Challenges in β_3-adrenoceptor agonist drug development

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Historical setting

In the latter part of the 1970s and the early 1980s three pharmaceutical companies were attempting to design drugs that would retain the thermogenic and antiobesity activity in rodents of nonselective sympathomimetic agents, while lacking the undesirable effects elicited by such agents, in particular increased heart rate and tremor. These companies were Lilly [Yen *et al.* 1984] Beecham Pharmaceuticals (for which this author worked) [Arch *et al.* 1984b], and Hoffman-La-Roche [Meier *et al.* 1984]. Others revealed their interest a little later [Holloway *et al.* 1991]. From this work, Beecham Pharmaceuticals identified compounds that activated an 'atypical' β-adrenoceptor in rodent brown and white adipocytes [Arch *et al.* 1984a]. From 1974 Zaagsma and colleagues had gathered evidence that the rat white adipocyte β-adrenoceptor was atypical but they had used only antagonists [Harms *et al.* 1974]. Antagonists mostly display low potency at the atypical β-adrenoceptor compared with β_1- and β_2-adrenoceptors. Even before Zaagsma's work, Furchgott had reviewed antagonist data that showed that the β-adrenoceptor in various parts of the gut is atypical [Furchgott, 1972]. Tan and Curtis-Prior suggested in 1983 that the adipocyte receptor should be called the β_3-adrenoceptor, but it was not until 1989 that I argued strongly for the use of this name to describe the atypical adipocyte β-adrenoceptor [Arch, 1989]. Soon afterwards, the human β_3-adrenoceptor was cloned [Emorine *et al.* 1989] and subsequent work showed that the rodent β_3-adrenoceptor is identical to the atypical β-adrenoceptor found in both adipocytes and the gut. It also became clear that the β_3-adrenoceptor is primarily responsible for thermogenesis (increased energy expenditure) and fat loss in response to sympathetic stimulation in obese rodents. Fuller versions of this sequence of events have been published previously [Arch, 2008; Arch and Kaumann, 1993].

Some words of caution are needed for those who have not followed events over the years: the terms 'atypical β-adrenoceptor' and a 'third β-adrenoceptor' [Kaumann, 1989] have been used to describe a β-adrenoceptor (or β-adrenoceptors) that is not the β_3-adrenoceptor. In particular, there is a form of the β_1-adrenoceptor that has atypical pharmacology and in some respects is similar to that of the β_3-adrenoceptor. For example, both receptors are stimulated by certain 'nonconventional β-blockers', such as (–)-pindolol, at concentrations much higher than those that block β_1- and β_2-adrenoceptors, and both receptors have low affinity for conventional β-blockers. One difference, however, is that the original (arylethanolamine) β_3-adrenoceptor agonists have low potency at the atypical β_1-adrenoceptor [Brahmadevara *et al.* 2003; Arch, 2002; Brawley *et al.* 2000]. After being called the 'third heart β-adrenoceptor' [Kaumann, 1989], the atypical β_1-adrenoceptor was for a few years known as the β_4-adrenoceptor [Kaumann, 1997] but studies in knockout mice demonstrated that its pharmacological detection depended on the presence of the β_1- but not the β_3-adrenoceptor gene [Kaumann *et al.* 2001, 1998; Cohen *et al.* 2000; Konkar *et al.* 2000]. Even though it is not a genetically distinct β-adrenoceptor, this 'low affinity β_1-adrenoceptor,' as it is now usually called, could conceivably offer a target for drugs, especially for the treatment of cardiovascular disease [Arch, 2004].

Returning to β_3-adrenoceptor agonists, it was soon discovered that they are remarkably effective in rodent models of type 2 diabetes, as well as obesity [Sennitt *et al.* 1985]. This activity is most

likely a consequence of their ability to increase fatty acid oxidation and thereby lower the concentration of fatty acid metabolites, such as fatty acyl CoA, diacylglycerol and ceramide [Darimont *et al.* 2004; Wilson *et al.* 1987]. These metabolites activate certain protein kinase C isozymes, such as PKC-θ, causing them to phosphorylate key serine residues of insulin receptor substrate-1. Fatty acids and their metabolites may also play a role in the inflammatory response through similar mechanisms [Schwartz *et al.* 2010; Kennedy *et al.* 2009; Schenk *et al.* 2008]. Antilipolytic agents or ablation of β_3-adrenoceptors in white adipose tissue reduces or prevents the thermogenic activity of β_3-adrenoceptor agonists [Arch, 2011, 2008]. However, it may be that while the 'push' of fatty acid supply is required for thermogenesis, this actually reduces insulin sensitivity because it tends to raise fatty acid metabolite levels. It is only when the 'pull' of increased fatty acid oxidation capacity exceeds the push that fatty acid metabolite levels fall and insulin sensitivity improves. Indeed, β-adrenoceptor agonists and natural catecholamines raise plasma nonesterified fatty acid (NEFA) levels and exacerbate insulin resistance initially. It is only on repeated dosing that NEFA levels fall and insulin sensitivity improves [Sugimoto *et al.* 2005; Liu *et al.* 1998; Virtanen *et al.* 1997]. This is at least partly because the capacity for fatty acid oxidation increases with repeated dosing; it is also possible that lipolysis decreases.

Translation from rodents to humans

Why then, over 25 years since the first β_3-adrenoceptor agonists were identified, is one not being used to treat obesity or type 2 diabetes in humans? The first setback for this author came with the discovery that the same compounds that were as effective as lipolytic agents in rodent white adipocytes had little or no lipolytic effect in human adipocytes. There were two possible explanations: either the β_3-adrenoceptor was poorly expressed in human white adipocytes, or those compounds that activated the rodent receptor had little efficacy at the human receptor. Both explanations turned out to be true. When the human and rodent receptors were cloned and expressed in cell lines it became clear that their pharmacologies differed. Moreover, lipolysis in human white adipocyte was mediated mainly by classical β-adrenoceptors [Lafontan and Berlan, 1993].

The problem of different β_3-adrenoceptor agonists being required to activate the human and rodent receptor seemed like a relatively simple exercise in medicinal chemistry, using the then new screening technologies based on human cloned receptors. At SmithKline Beecham, we did not concern ourselves with using tool compounds to demonstrate efficacy in rodent models of obesity and diabetes; that had already been done and it did not worry us that our human β_3-adrenoceptor-selective compounds could not be used in rodents. However, we were confronted by the problem that none of the compounds that we identified as highly selective for the human receptor displayed good oral bioavailability. This is clearly a problem that was encountered by others, as illustrated by a stream of publications by authors from Merck that have a strong emphasis on the need to achieve good oral bioavailability [Goble *et al.* 2010; Stearns *et al.* 2002].

A few β_3-adrenoceptor agonists that were both highly selective and had good oral bioavailability and pharmacokinetics for an oral drug intended to raise energy expenditure over 24 h may have failed for reasons of toxicity or perhaps because they were β_1- or β_2-adrenoceptor antagonists, which bring their own problems. With other compounds there may have been some compromise on selectivity as agonists to achieve suitable oral bioavailability and pharmacokinetic profile. In the experience of this author, even slight agonist activity at cloned β_1- or β_2-adrenoceptors may translate into significant agonist activity in human right atrial appendage [Sennitt *et al.* 1998]. These compounds may therefore have failed in the clinic owing to their β_1- or β_2-adrenoceptor-mediated cardiovascular effects. Added to this they may have elicited some cardiovascular effects via β_3-adrenoceptors in the heart and in vascular smooth muscle. The article by Bhadada *et al.* (2011) discusses the role of β_3-adrenoceptors in these tissues.

The other question is whether β_3-adrenoceptors play the same role in humans as in rodents. Studies using highly selective β_3-adrenoceptor agonists suggested that there are functional β_3-adrenoceptors in human white adipocytes but their role is minor [Sennitt *et al.* 1998]. Fortunately, human brown adipocytes express β_3-adrenoceptors [Deng *et al.* 1997; Krief *et al.* 1993], but is there enough brown adipose tissue

in humans to make these a suitable target for drugs?

Compared with humans, rodents have a much higher surface area to volume ratio, they are more exposed to variations in ambient temperature and they do not wear clothes. They depend upon sympathetically driven 'nonshivering thermogenesis' to maintain body temperature when the ambient temperature is below the thermoneutral range. A significant proportion of nonshivering thermogenesis takes place in brown adipose tissue and is mediated primarily by the β_3-adrenoceptor. Moreover, when heat loss increases, for example when ambient temperature falls, the capacity for nonshivering thermogenesis increases and most – some say all – of this increased capacity is in brown adipose tissue [Golozoubova *et al.* 2006]. A single dose of a β_3-adrenoceptor agonist can at least double energy expenditure in a mouse maintained at about 21°C, and in *ob*/*ob* mice, which have low sympathetic activity (or if the capacity for thermogenesis has been increased by keeping animals in a cold environment and then they are returned to the warm to inhibit endogenous sympathetic activity) the increase can be much greater [Feldmann *et al.* 2009; Wilson *et al.* 1984]. By contrast in humans, sympathomimetic agents raise energy expenditure by no more than about 30% [Schiffelers *et al.* 2000], whilst β_3-adrenoceptor agonists may achieve less than 10% [van Baak *et al.* 2002].

There has long been evidence that adult humans have some brown adipose tissue [Lean, 1989, Heaton, 1972]. The conventional view, however, has been that only babies have a significant amount of the tissue. For some, this provided an easy explanation for the failure of β_3-adrenoceptor agonists as drugs for obesity or type 2 diabetes. This belief has been challenged in recent years by reports that active brown adipose tissue had been detected in adult humans using positron-emission tomography to detect uptake of ^{18}F-fluorodeoxyglucose [Nedergaard *et al.* 2007]. Such work was first conducted by oncologists to detect tumours. For oncologists, brown adipose tissue is a nuisance: tumours do not form symmetrically across the shoulders and down each side of the spine! There is a strong relationship between the activity of brown adipose tissue in humans and their percentage body fat [van Marken Lichtenbelt *et al.* 2009]. This raises the possibility that low brown adipose tissue activity is a cause of obesity. Alternative explanations should not be discounted: low sympathetic activity may be a cause (or marker) of obesity, and brown adipose tissue activity a marker of sympathetic activity.

Whatever the explanation for the inverse relationship between brown adipose tissue activity and obesity, a key question is whether there is enough brown adipose tissue in obese humans to make it a target for drugs for obesity or diabetes. It is likely that more than half of all men and women have 10 g or more of brown adipose tissue [Cypess *et al.* 2009]. Unfortunately, this means that most obese people, who are in the lower half, have less than 10 g of fat. Calculations suggest that full activation of less than 10 g of brown adipose tissue would increase energy expenditure by less than 4%. Therefore, if β_3-adrenoceptor agonists are to depend on brown adipose tissue to achieve useful efficacy, not only must they be delivered to brown adipose tissue effectively, but probably also the amount or oxidative capacity of brown adipose tissue in the typical obese person must be increased.

One way to increase the effective amount of brown adipose tissue may simply be to give a β_3-adrenoceptor agonist chronically. This works in rodents and it may work in humans because patients with catecholamine-secreting tumours have more brown adipose tissue than most people [Lean, 1989]. Another approach might be to coadminister a β_3-adrenoceptor agonist with another drug. For example, a β_3-adrenoceptor agonist might be combined with an agonist of the bile acid receptor TGR5, which is expressed in brown adipose, among other tissues, and like the β_3-adrenoceptor is coupled to G_s [Watanabe *et al.* 2006].

A rather different approach might address the problem of both drug delivery and activation of brown adipose tissue. Zinc α_2-glycoprotein (ZAG), also known as lipid-mobilizing factor, is a protein secreted by some tumours that appears to be partly responsible for cachexia. It is also secreted by white adipose tissue, with there being lower blood levels in obesity. ZAG has similar efficacy in rodent models of obesity and type 2 diabetes to β_3-adrenoceptor agonists, except that it appears more like the β_2-adrenoceptor agonist clenbuterol in its ability to increase skeletal muscle mass [Russell and Tisdale, 2010]. ZAG reduces metabolic efficiency like

β_3-adrenoceptor agonists do and it is claimed to actually be a β_3-adrenoceptor agonist [Russell *et al*. 2002]. However, the lipolytic effects of isoprenaline and a β_3-adrenoceptor agonist were reduced to similar extents in adipocytes from ZAG-deficient mice, suggesting that ZAG enhances β-adrenoceptor signalling [Rolli *et al*. 2007]. ZAG increases Gs_α expression [Islam-Ali *et al*. 2001], which could be a way of achieving this. Interestingly, ZAG has not been reported to increase energy expenditure acutely in the way that a β_3-adrenoceptor agonist does. So while it may not strictly be a β_3-adrenoceptor agonist, ZAG may activate brown adipose tissue and perhaps increase β_3-adrenoceptor expression. It would have to be injected, side-stepping the issue of oral bioavailability, and could be modified to solve any pharmacokinetic (and patent protection) issue.

Alternative directions

I have focussed on the challenges of developing β_3-adrenoceptor agonists for metabolic diseases, especially obesity and diabetes. β_3-adrenoceptors offer potential targets for other diseases. The article by Bhadada *et al*. (2011) discusses their relevance to cardiometabolic diseases and the potential of antagonists as well as agonists. It also alludes to the point that different agonists (of any receptor) may activate different signalling pathways and so have different therapeutic benefits [Evans *et al*. 2010], although the suggestion that this might be due to selective activation of the β_{3a}- and β_{3b}-adrenoceptor splice variants is unlikely because no variants have been detected in humans [Evans *et al*. 1999]. Others have focussed on the potential of β_3-adrenoceptor agonists for the treatment of irritable bladder and other urinogenital disorders [Ursino *et al*. 2009].

Some of these other indications have the advantage that the β_3-adrenoceptor is more highly expressed in the relevant human tissues than in 'metabolic tissues'. There remains, however, the issue that compounds selective for the human β_3-adrenoceptor tend not to have good oral bioavailability or a long plasma half life. Moreover, there may now be limited 'chemical space' for designing new patentable molecules.

Some targets have been known for over 20 years before they have yielded a drug, so there is still hope, but the β_3-adrenoceptor will certainly not yield to the faint hearted.

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Conflict of interest statement

None declared.

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