European Journal of Pharmacology 628 (2010) 268–273

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Endocrine Pharmacology

Insulin-releasing and metabolic effects of small molecule GLP-1 receptor agonist 6,7-dichloro-2-methylsulfonyl-3-N-*tert*-butylaminoquinoxaline

Nigel Irwin [a,*], Peter R. Flatt [a], Steven Patterson [a], Brian D. Green [b]

[a] SAAD Centre for Pharmacy and Diabetes, School of Biomedical Sciences Research Institute, University of Ulster, Coleraine BT52 1SA, United Kingdom
[b] Institute of Agri-Food and Land Use, Queens University, Belfast, United Kingdom

ARTICLE INFO

Article history:
Received 13 June 2009
Received in revised form 7 October 2009
Accepted 10 November 2009
Available online 14 November 2009

Keywords:
Glucagon-like peptide-1 (GLP-1)
Glucose homeostasis
Small molecule
Insulin secretion
Diabetes

ABSTRACT

Much recent attention has focused on the GLP-1 receptor as a potential target for antidiabetic drugs. Enzyme resistant GLP-1 mimetics such as exenatide are now employed for the treatment of type 2 diabetes, but must be administered by injection. The present study has examined and compared the *in vitro* and *in vivo* metabolic actions of a small molecule GLP-1 receptor agonist 6,7-dichloro-2-methylsulfonyl-3-N-*tert*-butylaminoquinoxaline (DMB), with native GLP-1, exenatide and liraglutide. DMB significantly stimulated *in vitro* insulin secretion from BRIN-BD11 cells but with decreased molar potency compared to native GLP-1 or related mimetics. Administration of DMB in combination with glucose to mice significantly ($P<0.05$) decreased the overall glucose excursion compared to controls. Exenatide and liraglutide evoked similar ($P<0.001$) reductions of the overall glycaemic excursion, but were significantly ($P<0.001$ and $P<0.05$; respectively) more effective than DMB. These observations were associated with prominently ($P<0.05$) enhanced glucose-mediated insulin release by exenatide and liraglutide, but not by DMB. Combined injection of DMB with either liraglutide or exenatide did not substantially improve glucose-lowering or insulin-releasing responses. However, administration of DMB in combination with exendin(9–39) did not impair its glucoregulatory actions. These results provide evidence to support the development and potential use of low molecular weight GLP-1 receptor agonists for the treatment of type 2 diabetes.

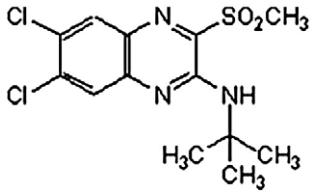
1. Introduction

Glucagon-like peptide-1 (GLP-1) is an endogenous hormone secreted from the enteroendocrine L-cells with well established potent effects on pancreatic beta-cell insulin secretion (Baggio and Drucker, 2007). This action combined with a number of other important effects including reduction in glucagon secretion, delayed gastric emptying and induction of satiety have prompted great interest in the use of GLP-1 in the treatment of type 2 diabetes (Baggio and Drucker, 2007). Patients with impaired glucose tolerance and type 2 diabetes also exhibit diminished secretion of native GLP-1 as glycaemic control deteriorates (Toft-Nielsen et al., 2001). However, the neural and cellular mechanisms responsible for nutrient-stimulation of GLP-1 secretion together with the deficit in diabetes are, as yet, poorly understood (Tolhurst et al., 2009). Furthermore, the native peptide has an extremely short biological half-life due to degradation by the ubiquitous enzyme dipeptidyl peptidase IV (DPP IV), limiting therapeutic utility of exogenous GLP-1 administration (Baggio and Drucker, 2007). Thus, DPP IV inhibitors and enzyme resistant GLP-1 mimetics, such as exenatide, are now being employed for the treatment of type 2 diabetes (Baggio and Drucker, 2007; Flatt et al., 2009). In addition, liraglutide is a long acting fatty acid derivatised human GLP-1 analogue which has recently received approval in Europe (EMEA) as second line treatment for type 2 diabetes.

Following parenteral administration, these GLP-1-based peptides circulate in the bloodstream at supraphysiological levels, bind to and activate the GLP-1 receptor, and mimic the biological effects of the intact native peptide. However, because they are peptides, exenatide, liraglutide and similar GLP-1 mimetics must be administered by injection. Thus, to improve therapeutic utility of the GLP-1 receptor target and avoid parenteral administration, development of small molecule GLP-1 receptor agonist ligands suitable for oral administration would be an advantage. To date there are a small number of published reports characterising low molecular weight agonists and antagonists of the GLP-1 receptor (Tibaduiza et al., 2001; Knudsen et al., 2007; Chen et al., 2007). One such molecule, 6,7-dichloro-2-methylsulfonyl-3-N-*tert*-butylaminoquinoxaline (DMB; Scheme 1), is a non-peptidic quinoxaline compound that acts as a GLP-1 receptor agonist and an ago-allosteric modulator of GLP-1 receptor binding. Thus, DMB functions independently as a GLP-1 receptor agonist and also as a positive allosteric modulator of conventional GLP-1 peptide/receptor binding. DMB has been shown to induce GLP-1 receptor mediated cAMP production *in vitro* and potentiate glucose-dependent insulin release in

* Corresponding author. School of Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA, Northern Ireland, United Kingdom. Tel.: +44 0 28 70 324313; fax: +44 0 28 70 324965.
E-mail address: n.irwin@ulster.ac.uk (N. Irwin).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.11.022



Scheme 1. Structure of 6,7-dichloro-2-methylsulfonyl-3-N-*tert*-butylaminoquinoxaline (DMB) molecular formula $C_{13}H_{15}Cl_2N_3O_2S$; molecular weight is 348.3.

pancreatic islets derived from wild-type, but not from GLP-1 receptor knockout mice (Knudsen et al., 2007). DMB has an EC_{50} of 101 nM with a bell-shaped dose–response curve, and is able to evoke insulin release from primary islets at 1 μM under 10 mM gluco-stimulatory conditions (Knudsen et al., 2007). The potential of small molecules is further supported by the use of DPP IV inhibitors, such as sitagliptin and vildagliptin for the treatment of type 2 diabetes (Flatt et al., 2009). In addition to DMB, a recent study has described a small molecule (3-(1-methylethyl)-9b-phenyl-[1,3]oxazolo[2,3-a]isoindole-2,5(3H.9bH)-dione) that induces GLP-1 secretion from the enteroendocrine L-cells, with possible therapeutic applications (Eiki et al., 2009).

The present study has assessed the *in vitro* insulinotropic and *in vivo* glucose-lowering and insulin-releasing actions of the recently described low molecular weight selective GLP-1 receptor agonist DMB. Biological activity of DMB has been compared for the first time against native GLP-1, exenatide and liraglutide. Results indicate that DMB stimulates insulin secretion and lowers blood glucose concentrations, but is considerably less potent than native GLP-1 or stable peptide GLP-1 receptor mimetics.

2. Materials and methods

2.1. Peptide synthesis

Native GLP-1, exenatide, liraglutide and exendin(9–39) were obtained from GL Biochem Ltd. (Shanghai, China). All peptides were characterised using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry as described previously (Gault et al., 2003). 6,7-dichloro-2-methylsulfonyl-3-N-*tert*-butylaminoquinoxaline (DMB) was purchased from Calbiochem (Nottingham, UK) and dissolved in dimethyl sulfoxide (20 mg/ml).

2.2. Effects of DMB and GLP-1 related peptides on in vitro insulin secretion

BRIN-BD11 cells, characterised previously (McClenaghan et al., 1996), were cultured using RPMI-1640 tissue culture medium containing 10% foetal bovine serum, and 1% (v/v) antibiotics (100 U/ml penicillin, 0.1 mg/ml streptomycin). All cells were maintained in sterile tissue culture flasks (Corning Glass Works, UK) at 37 °C in an atmosphere of 5% CO_2 and 95% air using a LEEC incubator (Laboratory Technical Engineering, Nottingham, UK). For insulin secretion studies, BRIN-BD11 cells were seeded into 24-multiwell plates at a density of 1.0×10^5 cells per well, and allowed to attach overnight at 37 °C. Acute tests for insulin release were preceded by 40 min pre-incubation at 37 °C in 1.0 ml Krebs Ringer bicarbonate buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 10 mM $NaHCO_3$, 0.5% (w/v) BSA, pH 7.4) supplemented with 1.1 mM glucose. Test incubations were performed in the presence of 5.6 mM glucose with a range of concentrations of GLP-1, exenatide and liraglutide (10^{-12} to 10^{-6} M) or DMB (10^{-8} to 10^{-3} M). After 20-min incubation, the buffer was removed from each well and aliquots (200 μl) were used for measurement of insulin. In a separate series of experiments the additive insulinotropic effects of a half maximal stimulatory concentration of DMB (1×10^{-4} M) in combination with native GLP-1 (10^{-12} to 10^{-6} M) was assessed in a similar manner.

2.3. Effects of DMB and GLP-1 related peptides on glucose-lowering and insulin release in mice

The effects of DMB, GLP-1, exenatide and liraglutide on plasma glucose and insulin concentrations were examined in 14- to 18-week-old normal mice, derived from the colony originally maintained at Aston University. The genetic background and characteristics of the colony used have been outlined in detail elsewhere (Bailey et al., 1982). The animals were housed individually in an air-conditioned room at 22±2 °C with a 12 h light:12 h dark cycle. Drinking water and a standard rodent maintenance diet (Trouw Nutrition, Cheshire, UK) were freely available until 18 h before acute tests. Mice ($n=8$) received an intraperitoneal injection of glucose alone (18 mmol/kg body weight) or in combination with DMB (5000 nmol/kg body weight) or related GLP-1 peptides (each at 25 nmol/kg body weight). These doses were selected on the basis of pilot studies and our previous publications (Irwin et al., 2009), respectively. Our previous studies have shown that dissolving drugs in dimethyl sulfoxide at a concentration of 20 mg/ml does not affect the glycaemic or insulinotropic responses to glucose when administered under the current experimental conditions (data not shown). In a second series of experiments glucose (18 mmol/kg) and DMB (5000 nmol/kg) were administered (i.p.) in the presence and absence of liraglutide, exenatide or exendin(9–39) (each at 25 nmol/kg). All test solutions were administered in a final volume of 8 ml/kg body weight. All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.4. Biochemical analysis

Blood samples were collected from the cut tip on the tail vein of conscious mice into chilled fluoride/heparin glucose microcentrifuge tubes (Sarstedt, Nümbrecht, Germany) immediately prior to injection and at 15, 30 and 60 min post injection. Plasma was aliquoted and stored at −20 °C prior to glucose and insulin determinations. Plasma glucose was assayed by an automated glucose oxidase procedure using a Beckman Glucose Analyzer II. Plasma insulin was assayed by dextran–charcoal RIA as described previously (Flatt and Bailey, 1981).

2.5. Statistics

Results are expressed as means±S.E.M. and data compared using ANOVA, followed by a Student–Newman–Keuls *post hoc* test. Incremental areas under plasma glucose and insulin curves (AUC) were calculated using a computer-generated program employing the trapezoidal rule with baseline subtraction. Groups of data from both were considered to be significantly different if $P<0.05$.

3. Results

3.1. Stimulation of in vitro insulin secretion by DMB and GLP-1 related peptides

Fig. 1A shows the effect of increasing concentrations of GLP-1, exenatide and liraglutide on insulin secretion from clonal BRIN-BD11 cells. All peptides stimulated insulin release by up to 2.7-fold in a concentration-dependent manner. Exenatide was significantly ($P<0.05$ to $P<0.001$) more potent than native GLP-1 and liraglutide at all concentrations examined (Fig. 1A). Fig. 1B depicts the insulinotropic effect of DMB in BRIN-BD11 cells. DMB evoked significant ($P<0.001$) insulin release only at the highest concentrations (10^{-4} and 10^{-3} M) examined. Combined incubation with DMB (10^{-4} M) and native GLP-1 (10^{-12} to 10^{-6} M) revealed enhanced insulinotropic effects compared to either compound alone (Fig. 1C).