ORIGINAL ARTICLE

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Lipophilicity predicts the ability of nonsulphonylurea drugs to block pancreatic beta-cell K_{ATP} channels and stimulate insulin secretion; statins as a test case

Joana Real[1] | Caroline Miranda[1] | Charlotta S. Olofsson[1] | Paul A. Smith[2] (iD)

[1]Department of Physiology/Metabolic Physiology, Institute of Neuroscience and Physiology, Göteborg, Sweden

[2]School of Life Sciences, University of Nottingham, Nottingham, UK

Correspondence
Paul A. Smith, School of Life Sciences, University of Nottingham, Nottingham, UK.
Email: paul.a.smith@nottingham.ac.uk

Summary

Aims: K_{ATP} ion channels play a key role in glucose-stimulated insulin secretion. However, many drugs block K_{ATP} as "off targets" leading to hyperinsulinaemia and hypoglycaemia. As such drugs are often lipophilic, the aim was to examine the relationship between drug lipophilicity (P) and IC_{50} for K_{ATP} block and explore if the IC_{50}'s of statins could be predicted from their lipophilicity and whether this would allow one to forecast their acute action on insulin secretion.

Materials and methods: A meta-analysis of 26 lipophilic, nonsulphonylurea, blockers of K_{ATP} was performed. From this, the IC_{50}'s for pravastatin and simvastatin were predicted and then tested experimentally by exploring their effects on K_{ATP} channel activity via patch-clamp measurement, calcium imaging and insulin secretion in murine beta cells and islets.

Results: Nonsulphonylurea drugs inhibited K_{ATP} channels with a Log IC_{50} linearly related to their logP. Simvastatin blocked K_{ATP} with an IC_{50} of 25 nmol/L, a value independent of cytosolic factors, and within the range predicted by its lipophilicity (21-690 nmol/L). 10 µmol/L pravastatin, predicted IC_{50} 0.2-12 mmol/L, was without effect on the K_{ATP} channel. At 10-fold therapeutic levels, 100 nmol/L simvastatin depolarized the beta-cell membrane potential and stimulated Ca^{2+} influx but did not affect insulin secretion; the latter could be explained by serum binding.

Conclusions: The logP of a drug can aid prediction for its ability to block beta-cell K_{ATP} ion channels. However, although the IC_{50} for the block of K_{ATP} by simvastatin was predicted, the difference between this and therapeutic levels, as well as serum sequestration, explains why hypoglycaemia is unlikely to be observed with acute use of this statin.

KEYWORDS
beta-cell, insulin, K_{ATP} channel, simvastatin

1 | INTRODUCTION

The role of the pancreatic beta-cell K_{ATP} ion channel in the regulation of insulin secretion is well characterized.[1] An extensive range of compounds is known to block the K_{ATP} channel; canonically these are sulphonylureas, such as tolbutamide,[2] gliclazide,[3] glipizide[4] and glibenclamide[2,4]; and glitinides, such as meglitinide[2] and repaglinide.[5] The ability of these drugs to selectively block K_{ATP} in pancreatic beta cells underlies their use in the treatment of hyperglycaemia.[4] However, many other structurally diverse drugs can also block K_{ATP} as an "off target"; where inadvertent block of the pancreatic beta-cell K_{ATP} channel can lead to the adverse effects of hyperinsulinaemia and hypoglycaemia seen in the clinic, for example following over dosage with quinolones in the treatment of malaria.[6] Indeed, most studies that have investigated the ability of nonsulphonylurea drugs to block beta-cell K_{ATP} channel activity and stimulate insulin secretion have arisen from clinical reports of adverse hypoglycaemic episodes during their acute usage; as reported for thiazolidinediones,[7,8] quinolones,[6,9] fluoroquinolones[10] and pheniramines.[11] Although no obvious common structural chemical moieties underlie the "off target" action of these drugs, they do all share the common property of lipophilicity. As a trend between the potency to block K_{ATP} and lipophilicity is noted for barbiturates[12] whether this also extends to other, nonsulphonylurea, blockers of K_{ATP} is unknown.

Statins, that is 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors, are compounds currently used to treat hypercholesterolaemia. These drugs widely differ in their water solubility, and clinical trials have highlighted an association between their lipophilicity and risk of glucose intolerance and diabetes with chronic use[13]; however, these drugs, unlike other lipophilic drugs, are not reported to produce hypoglycaemia with acute use. The question arises as to whether statins can block K_{ATP} and if lipophilicity is indeed a useful predictor for the potency of a compound to block this ion channel? If so, we can then ask the questions as to whether statins promote Ca^{2+} influx, and if so, query their inability to stimulate insulin release in vivo?

The primary aim of this study was to determine the relationship between drug lipophilicity and block of K_{ATP} by a meta-analysis and then to test whether this relationship could reliably predict the potency of other drugs to affect the activity of pancreatic β-cell K_{ATP} channels and insulin secretion.

2 | MATERIALS AND METHODS

2.1 | Relationship between Log IC_{50} and the logP for the block of K_{ATP}

The Log IC_{50} of 26 compounds that block beta-cell K_{ATP} channel activity was extracted from publications given in Figure 1. IC_{50} values were previously determined by inside-out or standard whole-cell patch-clamp recording methods under similar experimental conditions of temperature and pH and the presence of intracellular Mg^{2+};
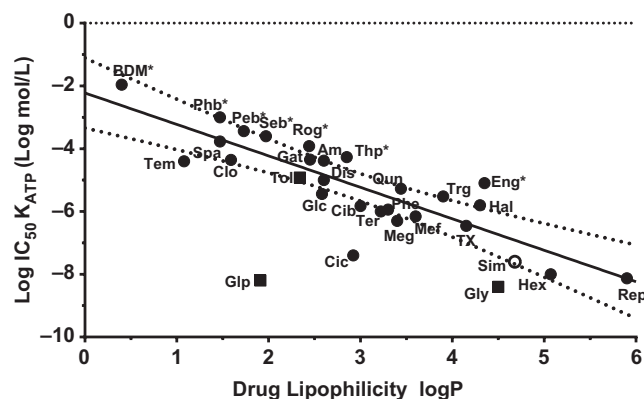

FIGURE 1 Relationship between Log IC_{50} of K_{ATP} and logP values for compounds that block beta-cell K_{ATP} channel activity. Key: Am, amantadine[37]; BDM, 2,3-butanedione monoxime[18]; Cib, cibenzoline[38]; Cic, ciclazindol[28]; Clo, clonidine[9]; Dis, disopyramide[11,27]; Eng, englitazone[8]; Gat, gatifloxacin[10]; gen, genistein[32]; Glp, glipizide; Gly, glibenclamide[4]; Glc, gliclazide[3]; Hal, haloperidol[29]; Hex, 2-n-hexyl-4-benzoxazine[30]; Mef, mefloquine[6]; Meg, meglitinide[4]; Peb, pentobarbitone[12]; Phb, phenobarbitone[12]; Phe, phentolamine[20,31]; Qun, quinine[9]; Rep, repaglinide[5]; Rog, rosiglitazone[7]; Seb, secobarbitone[12]; Sim, simvastatin; Spa, sparteine[37]; Tem, temafloxacin[10]; Ter, terfenadine[39]; Thp, thiopentone[12]; Tb, tolbutamide[4]; Trg, troglitazone[40]; TX, Triton X-100.[24] Log IC_{50} values are from respective references. logP values are as published on DrugBank, except for cibenzoline, englitazone and Triton X-100, which were calculated using Chemicalize. Solid line is linear regression fit (r^2 .78) of the equation: Log IC_{50} = −logP−2.22 to only data indicated by ●. Dotted lines are the 95% confidence intervals. Log IC_{50} determined in the absence of intracellular Mg^{2+} are suffixed by *

except where Mg^{2+} did not affect drug action. Only compounds with logP values, where P is the octanol/water partition coefficient, >0 were included to avoid situations where the drug would exist in a predominantly charged state at physiological pH and inhibit K_{ATP} by an open-channel block mechanism. Experimentally determined logP values (Log D at pH 7.4,) were extracted from www.DrugBank.ca and were confirmed from the original source references to be within the physiological pH range 7-8 to account for neutral and ionized species in the aqueous phase. When an experimental logP was unavailable, as was the case for cibenzoline, ciclazindol, englitazone, 2-n-hexyl-4-benzoxazine, temafloxacin, terfenadine and Triton X-100, it was calculated from quantitative structural activity relationship calculations (QSAR) at www.Chemicalize.com. To determine whether a relationship existed between Log IC_{50} and logP Pearson correlation was performed, and the data were described by linear regression with PRISM version 7 (GraphPad, San Diego, CA, USA).

2.2 | Preparation of β cells

Primary pancreatic β cells were dissociated from islets of male CD1 mice (30-50 g; 3-6 months old).[14] All animal care and experimental procedures were carried out in accordance with either the UK

Home Office Animals (Scientific Procedures) Act (1986) or Swedish ethical review board. Mice were killed by cervical dislocation and exsanguinated by decapitation. Islets were extracted either by type V collagenase (Sigma) or Liberase TM (Roche) digestion. Single cell was liberated by dissociation with trypsin-EDTA[14] and maintained in RPMI 1640 media, supplemented with 11 mmol/L glucose, 10% FBS, 10 mmol/L HEPES, 50 μg/mL penicillin and 50 μg/mL streptomycin and kept up to 2 days in a humidified atmosphere of 5% CO_2/95% at 37°C. To reduce and replace animal usage, the murine β-cell line MIN6[15,16] was used to test the effect of drugs on K_{ATP} in inside-out patches as well as intracellular Ca^{2+} levels. MIN6 cells, passage 35-40, were maintained as for the primary β cells but without antibiotics.[16]

2.3 | Insulin measurement

Islets were recovered in 11 mmol/L glucose RPMI for 2 hours and then incubated for 45 minutes in 3 mmol/L glucose Krebs buffer (in mmol/L): 120 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$ and 10 HEPES supplemented with 0.1% BSA, prior to experimentation. Islets in groups of 10 were picked at random and incubated at 37°C for 1 hour under conditions indicated. Supernatant was collected, protease activity inhibited with 0.05 mg/mL aprotinin (Sigma-Aldrich), and insulin measured by ELISA (Mercodia, Sweden). Insulin is expressed in relation to total protein content (Pierce BCA protein kit; Thermo Scientific, USA).

2.4 | Intracellular Ca^{2+} measurement

Intracellular Ca^{2+}, $[Ca^{2+}]_i$, was monitored by epifluorescent microscopy with FLUO-4 as described previously.[14] Experiments were performed in a modified Hanks solution (in mmol/L): 137 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 1.2 NaH_2PO_4, 4.2 $NaHCO_3$, 10 HEPES (pH 7.4 with NaOH) and 2 mmol/L glucose. Images were captured at 1 Hz with a CoolSNAP HQ2 camera (Photometrics, UK). Only cells that responded to tolbutamide (20 μmol/L) were chosen for analysis. Regions of interest (ROI) were corrected for background fluorescence by subtraction, the average fluorescence intensity per ROI calculated and normalized to that measured with an extracellular $[K^+]$ of 50 mmol/L; a condition that elicits maximal voltage-gated Ca^{2+} influx.

2.5 | Measurement of K_{ATP} ion channel activity

K_{ATP} channel activity in cell-attached experiments was measured in Hanks solution, and this allows the membrane potential (Vm) to vary such that Vm changes (ΔVm) can also be estimated from fluctuations in the single-channel current amplitude, Δi, via ΔVm = (Δi)/γ; where γ is the K_{ATP} single-channel chord conductance. To arrest rundown of excised K_{ATP} channels,[17,18] concentration-effect relationships were made with inside-out patches in a low intracellular Mg^{2+} solution (in mmol/L): KCl 140, $CaCl_2$ 4.6, EDTA 10, HEPES 10 (free $[Mg^{2+}]$ <6 nmol/L; free $[Ca^{2+}]$ <30 nmol/L; pH 7.2 with KOH). For both

configurations, the pipette solution contained (in mmol/L) 140 KCl, $CaCl_2$ 2.6, $MgCl_2$ 1.2 and HEPES 10 (pH 7.4 with NaOH).

Patch pipettes, resistances 2-4 MΩ, were drawn from GC150TF capillary glass (Harvard Instruments), coated with dental wax (Kerr) and fire polished before use. Currents were measured using an Axopatch 1D patch-clamp amplifier (Molecular Devices). The zero-current potential of the pipette was adjusted with the pipette in the bath just before seal establishment. No corrections have been made for liquid junction potentials (<4 mV). Currents were low-pass filtered at 2 kHz (−3 db, 8 pole Bessel) and digitized at 10 kHz using pClamp 8.3 (Axon Instruments, Foster City, USA). Single-channel data were analysed with half-amplitude threshold techniques as implemented in Clampfit Ver. 10.6 (Axon Instruments). For cell-attached recording, the pipette potential, Vp, was held at 0 mV and for inside-out patches +70 mV. Channel activity, NPo, was recorded for 3-minute periods, first in basal then after a 10-minute incubation in drug. To control for intrapatch variability in basal NPo, the effects of drug are quantified as the fraction of basal NPo measured prior to compound addition.

To monitor the time course of block and recovery of K_{ATP} channel activity by simvastatin, standard whole cell (WC-K_{ATP}) was used as described.[17,18] For these experiments, the pipette contained the low Mg^{2+} intracellular solution and cells continuously perifused with Hanks solution. K_{ATP} activity was monitored by the current elicited in response to 10 mV pulses of alternate polarity, 200 ms duration, applied at 0.5 Hz from a holding potential of −70 mV.[17,18] Electrophysiological experiments were performed at 21-23°C.

Simvastatin and pravastatin were obtained from Tocris Bioscience, Bristol, UK. Simvastatin was dissolved in ethanol or DMSO, pravastatin in H_2O. Drug additions were made from serial diluted stocks such that the vehicle was always applied at the same final concentration: 0.1% vol/vol.

2.6 | Statistical analysis

Statistical analysis was performed using PRISM, and data were checked for normality with the D'Agostino & Pearson omnibus normality test and the appropriate statistical test used. Unless stated otherwise, pairwise comparison was by unpaired T test or Wilcoxon signed-rank test and multiple comparisons by ANOVA or Kruskal-Wallis. The concentration-response for K_{ATP} block by simvastatin was quantified by fitting the data with the equation:

$$Y = Y_{max}/(1 + ([S]/IC50)^h),$$

where Y is the fractional K_{ATP} activity relative to control, Y_{max}, h is the slope index, [S] is the simvastatin concentration, and IC_{50} is the concentration that produces half-maximal inhibition. IC_{50} was estimated from the block of WC-K_{ATP} by a single drug concentration via rearrangement of this equation with h values of 1 for tolbutamide[4] and 0.7 for simvastatin.

Data are given as the mean ± SEM or median with 5%-95% confidence intervals (C.I.), with n the number of experimental units. Statistical significance is defined as $P < .05$ and is flagged as * in graphics.

3 | RESULTS

3.1 | Relationship between Log IC_{50} and the logP for the block of K_{ATP}

Figure 1 shows that, with exception of the second-generation sulphonylureas (glipizide and glibenclamide), the potency (Log IC_{50}) of drugs to block K_{ATP} channel activity was linearly correlated with lipophilicity (logP) with a slope of −1 (1.26 to −0.74, 95% C.I.) and a Log y intercept of −2.23 (−3.0 to −1.4 95% C.I.; Pearson $r = −.81$, $P < .001$). From this regression analysis, simvastatin (logP 4.68) was predicted to have an IC_{50} between 21 and 690 nmol/L (95% C.I.) and pravastatin (logP 0.59) an IC_{50} between 0.2 and 12 mmol/L (95% C.I.)

3.2 | Simvastatin potently blocks K_{ATP} channel activity

In the absence of glucose, cell-attached patches displayed single-channel currents with biophysical metrics characteristic for K_{ATP} channels (Figure 2A): single-channel current amplitude (i) 4.7 ± 0.2 pA, chord conductance (γ) ~67 ± 2 pS, burst kinetics with a mean open-channel dwell time of 1.2 ± 0.1 ms (n = 10). Within 3 minutes of perifusion, 5 mmol/L glucose significantly reduced NPo by 56% (33%-87%, 95% C.I.; n = 10) and depolarized Vm by 5.2 ± 0.8 mV (n = 10; Figure 2B); observations consistent with a K_{ATP} channel identity.[16,18-20] Addition of 10 µmol/L simvastatin inhibited the remaining channel activity by a further 76% (39%-98%, 95% C.I.; n = 5; Figure 2C), an action associated with depolarization of Vm by 31 ± 6 mV (n = 6); effects consistent with K_{ATP} block. The vehicle control, 0.1% vol/vol DMSO, neither affected NPo nor Vm (n = 5).

Comparable results were observed in cell-attached patches but in the absence of glucose, 10 µmol/L simvastatin ($P < .05$, ANOVA), but not 10 µmol/L pravastatin, significantly decreased K_{ATP} channel activity and depolarized Vm (31 ± 5 mV; n = 9) relative to vehicle controls (Figure 3A). To check that DMSO itself did not modify the effect of simvastatin; 10 µmol/L simvastatin still significantly inhibited K_{ATP} channel activity with ethanol (0.1% vol/vol) as the vehicle (Figure 3A). In inside-out patches from primary beta cells (Figure 2D-E), 10 µmol/L simvastatin, but not pravastatin, inhibited channel activity (Figure 3A). In inside-out patches, simvastatin neither affected single-channel current amplitude nor open-channel dwell time (Figure 2D-F) but inhibited K_{ATP} channel activity (Figure 3B) with an IC_{50} of 26 nmol/L (18-37 nmol/L, 95% C.I.) and h of 0.73 (0.53-0.94, 95% C.I.). These data demonstrate that the effect of simvastatin is independent of glucose metabolism, intracellular Mg^{2+} and cytosolic

factors. 20 µmol/L tolbutamide and 100 nmol/L simvastatin inhibited K_{ATP} channel activity in standard whole cell by 43 ± 3.3% (n = 9) and 47 ± 3.1%, respectively (n = 5; Figure 3C-E). The IC_{50} for simvastatin estimated from the whole-cell data: 31 nmol/L (20-42 nmol/L, 95% C.I.) was almost identical to that measured for the IO patches (26 nmol/L; Figure 3F). Both the onset and washout of tolbutamide block were complete within 1 minute, unlike that for simvastatin, which took at least 10 minutes to achieve steady-state block and was irreversible.

3.3 | Simvastatin stimulates Ca^{2+} influx

In the presence (Figure 4A), but not the absence (Figure 4D), of 2 mmol/L glucose, 100 nmol/L simvastatin elicited an increase in $[Ca^{2+}]_i$ similar to that with 20 µmol/L tolbutamide (Figure 4A). An observation of an increase in $[Ca^{2+}]_i$ by 100 nmol/L simvastatin over a 15-minute incubation had an odds ratio of 10.8 (3-39, 95% C.I., $P = .0001$) relative to its DMSO vehicle control.

3.4 | Simvastatin failed to affect insulin secretion

Neither pravastatin nor simvastatin affected basal or glucose-stimulated insulin secretion from mouse pancreatic islets at the 2 concentrations tested (100 nmol/L and 1 µmol/L; Figure 5A,B). As the failure of simvastatin to effect insulin secretion may be due to serum sequestration, the effect of BSA on the efficacy of the drug to block K_{ATP} was explored. In whole-cell records, 0.5% BSA significantly decreased the efficacy of 1 µmol/L simvastatin by 10-fold to an amount similar to that found with 100 nmol/L of the drug (Figure 5C). In cell-attached patches, 0.1% BSA abolished the ability of 100 nmol/L simvastatin to inhibit K_{ATP} activity (NPo) and depolarize Vm (Figure 5D).

4 | DISCUSSION

The Log IC_{50} for the block of K_{ATP} by lipophilic drugs, except for the second-generation sulphonylureas, was positively correlated with their logP. Moreover, this relationship accurately predicted the Log IC_{50} for the block of K_{ATP} by simvastatin and pravastatin.

The observation that Log IC_{50} of K_{ATP} block by a drug is related to its logP has already been described for barbiturates.[12] However, we now show that this relationship holds for a far greater range of structurally diverse compounds. Two compounds, englitazone and ciclazindol, fell outside the 95% confidence limits of the linear model, and the reasons for this are unclear but may relate to the accuracy of the logP values used. The 95% confidence intervals of our linear model encompass a Log_{10} range from 1 to 2; with a 100-fold difference between the confidence limits for the smallest and largest logP values, but only a 10-fold difference at its mid-point where the majority of data lay.

The pancreatic β-cell K_{ATP} channel is an octamer composed of 4 Kir6.2 and 4 SUR1 subunits, the latter associated with the specific
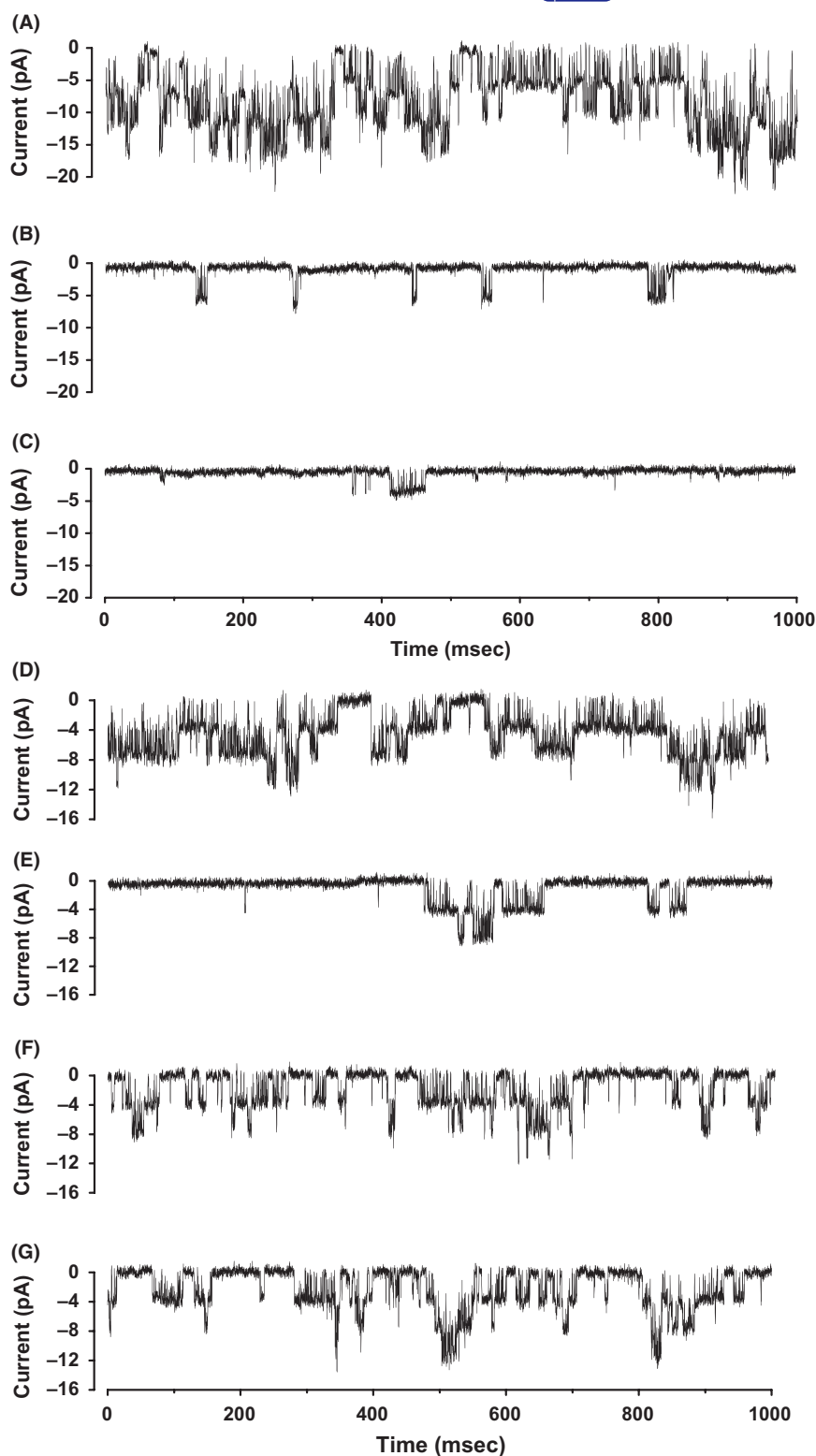
FIGURE 2 Representative recordings of single-channel K_{ATP} activity from primary mouse beta cells identified on basis of cell morphology and block by glucose. Openings are shown downward. A-C, Cell-attached recording under basal conditions (A), then after the addition of 5 mmol/L glucose (B) followed by 10 μmol/L simvastatin (C); pipette potential 0 mV. Note decrease in single-channel current amplitude associated with depolarization of the plasma membrane potential. D-F, Records from 2 different inside-out patches at a pipette potential +70 mV. D, Control record and then after 10 min in 10 μmol/L simvastatin (E). F, Control record in a different patch and then after perifusion of 0.1% vol/vol DMSO (G)

binding of sulphonylureas.[21-23] Expression studies with the truncated Kir6.2 pore subunits Kir6.2ΔC26 or Kir6.2ΔC36, which form functional inwardly rectifying K$^+$ channels in the absence of SUR1 subunits, have demonstrated that it is the pore construct itself that possesses the predominant binding site for all lipophilic drugs tested to date: detergents,[24] imidazoles,[20,25] barbiturates,[26] pheniramines,[27] glitazones,[7,28] phenyl-piperidinyl-butyrophenones,[29] quinolones[6] and 2-n-4-benzoxazines,[30] but not high-affinity sulphonylureas.[22,23] A decrease in intracellular Mg^{2+} is established to reduce the potency of glitinides and sulphonylureas to inhibit K_{ATP} activity but not that of other drugs[2,4,22]; an effect thought to be due to disruption of the interaction between the SUR1 and Kir6.2 subunits. Consequently,
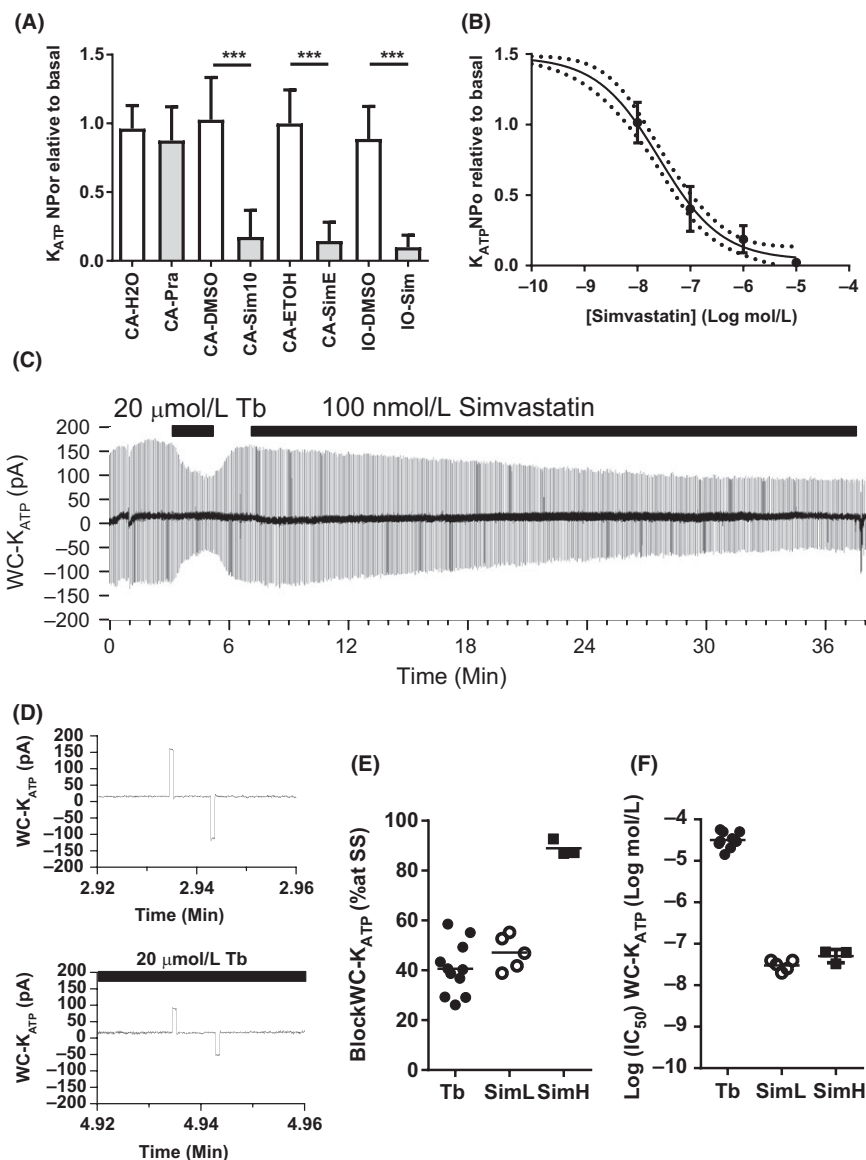
FIGURE 3 Effects of various treatments on K_{ATP} channel activity in MIN6 cells. A, In cell-attached mode (CA-Prefix): 10 µmol/L pravastatin (CA-Pra, n = 7) and its H_2O vehicle control (CA-H20, n = 8); 10 µmol/L simvastatin (CA-SimD, n = 9) and DMSO vehicle control (CA-DMSO, n = 5); 10 µmol/L simvastatin (CA-SimE, n = 10) and ethanol vehicle control (CA-ETOH, n = 5). In inside-out mode (IO-Prefix): 10 µmol/L simvastatin (IO-Sim, n = 8) and its DMSO vehicle control (IO-DMSO, n = 5). Data mean ± SD. B, Concentration-effect relationship for the block of K_{ATP} channel activity (NPo) in inside-out patches by simvastatin relative to basal. Note control value is greater than unity due to "run up" of channel activity. Data are mean ± SD (n = 7-8). Solid line is drawn with equation 1 using values given in the text. Dotted lines are the 95% confidence of the fit. C, Representative whole-cell K_{ATP} current elicited by 10 mV steps of alternate polarity in response to tolbutamide (Tb) and simvastatin. D, Expansion of record shown in (C) showing control trace and effect of tolbutamide (Tb, bottom trace) (E) Individual and mean values (horizontal lines) for the block of whole-cell K_{ATP} by 20 µmol/L tolbutamide (Tb, n = 11), 100 nmol/L simvastatin (SimL, n = 5) and 1 µmol/L simvastatin (SimH, n = 3). F, Individual and mean (horizontal line) for the IC_{50} of whole-cell K_{ATP} block by tolbutamide and for simvastatin calculated from the data shown in (E)

drug classes which block K_{ATP} channel activity with an IC_{50} unaffected by Mg^{2+} are those already shown to inhibit K_{ATP} by a direct interaction with the Kir6.2 pore subunit, notably the thiazolidinediones[2,7,8]; quinolones[6,9]; imidazoles[2,9,20,25,31]; disopyramide[27]; barbiturates[12,26]; detergents[24]; haloperidol[29]; fluoroquinolones[10]; 2-n-hexyl-4-benzoxazines[30]; and terfenadine.[2] As the block of K_{ATP} channel activity by simvastatin was also unaffected by intracellular $[Mg^{2+}]_i$, it supports the idea that its block of K_{ATP} is by a direct interaction with the Kir6.2 pore subunit. The fact that neither lipophobic pravastatin (logP 0.59) nor DMSO (logP −1.35) inhibited K_{ATP} channel activity lends support to an intramembrane effect for simvastatin and the other lipophilic drugs.

The slowness and apparent irreversibility for the block of K_{ATP} by simvastatin are consistent with an intramembrane effect, especially as other slow blockers of K_{ATP} of similar lipophilicity, for example englitazone[8] and Triton X-100,[24] are similarly slow in onset and irreversible.

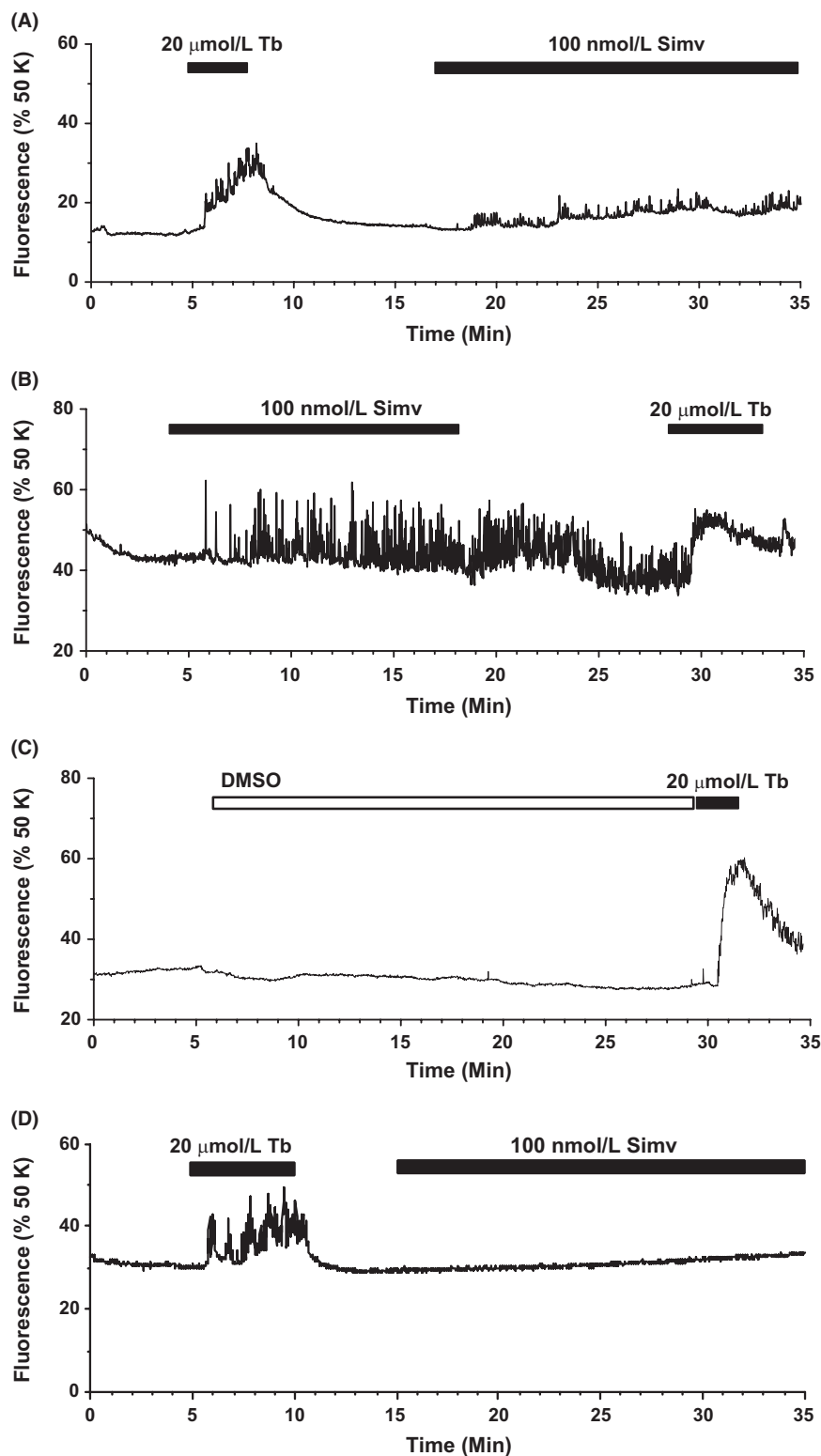**FIGURE 4** Representative records for the effect of 100 nmol/L Simvastatin, DMSO and 20 μmol/L tolbutamide on intracellular Ca^{2+} within single MIN6 cells in either the presence (A-C) or absence (D) of 2 mmol/L glucose. Intracellular Ca^{2+} is normalized as a percentage of that measured in 50 mmol/L extracellular K^+ perifused at experiment end. A, Addition of tolbutamide followed by a 10-min wash before the addition of simvastatin. B, Addition of simvastatin followed by a 10-min wash before the addition of tolbutamide. C, Addition of DMSO followed by the addition of tolbutamide. D, Addition of tolbutamide followed by a 10-min wash before the addition of simvastatin in the absence of glucose

What is surprising is that logP, a facile characteristic of a drug physico-chemical profile and considered a rudimentary parameter for drug-lipid bilayer interactions, is sufficient alone to predict its IC_{50} for the block of K_{ATP} without the need to revert to more complex QSAR.

Insight into the underlying mechanism by which simvastatin and the other compounds may affect channel function may be inferred

from the action of genistein. Genistein is an isoflavonoid that blocks the K_{ATP} current of smooth muscle (an octamer of 4 Kir6.x and 4 SUR2B) with an IC_{50} of ~5.5 μmol/L,[32] a value that also lies within the range predicted on the basis of its logP of 3.08 (1.8-14 μmol/L; 99% C.I.). Subsequent studies with genistein on the gating of grami-cidin ion channel in model bilayer membranes have given rise to the
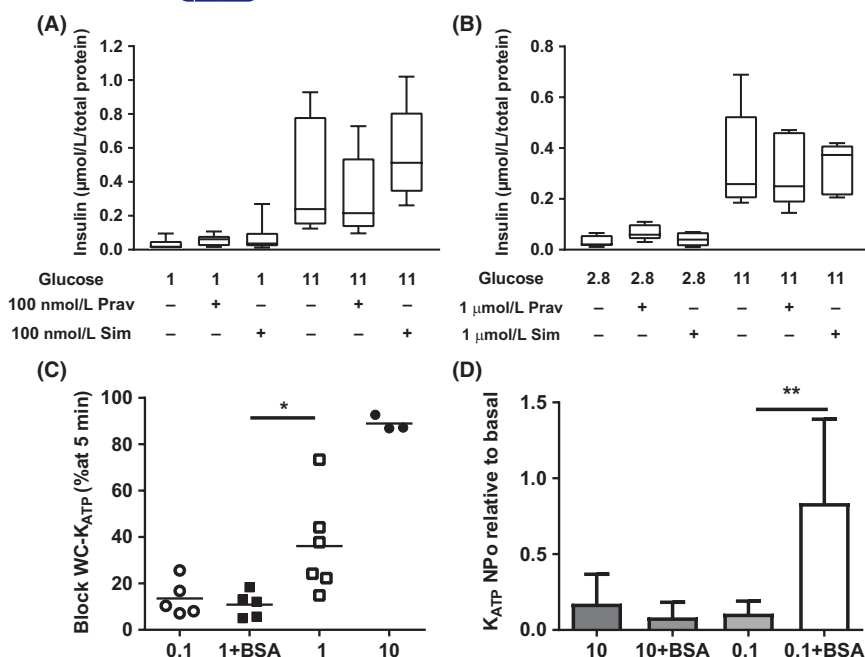
FIGURE 5 Effects of various treatments on insulin secretion and K_{ATP} channel activity (A-B) Effects of simvastatin and pravastatin on insulin secretion normalized to total islet protein content. A, Effect of 100 nmol/L of drug as indicated, on basal (1 mmol/L glucose) and 11 mmol/L glucose-stimulated insulin secretion (n = 6 from 6 animals). B, Effect of 1 µmol/L of drug as indicated, on basal (2.8 mmol/L glucose) and 11 mmol/L glucose-stimulated insulin secretion (n = 6 from 3 animals). C, Block of whole-cell K_{ATP} by simvastatin at the concentrations shown (µmol/L) in the absence or presence of 0.5% (wt/vol) BSA (n = 3-5). D, Block of K_{ATP} activity measured in the cell-attached patch configuration at the simvastatin concentrations shown (µmol/L) either in the absence or presence of 0.1% (wt/vol) BSA (n = 3-11). Data are mean values ± SD

idea that lipophilic compounds affect ion channel gating by altering the bilayer elastic properties and the ability of an ion channel to reorganize its protein structure during gating transitions,[33] in this case, leading to increased occupancy of the closed channel state: inhibition.

We found that, in the absence of BSA, 100 nmol/L simvastatin induced an increase in $[Ca^{2+}]_i$ with temporal characteristics similar to those seen with other drugs that block K_{ATP} channel activity and stimulate insulin secretion such as tolbutamide; however, we did not observe stimulation of insulin secretion; a fact we ascribe due to sequestration of this drug by serum in the secretion experiments which substantially reduces its free concentration. In contrast, simvastatin concentrations of 200 nmol/L and greater, but not pravastatin, have previously been shown to reversibly block glucose-stimulated Ca^{2+} influx and insulin secretion in pancreatic beta cells[34]; an action explained by a direct block of L-type Ca^{2+} channels. Why Yada et al[34] observed simvastatin-induced changes in glucose-stimulated insulin secretion, where we did not, remains a mystery. Although this difference may relate to them using a lower concentration of serum; however, unfortunately, they do not state the amount of serum used in this or in their previous papers.

At therapeutic levels, 0.1-10 nmol/L,[35] sequestration of simvastatin by serum coupled with its low affinity for the K_{ATP} channel probably explains why simvastatin does not cause a substantive block of this channel and hyperinsulinaemia during clinical use; moreover, the same argument also abrogates an interaction of statins with K_{ATP} channels as an explanation for the adverse metabolic actions seen with chronic use of these drugs. We also expect little effect on the Kir6.2 pore subunit expressed in skeletal and cardiac muscle, as these channels are majorly closed under physiological conditions.[36] To conclude, we demonstrate that logP, a measure of lipophilicity, can predict the potency of a drug to block K_{ATP} channels without the need to consider complex quantitative structural activity relationships and suggest that the logP can be used as a simple aid to predict the potential of a drug, new or old, to inhibit beta-cell K_{ATP}. However, whether such drugs cause hyperinsulinaemia appears dependent on their absolute IC_{50} and free plasma concentration.

CONFLICT OF INTEREST

One of the authors have a conflict of interest that might bias their work and have nothing to declare.

WEBLINKS

Chemicalize [Online] Available from http://www.chemicalize.org [Accessed: 20 April 2016].

DrugBank [Online] Available from http://www.drugbank.ca [Accessed: 20 April 2016].

ORCID

Paul A. Smith http://orcid.org/0000-0002-8710-6830

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