EDITORIAL

For reprint orders, please contact: reprints@futuremedicine.com

M_3-muscarinic receptor signaling pathways: therapeutic targets for diabetes?

Kok Choi Kong[1]

Andrew B Tobin[†1]

"The question is, does the determination of the role played by the M3R in insulin release provide a novel target for the treatment of diabetes?"

The global incidence of diabetes mellitus, specifically Type 2 diabetes, is increasing at such an alarming rate that it has been cited by many as a global epidemic alongside and intertwined with the obesity epidemic [1,2]. The disease is caused by defects in both insulin signaling and insulin secretion. The roles of the parasympathetic system and cholinergic input in the regulation of insulin secretion have long been established [3,4]. However, it is only recently, with the use of transgenic and knockout mice technology, that the M_3-muscarinic receptor (M3R) has been identified as the *bona fide* acetylcholine receptor that is responsible for enhancing glucose-dependent insulin release in the β-cells of the islets of Langerhans in the pancreas [5]. The question is, does the determination of the role played by the M3R in insulin release provide a novel target for the treatment of diabetes?

The determination of the involvement of the M3R in insulin release has occurred alongside the discovery of the cellular signaling cascades by which the M3R mediates glucose-induced insulin release. The outcome of these studies has been the intriguing observation that the M3R appears able to regulate insulin secretion via a number of distinct signaling cascades. One of these involves the protein kinase PKD1, which is activated by the phosphorylated form of the M3R in a process that results in secretory vesicle priming [6]. This protein kinase is also negatively regulated by the mitogen-activated protein kinase p38δ – in this case, the M3R is proposed to stimulate insulin release by inhibiting p38δ activity [7].

In another mechanism, the ability of the M3R to mediate insulin release via inositol 1,4,5-triphosphate (IP_3)/calcium-dependent signaling has been demonstrated to be modulated by the adaptor protein ankyrin-B. Here, ankyrin-B modulates M3R-mediated insulin release by binding to, and thus stabilizing, IP_3 receptors in β-cells [8]. Pancreatic islets from heterozygous ankyrin-B-mutant (*ankB*$^{+/-}$) mice exhibited a reduction in both basal and carbachol-stimulated intracellular calcium release [8], suggesting that the IP_3 receptor is stabilized in the open state.

The M3R has also been demonstrated to activate a sodium channel, designated the sodium leak channel nonselective

"...the discovery that the M3R can potentiate insulin release through an arrestin-dependent mechanism suggests that biased agonists that direct muscarinic receptor signaling via arrestins would be of therapeutic benefit."

[1]Department of Cell Physiology & Pharmacology, University of Leicester, LE1 9HN, UK
[†]Author for correspondence: Tel.: +44 116 252 2935; Fax: +44 116 252 2921; tba@le.ac.uk

part of Future Medicine fsg

(NALCN) and formerly named Rb21, then VGCNL1. This channel belongs to the four-domain ion channel family. Importantly, M3R activation of the NALCN channel in the model pancreatic β-cell line, MIN-6, does not occur via receptor signaling to the heterotrimeric G-protein-dependent pathway but, rather, occurs through a pathway dependent on a Src family of tyrosine kinases-dependent pathway [9]. In addition, the regulators of the G-protein signaling protein, RGS4, negatively modulate M3R-mediated insulin secretion in β-cells due to their selective inhibition of M3R signaling in β-cells [10].

These regulatory mechanisms appear to act in a co-ordinated fashion *in vivo* to mediate parasympathetic control of insulin release. The *in vivo* effects of muscarinic receptor agonists in regulating glucose-stimulated insulin secretion were first examined over a decade ago [11]. Intravenous administration of carbachol-potentiated glucose-stimulated insulin secretion in mice fed with either a control or a high-fat diet. Carbachol also normalized glucose-stimulated insulin secretion and glucose tolerance in mice subjected to a high-fat diet [11]. These data led to the proposal that the development of islet-specific muscarinic agonists might be a feasible target to improve insulin secretion in Type 2 diabetes. However, it was not until recently that this proposal was vigorously investigated. Gautam and colleagues performed a series of studies, that concluded with the generation of transgenic mice that specifically expressed a constitutively active mutant of M3R in pancreatic β-cells, and, by doing so, mimicked the effects of a drug that chronically stimulated β-cell M3Rs [12]. These mutant mice exhibited markedly improved glucose tolerance and increased serum insulin levels, as well as resistance to diet-induced glucose intolerance and hyperglycemia [12]. These studies strongly support the hypothesis that chronic, continuous activation of β-cell M3Rs might produce beneficial effects on glucose homeostasis. These studies further established the therapeutic potential for M3R selective agonists for the treatment of Type 2 diabetes. However, the use of such agonists may be limited by their possible side effects owing to other peripheral actions of M3Rs, such as smooth muscle contraction and saliva secretion.

If targeting M3R receptors is to be successful then ligands that are selective to the M3R subtype, and that do not also stimulate the other muscarinic receptor subtypes, namely M1, M2, M4 and M5, will have to be developed. The search for such ligands has been unsuccessful so far, largely owing to the fact that the acetylcholine binding sites on the muscarinic receptors are very similar between the five receptor subtypes. However, hope that pharmacologists can selectively target the muscarinic receptors has recently emerged, owing to the discovery of ligands that interact with allosteric sites on the receptors [13,14]. These so-called allosteric modulators target variant regions of the receptor and can therefore show subtype selectivity. It is anticipated that positive allosteric modulators, which increase the affinity and/or efficacy of acetylcholine to the M3R, could be used to enhance the effects of the natural ligand acetylcholine selectively at the M3R.

In addition to allosteric modulators, the discovery that the M3R can potentiate insulin release through an arrestin-dependent mechanism suggests that biased agonists that direct muscarinic receptor signaling via arrestins would be of therapeutic benefit [6]. The potential of biased agonists has been revealed for the β-adrenoceptor, where the therapeutic efficacy of carvedilol in the treatment of heart disease has been attributed to the fact that this ligand can direct signaling of the β-adrenoceptor via arrestin-dependent pathways [15]. Similar biased ligands that direct signaling of the M3R via arrestin signaling would be expected to potentiate insulin release from β-islets in a manner that results in reduced side effects, due to G-protein-dependent calcium signaling being minimized.

Alternatively, signaling proteins downstream of M3Rs in β-cells may be targeted. Carbachol augmentation of glucose-induced insulin secretion was significantly impaired in islets prepared from *ankB*$^{+/-}$ mice or in rat islets following siRNA knockdown of ankyrin-B [8]. In addition, *ankB*$^{+/-}$ mice exhibited hyperglycemia after oral ingestion of glucose, and the R1788W mutation of ankyrin-B impaired its function in islets and is associated with Type 2 diabetes in Caucasians and Hispanics [8]. Although homozygous *PKD1*-knockout (*PKD1*$^{-/-}$) mice are embryonically lethal [16], deletion of PKD1 in INS1 insulinoma cells completely abolished the insulin release induced by glucose and carbachol [7]. siRNA knockdown of *PKD1* in mouse islets also impaired the M3R-mediated augmentation of

glucose-induced insulin secretion [6]. Although siRNA targeting of signaling pathways in islets is never going to be therapeutically feasible, these studies demonstrate that targeting the signaling pathways downstream of the M3R can effectively modulate insulin release.

Finally, NALCN may also appear to be a potentially attractive drug target for Type 2 diabetes; since the NALCN current is too small to induce insulin release by itself but, instead, potentiates glucose-induced insulin secretion [17], compounds that activate NALCN would be superior to the sulfonylureas, as such compounds would depolarize β-cells and stimulate insulin secretion only at normal and elevated blood glucose levels [9].

So much is now known about the M3R-mediated insulin secretion pathway, yet so much more must still be done. Most, if not all, of the information gathered so far has been obtained from rodents. Although the physiological role of M3Rs in β-cells appears to be conserved between species, further studies are warranted to determine the effectiveness of β-cell M3Rs and/or downstream signaling components as drug targets for the treatment of Type 2 diabetes in humans.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Bibliography

1 Colagiuri R, Colagiuri S, Yach D *et al.*: The answer to diabetes prevention: science, surgery, service delivery, or social policy? *Am. J. Public Health* 96(9), 1562–1569 (2006).

2 Yach D, Stuckler D, Brownell KD: Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat. Med.* 12(1), 62–66 (2006).

3 Gilon P, Henquin JC: Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. *Endocr. Rev.* 22(5), 565–604 (2001).

4 Malaisse WJ: Stimulus-secretion coupling in the pancreatic B-cell: the cholinergic pathway for insulin release. *Diabetes Metab. Rev.* 2(3–4), 243–259 (1986).

5 Gautam D, Han SJ, Hamdan FF *et al.*: A critical role for β cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis *in vivo*. *Cell Metab.* 3(6), 449–461 (2006).

6 Kong KC, Butcher AJ, McWilliams P *et al.*: M3-muscarinic receptor promotes insulin release via receptor phosphorylation/arrestin-dependent activation of protein kinase D1. *Proc. Natl Acad. Sci. USA* 107(49), 21181–21186 (2010).

7 Sumara G, Formentini I, Collins S *et al.*: Regulation of PKD by the MAPK p38δ in insulin secretion and glucose homeostasis. *Cell* 136(2), 235–248 (2009).

8 Healy JA, Nilsson KR, Hohmeier HE *et al.*: Cholinergic augmentation of insulin release requires ankyrin-B. *Sci. Signal.* 3(113), RA19 (2010).

9 Swayne LA, Mezghrani A, Varrault A *et al.*: The NALCN ion channel is activated by M3 muscarinic receptors in a pancreatic β-cell line. *EMBO Rep.* 10(8), 873–880 (2009).

10 Ruiz de Azua I, Scarselli M, Rosemond E *et al.*: RGS4 is a negative regulator of insulin release from pancreatic β-cells *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* 107(17), 7999–8004 (2010).

11 Ahren B, Sauerberg P, Thomsen C: Increased insulin secretion and normalization of glucose tolerance by cholinergic agonism in high fat-fed mice. *Am. J. Physiol.* 277(1 Pt 1), E93–E102 (1999).

12 Gautam D, Ruiz de Azua I, Li JH *et al.*: Beneficial metabolic effects caused by persistent activation of β-cell M3 muscarinic acetylcholine receptors in transgenic mice. *Endocrinology* 151(11), 5185–5194 (2010).

13 Conn PJ, Christopoulos A, Lindsley CW: Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discov.* 8(1), 41–54 (2009).

14 May LT, Leach K, Sexton PM *et al.*: Allosteric modulation of G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* 47, 1–51 (2007).

15 Wisler JW, DeWire SM, Whalen EJ *et al.*: A unique mechanism of β-blocker action: carvedilol stimulates β-arrestin signaling. *Proc. Natl Acad. Sci. USA* 104(42), 16657–16662 (2007).

16 Fielitz J, Kim MS, Shelton JM *et al.*: Requirement of protein kinase D1 for pathological cardiac remodeling. *Proc. Natl Acad. Sci. USA* 105(8), 3059–3063 (2008).

17 Gilon P, Rorsman P: NALCN: a regulated leak channel. *EMBO Rep.* 10(9), 963–964 (2009).