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Physiological Role, Distribution and Pharmacological Characteristics of β_3-Adrenoceptors: An Overview.

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The existence of an atypical (non β_1, non β_2) form of β-receptors in various tissues has been reported in several studies. Reports of responses produced by β-adrenoceptor antagonists suggested the existence of an atypical β-adrenoceptor (β_3). Interest is currently growing in the future heterogeneity of β-adrenoceptors. Bearing in mind the additional potential for therapeutic advancement, this review summarizes the structure-function relationship, physiological role and tissue distribution of β_3-adrenoceptors. It also gives the introductory overview of pharmacological actions and clinical indications for treatment with β_3-adrenoceptor agonists.

The first distinction of adrenoceptors into α and β subtype on the basis of different sensitivity to natural and artificial catecholamines[1] was substantiated by the discovery of β-antagonists[2]. Ahlquist[1] rationalized a large number of observations by his conjecture that catecholamines acted via two principal receptors. He termed these receptors α and β. α-Receptors are those that exhibit the potency series epinephrine≥norepinephrine≥isoprenaline. β-Receptors have the potency series isoprenaline>epinephrine ≥norepinephrine. These drugs soon developed as life saving major cardiovascular drugs. Later subdivision of β-adrenoceptor into β_1- and β_2-subtypes[3] likewise relied selectivity of activity of appropriate agonists and antagonists, with potential of β_2-selective agonists being recognized as powerful bronchodilators in management of obstructive lung disease.

Comprehensive pharmacological data as discussed in several reviews[4-6] and analysis of responses produced by β-adrenoceptor antagonists suggested the existence of atypical β-adrenoceptor (β_3). Such responses include lipolysis in rat adipose tissue[7,8], relaxation of rat proximal[9] and distal colon[10,11]. The existence of atypical (β_3) adrenoceptors have been also demonstrated in gastric fundus[12], guinea pig ileum[13], vascular smooth muscle[14], rat brain[15], rat oesophageal muscularis mucosae[16] and rat isolated skeletal muscle[17]. There is also a strong evidence of presence of adrenoceptor population in the microvasculature of the antrum[18].

Genes reportedly coding for human and rodent β_3-adrenoceptors have now been isolated[19-23]. Emorine *et al.*[19] provided the first molecular evidence for the existence of a third β-adrenoceptor subtype by cloning a human gene which encoded a resistance to receptor blockade by conventional β-antagonists and susceptibility to the novel thermogenic β-agonists. Expression of this gene was demonstrated in human brown adipose tissue (BAT) by cDNA cloning as well as in SK-N-MC human neuroblastoma cells by northern blot hybridization analysis[24]. In human adipose tissue, colon and gall bladder, the presence of β_3-adrenoceptor has been proved by polymerase chain reaction assays[25], while presence of functional β_3-adrenoceptors in human fat cells was indicated by lipolysis stimulation studies[26]. The murine gene[27] and the rat cDNA[20] coding for the β_3-adrenoceptor were also cloned and their functional characterization further established their similarity with the rodent atypical β_3-site[27-29]. The presence of β_3-mRNA in a tissue

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indirectly provides evidence for presence of β_3-adrenoceptor protein. β_3-mRNA was detected in region of rat gastrointestinal tract corresponding to sites where atypical β_3-adrenoceptor responses have been observed, namely stomach, ileum and colon. The high level of β_3-adrenoceptor mRNA expression in ileum[30], colon[9,31] and stomach[12,32-34] suggest that these receptors play an important role in regulation of gut motility by mediating smooth muscle relaxing effect[9,30,31].

Structure- function relationship of β_3-adrenoceptors:

All 3 subtypes of β-adrenoceptors (β_1, β_2 and β_3) belong to the R_7G superfamily of G protein coupled receptors characterized by seven transmembrane domains (TMS) of about 22-28 amino acid residues each[35]. The N-terminal region of these receptors is extracellular, of variable length and glycosylated. The C-terminal is intracellular and contains as in β_1- and β_2-adrenoceptors, several sites for phosphorylation by at least two different kinases: protein kinase A and β_3-adrenoceptor kinase. Although all three β-adrenoceptor subtypes are coupled to the G_s protein and stimulate adenylate cyclase, it is possible that one or each of the receptors actually interacts with different combinations of α_s subunit with either of one of the seven or eight gene products encoding the β or γ subunits. Adenylate cyclase itself exists in at least eight isoforms and it may be possible that β_3-adrenoceptors, for example, would be linked to a different isoenzyme compared to other β adrenoceptor subtypes[36]. Computer modeling helped to define a composite image of the β_3-adrenoceptors ligand-binding site[35]. At least four of the 7TMS are essential for ligand binding and two of the three others (TM2, which contains Asp 83 and TM7, which contains Tyr 336) are involved in α_s activation. The amino acid residues that are involved in the ligand binding were identified by site directed or photoaffinity labeling.

Physiological role of the β_3-adrenoceptors:

The role of β_3-adrenoceptors in normal physiology is, however, still not completely defined. It is expressed primarily in adipose tissue, where it is likely to mediate noradrenaline-induced changes in energy metabolism and thermogenesis. It is present in the highly innervated brown adipose tissue, at much higher levels than in white fat, especially subcutaneous. However, brown fat is present as an organized tissue only in neonates, and can hardly be detected a few weeks after birth, except in rodents. Individual brown adipocytes are present throughout the whole life time, and may serve as memory cells that can proliferate under special circumstances such as intense cold or when large amounts of catecholamines are secreted, for e.g., by a tumor of the adrenal gland (pheochromocytoma).

Evidence for the role of the β_3-adrenoceptors in modulating energy metabolism and thermogenesis comes from several origins. Firstly, studies on rodents established that the β_3-adrenoceptor is the predominant β-adrenoceptor subtype expressed in white[37] and brown[7] adipocytes and prolonged administration of β_3-adrenoceptor agonists considerably reduces diet- induced obesity. Secondly, long term treatment of adult dogs with β_3-adrenoceptors agonists leads to reduction of weight accompanied by the reappearance of brown adipose tissue[38]. This is the main tissue responsible for thermogenesis and is normally undetectable in adult mammals other than rodents. In addition, expression of the β_3-adrenoceptors has now been well documented in isolated human mammary white[25] and immortalized differentiated human brown adipocytes[39] where it was shown to regulate lipolysis.

Yet further evidence for the central role of the β_3-adrenoceptors in regulating body fat composition was provided by a study on mice in which the gene encoding one of the regulatory subunits of cAMP-dependent protein kinase A (PKA) was disrupted[40]. In these animals the PKA activity in brown adipocytes is increased, and the treatment with a β_3-adrenoceptors agonist resulted in enhanced energy expenditure, leanness and a marked resistance to diet-induced obesity.

Pharmacological properties mediated by β_3-adrenoceptors:

G_s protein mediates the regulation of agonist action for all subtypes of β-adrenoceptor. β_3-adrenoceptor share with β_1- and β_2-adrenoceptor subtypes, the recognition of the two natural catecholamines (adrenaline and noradrenaline) and the stimulation of adenylate cyclase in response to binding of these agonists, as well as to synthetic ligands of which isoprenaline is the most powerful (Tables 1 and 2).

Initial pharmacological characterization (ligand binding and adenylate cyclase activation) of the β_3-adrenoceptor was performed on transfected cells of various species including Chinese hamster ovary (CHO) cells and fibroblasts, murine L cells or on untransferred fibroblast-derived adipocyte like 3T3 F442A cells in which the β_3-adrenoceptor is the main receptor[41]. The β_3-adrenoceptors have a higher affinity for noradrenaline than for adrenaline, in contrast to the β_2-adrenoceptor subtype, which has a higher affinity for adrenaline, and to the β_1-adrenoceptor subtype which binds

both ligands equally well (Tables 1, 2 and 3). The low affinity of the β_3-adrenoceptor for the natural agonists led to the early suggestion that β_1- and β_2-adrenoceptors mediate the effects of circulating catecholamines and the β_3-adrenoceptors regulate the response to much higher concentrations present, e.g., in the synaptic cleft during nerve stimulation. This would be the case in highly innervated brown adipose tissue where the β_3-adrenoceptors is abun-

TABLE 1: BINDING CONSTANTS OF THE HUMAN β_1-, β_2- AND β_3-ADRENOCEPTORS EXPRESSED IN CHINESE HAMSTER OVARY CELLS.

Ligand	β_1-adrenoceptor Binding K_i (nM)	β_2-adrenoceptor Binding K_i (nM)	β_3-adrenoceptor Binding K_i (nM)
β-adrenoceptor agonists			
Noradrenaline	24100±14100	11800±5800	475±75
Adrenaline	18900±6400	4320±1160	20650±2810
Isoprenaline	2020±900	254±30	620±220
Salbutamol	—	—	53000±10000
BRL 37344	1750±3109	1120±380	287±92
LY 79771	—	—	555±71
SR 58611A	38500±13400	187±26	6640±960
SM 11044	1800±1700	4100±200	1300±200
Cimaterol	—	—	4700±1710
Clenbuterol	190±30	60±9	1100±200
β_3-adrenoceptor- selective agonists			
Bucindolol	0.20±0.04	0.10±0.03	23±10
ICI 201651	549±200	2860±750	85±12
CGP 12177A	0.9±0.1	4±2	88±22
Carazolol	—	—	2.0±0.2
Oxprenolol	5.4±1.3	1.5±0.4	70±10
Pindolol	3.4±0.7	2.3±0.9	11±2
Cyanopindolol	—	—	—
Alprenolol	8.8±0.2	1.5±0.3	110±30
Nadolol	40±6	14±5	636±72
CL 316243	—	—	14000
β-adrenoceptor antagonists			
(-) Bupranolol	1.7±0.3	0.4±0.1	50±14
ICI 118551	120±3	1.2±0.2	257±34
CGP 20712A	1.5±0.2	1800±400	2300±450

Binding constants (K_i- inhibition constant) were deducted from concentration-response curve.

dantly expressed.

Human, murine, bovine and rodent β_3-adrenoceptors, expressed in Chinese hamster ovary cells appear to share very similar pharmacological properties (Tables 4 and 5). These are distinct from that of the β_1- and β_2-adrenoceptor

TABLE 2: ADENYL CYCLASE STIMULATION OF THE HUMAN β_1-, β_2- AND β_3-ADRENOCEPTORS EXPRESSED IN CHINESE HAMSTER OVARY CELLS.

Ligand	β_1-adrenoceptor Effect K_{act} (nM)	β_2-adrenoceptor Effect K_{act} (nM)	β_3-adrenoceptor Effect K_{act} (nM)
β-adrenoceptor agonists			
Noradrenaline	0.8±0.3	36±0.4	6.3±0.7
Adrenaline	2.7±0.8	2.2±0.5	49±5
Isoprenaline	0.19±0.04	2.5±0.8	3.9±0.4
Salbutamol	74±15	5.2±0.6	266±23
BRL 37344	112±28	177±47	15±3
LY 79771	86±8	325±121	18±3
SR 58611A	12000±600	36±19	25±5
SM 11044	190±20	62±6	84±10
Cimaterol	0.64±0.15	0.57±0.002	17±3
Clenbuterol	—	1.0±0.2	1050±130
β_3-adrenoceptor- selective agonists			
Bucindolol	Antagonist	Antagonist	7.0±1.2
ICI 201651	Antagonist	Antagonist	20±9
CGP 12177A	Antagonist	Antagonist	139±44
Carazolol	Antagonist	Antagonist	11.3±1.2
Oxprenolol	Antagonist	Antagonist	77±13
Pindolol	Antagonist	Antagonist	153±12
Cyanopindolol	Antagonist	Antagonist	174±58
Alprenolol	Antagonist	Antagonist	219±46
Nadolol	Antagonist	Antagonist	1120±350
CL 316243	Antagonist	Antagonist	68
β-adrenoceptor antagonists			
(-) Bupranolol	Antagonist	Antagonist	Antagonist
ICI 118551	Antagonist	Antagonist	Antagonist
CGP 20712A	Antagonist	Antagonist	Antagonist
Receptor/ Cell	150,000	240,000	150,000

Adenylate cyclase stimulation (K_{act}- activation constant) was deducted from concentration- response curve.

subtypes, despite their overall structural homology. However, the species difference do exist, and these include high efficacies for BRL 37344 and CL 316243 for rodent β_3-adrenoceptors compared to the human β_3-adrenoceptors (K_i BRL 37344=0.4±0.1 Vs 15±3; K_{act} CL 316243=0.71±0.2 Vs 68). Such species specific variations highlight the considerable importance of performing an extensive ligand binding and adenylate cyclase activation analysis in the targeted receptor of the species to be treated by the β_3-adrenoceptor agonist of choice.

BRL 37344, a β_3-adrenoceptor agonist, exhibited a high potency in stimulating adenylate cyclase in human and murine β_3-adrenoceptors expressed in Chinese hamster ovary cells and is described as potent activator of lipolysis and thermogenesis in white[42] and brown[7] adipose tissue. LY 79771 is a potent and selective thermogenic antiobesity agent[43]. ICI 201651, the *in vitro* metabolized form of ZD 7114 (initially described as ICI D7114) is reported to stimulate oxygen consumption in brown tissue[44].

TABLE 3: INTRINSIC ACTIVITY OF THE HUMAN β_1-, β_2- AND β_3-ADRENOCEPTORS EXPRESSED IN CHINESE HAMSTER OVARY CELLS.

Ligand	β_1-adrenoceptor Intrinsic Activity	β_2-adrenoceptor Intrinsic Activity	β_3-adrenoceptor Intrinsic Activity
β-adrenoceptor agonists			
Noradrenaline	—	—	1.00
Adrenaline	—	—	1.00±0.04
Isoprenaline	1.08±0.06	1.01±0.03	0.90±0.02
Salbutamol	—	—	0.77±0.008
BRL 37344	1.30±0.11	0.80±0.04	1.11±0.12
LY 79771	1.42±0.30	0.22±0.03	1.06±0.04
SR 58611A	0.96±0.07	0.87±0.07	1.23±0.23
SM 11044	1.50±0.21	1.03±0.08	0.98±0.19
Cimaterol	1.20±0.06	0.98±0.03	1.15±0.08
Clenbuterol	—	0.91±0.02	0.72±0.07
β_3-adrenoceptor- selective agonists			
Bucindolol	—	—	1.01±0.10
ICI 201651	—	—	1.14±0.14
CGP 12177A	—	—	0.68±0.02
Carazolol	—	—	1.02±0.06
Oxprenolol	—	—	0.53±0.07
Pindolol	—	—	0.55±0.05
Cyanopindolol	—	—	0.82±0.04
Alprenolol	—	—	0.97±0.07
Nadolol	—	—	0.80±0.05
CL 316243	—	—	1.6

Intrinsic activity were deducted from concentration- response curve.

Oxyprenolol, CGP 12177A and pindolol initially described as displaying sympathomimetic activities in tissues[45] indeed behaved as agonists at the human and murine β_3-sites. They were less potent than most of the above described full agonists at β_3-adrenoceptors (1 to 3 log units higher K_a values) and exhibited only partial activities relative to isoprenaline (IA=0.35 to 0.75).

Isoprenaline stereoselectivity indices (SSI) are 1.45,

TABLE 4: COMPARATIVE ANALYSIS OF THE BINDING PARAMETERS OF THE β_3-ADRENOCEPTOR IN VARIOUS SPECIES.

Ligands	Rodent CHO β_3-adrenoceptor	Murine 3T3-F442A	Human CHO β_3-adrenoceptor	Bovine CHO β_3-adrenoceptor
	Binding K_i (nM)	Binding K_i (nM)	Binding K_i (nM)	Binding K_i (nM)
β-adrenoceptor agonists				
(-) Isoprenaline	2710± 820	6500±1500	620±220	84±81
(-) Adrenaline	4600±1850	237500±81000	20650±2810	11105±7345
(-)Noradrenaline	1840±600	121000±50000	475±75	423±255
BRL 37344	290±136	137±71	287±92	2.13±1.14
SM 11044	1030±60	—	1300±200	—
β_3-adrenoceptor-selective agonists				
CGP 12177A	152±19	152±48	88±22	218±161
ICI 201651	239±104	—	85±12	27.7±24
CL 316243	1000±200	—	14000	—
Bucindolol	21±5	—	23±10	73±42
Carazolol	18	—	2	0.39±0.29
Alprenolol	91±1	—	110±30	340
Pindolol	315±40	—	11±2	—
BRL 28410	14,600±7100	—	14800±4200	—
β-adrenoceptor partial agonists/ antagonists				
(-) Propranolol	150±22	589±74	145±8	589±74
(-) Bupranolol	42±19	85±40	50±14	85±40
β-adrenoceptor antagonists				
ICI 118551	2100±920	—	257±34	—
CGP 20712A	13000±7100	—	2300±450	—

Competition binding assays (Binding) were performed on intact rodent, human and bovine β_3-adrenoceptors in Chinese Hamster ovary (CHO) cells. K_i, inhibition constant; K_{act}, activation constant.

TABLE 5: COMPARATIVE ANALYSIS OF THE ADENYLATE CYCLASE PARAMETERS OF THE β_3-ADRENOCEPTOR IN VARIOUS SPECIES.

Ligands	Rodent CHO β_3-adrenoceptor	Murine 3T3-F442A	Human CHO β_3-adrenoceptor	Bovine CHO β_3-adrenoceptor
	↑ [cAMP] K_{act} (nM)	↑ [cAMP] K_{act} (nM)	↑ [cAMP] K_{act} (nM)	↑ [cAMP] K_{act} (nM)
β-adrenoceptor agonists				
(-) Isoprenaline	4.5±1.8	1860±190	3.9±0.4	14±4.8
(-) Adrenaline	23±0.3	6800±550	49±5	50.7±5.3
(-)Noradrenaline	13±4	4000±600	6.3±0.7	54±7.43
BRL 37344	0.4±0.1	180±20	15±3	0.3±0.07
SM 11044	64±9	—	84±10	—
β_3-adrenoceptor-selective agonists				
CGP 12177A	41±9	480±30	139±44	1.41±0.5
ICI 201651	15±1	—	20±9	—
CL 316243	0.71±0.2	—	68	—
Bucindolol	40±14	—	7.0±1.2	12.8±5.2
Carazolol	25	—	10	0.4±0.1
Alprenolol	827±89	—	219±46	—
Pindolol	999±187	10000±3500	153±12	—
BRL 28410	224± 22	—	2710±620	—
β-adrenoceptor partial agonists/ antagonists				
(-) Propranolol	Antagonist 406±98	661±78	1490±550	661±78
(-) Bupranolol	Antagonist 12±1	507±75	Antagonist	507±75
β-adrenoceptor antagonists				
ICI 118551	Antagonist 4969±137	—	Antagonist	—
CGP 20712A	Antagonist 6425±584	—	Antagonist	—

cAMP accumulation {↑[cAMP]} were performed on intact rodent, human and bovine K_{act}, activation constant β_3-adrenoceptors expressed in CHO cells.

1.34 and 1.28 in CHO-Hu-β_3-adrenoceptor, CHO-Mo-β_3-adrenoceptor and rat WAT[46] respectively compared to those (SSI=2-3) reported for conventional β_1- and β_2-adrenoceptors[47]. The β_1-selective antagonist CGP 20712A and the β_2-selective antagonist ICI 118551 exhibited low potencies in inhibiting isoprenaline stimulated adenyl cyclase in CHO-Hu-β_3-, CHO-Mo-β_3- and murine 3T3-F442A adipocytes[28].

Pharmacological criteria for the β_3-adrenoceptor may be summarized as higher selectivity for a novel class of compounds, initially described as potent activators of lipolysis and thermogenesis in white and brown adipose tissues whereas atypically low affinities and efficacy for conventional β-adrenoceptor agonists and antagonists. Pharmacological studies performed in the β_3-adrenoceptor deficient mice showed that lipolysis in adipocytes, serum insulin levels, whole body energy expenditure and reduction of food intake, may all be to some extent, mediated by the β_3-adrenoceptors[40].

Interaction of β_3-adrenoceptors with other receptors:

5-HT and isoprenaline induced- relaxation of isolated oesophageal muscularis mucosae of rat are mediated by 5-HT_4- receptors and β_3-adrenoceptors respectively. Eaglen *et al.*[48] have shown that activation of muscularis M_3-receptors of rat oesophageal muscularis functionally opposes relaxant responses via 5-HT_4 and β_3-adrenoceptor activation.

TISSUE DISTRIBUTION OF β_3-ADRENOCEPTORS

Adipose tissue:

Adipocyte β-adrenoceptors were first classified as β_1-subtype by relative lipolytic potencies of several classical β-agonists[3] as well as by radioligand binding studies[49,50]. Pharmacological analysis of the β-adrenoceptor mediated metabolic responses of rat adipocytes suggested the existence of a third β-adrenoceptor, often called atypical β-adrenoceptor according to its metabolic and pharmacological features[42,51,52].

The β-adrenoceptor classification has been last modified since the gene coding for the human[19], rat[53] and mouse[27] β_3-adrenoceptor was cloned and expressed in Chinese hamster ovary cells. The coexistence of three β-adrenoceptor subtypes is sustained by the identification of three subtypes of β-adrenoceptor mRNA being expressed predominantly[21]. However, functional studies have demonstrated that only the β_1-and β_3-adrenoceptor subtypes can stimulate lipolysis in rat[54]. Furthermore, catecholamine-induced lipolysis is mediated predominantly by β_3-adrenoceptors in rat white adipocytes[55,56].

In humans, brown adipocytes are quite difficult to isolate and study, and white fat deposits appear to vary extensively in their expression of the β_3-adrenoceptor depending on the origin of the tissue or even of the individual[25]. A number of pharmacological studies have been performed and demonstrated the presence of β_3-adrenoceptor in isolated human fat cells[26] and directly on human omental fat tissue[56]. Recently, it was found that β_3-adrenoceptors are present in human mesenteric, omental and subcutaneous adipocytes where they mediate noradrenaline-induced lipolysis[57,58]. Further, it was suggested that the β_3-adrenoceptors are expressed during the course of differentiation in brown adipocytes and plays a significant role in the responses of glucose transport to adrenergic stimulation[59-62].

The evidence for the central role of the β_3-adrenoceptors in regulating body fat consumption was provided by a study on mice, in which the gene encoding one of the regulatory subunits of cAMP dependent protein kinase A was disrupted[40]. In these animals the protein kinase A activity in brown adipocytes is increased and treatment with β_3-adrenoceptor agonists resulted in enhanced energy expenditure, leanness and a marked resistance to diet induced obesity.

Gastrointestinal tract:

The sympathetic nervous system has an inhibitory effect on human gastrointestinal motility. Previous studies in human isolated gastrointestinal preparations have revealed that stimulation by β-adrenoceptor agonists induces relaxation of muscle of ileum[63,64], jejunum[65] and colonic circular[66] and longitudinal smooth muscle[67]. The relative activity of noradrenaline and adrenaline in various human gastrointestinal smooth muscle preparations has been suggested that both β_1-and β_2-adrenoceptors are present in this tissues[68].

Since the introduction of the β_3-adrenoceptor agonists, the same criteria for adipose tissue have been used to identify β_3-adrenoceptor throughout the gastrointestinal tract of several species. Recent studies have indicated that atypical β-adrenoceptors mediate relaxation in gastrointestinal tissues of a variety of species, including guinea pig ileum and gastric fundus; rat gastric fundus, ileum, jejunum, colon and oesophagus[6,10,12,69]. In addition of relaxation, it is suggested that stimulation of atypical β-adrenoceptors produces rise in gastric acid secretion in stomach[70], bicarbonate secretion in colon[71]. But number of studies has shown

that selective β_3-adrenoceptor agonists cause inhibition of gastric acid secretion and gastroprotective effects[72-78]. The characteristics of the atypical β-adrenoceptor in these tissues resemble those of the cloned β_3-adrenoceptor. Recently localisation of β_3-adrenoceptors in human gastrointestinal tract using immuno-histochemical study confirmed the existence of β_3-adrenoceptor in the vasculature and nonvascular smooth muscles of oesophagus, stomach, duodenum, ileum, caecum, rectum and gall bladder[79].

Spontaneous activity in human colonic circular smooth muscle is inhibited by isoprenaline and noradrenaline and these effects are blocked by propranolol with an affinity lower than expected for human β_1-and β_2-adrenoceptors[80]. However, these preparations do not respond to BRL 37344 and responses to isoprenaline are blocked by the β_1-selective antagonist, betaxolol. A more recent study with human colonic circular muscle showed that isoprenaline relaxation in the presence of the β_1-and β_2-adrenoceptor antagonists remained uninhibited. The same was inhibited by the presence of β_3-adrenoceptor antagonist, SR 59320A[12]. The relaxation produced by SR 58611A and isoprenaline showed functional evidence for the β_3-adrenoceptors in guinea pig common bile duct and distal colon. This relaxation caused by isoprenaline was not inhibited by the presence of conventional β-antagonist, CGP20712A, ICI 118551 and propranolol (at concentrations saturating β_1- and β_2-adrenoceptors). However isoprenaline was about ten times as potent as SR 58611A on stimulating β_3 adrenoceptors[81].

Kuratani *et al.*[18] showed that a range of β_3-adrenoceptor agonists can enhance gastric antral mucosal blood flow in the halothane-anaesthetised rat. It has been speculated that this vasodilator action accounts for the gastroprotective response in the conscious rat. This result was substantiated by the studies of Bahl *et al.*[72], who found that β_3-adrenoceptor agonists BRL 37344 and CL 316243 inhibit indomethacin-induced gastric antral ulceration in rats. Both agonists were approximately 100 fold more potent than salmeterol as gastroprotective agents, but unlike salmeterol, their effects was not blocked by co-administration of propranolol or of ICI 118551 (up to 10 mg/kg, po). However, a review by Jacobsen[82] revealed that there is poor correlation between the abilities of a range of compounds to increase mucosal blood flow and afford gastroprotection. A possible alternative or additional site action by which β_3-adrenoceptor agonists may produce this action is by virtue of their profound inhibitory effects on gastrointestinal motility[83]. A recent study with CGP 12177A and ZD 7114 showed that the underlying mechanism for the significant gastroprotective effects was due to the enhancement of gastric mucosal blood flow[84]. Daly[85] proposed that β-adrenoceptor involvement in gastrin release and gastric acid secretion may only be important under conditions of severe stress. Another β_3-adrenoceptor agonists CGP 12177A and ZD 7114 also showed significant antiulcer activity various gastric ulcer models[75,77,78]. The involvement of reduction in lipid peroxidation by these agents has been ruled out. In these studies β_3-adrenoceptor agonists was found to be potent inhibitor of gastric secretion.

Mersereau and Hinchey[86] demonstrated marked enhanced basal myoelectrical activity of rat gastric smooth muscle with like indomethacin. They proposed that this altered smooth muscle state, in combination with the inhibition of prostaglandin synthesis renders the mucosa vulnerable to injury by increased peristaltic action. Increase in the peristaltic activity (by field stimulation) of rat stomach leads to a reduction in blood flow that is particularly profound in the antral area. Thus, it possible that β_3-adrenoceptor agonists confer gastroprotection in two ways. The first by directly inhibiting the elevated level of contractile activity of gastric smooth muscle induced by indomethacin and the second by reversing the reduction in blood flow caused by this hypermotile state[87].

Cardiovascular system:

Clark and Bertholet[88] demonstrated a vasorelaxant effect of pindolol. A non-specific β-adrenoceptor antagonist with significant intrinsic sympathomimetic activity in canine isolated perfused mesenteric vessels. The vasorelaxant effect of pindolol was not significantly inhibited by propranolol, thus suggesting the presence of an atypical β-adrenoceptor subtype different from the conventional β_1-and β_2-adrenoceptors. Recently β_3-adrenoceptor transcripts were detected in human ventricle by polymerase chain reaction assay[89]. However, there is still uncertainty as to whether the third β-adrenoceptor proposed function in mammalian heart is identical with the cloned β_3-adrenoceptor or is a distinct atypical β-adrenoceptor[90].

In mammalian heart the coexistence of a third β-adrenoceptor population, in addition to β_1-and β_2-adrenoceptors, has been suggested. The proposal was based on the properties of certain non-conventional partial agonist, the *in vitro* cardiostimulant potencies of which in isolated tissues of rat, guinea pig and cat were continuously lower than their corresponding affinities for β_1-and β_2-adrenoceptor[45]. In man, evidence for a third cardiac β-adrenoceptor population is elusive. The non-conventional partial agonist, pindolol, can cause tachycardia in man[91]

and it has been suggested that this may be due in part to activation of a third sinoatrial β-adrenoceptor population[45]. However Kaumann and Lobning[92] failed to detect positive inotropic effects of pindolol in human isolated atrium, presumably because the efficacy of pindolol for cardiac atypical β-adrenoceptor is low compared to other non-conventional partial agonists[5,93]. But recently, the existence of minor third β-adrenoceptor population, possibly related to β_3-adrenoceptors is suggested by the potent positive inotropic effects of CGP 12177A and their resistance to blockade by propranolol by antagonism by bupranolol[94].

CGP 12177A behaved as a full agonist producing the same maximum relaxation of the precontracted carotid artery and was equipotent with isoprenaline (in the presence of propranolol 10^{-7} M to block β_1-and β_2-adrenoceptors). The relaxant effect of CGP 12177A was not antagonized by propranolol (10^{-7} M). Thus suggesting the interaction of CGP 12177A with atypical β-adrenoceptors in the carotid artery[95]. The β_3-mediated vasodilatation of BRL 37344 was seen in vessels of skin and fat in conscious dogs resulting in hypotensive effect[96].

Other Systems:

Presence of β_3-adrenoceptor was shown in tracheal epithelium[97]. β_3-adrenoceptor agonists BRL 37344 & SR 58611A, caused concentration dependent reduction of the NANC excitatory component by inhibiting the releasing of neuropeptides from NANC fibres in the guinea pig isolated main bronchus[98-100]. This suggests that β_3-adrenoceptor agonists might exert a pre-junctional inhibitory action on NANC contraction. However, the same agonists were ineffective in human and sheep bronchi[101]. β_3-adrenoceptors are also involved in stimulation of ciliary motility in rabbit and canine bronchial epithelium[102-103]. On the basis of pharmacological and molecular biological studies it have been shown that β_3-adrenoceptors exist in rat detrusor muscle besides β_1- and β_2-adrenoceptors as a mixed population consisting of three subtypes[104].

β_3-adrenoceptor mRNA was detected in hippocampus, cortex and striatum by using a reverse transcription polymerase chain reaction method. The levels are low compared to those in brown adipose tissue[15]. Although studies in man failed to detect β_3-adrenoceptor mRNA in brain[105], a report indicates low levels of β_3-adrenoceptor mRNA in several brain regions and markedly (100 fold) higher levels in infants[106]. Functional evidence for presence of β_3- or atypical β-adrenoceptors in the CNS has been demonstrated by the effects of β_3-adrenoceptor agonist SR 58611A in several animal models of depression including antagonism of apomorphine or reserpine- induced hypothermia, potentiation of yohimbine toxicity and reversal of learned helplessness[107]. The effects were not antagonized by selective β_1- and β_2-adrenoceptor antagonists.

It has been shown that β_3-adrenoceptor agonist BRL 35135 on chronic treatment improves insulin sensitivity and glucose tolerance[108]. It is suggested that β_3-adrenoceptor is involved in the stimulation of skeletal muscle glucose uptake induced by the agonist. These results were confirmed by a series of experiments carried out by Liu and Stock[109]. The stimulation was resistant to the selective β_1-adrenoceptor antagonist atenolol and was inhibited only by very high doses of the non-selective β-adrenoceptor antagonist, propranolol. Further, the β_3-adrenoceptor agonists have been shown to increase blood flow[95,110,111], which in itself can modulate insulin-mediated glucose uptake[112]. This makes difficult to decide whether BRL 35135 is acting on the atypical β-adrenoceptor or the β_2-adrenoceptor on skeletal muscle and/or on vascular smooth muscle. Moreover, apart from one report[113], there is little molecular evidence for β_3-adrenoceptors in skeletal muscle, although both functional[114,115] and ligand binding studies[116,117] indicate the presence of an atypical β-adrenoceptors.

CLINICAL INDICATIONS FOR TREATMENT WITH β_3-ADRENOCEPTOR AGONISTS

As antiobesity and antidiabetic agents:

Several studies have shown that β_3-adrenoceptor agonists (for e.g. BRL 35135, BRL 37344, CL 316243, CGP 12177A elicit a reduction in weight gain or as decrease in body weight of obese, but not lean rodents. This always occurred without a concomitant decrease in food intake, suggesting that it was a consequence of increased thermogenesis. This chronic treatment of obese animals with β_3-adrenoceptor agonists cause a sustained increase in metabolic rates, body lipid content, consistent with the potent lipolytic activity in rodent white and brown fat and with the dependence of thermogenesis on fat oxidation[118].

In addition to the antiobesity properties, β_3-adrenoceptor agonists also exhibit antidiabetic effects in rodent models of NIDDM. In genetically obese mice, repeat dosing with β_3-adrenoceptor agonists improves glycaemic control and insulin sensitivity, as measured by reduction in plasma glucose and insulin levels. In genetically diabetic mice, hyperglycemia and hyperinsulinaemia are similarly reduced. The antidiabetic effect of both BRL 35135 and CL

316243 are seen at doses that have little or no effect on body weight and are therefore, unlikely to secondary to weight loss.

The mechanism of the antidiabetic effect of β_3-adrenoceptor agonists is unclear. As with the thiazolidinediones, the *in vivo* reduction in plasma glucose and insulin levels elicited by BRL 35135, CL 316498 or other β_3-adrenoceptor agonist increases with time, suggesting that induction of gene transcription may be involved. BRL 35135 has been shown to increase glucose uptake into diaphragm, heart and brown adipose tissue (BAT) of obese mice and skeletal muscle in rats. Treatment of obese mice with BRL 26830 was in addition has been found to correct insulin receptor number and kinase activity in adipose tissue and skeletal muscle and to increase transporter numbers[118].

Other potential therapeutic areas

β_3-adrenoceptor agonists may be useful for intestinal hypermotility disorders[9]. Abnormal colonic motility is believed to play a major role in many alimentary disorders. Sympathetic nerves reach all regions of the human gastrointestinal tract and nerve stimulation causes relaxation of human ileum and of both ascending and sigmoid colon[119]. It has been suggested that β_1-adrenoceptors are located within ganglionic plexuses while β_2-adrenoceptors are on smooth muscle cells[120]. β-Adrenoceptor stimulation is known to relax smooth muscle, sympathetic hyperactivity and increased catecholamines during surgery inhibit gastrointestinal motility in the stomach and colon[121].

The antidepressant effects of SR 58611A were studied in five rodent models of depression. It was effective in these models at minimally effective dose of 0.1-0.3 mg/kg, ip. These effects were not blocked by selective β_1-and β_2-adrenoceptor antagonists, but were by high doses of propranolol and alprenolol[108]. It is also reported that obese patients treated with β_3-adrenoceptor agonist BRL 26830 had a significant reduction in the somatic symptoms of anxiety that appeared to be independent of weight loss[122].

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