BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2010.01005.x
www.brjpharmacol.org

COMMENTARY

Are there functional β_3-adrenoceptors in the human heart?

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Keywords
β_3-adrenoceptors; heart, human; inotropy; calcium current

Received
29 July 2010
Accepted
09 August 2010

β_3-Adrenoceptor mRNA is expressed in the human heart, but corresponding receptor protein has not yet consistently been demonstrated. Furthermore, their physiological role remains highly controversial. For example, in human atria these receptors apparently do not promote cAMP formation. Evidence presented in this issue of the *BJP* suggests that a previously reported β_3-adrenoceptor-mediated stimulation of Ca^{2+} channels at room temperature is absent at physiological temperatures, and that β_3-adrenoceptors have no effect on atrial contraction. Drugs classified as β_3-adrenoceptor agonists cause contraction in human atria but in most cases this involves β_1- and/or β_2-adrenoceptors. In contrast, in human ventricles β_3-adrenoceptor agonists can exhibit negative inotropic effects, potentially involving *Pertussis* toxin-sensitive G-proteins and activation of a NO synthase. However, firmer pharmacological evidence is required that these effects indeed occur via β_3-adrenoceptors. Whether the expected future use of β_3-adrenoceptor agonists in the treatment of urinary bladder dysfunction is associated with adverse events related to cardiac function remains to be determined from clinical studies.

LINKED ARTICLE
This article is a commentary on Christ *et al.*, pp. 823–839 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2010.00996.x

β-Adrenoceptors are important regulators of human cardiac function and the roles of β_1- and β_2-adrenoceptors in this regard are well defined (Brodde and Michel, 1999). Although the existence of a third β-adrenoceptor subtype, the β_3-adrenoceptor, was formally recognized based on its cloning more than 20 years ago (Emorine *et al.*, 1989), its existence as a functional receptor in the human heart remains controversial, as highlighted by a paper in this issue of the *BJP* (Christ *et al.*, 2011). A major part of this controversy may relate to the lack of selectivity of pharmacological tools, such as the agonist BRL 37344 or the antagonist SR 59230, used to probe the involvement of this subtype in a given response (Vrydag and Michel, 2007). Furthermore, interpretation of the data is further clouded by the existence of an atypical low affinity state of the β_1-adrenoceptor, which is activated by some compounds used to probe β_3-adrenoceptor function, for example CGP 12177 (Kaumann and Molenaar, 2008). Against this background we will review, briefly, the current evidence for and against functional β_3-adrenoceptors in the human heart. For reasons of scope, animal studies will not be covered here but have been reviewed previously (Rozec and Gauthier, 2006; Galindo-Tovar *et al.*, 2009).

Cardiac β_3-adrenoceptor message and protein expression

β_3-Adrenoceptor mRNA has been detected repeatedly in the human heart (Krief *et al.*, 1993; Gauthier *et al.*, 1996) although with much lower abundance than that of β_1- or β_2-adrenoceptors (Moniotte *et al.*, 2001b). In contrast to β_1-adrenoceptor mRNA, β_3-adrenoceptor mRNA was reported not to be down-regulated in heart failure patients (Moniotte *et al.*, 2001b), potentially shifting the relative role of the two subtypes in disease states. Whether β_3-adrenoceptor protein is also expressed in the human heart is less clear as no suitable radioligand exists (Vrydag and Michel, 2007). Antibody-based detection of β_3-adrenoceptor protein has been reported in one study with a validated antibody (Chamberlain *et al.*, 1999). Two other studies using a

Table 1

Affinity of frequently used drugs at cloned human β_1-, β_2- and β_3-adrenoceptors expressed in Chinese hamster ovary cells

	β_1-adrenoceptor	β_2-adrenoceptor	β_3-adrenoceptor	Reference
BRL 37344	4.4–5.2	5.1–6.5	6.4–6.5	Hoffmann *et al.*, 2004; Baker, 2010
CGP 12177	8.3–9.2	8.4–9.4	7.0–7.1	Hoffmann *et al.*, 2004; Baker, 2005
CL 316243	<3	4.1	5.1	Baker, 2005
L-748337	5.4–6.4	6.5–6.7	8.0–8.4	Candelore *et al.*, 1999; Baker, 2010
SR 58611	<4.5	6.3	8.5	Bianchetti and Manara, 1990
nadolol	7.2	8.6	6.2	Baker, 2005)
SR 59230	7.5–7.8	7.2–8.5	6.9–7.4	Hoffmann *et al.*, 2004; Baker, 2010

Data are pK_i values in radioligand binding studies (except for SR 58611 which are pEC_{50} data for guinea pig atrium, rat uterus and rat colon).

different non-validated antibody have also detected β_3-adrenoceptor protein in immunohistochemistry and/or immunoblotting (Moniotte *et al.*, 2001a; Napp *et al.*, 2009). However, the latter findings are more difficult to interpret as recent data demonstrate that many β-adrenoceptor subtype antibodies lack selectivity for their cognate receptor (Hamdani and van der Velden, 2009; Pradidarcheep *et al.*, 2009).

Lack of suitable ligands for functional studies

The uncertainty regarding expression of β_3-adrenoceptor protein places the burden of proof on functional studies. Most of these studies have relied on agonists such as BRL 37344, CGP 12177, CL 316243 or SR 58611 or antagonists such as SR 59230 and L-748337. However, interpretation of the results of these studies is hampered by the lack of widely available, truly selective or highly preferential β_3-adrenoceptor ligands (see Table 1). For example, BRL 37344 has little selectivity for β_3- over β_1- and/or β_2-adrenoceptors and also displays several non-adrenoceptor effects (Vrydag and Michel, 2007; Ngala *et al.*, 2009; Baker, 2010). Within a single experiment, it may cause some of its effects via β_3- but others via β_1/β_2-adrenoceptors (Mori *et al.*, 2010). CGP 12177 is a potent antagonist at β_1- and β_2-adrenoceptors, a low potency agonist at the atypical site of β_1-adrenoceptors and a partial agonist at β_3-adrenoceptors (Kaumann and Molenaar, 2008). CL 316243 preferentially works on rodent receptors and has only a 10-fold selectivity for human β_3- over β_2-adrenoceptors (Baker, 2005). SR 58611 is less well characterized for its selectivity for human subtypes but at least some of its effects apparently are not sensitive to β_3-adrenoceptor antagonism (Brahmadevara *et al.*, 2003). Among antagonists, SR 59230 is widely used but has little selectivity for the β_3-subtype (Vrydag and Michel, 2007; Baker, 2010). Moreover, it can be an agonist for some β_3-adrenoceptor responses (Michel and Alewijnse, 2007; Evans *et al.*, 2010). L-748337 has the greatest selectivity for human β_3- versus β_1- and β_2-adrenoceptors (K_i 4 vs. 390 and 204 nM respectively) (Candelore *et al.*, 1999). A more recent study reports an even greater β_3-selectivity (Ki 9 vs. 3631 and 3388 nM respectively) but also detected a second low affinity site on β_3- but not β_1- or β_2-adrenoceptor-expressing cells (Baker, 2010). The lack of suitable pharmacological ligands needs to be taken into consideration in the interpretation of the functional data discussed below, particularly as several studies have relied on a single agonist or antagonist concentration.

Evidence for cardiac β_3-adrenoceptors from functional studies

cAMP studies

Stimulation of cAMP formation is the prototypical signalling response of β-adrenoceptors. However, adenylyl cyclase stimulation by noradrenaline and adrenaline in human right atrium was entirely mediated by β_1- and β_2-adrenoceptors (Gille *et al.*, 1985). Confirming these findings, the cAMP accumulation in atrial strips in response to isoprenaline was fully suppressed by 100 nM propranolol (Ikezono *et al.*, 1987).

Ca^{2+}channel studies

Cardiac β-adrenoceptors can also couple to activation of L-type Ca^{2+} channels and such activation has been demonstrated in isolated human atrial myocytes for three agonists which can activate

β_3-adrenoceptors, that is, BRL 37344, CGP 12177, and SR 58611 (Skeberdis *et al.*, 2008). Such responses were blocked by 1 μM of the β_3-antagonist L 748337, but this concentration may also inhibit β_1-/β_2-adrenoceptors (Table 1). Of note, these electrophysiological experiments had been carried out at room temperature, and Christ *et al.* confirm these observations for BRL 37344 and SR 58611 (Christ *et al.*, 2011). On the other hand, the Ca^{2+} current activation by CGP 12177 at room temperature was not antagonized by 1 μM L-748337 in their hands but rather by 1–10 μM (-)-bupranolol, suggesting mediation via the atypical site of the β_1-adrenoceptor. Importantly, when the channel recordings were performed at 37°C, CGP 12177 no longer affected Ca^{2+}-channel function except for a small, bupranolol-sensitive effect when signals were amplified by the presence of the phosphodiesterase inhibitor isobutylmethylxanthine. Taken together these findings demonstrate β_3-adrenoceptor stimulation of Ca^{2+}-channels in human atrium at lower temperatures, incompatible with mammalian life, but not at a physiological temperature. BRL 37344 was also reported to cause Ca^{2+} elevations as assessed by the fluorescent indicator dye Fura-2 (Pott *et al.*, 2003), but unfortunately the temperature at which this was measured was not reported. Interestingly, Christ *et al.*, (2011) speculate that the preferential β_3-adrenoceptor function at lower temperatures may relate to its phylogenetic role in brown adipose tissue and the shivering response (Arch, 2008). How these effects on Ca^{2+}-channel function relate to a reported reduction of action potential duration in endomyocardial biopsies by BRL 37344 (Gauthier *et al.*, 1996) remains unclear, particularly as the temperature at which these experiments were performed was not reported.

Cardiac contractility studies

Most data on possible human cardiac β_3-adrenoceptor function relate to the control of contractility. Unless specifically stated otherwise, all of these experiments were performed at about 37°C. While the endogenous catecholamines noradrenaline and adrenaline and the prototypical β-adrenoceptor agonist isoprenaline cause positive inotropy in both atria and ventricles (Brodde and Michel, 1999), the proposed effects of β_3-adrenoceptor agonists differ markedly between atria and ventricles.

Atrial contractility. In human atrial preparations, noradrenaline- and adrenaline-induced contractions were antagonized by propranolol with a potency consistent with mediation by β_1/β_2-adrenoceptors (Gille *et al.*, 1985). Similarly, responses to isoprenaline were also attenuated by 100 nM propranolol, although not completely suppressed (Ikezono *et al.*, 1987). BRL 37344 was reported to cause contraction of human atrium (partial agonism relative to isoprenaline) in most (Arch and Kaumann, 1993; Sennitt *et al.*, 1998; Pott *et al.*, 2003; Skeberdis *et al.*, 2008; Christ *et al.*, 2011) but not all studies (Kaumann *et al.*, 1997). These responses were typically blocked by propranolol and/or other antagonists with potencies compatible with β_1/β_2-adrenoceptors (Arch and Kaumann, 1993; Sennitt *et al.*, 1998; Pott *et al.*, 2003; Christ *et al.*, 2011), although one study failed to observe inhibition by 200 nM nadolol (Skeberdis *et al.*, 2008). Interestingly, the contractile effects of the β_3-adrenoceptor agonists, in contrast to those of isoprenaline, were no longer observed at 25°C, that is, the temperature where those compounds promote Ca^{2+} currents, indicating a dissociation between Ca^{2+} currents and contraction (Christ *et al.*, 2011). While these data suggest that positive inotropic effects of BRL 37344 occur primarily if not exclusively via β_1/β_2-adrenoceptors, this agonist can also activate NO synthase and promote NO release in human right atrium by a propranolol-resistant mechanism (Pott *et al.*, 2003; Brixius *et al.*, 2004). As shown in many studies, CGP 12177 causes inotropic effects in the human atrium via the atypical site of the β_1-adrenoceptor (for review see Kaumann and Molenaar, 2008), but one study failed to observe the expected inhibition of this response by 200 nM nadolol (Skeberdis *et al.*, 2008). Bucindolol may also cause small inotropic responses via the atypical site of the β_1-adrenoceptor (Bundkirchen *et al.*, 2002). β_3-Adrenoceptor agonists such as RO 363 (Molenaar *et al.*, 1997) or a range of agonists from the phenylethanolamine and aryloxypropanolamine class (Sennitt *et al.*, 1998) also cause their positive inotropic effects via β_1/β_2-adrenoceptors. With regard to SR 58611, one study has reported positive inotropic effects which were insensitive to 200 nM nadolol (Skeberdis *et al.*, 2008), whereas two other studies did not observe inotropic effects of this β_3-agonist (Kaumann *et al.*, 1997; Christ *et al.*, 2011). The β_1-antagonist and putative β_3-agonist nebivolol caused small negative inotropic effects when studied in the combined presence of 1 μM propranolol, a NO synthase inhibitor and a low concentration of forskolin (Bundkirchen *et al.*, 2002). Finally, a lack of inotropic effects in the human atrium has also been reported for the β_3-agonists ZD 2079 and CL 316243 as well as for SR 59230 (Kaumann *et al.*, 1997), the latter being an antagonist in most systems but having agonist properties for some cellular response (Michel and Alewijnse, 2007; Evans *et al.*, 2010). In conclusion, the majority of studies

indicate that positive inotropic responses to β_3-adrenoceptor agonists in human right atrium are not mediated by β_3-adrenoceptors but rather by β_1-(including atypical β_1) and β_2-adrenoceptors. Moreover, these effects are typically small and therefore may not be sufficiently robust to be detected consistently.

Ventricular contractility. A very different situation exists in human ventricles. As in the atria, the endogenous catecholamines noradrenaline and adrenaline cause positive inotropic effects via β_1/β_2-adrenoceptors (Brodde and Michel, 1999). While one study has reported positive inotropic effects of CGP 12177 or cyanopindolol in the presence of 200 nM propranolol in ventricular trabeculae when effects were enhanced by isobutylmethylxanthine (Kaumann *et al.*, 1997), two other studies reported negative inotropic effects of CGP 12177 in endomyocardial biopsies (Gauthier *et al.*, 1996; 1999). In the latter studies and one additional report from that group (Rozec *et al.*, 2009), BRL 37344, SR 58611, CL 316243 and nebivolol also exhibited negative inotropic effects which were not affected by 10 μM nadolol or 1 μM metoprolol whereas some inhibition was observed with 1 μM bupranolol or L 748337. The negative inotropic effects of BRL 37344 were sensitive to *Pertussis* toxin treatment. Negative inotropic effects of BRL 37344 (Napp *et al.*, 2009) or marginal negative inotropic effects of BRL 37344, SR 58611A and CL 316243 causing changes amounting to only 2.5% of basal force (Kaumann and Molenaar, 1997), were also reported by other investigators. Of interest, BRL 37344 can cause NO release in human ventricles (Brixius *et al.*, 2004) and the negative inotropic effects of both BRL 37344 and nebivolol were abolished in the presence of a NO synthase inhibitor (Napp *et al.*, 2009; Rozec *et al.*, 2009), in line with data from several other mammalian species (Moens *et al.*, 2010). The physiological relevance of ventricular β_3-adrenoceptors is difficult to judge but with cloned subtypes the response to β_3-preferring agonists is typically small relative to that to isoprenaline unless high expression levels of the receptor are used (Hoffmann *et al.*, 2004; Baker, 2010). This makes it difficult to estimate how relevant such negative inotropic effects may be *in vivo* in the presence of endogenous catecholamines.

Conclusions

In conclusion, the physiological relevance of β_3-adrenoceptor-mediated activation of L-type Ca^{2+}-channels in the human heart at room temperature remains unclear as it apparently is absent at physiological temperature. Contractile effects of CGP 12177 in human atrium appear to be mediated by the atypical site of the β_1-adrenoceptor, and most other β_3-adrenoceptor agonists may also act via β_1/β_2-adrenoceptors. In contrast, β_3-adrenoceptor agonists may cause negative inotropic effects in the human ventricle but the involvement of a β_3-adrenoceptor in these effects is not fully clear. Unlike the β_1- and β_2-adrenoceptors, little information is available on the spatial nature of β_3-adrenoceptor signalling, or even whether NO release is a direct effect on the ventricular myocyte or indirect through stimulation of neighbouring microvascular endothelial cells. Possibly the effects of β_3-adrenoceptors on contraction are subsidiary, and their main role is a metabolic, or another undetermined, effect. Two challenges for future research arise from these data. Firstly, the involvement of β_3-adrenoceptors in human cardiac (particularly ventricular) responses to β_3-adrenoceptor agonists needs to be established more firmly using quantitative pharmacology, that is, full concentration–response curves to agonists in the presence of multiple concentrations of subtype-preferential antagonists. Secondly, it remains to be explored whether selective β_3-adrenoceptor agonists such as mirabegron (Vrydag *et al.*, 2009) when used clinically for the treatment of the overactive bladder syndrome will be associated with cardiac adverse events. Limited data from two clinical studies with mirabegron (Chapple *et al.*, 2008; 2010) or solabegron (Grudell *et al.*, 2008) have not pointed towards such adverse events until now but a definitive judgement has to await further studies.

Conflict of interest

Work in this field in the laboratory of MCM is supported by Astellas. RAB and SEH report no conflict of interest.

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