared to [10^{-7} M] $b = **p < 0.01$, $***p < 0.001$ same dose, different time compared to 5 mins, two-way ANOVA).

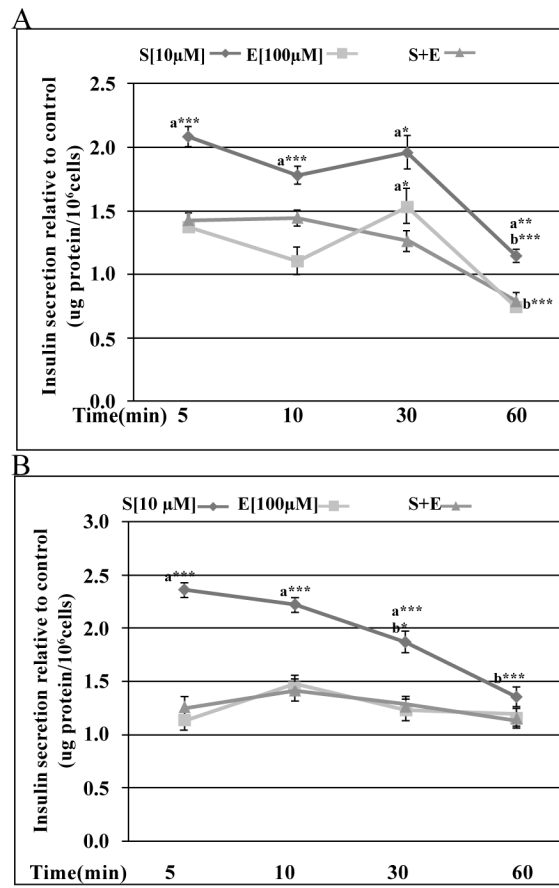


Figure 3. Effects of Efaroxan (E) on S43126(S)-induced insulin release. I₁-imidazoline receptor antagonist Efaroxan [100 µM] attenuated the insulinotropic response of Min6 cells to S41326 under (2A) low and (2B) high glucose. (2A) Data are presented as mean percent change +/- standard error from three separate experiments run in duplicate. (a= **p< 0.01, ***p< 0.001 same time point, compared to **E** and b= ***p< 0.001 same dose, different time compared to 5 mins, ANOVA). (2B) Data are presented as mean percent change +/- standard error from three separate experiments run in duplicate. (a= ***p< 0.001 same time point, compared to **E** and b= ***p< 0.001 same dose, different time compared to 5 mins, two-way ANOVA).

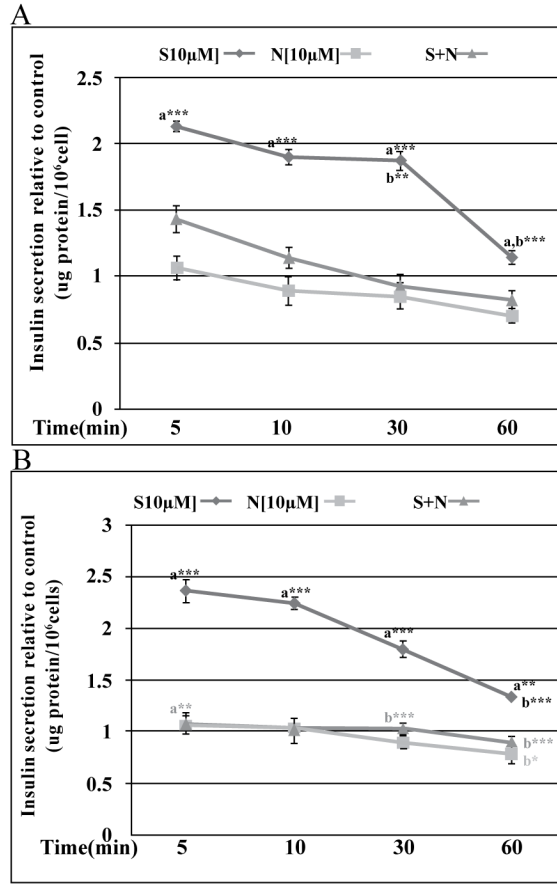


Figure 4. Effects of Nifedipine (N) on S43126(S)-induced insulin release. Voltage-gated L-type calcium channel blocker nifedipine [10 μM] attenuated the insulinotropic response of Min6 cells to S43126 under (3A) low and (3B) high glucose. (3A) Data are presented as mean percent change +/- standard error from three separate experiments run in duplicate. (*a*=**p*< 0.05, ****p*< 0.001 same time point, compared to N and *b*= ***p*< 0.01, ****p*< 0.001 same dose, different time compared to 5 mins, ANOVA). (3B) Data are presented as mean percent change +/- standard error from three separate experiments run in duplicate. (*a*= ****p*< 0.001 same time point, compared to N and *b*= ***p*< 0.01, ****p*< 0.001 same dose, different time compared to 5 mins, two-way ANOVA).

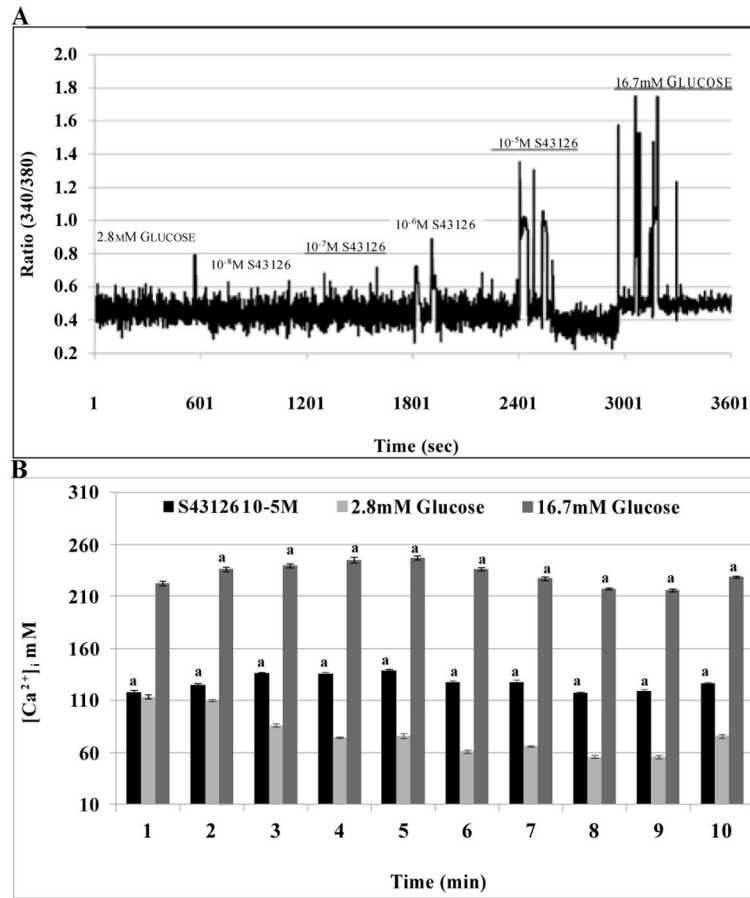


Figure 5. Effects of S43126 on Ca²⁺ oscillations. S43126 evoked a dose-dependent increase in cytosolic Ca²⁺ under (3A) conditions of basal glucose (2.8 mM). (B) S43126 [10^{-5} M] caused an increase in [Ca²⁺]_i that was greater than the influx caused by 2.8 mM glucose but less than the influx caused by 16.7 mM glucose at all time points studied. Data are presented as mean percent change \pm standard error from eight separate experiments (***) $p < 0.001$ 2.8 mM Glucose (control) vs S43126 [10^{-5} M] & 16.7mM Glucose at the same time, one-way ANOVA).

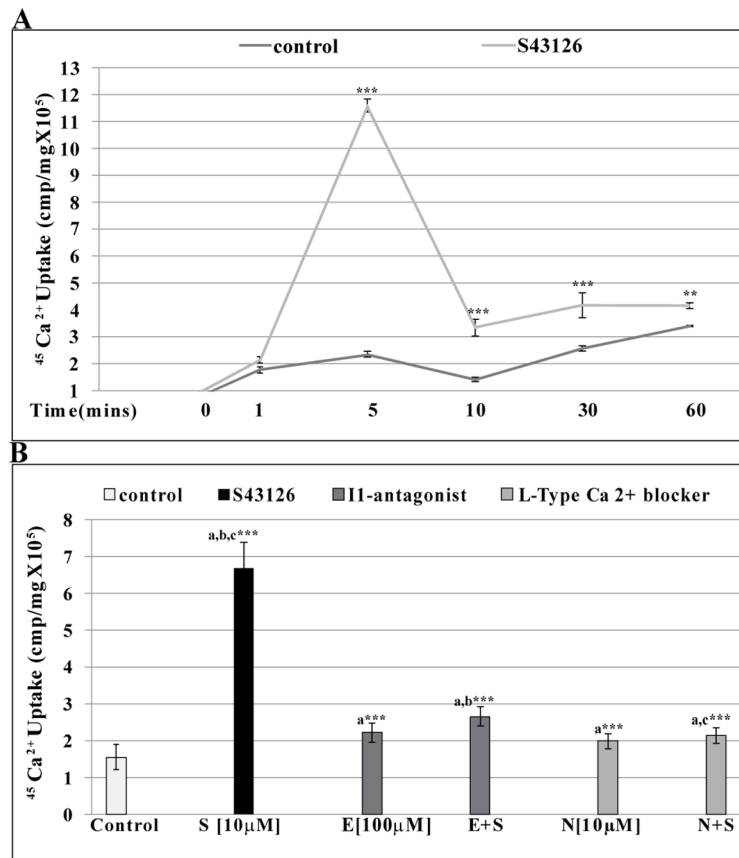


Figure 6. Effects of S43126 on $^{45}\text{Ca}^{2+}$ uptake. (A) S43126 [10^{-5}M] induced a time-dependent increase in $^{45}\text{Ca}^{2+}$ influx up to 5 mins, followed by a decline at 10 min and then a plateau between 30–60 mins. Data are presented as mean percent change \pm standard error from four separate experiments run in duplicate. (***) $p < 0.001$ Control vs S43126 [10^{-5}M] at the same time, two-way ANOVA). (B) The $^{45}\text{Ca}^{2+}$ influx mediated by S43126 [10^{-5}M] was attenuated in the presence of efaroxan [$100\ \mu\text{M}$] and nifedipine [$10\ \mu\text{M}$]. The data are presented as mean percent change \pm S.E. from four separate experiments run in duplicate. (a= *** $p < 0.001$ Control vs S, E, E+S, N, N+S, b= *** $p < 0.001$ E vs S, E+S and c= *** $p < 0.001$ N vs S, N+S, one-way ANOVA).

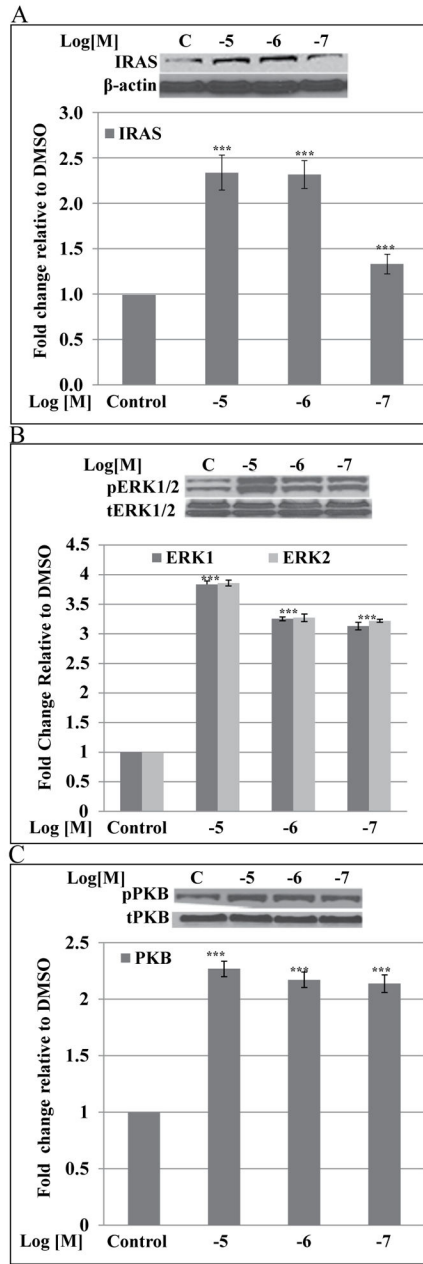


Figure 7. Dose-Response WB. Effects of S43126 [10^{-7} 10^{-5} M] on (A) IRAS protein expression (B) ERK1/2 phosphorylation (C) PKB (Akt) phosphorylation. S43126 [10^{-7} 10^{-5} M] caused an increased induction of IRAS protein, and a dose-dependent increase in the phosphorylation of both ERK1/2 and PKB. The relative protein expression of IRAS was defined by the ratio of the β -actin while the relative phosphorylation of ERK1/2 and PKB was defined by the ratio of phosphorylated active form to total immunoreactive protein, in arbitrary absorbance units. Data are presented as mean percent change \pm standard error from two separate

experiments run in duplicate. (***) $p < 0.001$ Control vs Dose [10^{-7} – 10^{-5} M], one-way ANOVA).

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