The brown adipocyte β-adrenoceptor

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The essential feature of brown adipose tissue (BAT) is its capacity to oxidize fat, producing heat, but without synthesizing ATP from ADP. The main regulator of this activity is noradrenaline, which acts primarily via the β-adrenoceptor (Arch *et al.* 1986). An understanding of the nature of the β-adrenoceptor in brown adipocytes is important both for the development of thermogenic anti-obesity drugs, and when using agonists or antagonists to investigate the role of the receptor in mediating functional responses in BAT.

When β-adrenoceptors were first divided into two (β_1- and β_2-) subtypes by Lands *et al.* (1967) on the basis of potency orders for a series of catecholamine agonists, white adipose tissue receptors were classified along with cardiac and gut receptors as belonging to the β_1-subtype. Despite the clear demonstration by, in particular, Harms, Zaagsma, Nahorski and their co-workers (Harms *et al.* 1977; Bojanic *et al.* 1985) that white adipocyte and cardiac β-adrenoceptors are not identical, this view has remained entrenched in the literature, supported by many studies in which the workers have failed to appreciate the limitations of the pharmacological agents and approaches that they have adopted. These same approaches, with their serious limitations, have been used since 1978 in the classification of the brown adipocyte β-adrenoceptor. Indeed, it has often been assumed from the outset of these studies that the receptor is a β_1- or β_2-adrenoceptor, or perhaps a mixture of these subtypes, and the majority view prevailing is that the receptor is of the β_1-subtype. The objective of the present paper is to demonstrate the inadequacies of many studies on the classification of brown adipocyte β-adrenoceptors, and to show that by using appropriate pharmacological agents and a suitable analysis of the results brown adipocyte β-adrenoceptors can be differentiated from classical β_1- and β_2-adrenoceptors.

Inadequacies of some methods used to subclassify brown adipocyte β-adrenoceptors

Some workers have classified brown adipocyte or BAT β-adrenoceptors on the basis of potency orders for stimulation of a functional response (e.g. BAT temperature or adipocyte respiration) by a series of β-adrenoceptor agonists (Table 1). These potency orders have been compared with what is known of the potency orders for the agonists in

Table 1. *Classification of brown adipocyte and brown adipose tissue (BAT) β-adrenoceptors by potency order of agonists*

Species	Measurement	Potency order	Authors' classification	References
Rat	Respiration	Noradrenaline >adrenaline	β_1	Bukowiecki *et al.* (1980)
Rat	BAT temperature–GDP binding	Noradrenaline >clenbuterol ≧ prenalterol	β_1 and β_2	Rothwell *et al.* (1983)
Rabbit	BAT temperature	Isoprenaline ≫ salbutamol, terbutaline (both ineffective)	β_1	Harris *et al.* (1986)
Hamster	Respiration	Noradrenaline = adrenaline	β_1	Mohell *et al.* (1983)

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other tissues. This approach was used by Lands *et al.* (1967), but it can be misleading if some of the agonists have a lower efficacy than others and absolute responses are compared. For example, the supposed β_1-selective agonist prenalterol might have little effect in BAT, not because it binds poorly to the β-adrenoceptors, but because it has low efficacy and BAT is a tissue where receptor–response coupling is poor (see Kenakin, 1984). It is better to use a β-agonist of high efficacy such as denopamine (Naito *et al.* 1985) or, failing this, express EC_{50} values relative to the compounds' own maximum responses, not relative to the maximum response of a full agonist. With these precautions it may be possible to argue for differences in receptors on the basis of marked changes in the relative potencies of agonists, even if their potency order is not reversed between two tissues.

A more important criticism of the work conducted on BAT or brown adipocytes is that the range of agonists studied has been very limited. In two cases only the relative potencies of noradrenaline and adrenaline were considered in subclassifying the β-adrenoceptor, the potencies of the other agonists used (isoprenaline, phenylephrine) merely showing that the receptor was a β-adrenoceptor and not an α-adrenoceptor. Since noradrenaline was as potent as, or more potent than, adrenaline, the receptor was classified as β_1. If adrenaline had been more potent than noradrenaline, it would have been classified as β_2. Such an approach cannot identify atypical β-adrenoceptors or, indeed, show the presence of a mixture of β_1- and β_1-adrenoceptors.

Rather than use agonists, whose differing efficacies can be misleading, pharmacologists prefer to classify receptors using antagonists, which have zero efficacy. Ideally, this approach demands the construction of full dose–response curves for an agonist in the presence of at least three different concentrations of an antagonist and in its absence. From these results it is possible to calculate the affinity of the antagonist for the receptor to which the agonist binds to produce the response. A problem arises if the agonist stimulates two receptors, because if the antagonist has differing affinities for these receptors, its apparent affinity will vary with its concentration. It is, therefore, necessary to use agonists selective for each receptor subtype in combination with antagonists of various selectivities (O'Donnell & Wanstall, 1981). 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.

Most studies fall far short of these stringent criteria (Table 2). Apart from our own results and those of Jones *et al.* (1989) and Stock & Sudera (1988), which are described

Table 2. *Classification of brown adipocyte and brown adipose tissue (BAT) β-adrenoceptors using antagonists*

Species	Measurement	Agonist(s)	Potency/efficacy of antagonists	Authors' classification	References
Rat	BAT GDP binding	Noradrenaline	Propranolol > atenolol or ICI 118,551	β_1 and β_2	Rothwell *et al.* (1982)
Rabbit	BAT temperature	Isoprenaline	Atenolol effective	β_1	Harris *et al.* (1986)
Hamster	Respiration	Isoprenaline, noradrenaline, adrenaline	K_d for propranolol 0·3 μM (pA_2 6·5)	β_1!	Mohell *et al.* (1983)

K_d, dissociation constant; $pA_2 = -\log_{10}K_d$.

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later (pp. 218–219), only Mohell *et al.* (1983) calculated the affinity of an antagonist (propranolol) for the β-adrenoceptor. They used a single concentration of propranolol (6 μM) but, more importantly, failed to note that propranolol had a 100-fold lower affinity for hamster brown adipocyte β-adrenoceptors than for β_1-adrenoceptors in heart or β_2-adrenoceptors in the lung or uterus.

A number of authors have classified brown adipocyte β-adrenoceptors by ligand-binding studies. This involves displacing a radiolabelled β-adrenoceptor antagonist from whole cells or membranes using a series of agonists or antagonists. Two approaches to receptor classification by binding studies have been used (Table 3). One has been simply to consider the relative potencies of noradrenaline and adrenaline in displacing the labelled ligand. This approach is similar to that described previously, where the relative potencies of the natural agonists in stimulating a functional response are considered. It can only lead to the conclusion that the receptor is of the β_1- or β_2-subtype; it cannot identify a novel receptor subtype or point to the presence of a mixture of β_1- and β_2-adrenoceptors. Using this approach most groups have concluded that the brown adipocyte receptor is of the β_1-subtype, but one group (Baresi *et al.* 1986) has argued for the β_2-subtype.

The second, more sophisticated use of receptor-binding studies involves the use of selective antagonists to displace the labelled ligand. Provided the labelled ligand binds to all β-adrenoceptor subtypes, Scatchard plots of the results will reveal the affinity of the antagonist for each subtype and also the number of receptors of each subtype. The use of unlabelled antagonists of different selectivities is preferable to using a single antagonist; each antagonist should reveal the same proportions of the various receptors. Two groups have used this approach and reported β_1-:β_2-adrenoceptor values of about 60:40 and 80:20 for rat BAT (Table 3).

There are two problems that affect all binding studies. The first is that 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 agonist acts, binding studies cannot detect receptors that do not bind the labelled ligand. These problems are especially pertinent to adipocyte β-adrenoceptor binding studies and are discussed further later (pp. 220–221).

Table 3. *Classification of brown adipocyte and brown adipose tissue (BAT) β-adrenoceptors in receptor-binding studies*

Species	Potency order or other evidence	Authors' classification	References
Rat	Noradrenaline ≧ adrenaline	β_1	Bukowiecki *et al.* (1978)
Rat	Noradrenaline ≧ adrenaline	β_1	Senault *et al.* (1984)
Rat	Noradrenaline > adrenaline	β_1	Kurahashi & Kuroshima (1981)
Rat	Adrenaline > noradrenaline	β_2	Baresi *et al.* (1986)
Hamster	Noradrenaline ≧ adrenaline	β_1	Svoboda *et al.* (1979)
Alaskan vole (*Clethrionomys rutilus*)	Noradrenaline ≧ adrenaline	β_1	Feist (1983)
Rat	Scatchard analysis: atenolol and ICI 118,551	$\beta_1$59:$\beta_2$41	Rothwell *et al.* (1985)
Rat	Scatchard analysis: seven ligands	$\beta_1$80:$\beta_2$20	Levin & Sullivan (1986)

Physiological significance of β-adrenoceptor subtypes

It is not difficult to propose advantages for the organism in having more than one type of receptor that responds to a natural messenger molecule, provided the receptors mediate different responses. For example, since stimulation of β-adrenoceptors promotes lipolysis, whilst stimulation of α_2-adrenoceptors inhibits lipolysis, adipose tissue sites with different proportions of α- and β-adrenoceptors can differ in their responses to noradrenaline or adrenaline. These opposing responses are possible because stimulation of α_2- and β_1-adrenoceptors produces different second messenger responses (depression and elevation of cyclic AMP levels respectively). It is more difficult to see an advantage in there being subtypes of β-adrenoceptor, because the second messenger response for all known subtypes involves an elevation of cyclic AMP levels, and the receptor subtypes never mediate different responses. However, the natural agonists have different potencies at β_1- and β_2-adrenoceptors, and so, by having different types of β-adrenoceptor, two tissues might show different sensitivities to the neural (noradrenaline) and humoral (adrenaline) components of the sympathetic nervous system. In addition, there is evidence that responses mediated by β_1- and β_2-adrenoceptors can be differentially regulated, so that the relative influences of noradrenaline and adrenaline can change within one tissue (O'Donnell & Wanstall, 1987).

Extending this line of thought, it has been proposed that β_1-adrenoceptors are innervated by sympathetic nerves and normally stimulated by noradrenaline, whereas β_2-adrenoceptors are non-innervated and stimulated by circulating adrenaline (Ariëns & Simonis, 1983). Thus, noradrenaline tends to be more potent than adrenaline at β_1-receptors, but adrenaline is more potent at β_2-receptors. This idea has been investigated by depleting neuronal catecholamines using reserpine or 6-hydroxydopamine, with the expectation that innervated tissues will then become supersensitive to β-agonists. This expectation has been confirmed. Thus BAT, which has a rich sympathetic innervation and is influenced primarily by noradrenaline (Rothwell *et al.* 1982; Young *et al.* 1984) became supersensitive to isoprenaline (Grassby *et al.* 1987). For the most part the tissues that have a good sympathetic innervation and become supersensitive to β-agonists are more sensitive to noradrenaline than adrenaline, whilst other tissues are more responsive to adrenaline. The one exception is white adipose tissue, which has a poor innervation and does not become supersensitive, but is at least as responsive to noradrenaline as it is to adrenaline. This might be interpreted as meaning either that not all β_1-adrenoceptors are innervated or that white adipocytes do not possess β_1-adrenoceptors.

For workers interested only in the physiological significance of β-adrenoceptor subtypes, it may be appropriate to adopt methods that only allow for two subtypes. However, it does not follow that each subtype is homogeneous.

pA_2 values for antagonists

The pA_2 value of a competitive antagonist is related to its dissociation constant K_d for the receptor at which it antagonizes the agonist by the equation $pA_2 = -\log_{10}K_d$. In our own studies we have determined pA_2 values for antagonists of various selectivities using, in general, three or four concentrations of each antagonist. As agonists we have used both isoprenaline and the Beecham compounds BRL 28410 and BRL 37344, which selectively stimulate brown adipocyte β-adrenoceptors (see p. 220). The functional response measured was glycerol release. We have not found that our apparent pA_2 values vary according to the concentration of antagonists. Propranolol had a similar pA_2 value for β_1- and β_2-adrenoceptor-mediated responses (atrial and tracheal tension

respectively) whether isoprenaline or BRL 28410 was the agonist, which confirms that it has a similar affinity for β_1- and β_2-adrenoceptors (Table 4). However, its pA_2 value for rat brown adipocyte lipolysis was at least one unit lower, indicating a tenfold lower affinity for the brown adipocyte receptor compared with β_1- and β_2-adrenoceptors. This, by itself, shows that the brown adipocyte receptor can be distinguished from β_1- and β_2-adrenoceptors. Nevertheless, it was shown that the β_1-selective antagonist atenolol has a pA_2 value two units lower for brown adipocyte lipolysis than for right atrial tension. This confirms that the brown adipocyte is not a β_1-adrenoceptor. Similarly, it was shown that the β_2-selective antagonist ICI 118,551 has a pA_2 value two units lower for brown adipocyte lipolysis than for tracheal tension, which confirms that the brown adipocyte receptor is not a β_2-adrenoceptor. These results have been supported by Stock & Sudera (1988), who measured rat brown adipocyte respiration rather than lipolysis, and determined pA_2 values for all three antagonists (propranolol, atenolol and ICI 118,551) against both isoprenaline and BRL 37344. For the three combinations of agonist and antagonist where direct comparison is possible, their pA_2 values are within 0·1 units of those shown in Table 4.

The atypical β-adrenoceptor of brown adipocytes is not confined to the rat. The results of Mohell *et al.* (1983) indicate that the hamster receptor is atypical, and Jones *et al.* (1989) have obtained pA_2 values of 6·8 for antagonism of isoprenaline-stimulated neonatal rabbit brown adipocyte respiration and lipolysis by propranolol. Furthermore, our unpublished findings (M. A. Holland and J. R. S. Arch) indicate that propranolol has a low pA_2 value (about 6·4) for antagonism of lipolysis and respiration in mouse brown adipocytes.

Propranolol, atenolol and ICI 118,551 have low pA_2 values whether isoprenaline, BRL 28410 or BRL 37344 is the antagonist, which shows that all three agonists act via an atypical receptor. However, Stock & Sudera (1988) found lower pA_2 values for all three antagonists when BRL 37344 was the agonist than when isoprenaline was the agonist. Similarly, Jones *et al.* (1989) found lower pA_2 values for propranolol with BRL 28410 as the agonist than with isoprenaline as the agonist. This trend was also apparent in our own studies (Table 4), but was not statistically significant. One possible explanation for these results is that the bulky N-substituent of BRL 37344 or BRL 28410 binds to an accessory site and allosterically reduces the affinity of the main binding site for standard antagonists (see Ariëns *et al.* 1979; Ehlert, 1986). Thus, the main binding site for the agonists may be at the same point, even though pA_2 values for antagonists vary according to the agonist.

Unfortunately, there are no reports of antagonists that selectively block brown adipocyte β-adrenoceptors, though studies with white adipocytes suggest that some antagonists might discriminate against brown adipocyte β-adrenoceptors to a lesser extent than propranolol, atenolol or ICI 118,551. Such antagonists might include the d-enantiomers of standard β-blockers (Harms *et al.* 1977).

Table 4. *Values of pA_2 for antagonism of brown adipocyte lipolysis: comparison with tissues that contain β_1- or β_2-adrenoceptors*

(Values taken from Arch *et al.* (1984) and Wilson *et al.* (1984))

Antagonist . . .	Propranolol	Propranolol	Atenolol	ICI 118,551
Agonist . . .	Isoprenaline	BRL 28410	BRL 37344	BRL 37344
Rat brown adipocyte lipolysis	7·3	6·8	5·2	5·7
Rat right atrial tension	8·7	8·7	7·2	7·2
Guinea-pig tracheal tension	8·3	8·6	5·7	8·7

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Table 5. *Potency orders of β-adrenoceptor agonists*

(Compounds were ordered by potency, expressed as EC_{50} value relative to each compound's own maximum effect)

Rat brown adipocyte lipolysis	BRL 37344 > isoprenaline > fenoterol > salbutamol = BRL 28410 = prenalterol
Rat atrial rate and tension (β_1)	Isoprenaline > prenalterol ≧ fenoterol > salbutamol ≧ BRL 37344 > BRL 28410
Rat uterine tension (β_2)	Isoprenaline > fenoterol > salbutamol > BRL 37344 > BRL 28410 > prenalterol
Guinea-pig tracheal tension (β_2)	Isoprenaline ≧ fenoterol > salbutamol ≧ BRL 37344 > prenalterol > BRL 28410

Novel selective agonists

The existence of atypical β-adrenoceptors in brown adipocytes has been confirmed by the discovery of agonists, such as BRL 28410 and BRL 37344, that selectively stimulate these receptors. Isoprenaline is more potent as a stimulant of right atrial rate or tension (mediated by β_1-adrenoceptors) or as a relaxant of uterine or tracheal tension (mediated by β_2-adrenoceptors) than as a stimulant of rat brown adipocyte lipolysis. In contrast, BRL 28410 and BRL 37344 are more potent as stimulants of lipolysis (Arch *et al.* 1984). This shows that the brown adipocyte β-adrenoceptor can be distinguished from β_1- and β_2-adrenoceptors. Furthermore the β_1-selective agonist prenalterol is 100-fold more potent as a stimulant of atrial rate than as a stimulant of lipolysis, and the β_2-selective agonists fenoterol and salbutamol are respectively 50- and 100-fold more potent as relaxants of the uterus and trachea.

Potency orders summarizing these results are shown in Table 5. The supremacy of BRL 37344 as lipolytic stimulant compared with its low placing for the other responses illustrates the atypical nature of the brown adipocyte β-adrenoceptor. BRL 28410 has a low potency even as a stimulant of lipolysis, but its potency relative to the other agonists (excluding BRL 37344) is much higher for rat brown adipocyte lipolysis than the other responses.

The evidence that BRL 28410 selectively stimulates brown adipocyte β-adrenoceptors has been extended to the rabbit by Jones *et al.* (1989). Comparison of their results for stimulation of brown adipocyte respiration with those of Wilson & Lincoln (1984) for stimulation of rabbit right atrial rate shows that BRL 28410 is 2·5-fold more potent as a stimulant of respiration than as a stimulant of atrial rate. In contrast, isoprenaline is about 450-fold more potent as a stimulant of atrial rate.

Can the atypical receptor be detected by binding studies?

The receptor-binding studies summarized in Table 3 have failed to reveal the atypical β-adrenoceptor in brown adipocytes. Furthermore, Levin & Sullivan (1986) found that another of the Beecham compounds that selectively stimulate brown adipocyte lipolysis, BRL 35113 (Arch *et al.* 1984), displaced the labelled antagonist CGP-12177 from only two subtypes of β-adrenoceptor in rat brown adipocyte membranes. These subtypes were assumed to be β_1- and β_2-adrenoceptors, which may be correct, though Levin & Sullivan (1986) did not measure the affinity of BRL 35113 for receptors in tissues known to contain primarily β_1- or β_2-adrenoceptors. We have not studied the binding of the Beecham compounds to brown adipocyte membranes, but these compounds are also selective stimulants of white adipocyte lipolysis, and we have shown that BRL 28410 has

similar K_d values for the displacement of [^{3}H]dihydroalprenolol from rat lung, atrial and white adipose tissue membranes (23, 32 and 30 μM respectively in the absence of GTP) (P. Young and J. R. S. Arch, unpublished results).

The simplest explanation for these results and those of Levin & Sullivan (1986) on BRL 35113 is that the labelled ligand bound to β_1- or β_2-adrenoceptors, but it was used at too low a concentration to bind to atypical receptors. For example, we used 3 nM-[^{3}H]dihydroalprenolol. This is appropriate for binding to functional β_1- and β_2-adrenoceptors because the pA_2 value of l-alprenolol at these receptors indicates a K_d value of about 1 nM. However, the pA_2 value of l-alprenolol in white adipocytes indicates a K_d value for atypical receptors of about 150 nM. If a high concentration of the labelled ligand were used it might remain difficult to detect the atypical receptor against the background of binding to β_1- and β_2-adrenoceptors and non-specific sites. Detection of the atypical receptor by binding studies would appear to require the use of a labelled ligand that binds selectively to the atypical receptor.

An alternative explanation for the lack of selectivity of BRL 28410 in binding studies is that it selectively stimulates lipolysis, not because it binds selectively to the brown adipocyte receptor, but because it has higher efficacy at this receptor than at β_1- or β_2-adrenoceptors. Such an explanation may be proposed for the functional β_2-selectivity of fenoterol and salbutamol, since these compounds do not bind selectively to β_2-adrenoceptors (Minneman *et al.* 1981). However, the difficulty of interpreting binding studies in the β-adrenoceptor field is illustrated by a report that fenoterol does not show selective efficacy at β_2-adrenoceptors (O'Donnell & Wanstall, 1977). Furthermore, the selective β_2-agonists butoxamine and H35/25, which have zero efficacy at β_1- and β_2-adrenoceptors, also fail to bind selectively to β_2-adrenoceptors (Minneman *et al.* 1981).

The question remains to be addressed as to the role of the β_1- (and possibly also β_2-) adrenoceptors in brown adipocytes that are detected by receptor-binding studies. From their studies on rat white adipocytes Bojanic *et al.* (1985) concluded that the β_1-adrenoceptors are poorly coupled to adenylate cyclase (*EC* 4.6.1.1), or are greatly outnumbered by atypical receptors. The failure of the β_1-adrenoceptors to contribute significantly to the functional activity of isoprenaline was demonstrated by the finding that these receptors could be selectively inactivated using the photoaffinity antagonist *p*-aminobenzylcarazolol without affecting the adenylate cyclase response (Bojanic & Nahorski, 1984). It seems unlikely that β_1-adrenoceptors in adipocytes have no role, and one obvious possibility is that they are precursors or products of the atypical receptors.

Conclusion

Even though the receptors have not been detected by binding studies, there is overwhelming evidence from studies using both antagonists and agonists that the functional β-adrenoceptor in brown adipocytes can be differentiated from β_1- and β_2-adrenoceptors. Atypical adrenoceptors of this type were first discovered by Harms *et al.* (1977) in rat white adipocytes and similar or even more atypical receptors may mediate insulin secretion (Furman & Tayo, 1974; Ahren & Lundquist, 1981; M. V. Sennitt and M. A. Cawthorne, unpublished results), and relaxation of the gut (Coleman *et al.* 1987; Bond *et al.* 1988; Croci *et al.* 1988). Tan & Curtis-Prior (1983) proposed that the rat white adipocyte receptor should be described as β_3. The danger in introducing a term such as this is that it invites a proliferation of subtypes: β-adrenoceptors in brown and white adipocytes and in gut may be similar but not identical, there are almost certainly species differences, and there may be all shades of subtypes between β_1, β_2 and the extreme atypical receptor. Nevertheless, it is time to recognize that there is a group of similar

β-adrenoceptors, characterized by a low affinity for propranolol and other standard β-blockers, and marked responsiveness to BRL 37344, that cannot be described as β_1 or β_2. This group of receptors will attain their due recognition only if they are described as 'β_3-adrenoceptors'.

REFERENCES

Ahrén, B. & Lundquist, I. (1981). *European Journal of Pharmacology* **71,** 93–104.

Arch, J. R. S., Ainsworth, A. T., Cawthorne, M. A., Piercy, V., Sennitt, M. V., Thody, V., Wilson, C. & Wilson, S. (1984). *Nature* **309,** 163–165.

Arch, J. R. S., Brooks, B. J., Thurlby, P. L. & Wilson, S. (1986). *Biochemical Society Transactions* **14,** 230–233.

Ariëns, E. J., Beld, A. J., Rodrigues de Miranda, J. F. & Simonis, A. M. (1979). In *The Receptors*, pp. 33–91 [R. D. O'Brien, editor]. New York and London: Plenum Press.

Ariëns, E. J. & Simonis, A. M. (1983). *Biochemical Pharmacology* **32,** 1539–1545.

Baresi, L. A., Morley, J. E. & Scarpace, P. J. (1986). *Federation Proceedings* **45,** 351.

Bojanic, D., Jansen, J. D., Nahorski, S. R. & Zaagsma, J. (1985). *British Journal of Pharmacology* **48,** 131–137.

Bojanic, D. & Nahorski, S. R. (1984). *Journal of Receptor Research* **4,** 21–35.

Bond, R. A., Blue, D. R. & Clarke, D. E. (1988). *British Journal of Pharmacology* **93,** Proceedings Suppl., 21P.

Bukowiecki, L., Follea, N., Paradis, A. & Collet, A. (1980). *American Journal of Physiology* **238,** E552–E563.

Bukowiecki, L., Follea, N., Vallieres, J. & LeBlanc, J. (1978). *European Journal of Biochemistry* **92,** 189–196.

Coleman, R. A., Denyer, L. H. & Sheldrick, K. E. (1987). *British Journal of Pharmacology* **90,** Proceedings Suppl., 40P.

Croci, T., Cecchi, R., Tarantino, A., Aureggi, G., Bianchetti, A., Boigegrain, R. & Manara, L. (1988). *Pharmacological Research Communications* **20,** 147–151.

Ehlert, F. J. (1986). *Trends in Pharmacological Sciences* **7,** 28–32.

Feist, D. (1983). *American Journal of Physiology* **245,** R357–R363.

Furman, B. L. & Tayo, F. M. (1974). *Journal of Pharmacy and Pharmacology* **26,** 512–517.

Grassby, P. F., Arch, J. R. S., Wilson, C. & Broadly, K. J. (1987). *Biochemical Pharmacology* **36,** 155–162.

Harms, H. H., Zaagsma, J. & De Vente, J. (1977). *Life Sciences* **21,** 123–128.

Harris, W. H., Moore, L. A. & Yamashiro, S. (1986). *Canadian Journal of Physiology and Pharmacology* **64,** 133–137.

Jones, T. W. G., Brown, S., Carver, L. & Spencer, N. (1989). *Proceedings of the Nutrition Society* **48,** 36A.

Kenakin, T. P. (1984). *British Journal of Pharmacology* **81,** 131–141.

Kurahashi, M. & Kuroshima, A. (1981). *Biomedical Research* **2,** 126–133.

Lands, A. M., Luduena, F. P. & Buzzo, H. T. (1967). *Life Sciences* **6,** 2241–2249.

Levin, B. E. & Sullivan, A. C. (1986). *Journal of Pharmacology and Experimental Therapeutics* **236,** 681–688.

Minneman, K. P., Pittman, R. N. & Molinoff, P. B. (1981). *Annual Review of Neuroscience* **4,** 419–461.

Mohell, N., Nedergaard, J. & Cannon, B. (1983). *European Journal of Pharmacology* **93,** 183–193.

Naito, K., Nageo, T., Otsuka, M., Harigaya, S. & Nakajima, H. (1985). *Japanese Journal of Pharmacology* **38,** 235–241.

O'Donnell, S. R. & Wanstall, J. C. (1977). *British Journal of Pharmacology* **60,** 255–262.

O'Donnell, S. R. & Wanstall, J. C. (1981). *Journal of Autonomic Pharmacology* **1,** 305–312.

O'Donnell, S. R. & Wanstall, J. C. (1987). *Trends in Pharmacological Sciences* **8,** 265–268.

Rothwell, N. J., Stock, M. J. & Stribling, D. (1982). *Pharmacology and Therapeutics* **17,** 251–268.

Rothwell, N. J., Stock, M. J. & Sudera, D. K. (1985). *American Journal of Physiology* **248,** E397–E402.

Rothwell, N. J., Stock, M. J. & Winter, P. D. O'B. (1983). *Journal of Physiology* **340,** 62P–63P.

Senault, C., Le Comte, V. & Portet, R. (1984). *Biochimie* **66,** 573–578.

Stock, M. J. & Sudera, D. K. (1988). *British Journal of Pharmacology* **95,** Proceedings Suppl., 741P.

Svoboda, P., Svartengren, J., Snochowski, M., Houstek, J. & Cannon, B. (1979). *European Journal of Biochemistry* **102,** 203–210.

Tan, S. & Curtis-Prior, P. B. (1983). *International Journal of Obesity* **7,** 409–414.

Wilson, C. & Lincoln, C. (1984). *Journal of Cardiovascular Pharmacology* **6,** 1216–1221.
Wilson, C., Wilson, S., Piercy, V., Sennitt, M. V. & Arch, J. R. S. (1984). *European Journal of Pharmacology* **100,** 309–319.
Young, J. B., Rosa, R. M. & Landsberg, L. (1984). *American Journal of Physiology* **247,** E35–E40.

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