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Review Article

β₃-Adrenergic agonists

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Introduction

Classification of receptor systems which are activated by sympathomimetic amines has engaged the attention of many investigators for many years, and continues to do so. In 1948, Ahlquist suggested the convenient designations alpha (α) and beta (β) to distinguish major differences in the responses elicited in various organ systems by adrenergic agents (1). The concept of two kinds of adrenoceptor was based on the relative potencies of six sympathomimetic amines to relax the ureters and uterus, contract the nictitating membrane, inhibit the gut and stimulate the myocardium. Thirty years later Ahlquist looked back at adrenoceptors (2) and in 1984 Carrier and Shlafer looked back at the man and his work (3).

"α-Adrenoceptors were generally associated with contraction of smooth muscle. Some blood vessels are constricted, the radial muscle of iris contracts to produce mydriasis and the ureters and splenic capsule are contracted. The only organ system that did not fit the scheme that 'all alpha-mediated responses are excitatory' was the gut, which was relaxed by what we now call α-agonists.

The β-adrenoceptor was generally associated with inhibitory responses: blood vessels are dilated, bronchial smooth muscle is relaxed, and, as was the case with α-agonists, intestinal activity is inhibited. Just as the gut served to demonstrate that all α-mediated responses are not excitatory, the heart served to demonstrate that not all responses that are classified as 'β' were of an inhibitory nature" (3).

In 1967, Lands *et al.* obtained results which could not be explained easily on the assumption that the β type designates a single receptor population (4). Their studies showed that two distinct receptor subtypes could at that time be in-

cluded within the β type. They found a marked similarity between the rank order of potencies of fifteen catecholamine sympathomimetic amines for lipolysis in white adipose tissue and cardiac stimulation and also a similarity for bronchodilator and vasodepressor activity. There was a lack of correlation for other combinations of the four effects. The receptor mediating responses in the heart and lipolysis was designated β₁ and that mediating vasodepressor activity and bronchodilation was labelled β₂. Further studies based on the potencies of sympathomimetic amines classified the receptor responsible for inhibition in the rabbit small intestine as β₁ and that in the rat uterus and diaphragm muscle as β₂ (5).

While pharmacologists were engaged in the classification of adrenoceptors, medicinal chemists had been producing compounds which would act as agonists or antagonists of the various adrenoceptor types. Some of these agents were non-selective for the adrenoceptor subtypes but others had varying degrees of selectivity for a particular subtype. Examples are isoprenaline, a selective agonist for β-adrenoceptors rather than α-adrenoceptors but non-selective for either β₁- or β₂- adrenoceptors (6), and propranolol, a selective antagonist for β- rather than α-adrenoceptors but non-selective for either β₁- or β₂-adrenoceptors (7). Examples of agents with selectivity for β₁- rather than β₂-adrenoceptors are the agonist prenalterol (8, 9) and the antagonist atenolol (10); agents with selectivity for β₂- rather than β₁-adrenoceptors are the agonist salbutamol (11) and the antagonist ICI 118551 (12, 13). Such agents were key to confirming or questioning the classification and to extending it. In particular, the classification of the white adipose tissue β-adrenoceptor as the β₁ subtype has been questioned by many groups and this is discussed in the next section.

Arch has stressed the serious limitations of classifying receptors solely on the basis of rank order of potencies of agonists (14). The approach can be criticized if only a limited range of agonists is used and it can be misleading if some of the agonists have a lower efficacy than others and absolute responses are compared. Rather than use agonists, whose efficacies may differ, pharmacologists now prefer to classify receptors using antagonists, which have zero efficacy. It is necessary to use agonists selective for each receptor

subtype in combination with antagonists of various selectivities. A novel receptor type can only be detected using both an agonist that acts, at least in part, via this receptor and an antagonist that has a different affinity for the novel receptor compared with known receptors. He pointed out that most studies fall far short of these stringent criteria.

The adipocyte atypical β-adrenoceptor

The classification of the lipolytic β-adrenoceptor as a β_1 subtype was questioned by Harms et al. in 1974 (15, 16), who showed that antagonism of lipolysis could correlate with either β_1-adrenoceptor antagonism or β_2-adrenoceptor antagonism depending upon the structural type of the antagonist used. They proposed a hybrid receptor having characteristics of both the β_1- and β_2-adrenoceptor subtypes. Later studies in which isoprenaline was used as the β-adrenoceptor agonist and two series of analogs of tolamolol as antagonists supported the idea of a dualistic β_1 and β_2 character of the rat adipocyte β-adrenoceptor which could not be explained by the presence of two different populations of β_1- and β_2-adrenoceptors. Further, the rat adipocyte β-adrenoceptor population was shown to be homogeneous (17). These views on the hybrid nature of the β-adrenoceptor of the white adipose cell of the rat were supported by Tan and Curtis-Prior who, in 1983, proposed that it be termed a 'beta-hybrid' or 'β_3' adrenoceptor (18).

The hypothesis of a new and discrete β-adrenoceptor subtype (β_3) remained tenuous in the absence of agonists or antagonists selective for it. In 1984, however, Wilson et al. of Beecham Pharmaceuticals reported on the activities of three novel arylethanolamine β-adrenoceptor agonists, BRL 28410, BRL 35113 and BRL 35135*, which showed selectivity for rat white adipose tissue lipolysis, supporting the view that the rat lipolytic β-adrenoceptor is atypical, that is, it is distinct from either the β_1- or β_2-adrenoceptor subtype (19). Appropriate studies were carried out using available β-adrenoceptor antagonists; there was no antagonist with selectivity for the atypical β-adrenoceptor. Wilson et al. pointed out that the hybrid β_1/β_2 receptor proposed by Harms et al. and De Vente et al. adequately explained the results obtained by those authors but that the model did not readily explain their own results. It is difficult to envisage a lipolytic receptor with β_1- and β_2-adrenoceptor characteristics when, for example, BRL 35113 is a poor agonist at both β_1- and β_2-adrenoceptors yet is a potent agonist at the lipolytic β-adrenoceptor.

Concurrent studies by Arch et al. of the Beecham Group (20), who used BRL 28410, BRL 35113 and BRL 37344 (the carboxylic acid related to the methyl ester BRL 35135) in a

study of lipolysis in rat interscapular brown adipocytes, showed that in the rat the brown adipocyte receptor is neither a β_1- nor a β_2-adrenoceptor and that brown and white adipocytes "have similar, though not necessarily identical," β-adrenoceptors. The most potent compound, BRL 37344, stimulated lipolysis with 400- and 20-fold selectivity compared with atrial β_1 and tracheal β_2 responses, respectively. Stock and Sudera studied rat brown adipocyte respiration, rather than lipolysis, using isoprenaline, BRL 37344 and ICI 201651 as agonists and propranolol, atenolol and ICI 118551 as antagonists, and concluded that the interaction of the novel agonists with brown adipocyte β-adrenoceptors differed from that of isoprenaline, giving support to the work of the Beecham group (21).

Receptor binding studies using conventional β-adrenergic agents had failed to reveal the atypical β-adrenoceptor in brown adipocytes. Most studies suggested that the brown adipocyte receptor is of the β_1-subtype but two groups had reported β_1-: β_2-adrenoceptor populations of 59:41 (22) and 80:20 (23) for rat brown adipose tissue. Arch (14) has reviewed the binding studies and has pointed to the problems that affect them. First, the receptors studied by binding methods may not be the ones that mediate the functional response. Second, just as functional studies using antagonists can only detect receptors through which the ligand acts, binding studies cannot detect receptors that do not bind the labelled ligand. Detection of the atypical receptor by binding studies requires the use of a labelled ligand that binds selectively to the atypical receptor. Recently, Hollenga and Zaagsma have investigated the effects of the selective antagonists CGP 20712A (β_1) and ICI 118551 (β_2) on BRL 37344- and isoprenaline-induced lipolysis in rat white adipocytes (24). Their results show that the selective β_3-agonist BRL 37344 acts solely through atypical β-adrenoceptors, whereas isoprenaline, a non-selective β-agonist, acts predominantly through atypical β-adrenoceptors; β_1-adrenoceptors, detected in binding studies, play at most a small and subordinate functional role in isoprenaline-induced lipolysis. Similarly, adenyl cyclase activation in rat adipocytes by BRL 37344 is solely mediated by atypical (β_3 subtype) receptors and isoprenaline is predominantly mediated by atypical (β_3 subtype) receptors (25).

An important contribution was made by Emorine et al. in 1989 when they reported the isolation of a gene which coded for the human β_3-adrenoceptor (26). A human genomic DNA library was screened with the entire coding regions of the genes for the turkey β_1- and the human β_2-adrenoceptors. Sequences complementary to both receptor probes were found in fragments from 14 clones, one of which was entirely sequenced and shown to contain a gene coding for a polypeptide with an amino acid sequence 51% and 46% identical to those of β_1- and β_2-adrenoceptors, respectively. The receptor protein, which the authors called 'β_3-adrenoceptor', had clear atypical β-adrenoceptor properties when expressed in Chinese hamster ovary cells (CHO), which normally contain no β-adrenoceptor activity (26, 27). The CHO-β_3 cells were analyzed for ligand binding and cAMP production and compared with the similarly prepared

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* BRL compounds, including BRL 35135, occasionally have the suffix 'A' after the number. It is understood that this simply indicates that the substance is a salt and so the 'A' is omitted in this review. The pharmacological properties of these compounds and the clinical results on two of them are considered in a later section. Chemical structures are also shown in a later section.

CHO-β_1 and CHO-β_2 cells (28). They used β-adrenergic ligands known to interact selectively with β_1, β_2 or atypical receptors and confirmed that the human β_3-adrenoceptor, when expressed in CHO cells, is indeed related to the atypical β-adrenoceptors characterized in rodent adipose tissue. The potency order of β-adrenergic agonists for the β_3-adrenoceptor was clearly different from that for the β_1-adrenoceptor and for the β_2-adrenoceptor and resembled that for lipolysis stimulation in rodent brown adipose tissue. Also, classical β-antagonists displayed very low affinity for the β_3-adrenoceptor expressed in CHO cells. However, differences between the cloned human β_3-adrenoceptor and rodent atypical adrenoceptors led Zaagsma and Nahorski to question whether they were, in fact, homologous proteins (29). Also, experiments with the cloned rat β_3-adrenoceptor (30, 31) indicate that its pharmacological properties are virtually identical to those of the atypical adrenoceptor in rat brown fat but differ in several respects from those reported for the cloned human β_3-adrenoceptor (26-28).

Recently, Liggett (32) has expressed the rat and the human β_3-adrenoceptor in CHO cells and has determined key pharmacological properties in parallel studies. Typical catecholamine agonists were found to have a similar low affinity for both rat and human β_3-adrenoceptors. In contrast to catecholamine agonists, differences in agonist efficacy and/or potency were noted for each of the non-catecholamine atypical agonists tested. For example, BRL 37344* was a full agonist (intrinsic activity 1.0 relative to isoprenaline) for the rat β_3-adrenoceptor, but its intrinsic activity was only 0.60 for the human β_3-adrenoceptor; also, it was about 15 times less potent for the human than for the rat β_3-adrenoceptor. The data indicate that the molecular actions of atypical β-adrenoceptor agonists differ markedly between species and so the action of atypical agonists at rodent β_3-adrenoceptors may not be predictive of therapeutic potential in humans.

A feature of brown adipose tissue is that the response of the tissue to β_3-agonists increases on chronic agonist exposure (33, 34) in contrast to tissues containing predominantly β_1- or β_2-adrenoceptors which become desensitized (35, 36). In line with this is the recent finding that whereas both β_1- and β_2-adrenoceptor subtypes undergo agonist-dependent decreases in receptor expression during long-term agonist exposure, cells expressing the β_3-adrenoceptor subtype fail to undergo a decrease in receptor number after prolonged agonist exposure and, in fact, display increases in number over time (37). However, Revelli et al. have demonstrated that administration of the thermogenic β-adrenergic agonist Ro 16-8714 (see later) to lean and obese Zucker rats induces a marked down-regulation of the β_3-adrenoceptor of the interscapular brown adipose tissue over a time course of 72 hours. It was proposed that the down-regulation of the β_3-adrenoceptor observed in this

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*It would appear that BRL 34377 was reported in error when BRL 37344 was meant.

study 72 hours after beginning treatment with Ro 16-8714 is compensated in the whole animal by the trophic effect of the drug on the brown adipose tissue (38).

Distribution of atypical β-adrenoceptors

It is now generally accepted that the β-adrenoceptor mediating lipolysis in rat white adipose tissue and oxygen consumption in brown adipose tissue is of neither the β_1 nor the β_2 subtype, that it is atypical, and it is being referred to as the β_3-adrenoceptor. It is characterized by a marked responsiveness to atypical β-agonists like BRL 37344 compared with classical β_1- and β_2-agonists, and by having a low affinity for propranolol and standard β_1- and β_2-antagonists.

In this section, atypical β-adrenoceptors in other tissues are reviewed and inevitably the question is raised as to whether they should be designated β_3-adrenoceptors. It had been suggested earlier (39) that the β_1 and β_2 classification of Lands may represent two extremes of a variable spectrum of different "isoreceptors", in the same way as specific enzymes can exist in isoenzymic forms. The other atypical β-adrenoceptors may be similar but not identical to those designated β_3. Some authors have expressed caution at using the specific nomenclature β_3-adrenoceptor because of the complexities involved in making the assignment (40). On the other hand Arch (14) has suggested that this group of atypical β-adrenoceptors, having the characteristics described above, will obtain their due recognition only if they are described as β_3-adrenoceptors. In this review the original authors' nomenclature of "atypical" or "β_3" is used. However, the present author is clearly following Arch in grouping together atypical β-adrenoceptors, that is, those which do not fit into either the β_1 or the β_2 classification. Some further subdivision may prove necessary in the future. It is recommended that in each case the data on which the assignment was made be examined. The situation is complicated by the co-occurrence of β_1-, β_2- and atypical adrenoceptors in certain tissues, by species differences, and, as mentioned earlier by the agonists used perhaps having different efficacies in different tissues. MacKay (41) has indicated the problems that can arise in the determination of pA_2 values of antagonists, and in making comparisons of values obtained by different workers, while Jenkinson (42) has pointed out that there is still some confusion over terms and terminology.

The presence of an undefined adrenoceptor which functions to inhibit histamine-induced longitudinal muscle tension development in the guinea pig ileum had been evident from the failure of propranolol to block the action of β-adrenoceptor agonists. The question arose as to whether the adrenoceptor in the guinea pig ileum was the same as the β_3-adrenoceptor on adipocytes. Bond and Clarke (40) showed that the order and relative potency of agonists at the ileum receptor was BRL 37344 (20) >(-)-isoprenaline (8) > noradrenaline (1) > adrenaline (0.5) > fenoterol (0.35) > (+)-isoprenaline (0.27), similar to the more restricted series examined by Arch et al. (20) for lipolysis in brown fat, BRL 37344 (1) > isoprenaline (0.2) > fenoterol (0.008) and by

Wilson *et al.* (19) for lipolysis in white fat, BRL 35135 (which is hydrolyzed to the active free acid BRL 37344) (1) = isoprenaline (1) > fenoterol (0.03). Further, the guinea pig ileum adrenoceptor was totally resistant to propranolol at up to 10 μM. Bond and Clarke refer to the guinea pig ileum β-adrenoceptor as atypical (see their expressed caution above, reference 40) rather than β₃. One important piece of evidence which argues against the notion that the adipocyte and the ileal receptor are the same comes from data obtained with propranolol. The atypical β-adrenoceptor on fat cells is sensitive to blockade by propranolol but abnormally low pA_2 values (6.2-6.6) have been reported. In contrast, the adrenoceptor on guinea pig ileum is resistant to propranolol at concentrations that are at least 8-16 times its equilibrium dissociation constant for the adipocyte receptor. However, Bond and Clarke point out that it would be premature to discriminate the two receptors solely upon the basis of the propranolol result. Blue *et al.* have further characterized the atypical β-adrenoceptor in guinea pig ileum by showing that while it is resistant to antagonism by propranolol, it is competitively antagonized by (-)-alprenolol and (-)-dihydroalprenolol with pA_2 values of 6.47 and 6.43, respectively. These values are much lower than those, 8.2 and 8.81, respectively, for antagonism at the β₁-adrenoceptor. (-)-Alprenolol also exerted agonist activity at the atypical β-adrenoceptor in guinea pig ileum (43). Further, in the presence of sufficient propranolol (5 μM) to saturate β₁-adrenoceptors, cyanopindolol gave a pA_2 value of 7.63. Thus, cyanopindolol is the most potent antagonist so far identified for the atypical β-adrenoceptor and, in the presence of propranolol, may serve as a useful probe for studies at the atypical β-adrenoceptor (44).

In rat gastric fundus, the resistance of β-adrenoceptor relaxant responses to propranolol and the antagonism by cyanopindolol of BRL 37344- and isoprenaline-induced responses led McLaughlin and MacDonald to conclude that atypical β-adrenoceptors were present (45). The relatively low potency of BRL 37344 at this site compared with other atypical β-adrenoceptor sites may be due to differences in coupling efficiency or may indicate receptor heterogeneity. In guinea pig gastric fundus the relatively high potency of BRL 35135, together with the relatively weak antagonism of β-adrenoceptor agonists by propranolol, support the presence of atypical β-adrenoceptors similar to those in rat adipocytes (46, 47).

Evidence for an atypical, or β₃-adrenoceptor, in ferret tracheal epithelium has been reported by Webber and Stock (48). BRL 37344 was more potent by 4-5 orders of magnitude than the β₁-(prenalterol) and β₂-(salbutamol) adrenoceptor selective agonists in stimulating methacholine-induced albumin transport. The weak antagonism of the response to BRL 37344 by ICI 118551 was consistent with this response being mediated by an atypical β-adrenoceptor. The use of BRL 37344 has shown the presence of atypical β-adrenoceptors on rat jejunum (49). Several β₁- or β₂-agonists and antagonists were tested and it was found that non-selective and selective antagonists for β₁- or β₂-adrenoceptors showed a relatively low affinity, compared

to their affinity for β₁- or β₂-adrenoceptors. BRL 37344 was more potent, although a partial agonist (60% maximal response), compared to isoprenaline, whereas it was clearly less potent than isoprenaline on β₁- or β₂-adrenoceptors.

BRL 37344 and BRL 35135 have been used to provide evidence that an atypical β-adrenoceptor is present in guinea pig bronchi (50). BRL 35135 reduced non-adrenergic non-cholinergic contractions induced by electrical field stimulation and this inhibitory effect of BRL 35135 was not significantly altered by non-selective or β₁-selective antagonists. While BRL 35135 inhibited the contractile response to electrical field stimulation, it did not inhibit the contractile response to exogenous substance P, which led to the suggestion that it elicits its inhibitory effects pre-junctionally on neuronal terminals rather than post-junctionally on the tachykinin receptors of airway smooth muscles.

Just as the Beecham BRL compounds have contributed to the identification of atypical β-adrenoceptors, so has a group of phenylethanolaminotetralines reported by the Sanofi Group, *e.g.* SR 58306A (51), SR 58539B (52) and SR 58611A (53). *In vitro*, SR 58306A, unlike isoprenaline and the β₂-selective agonists salbutamol and ritodrine, potently inhibited rat colon spontaneous contraction at concentrations substantially lower than those only partially relaxing the guinea pig trachea; also, it had no chronotropic action on guinea pig atria. β₂-Selective agonists were used as comparators because they had shown promise for treating conditions of abnormally enhanced gastrointestinal motility; they could not be used therapeutically because of their concurrent cardiovascular effects. Non-selective β-adrenergic antagonists competitively antagonized the action of SR 58306A on the colon, which was not prevented by selective β₁- or β₂-antagonists. Only alprenolol competitively antagonized the action of isoprenaline on the colon; antagonism by either pindolol or propranolol was non-competitive. The results suggested that compounds such as SR 58306A inhibit colonic motility by selectively stimulating atypical β-adrenoceptors, while isoprenaline interacts also with β₁- and β₂-adrenoceptors which coexist with the atypical β-adrenoceptor in rat colon. *In vivo*, the minimal effective i.v. dose of SR 58539B for raising heart rate was five times its ED_{50} for inhibition of colon motility; at this multiple of ED_{50} it caused a rise in blood pressure. SR 58611A was the most selective potent compound of those studied *in vitro* on rat colon, rat uterus and guinea pig atrium.

The phenylethanolaminotetralines were tested for their ability to induce lipolysis in rat white adipocytes (54, 55). There was a good correlation (r = 0.97) between the lipolytic EC_{50}s of (-)- and (+)-isoprenaline and several phenylethanolaminotetralines and their inhibition of rat colon motility, suggesting that receptors of the same type may account for their actions on both preparations. McLaughlin and MacDonald (56) investigated the responses to noradrenaline, isoprenaline and BRL 37344 in the rat distal colon *in vitro* and provided a further link between the effects of BRL 37344 and the Sanofi compounds. Relaxant responses to BRL 37344 were only weakly antagonized by propranolol. Responses to isoprenaline, resistant to propranolol antagonism, were antagonized in the presence of propranolol by cyanopindo-

lol (pA$_2$ 7.12), which had earlier been suggested as a probe for atypical β-adrenoceptors (44). Manara *et al.* (57) have compared *inter alia* SR 58539B with BRL 37344 and shown that the former has the greater rat colon to rat uterus or guinea pig atrium selectivity. The same group has carried out binding assays with [^{3}H]-dihydroalprenolol and has failed to identify sites corresponding to the atypical β-adrenoceptors clearly evidenced by their functional studies in rat colon (58). The identification of a radioligand with sufficiently high affinity for atypical receptors is of paramount importance.

An atypical β-adrenoceptor in rat esophagus has been characterized using agonist and antagonist probes (59, 60). The order and relativity of agonist potency was BRL 37344 (36) > (-)-isoprenaline (7) > SR 58611A (5) > (-)-adrenaline (2) > (-)-noradrenaline (1) > (+)-isoprenaline (0.2) > fenoterol (0.02). The receptor site in rat esophagus exhibited an unusually low affinity for propranolol compared with that normally obtained at classical β-adrenoceptors. The failure of cyanopindolol to antagonize (-)-isoprenaline suggested that the site may differ from other atypical β-adrenoceptors.

Both SR 58611A and BRL 37344 stimulated acid secretion in rat stomach *in vitro* (61). The response to SR 58611A was reduced by propranolol but not by β$_1$- or β$_2$-selective antagonists. The lack of effect of alprenolol was considered surprising. This agent had been reported earlier to be particularly effective (but not selective for) atypical β-adrenoceptors (43). In a similar type of study, the same group showed that stimulation of bicarbonate secretion in rat cecum *in vitro* is mediated by atypical β-adrenoceptors (62). In this case the response to SR 58611A was reduced by alprenolol but not by propranolol, indicating that the atypical β-adrenoceptors mediating the two secretory processes, although similar, may not be identical.

SR 58611A has been reported to have an antidepressant-like effect in rodents (63). The effect was not antagonized by selective β$_1$- or β$_2$-adrenoceptor antagonists, but was blocked by high doses of propranolol and alprenolol, suggesting the presence of atypical β-adrenoceptors in rodent brain.

Atypical β-adrenoceptors may also occur in rat liver (26), in rat soleus muscle (64), in the heart (65), in the circular smooth muscle layers of the human colon (66) and in the dog hind limb (67). The antinociceptive action of several β-adrenoceptor agonists in the mouse abdominal constriction test has been attributed to stimulation of atypical β-adrenoceptors (68).

β$_3$-Adrenoceptor agonists as potential drugs

Therapeutic areas where it has been suggested that β$_3$-adrenoceptor agonists may provide drugs are obesity (20), diabetes (69), intestinal hypermotility disorders (54), inflammatory airways disease (48) and depression (63); they may also provide locally acting analgesic drugs (68). Patent applications suggest that β$_3$-adrenergic agonists may be used in the treatment of ocular hypertension and glaucoma (70), hypertension (71), hypertriglyceridemia, hypercholesterolemia and atherosclerosis (72), and may also be used as topical antiinflammatory agents and platelet aggregation inhibitors (73). Work in the first three of these areas has yielded novel compounds with selectivity for the β$_3$-adrenoceptor which have not only been used as tools to help characterize the β$_3$-adrenoceptor, as discussed above, but have been considered worthy of clinical investigation. None is as yet generally available for human use. The plan is now to discuss these compounds principally under the heading of the therapeutic area in which they originated.

Thermogenic drugs for the treatment of obesity and diabetes

A thermogenic function was first ascribed to brown adipose tissue (BAT) in 1963 (74). BAT appears to be present in most, if not all mammals, but rarely accounts for more than 2% of body weight (75). Initially its heat producing function was believed to be of importance only in certain species as part of the process of non-shivering thermogenesis (NST) during arousal from hibernation, during acclimation to cold or during the neonatal period (76-78). It was not until 1978 that BAT was established as the major but not necessarily the exclusive site of NST in rodents (79-81). In cold-adapted rats it was shown that during maximal noradrenaline stimulation the blood flow to BAT is equivalent to one-third of the cardiac output and, from measurements of oxygen extraction by BAT, it was shown that this tissue could account for over 60% of NST. Cold-adapted rats have an increased turnover and urinary excretion of noradrenaline, show an enhanced thermogenic response to catecholamines, and hypertrophy and hyperplasia of BAT is observed. The high metabolic rate of these animals can be inhibited by β-adrenergic blockade (82, 83). NST has been observed in larger adult mammals, including man (84).

Non-shivering heat production depends on the oxidation of primarily fatty acids by BAT. The capacity of the tissue for thermogenesis is dependent upon a 'proton conductance' pathway which is associated with an inner mitochondrial membrane 'uncoupling' protein unique to brown fat. Thermogenesis in BAT is initiated by the sympathetic release of noradrenaline which acts predominantly via β-adrenoceptors, now designated β$_3$-adrenoceptors, to cause an activation of adenyl cyclase which, by increasing the concentration of cAMP, stimulates a hormone sensitive lipase thereby releasing free fatty acids. These act both as the substrate for β-oxidation and to override the control exerted on respiration by purine nucleotides binding to the GDP- binding protein thermogenin (uncoupling protein), thereby uncoupling ATP synthesis from respiration.

Uncoupling protein has a high affinity for purine nucleotides and the specific binding of GDP is used to monitor the activity of the proton conductance pathway. In rats, where the activity of the BAT mitochondrial proton conductance pathway is low, a proportion of the uncoupling protein is 'masked' and unable to bind GDP. However, on activation of the proton conductance pathway, GDP-binding sites are 'unmasked'. Therefore, GDP-binding in isolated mitochondria reflects the thermogenic status of BAT (85).

A phenomenon which shares many of the features of cold-induced NST is diet-induced thermogenesis (DIT),

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