Biochemical Pharmacology, Vol. 36, No. 1, pp. 155–162, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
© Pergamon Journals Ltd.

BETA-ADRENOCEPTOR SENSITIVITY OF BROWN AND WHITE ADIPOCYTES AFTER CHRONIC PRETREATMENT OF RATS WITH RESERPINE

PAUL F. GRASSBY,*† JONATHAN R. S. ARCH,‡ CAMPBELL WILSON‡§ and KENNETH J. BROADLEY*¶

*Department of Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff CF1 3XF, and ‡Beecham Pharmaceuticals Research Division, Great Burgh, Epsom, Surrey KT18 5XQ, U.K.

(*Received* 25 *June* 1986; *accepted* 11 *August* 1986)

Abstract—The effect of pretreatment with reserpine (1.0 mg/kg i.p. daily for 7 days) on beta-adrenoceptor-mediated responses has been measured in epididymal white and interscapular brown adipocytes, left atria and vas deferens of rats in order to investigate the classification of the receptors and whether they are innervated. Lipolysis was measured in adipocytes, and from the same rats, the $beta_1$-adrenoceptor-mediated positive inotropic responses of isolated paced left atria and $beta_2$-adrenoceptor-mediated inhibition of field stimulation-induced contraction of the vas deferens were examined. The agonists used were isoprenaline, oxyfedrine (atria only) and (except in brown adipocytes) ritodrine, which was a partial agonist in white adipocytes, atria and vas deferens. Atria and brown adipocytes exhibited beta-adrenoceptor supersensitivity after reserpine pretreatment, whereas vas deferens and white adipocytes did not. Reserpine-induced reductions in food intake and body weight did not appear to influence beta-adrenoceptor-mediated lypolysis, since restriction of the diet equivalent to that of reserpine-treated rats produced no change in white adipocyte sensitivity. Responses mediated via $beta_1$-, but not $beta_2$-adrenoceptors, display supersensitivity after chronic depletion of neuronal catecholamines with reserpine and this is evidence for innervation of this receptor subtype. Thus, atrial $beta_1$-adrenoceptors are assumed to be innervated, whereas vas deferens $beta_2$-adrenoceptors are not. The present results are consistent with histochemical evidence that brown, but not white, adipocyte beta-adrenoceptors are innervated. However, they are not compatible with conventional receptor classification studies, which suggest that rat brown and white beta-adrenoceptors are similar—either both $beta_1$ or both atypical.

The physiological responses to sympathetic nerve stimulation and sympathomimetic amines are mediated via postjunctional alpha- and beta-adrenoceptors. Mammalian beta-adrenoceptors have been subclassified into the $beta_1$ and $beta_2$ subtypes [1]. A possible physiological basis for this subdivision is that $beta_1$-adrenoceptors are innervated by the sympathetic nerves whereas $beta_2$-adrenoceptors are non-innervated and stimulated by circulating adrenaline [2, 3]. In support of this concept is the fact that the neurotransmitter, noradrenaline, is as potent or more potent than adrenaline at $beta_1$-adrenoceptors, but adrenaline is more potent at $beta_2$-adrenoceptors [4].

An alternative approach to determining whether the beta-adrenoceptors of a tissue are innervated is the use of depletion-induced supersensitivity. Chronic depletion of neuronal catecholamines by reserpine or 6-hydroxydopamine results in a slowly developing increase in postjunctional sensitivity to sympathomimetic amines, which is due to the loss of endogenous neurotransmitter release [5]. Our previous studies have indicated that reserpine and 6-hydroxydopamine pretreatments induce supersensitivity for the responses of the heart and ileum mediated via $beta_1$-adrenoceptors, but not the responses which are mediated via $beta_2$-adrenoceptors, such as in lung, vas deferens and aorta [6–8]. Thus $beta_1$-adrenoceptors exhibit supersensitivity because they are under the direct influence of neurotransmitter release from sympathetic neurones and can be considered as innervated. The failure of $beta_2$-adrenoceptors to display supersensitivity indicates that they are not under the influence of the neurone.

In the present study we have extended this principle to the beta-adrenoceptors mediating lipolysis in rat brown and white adipocytes. There has been considerable debate concerning the nature of the beta-adrenoceptors involved in these responses. The original subclassification of Lands *et al.* [1] based on relative potencies of natural and synthetic catecholamines, placed the receptor that mediates lipolysis in rat white adipose tissue into the $beta_1$-category and this has been supported by use of radioligand binding techniques [9, 10]. Similar methods have suggested that the beta-adrenoceptors

† Present address: Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, B.C., Canada U6T 1W5.

§ Present address: Beecham Pharmaceuticals Research Division, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD, U.K.

¶ To whom correspondence should be addressed.

of rat brown adipose tissue are also of the $beta_1$-subtype [11, 12]. However, studies on the lipolytic response with selective agonists and antagonists have failed to support this subclassification. The $beta_2$-selective agonist salbutamol was found to induce lipolysis of rat white adipocytes to the same extent as isoprenaline, whilst the $beta_1$-selective agonist tazolol was ineffective [13, 14]. Studies with selective antagonists have shown that a correlation with either $beta_1$- or $beta_2$-adrenoceptor effects occurred depending on the structure of the antagonist used. To explain these results, a hybrid beta-adrenoceptor was proposed which displayed characteristics of both subtypes [15, 16]. A mixed population of $beta_1$- and $beta_2$-adrenoceptors in rat white adipocytes has been discounted since pA_2 values for inhibition of lipolysis or cAMP accumulation by selective antagonists were the same whether selective $beta_1$- (noradrenaline) or $beta_2$-agonists (salbutamol or fenoterol) were employed [16–18]. Further evidence for the unusual nature of the receptor that mediates lipolysis is that in white adipocytes this receptor shows less discrimination than atrial ($beta_1$) or diaphragm ($beta_2$) receptors between stereoisomers of standard antagonists [19] and in both white and brown adipocytes pA_2 values of antagonists for inhibition of lipolysis are generally low compared with pA_2 values for antagonism of $beta_1$- and $beta_2$-adrenoceptors [18–21]. It has therefore been suggested [18] that rat white adipocytes possess both $beta_1$-adrenoceptors, which are detected by conventional radioligand binding techniques but are not coupled to lipolysis, and atypical beta-adrenoceptors, which mediate lipolysis but, because of their low affinity for the radioligand antagonists, have not been detected by binding techniques. Finally, support for an atypical beta-adrenoceptor mediating lipolysis comes from the description of a series of novel agonists with selective lipolytic activity in rat white [20] and brown [21] adipocytes.

How then does this atypical beta-adrenoceptor conform to the hypothesis of innervated $beta_1$-adrenoceptors and non-innervated $beta_2$-adrenoceptors? In an attempt to answer this question we have examined lipolysis in white and brown adipocytes from reserpine-pretreated rats and compared this with $beta_1$-adrenoceptor-mediated responses of left atria and the $beta_2$-adrenoceptor-mediated relaxation responses of vas deferens removed from the same animals.

MATERIALS AND METHODS

Male Wistar rats (250–350 g) that had received reserpine (1 mg/kg/day i.p.) or no treatment for 7 days were killed by a blow to the head. Isolated left atria and vasa deferentia were removed and epididymal white and interscapular brown adipocytes were prepared. Atria and vas deferens were suspended in organ baths containing Krebs–bicarbonate solution (composition in mM: NaCl 118.4; KCl 4.7; $CaCl_2 \cdot 2H_2O$ 1.9; $NaHCO_3$ 25.0; $MgSO_4 \cdot 7H_2O$ 1.2; glucose 11.7; KH_2PO_4 1.2) gassed with 5% CO_2 in oxygen and maintained at 38 ± 0.5°. Metanephrine (10 μM) and phentolamine (5 μM) were present throughout to inhibit extraneuronal uptake and alpha-adrenoceptors respectively. The tissues were attached via cotton threads to transducers (Devices UF1, 57 g sensitivity range) and isometric tension was recorded on a Devices M19 polygraph (Lectromed).

Left atria. After opening the thorax to expose the heart, a cotton thread was passed through the atrial wall to secure the tissue to bipolar platinum electrodes. A second thread through the atrial appendage was passed to the transducer. A resting diastolic tension of 0.5–0.8 g was applied. The atria were paced at 2 Hz with square-wave pulses of 5 msec duration and a voltage of threshold + 50% delivered by an SRI stimulator (type 6053). After a 30-min stabilization period, during which the atria were washed several times, a cumulative concentration–response curve to isoprenaline was constructed by half-logarithmic increments in concentration. The tissue was washed to restore baseline rate and tension to the pre-isoprenaline level. A second concentration–response curve to either ritodrine or oxyfedrine was then constructed. Control experiments were performed, in which two consecutive concentration–response curves for isoprenaline were constructed. The total tension developed at each concentration of agonist was measured. In control experiments, the values on the second curve for isoprenaline were expressed as a fraction of those on the first curve. This factor was applied to the corresponding concentration of the isoprenaline curve of test experiments. Increases in tension were then calculated by subtracting the resting level (corrected in the case of the isoprenaline curve). These values were then expressed as a percentage of the corrected isoprenaline maximum response.

Vas deferens. Complete vas deferens were passed through bipolar ring electrodes, at the base of which was incorporated a tissue holder to which one end of the tissue was secured. The other end was attached via cotton thread to the transducer and a resting tension of 1 g was applied. Contractions were induced by transmural stimulation with 3-sec trains of square-wave pulses (10 Hz, 1 msec pulse width, supramaximal voltage) every 30 sec. When contractions of constant height were obtained, beta-adrenoceptor-mediated relaxation responses were obtained as inhibition of contraction height. A cumulative concentration–response curve to isoprenaline was followed, after washout, by one to ritodrine. At the maximum effect of ritodrine, a maximum concentration of isoprenaline was added. Changes in tension in response to ritodrine were then expressed as a percentage of this isoprenaline-induced maximum inhibition.

Preparation of adipocytes. White adipocytes were prepared from the epididymal fat pads according to the method of Rodbell [22]. The fat pads were chopped coarsely with scissors and incubated with collagenase (8 mg per g of tissue) in pregassed (CO_2 in oxygen) Krebs–bicarbonate solution modified to include 4% w/v bovine serum albumin faction V and adjusted to pH 7.4. The digestion was carried out for 1 hr at 37° in sealed plastic tubes in a shaking water bath. The resulting cell suspension was filtered through a plastic gauze strainer, the fat cells were allowed to float to the surface and the medium below was removed. The cells were washed twice and

diluted to a final volume of 5 ml per g of the original tissue with modified Krebs–bicarbonate solution.

Brown adipocytes were prepared from interscapular fat pads removed from groups of 12 animals. A similar method to that described above was used with three modifications. A greater quantity of collagenase was employed (12 mg per g of tissue), a trypsin inhibitor (4 mg per g) was included and the digestion was carried out for only 30 min, shaking the tubes vigorously by hand every 10 min.

Determination of the lipolytic response to beta-adrenoceptor agonists. The cells were stirred to maintain a homogeneous suspension whilst 450-μl portions were removed for incubation with beta-adrenoceptor agonists in a final volume of 500 μl. Incubations were carried out in sealed incubation tubes, pre-gassed with 5% CO_2 in oxygen and maintained at 37° in a shaking water bath. The reaction was stopped after 30 min by addition of 200 μl of 10% trichloroacetic acid and the resultant precipitate was separated by centrifugation at $2000 \times g$ for 15 min. Aliquots (150 μl) of supernatant were removed from each tube and transferred to a plastic cuvette for determination of the glycerol produced by the enzymatic method of Garland and Randle [23].

The lipolytic response to beta-adrenoceptor agonist was determined by incubation with a range of concentrations (half logarithmic increments) in the presence of phentolamine (5 μM) and metanephrine (10 μM). Each agonist concentration was used in triplicate for a single batch of cells, and the mean was used to obtain a single (N = 1) concentration–response curve. In brown adipocytes only isoprenaline was used as the agonist in view of the large number of animals required for a single concentration–response curve, whereas in white adipocytes, both isoprenaline and ritodrine were used for each batch of cells. Absolute rates of glycerol production were expressed relative to the weight of adipose tissue from which the cells were obtained (μmol/g of tissue/hr). The basal level of lipolysis, obtained in the absence of agonist, was subtracted from each agonist-induced production of glycerol. The values for both isoprenaline and ritodrine were then expressed as a percentage of the maximum response to isoprenaline in the same cell suspension.

Analysis of results. Left atria and vas deferens were taken from rats selected at random from control and pretreated groups. The sensitivity to isoprenaline of individual atria, vas deferens and batches of adipocytes was expressed as the EC_{50} value. Individual molar EC_{50} values for isoprenaline were obtained by linear interpolation between points on either side of the 50% of maximum response on uncorrected concentration–response curves. Geometric mean EC_{50} values were calculated together with their 95% confidence limits. The sensitivity to the partial agonists ritodrine or oxyfedrine was expressed as the mean maximum response (±SE) relative to the isoprenaline maximum (corrected from control experiments in the case of atria). Statistical comparisons between tissues from control and pretreated animals were made by Student's unpaired *t*-test. Bars on concentration–response curves show SE.

Drugs and reagents. The following sources were used: bovine serum albumin factor V (Sigma), collagenase (Boehringer Ingleheim), glycerokinase (Sigma), (±)-isoprenaline bitartrate dihydrate (Ward Blenkinsop), lactate dehydrogenase (Sigma), (±)-metanephrine hydrochloride (Sigma), (−)-nicotinamide adenine dinucleotide (NADH) (Sigma), (±)-oxyfedrine hydrochloride (Ciba-Geigy), phosphoenolpyruvate (Sigma), pyruvate kinase (Sigma), reserpine (BDH), (±)-ritodrine hydrochloride (Duphar) and trypsin inhibitor (Type II-S, Sigma). Drugs were freshly prepared in distilled water and ascorbic acid (1 g/ml) was added to the isoprenaline solutions. Solutions of reserpine were prepared by dissolving reserpine (250 mg) and an equal weight of citric acid and benzyl alcohol (2 ml). Tween 80 (10 ml) was added and the concentration adjusted to 2.5 mg/ml with distilled water.

RESULTS

Left atrial sensitivity

Isoprenaline, ritodrine and oxyfedrine produced concentration-dependent increases in left atrial tension. Ritodrine and oxyfedrine behaved as partial agonists with maxima of 35.3 ± 5.4 and 16.3 ± 6.3% of the isoprenaline maxima in atria from untreated rats (Fig. 1, Table 1). After pretreatment with reserpine, the sensitivity of the atria to isoprenaline was increased, as shown by the leftwards displacement of the concentration–response curve (Fig. 1) and the significant reduction ($P < 0.001$) of the mean EC_{50} value (Table 1). The maximum responses of ritodrine and oxyfedrine were also significantly elevated ($P < 0.001$) in the atria from reserpine-pretreated rats to 65.7 ± 3.5 and 89.0 ± 10.5%, respectively (Fig. 1, Table 1).

There was a signficant increase ($P < 0.01$) in the basal developed tension of atria from reserpine-pretreated rats, but the maximum response to isoprenaline measured in absolute units of total tension did not differ significantly ($P > 0.05$) between control and reserpine-pretreated animals (Table 1).

Vas deferens sensitivity

Isoprenaline and ritodrine had concentration-dependent inhibitory effects upon the twitch responses of the rat vas deferens. Ritodrine was a

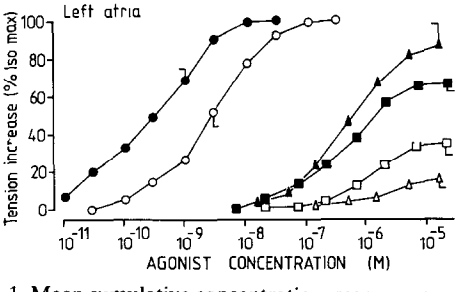

Fig. 1. Mean cumulative concentration–response curves for the increase in tension responses to isoprenaline (N = 12; ○, ●), ritodrine (N = 7; □, ■) and oxyfedrine (N = 5; △, ▲) of left atria from untreated (open symbols) and reserpine-pretreated rats (closed symbols).

Table 1. Mean EC_{50} values (with 95% confidence limits) for isoprenaline and maximum responses (as a percentage of the isoprenaline maximum) to partial agonists (±SE) in left atria and vas deferens from control and reserpine-pretreated rats

Tissue	Agonist	Untreated control	N	Reserpine pretreated	N
Left atria	Isoprenaline EC_{50} (nM)	3.13 (1.7–5.75)	7	0.38** (0.2–0.8)	6
	Ritodrine max. (%)	35.3 ± 5.4	7	65.7 ± 3.5**	6
	Oxyfedrine max. (%)	16.3 ± 6.2	5	89.0 ± 10.5**	6
	Basal developed tension (g)	0.11 ± 0.02	7	0.24 ± 0.04*	6
	Isoprenaline max tension (g)	0.25 ± 0.02	7	0.30 ± 0.02 (NS)	6
Vas deferens	Isoprenaline EC_{50} (nM)	3.67 (2.54–5.3)	7	2.81 (NS) (1.13–6.94)	6
	Ritodrine max. (%)	67.9 ± 2.0	5	70.4 ± 4.8 (NS)	6
	Resting twitch height (g)	1.56 ± 0.09	7	1.61 ± 0.16 (NS)	6
	Isoprenaline max inhibition (g)	1.02 ± 0.31	7	1.28 ± 0.1 (NS)	6

Comparisons between tissues from untreated and reserpine pretreated rats were made by Student's unpaired *t*-test; NS, not significant $P > 0.05$, *$P < 0.01$, **$P < 0.001$.

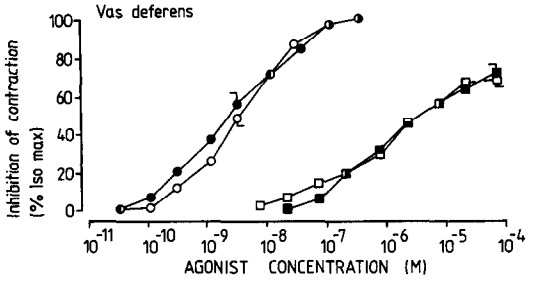

Fig. 2. Mean cumulative concentration–response curves for the inhibition by isoprenaline (N ≥ 6; ○, ●) and ritodrine (N ≥ 5; □, ■) of electrically induced contractions of the vas deferens of untreated (open symbols) and reserpine-pretreated rats (closed symbols).

partial agonist with a maximum response of 67.9 ± 2.0% of the isoprenaline maximum in vas deferens from untreated rats (Fig. 2, Table 1). After reserpine pretreatment, the concentration–response curves for both isoprenaline and ritodrine were superimposable upon those of untreated rats (Fig. 2). Neither the mean EC_{50} value for isoprenaline nor the maximum response to ritodrine (70.4 ± 4.8% of isoprenaline maximum) were significantly different ($P > 0.05$) from the corresponding values for untreated controls (Table 1).

The tension developed during resting twitch contractions and the maximum inhibition by isoprenaline were not significantly different ($P > 0.05$) between vas deferens from untreated and reserpine-pretreated rats (table 1).

Sensitivity of white adipocytes

Isoprenaline and ritodrine produced concentration-related increases in the rate of white adipocyte lipolysis. Ritodrine was a partial agonist producing a maximum response of 61.6 ± 3.3% of the isoprenaline maximum with adipocytes from untreated rats (Fig. 3A). The concentration–response curves obtained with adipocytes from reserpine-pretreated rats were superimposable upon the control curves (Fig. 3A) and there was no significant difference ($P > 0.05$) between the EC_{50} values of isoprenaline or between the maxima of ritodrine (Table 2). When plotted as the absolute change in glycerol released (μmol/g of tissue/hr), the concentration–response curves were again superimposable (Fig. 3B) and the absolute maxima of isoprenaline or ritodrine did not differ significantly ($P > 0.05$) between

Table 2. Mean EC_{50} values (with 95% confidence limits) for isoprenaline and maximum responses (as a percentage of the isoprenaline maximum) to ritodrine (±SE) for beta-adrenoceptor-mediated lipolysis in white and brown adipocytes from control and reserpine-pretreated rats.

	Isoprenaline EC_{50} (nM)				Ritodrine (% ISO max)			
	Control	N	Reserpine	N	Control	N	Reserpine	N
White adipocyte	20.9 (11.0–39.0)	6	17.6 NS (6.2–49.8)	4	61.6 ± 3.3	4	69.5 ± 7.8 NS	4
Brown adipocyte	8.4 (4.9–14.2)	4	1.45* (0.17–12.1)	4	—	—	—	—

Comparisons between adipocytes from control and reserpine-pretreated rats were made by Student's unpaired *t*-test, NS, not significant $P > 0.05$, *$P < 0.05$.

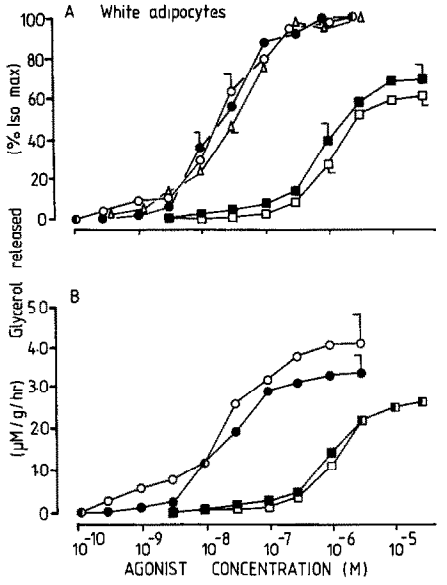

Fig. 3. Mean ($N \geq 4$) concentration–response curves for the lipolysis responses to isoprenaline (○, ●) and ritodrine (□, ■) of white adipocytes from untreated (open symbols) and reserpine-pretreated rats (closed symbols). Responses are plotted as increases in glycerol released expressed as (A) a percentage of the isoprenaline maximum response and (B) absolute units of μmol/g of tissue/hr. Also shown is the curve for isoprenaline obtained in diet-restricted rats (△).

white adipocytes of control (4.12 ± 0.75 and 2.72 ± 0.53 μmol/g/hr) and reserpine-pretreated rats (3.45 ± 1.15 and 2.54 ± 0.97 μmol/g/hr, respectively). The basal level of lipolysis was also not significantly different ($P > 0.05$) between untreated and reserpine-pretreated rat white adipocytes (Fig. 4).

Sensitivity of brown adipocytes

Isoprenaline increased the rate of lipolysis in brown adipocytes in a concentration-related manner (Fig. 5). Brown adipocytes from reserpine-pretreated rats were supersensitive to isoprenaline as shown by the leftwards shift of the concentration–response curve (Fig. 5A) and the significantly reduced ($P < 0.05$) EC_{50} value (Table 2). When

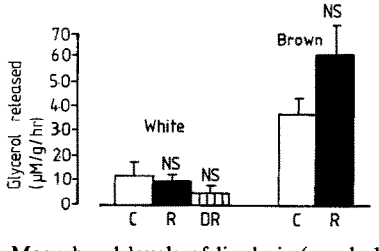

Fig. 4. Mean basal levels of lipolysis (μmol glycerol/g of tissue/hr) in brown and white adipocytes from control (C), reserpine-treated (R) and diet-restricted rats (DR). NS, no significant difference between reserpine-pretreated or diet-restricted rats and controls ($P > 0.05$).

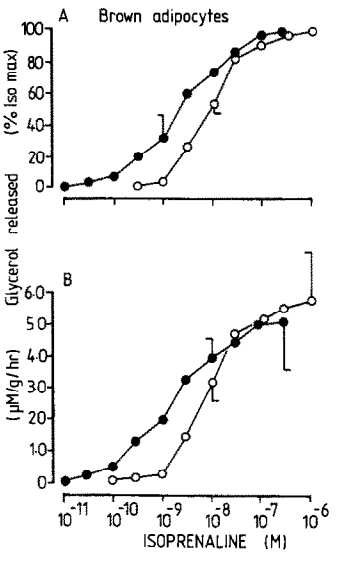

Fig. 5. Mean ($N = 4$) cumulative concentration–response curves for the lipolysis response to isoprenaline of brown adipocytes from untreated (○) and reserpine-pretreated rats (●). Responses are plotted as increases in glycerol released expressed as (A) a percentage of the maximum response and (B) absolute units of μmol/g of tissue/hr.

expressed as the absolute change in glycerol released (μmol/g of tissue/hr), the concentration–response curve was again displaced to the left by reserpine pretreatment (Fig. 5B) but with no significant change ($P > 0.05$) in the absolute maximum response. The basal level of lipolysis was greater in brown than in white adipocytes (Fig. 4), however, the values obtained with brown adipocytes from control and reserpine-pretreated rats were not significantly different (Fig. 4).

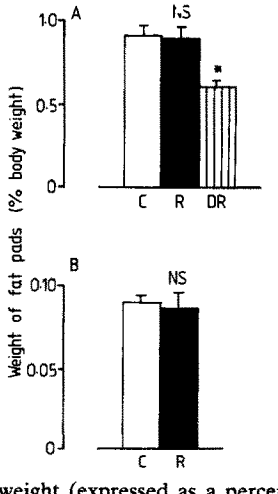

Fig. 6. Mean weight (expressed as a percentage of total body weight) of (A) epididymal white and (B) interscapular brown fat pads from untreated control (C), reserpine-pretreated (R) and diet-restricted (DR) rats. Difference between control and reserpine-pretreated or diet-restricted rats. NS, not significant; *$P < 0.05$.

Effect on lipolysis of the weight loss associated with reserpine pretreatment

A major effect associated with reserpine pretreatment is a loss of total body weight. The possible effect of this weight loss upon the mass of the epididymal white and interscapular brown fat pads and the sensitivity to isoprenaline was therefore examined. Rats pretreated with reserpine for 7 days incurred a weight loss of 14.6 ± 2.3% (N = 10). The mass of epididymal and interscapular fat pads was reduced in proportion, so that when expressed as a percentage of body weight, the values were the same for control and reserpine-pretreated animals (Fig. 6).

To mimic this weight loss, the diet of a group of rats (N = 6) was restricted to match the food intake of a group (N = 6) of reserpine-pretreated animals. The food intake during the 7-day period of reserpine pretreatment was reduced overall by 49.6%. The mean weight loss of untreated rats receiving this restricted food profile (8.6 ± 0.2%) was significantly less ($P < 0.05$) than after reserpine treatment. However, the mass of epididymal fat pad removed from these animals was significantly lower ($P < 0.05$) than from either control or reserpine-pretreated rats (when expressed as a percentage of total body weight) (Fig. 6).

The sensitivity to isoprenaline of white adipocytes from fasted animals was similar to that of white adipocytes from control and reserpine-pretreated rats. The concentration–response curves were superimposable (Fig. 3A) and the EC_{50} value [35.0(10.4–118)nM] was not significantly different ($P > 0.05$) from either the control or reserpine-pretreated values. Furthermore, the basal level of lipolysis was not significantly different ($P > 0.05$) from that of controls or pretreated rats (Fig. 4).

DISCUSSION

Chronic pretreatment of rats with reserpine resulted in supersensitivity of the atrial beta$_1$-adrenoceptor-mediated effects of isoprenaline, ritodrine and oxyfedrine. This confirms previous studies with guinea-pig and rabbit cardiac tissue [24–26]. In contrast, white adipocytes from these animals exhibited the same sensitivity to the lipolytic effects of isoprenaline and ritodrine as untreated animals. Ritodrine was a partial agonist, the maximum response being identical in adipocytes of both untreated and pretreated animals. The possibility was considered that the loss of body weight arising from reserpine pretreatment may have resulted in a reduced white adipose tissue mass and a subsensitivity to the lipolytic effect of isoprenaline; this would offset any supersensitivity. However, diet restriction of rats caused a loss of white adipose tissue mass greater in proportion to body weight than after reserpine pretreatment and yet the white adipocytes were equally sensitive to the lipolytic effect of isoprenaline. Thus it is unlikely that the failure to exhibit supersensitivity was due to an opposing subsensitivity due to the anorexic effect of reserpine.

In contrast to white adipocytes, brown adipocytes were supersensitive to isoprenaline after reserpine pretreatment. The supersensitivity in smooth and cardiac muscle that occurs after chronic depletion of catecholamines by reserpine is attributed to the loss of endogenous neurotransmitter release into the synaptic cleft [5]. A compensatory increase in sensitivity of the effector organ occurs with an upregulation of adrenoceptors, possibly through receptor proliferation [27]. This suggests that the beta-adrenoceptors mediating lipolysis in brown adipose tissue are under the direct influence of sympathetic innervation, whereas those in white adipose tissue are not.

Histochemical evidence for the innervation of brown adipose tissue is good. A rich supply of sympathetic fibres has been shown to innervate the blood vessels with a network of terminals surrounding the brown adipocytes in many species including the rat [28–31]. The situation with white adipose tissue is less clear. Early studies indicated that it was only the larger blood vessels of rat white adipose tissue that received a sympathetic innervation [32–34]. This contrasts with brown adipose tissue where there are abundant nerve terminals around the adipocytes [35]. More recently, morphological evidence has shown that the adipocytes do receive a sparse sympathetic innervation in the rat [31, 34, 36, 37]. This poor innervation does not explain why sympathetic nerve stimulation to white adipose tissue of rats *in vitro* [38] causes a lipolytic response. A possible reason for this is that a wave of membrane depolarization may spread from a few innervated cells to adjacent non-innervated cells [37]. Alternatively, noradrenaline released from neurones innervating blood vessels may reach the white adipocytes via the blood stream [32].

Section of the sympathetic nerves supplying canine white adipose tissue has been shown to induce supersensitivity of the lipolytic response to noradrenaline [39]. However, this was probably a presynaptic effect due to uptake inhibition rather than an effect at postjunctional beta-adrenoceptors, since isoprenaline, which is not a substrate for neuronal uptake, did not display supersensitivity [39]. This agrees with the present experiments where reserpine was the depleting agent. The lack of supersensitivity to isoprenaline in rat white adipocytes is therefore consistent with the adipocytes receiving a sparse innervation in comparison with brown adipocytes, where supersensitivity occurred and which are known to be densely innervated. Indeed, the beta-adrenoceptor agonist, Ro 16–8714, has been shown to induce a brown adipose tissue-dependent calorigenic response in rats which exhibited supersensitivity after catecholamine depletion by reserpine and 6-hydroxydopamine [40].

Previous studies have indicated that depletion-induced supersensitivity occurs for responses mediated via beta$_1$-adrenoceptors but not those mediated via beta$_2$-adrenoceptors [6–8]. The present results with the left atria and vas deferens of the rat confirm this finding. The positive inotropic responses of the rat left atria are mediated via a homogeneous population of beta$_1$-adrenoceptors [41] and exhibited supersensitivity to isoprenaline, oxyfedrine and ritodrine. The inhibition of vas deferens contractions, in contrast, is a beta$_2$-adrenoceptor-mediated response [42] which did not display supersensitivity. This conforms to the hypothesis of Ariëns [2, 3] that beta$_1$-

adrenoceptors are innervated receptors stimulated primarily by neurotransmitter ($beta_T$-receptors) since loss of endogenous transmitter by depletion would directly influence receptor sensitivity. In contrast, $beta_2$-adrenoceptors are considered to be non-innervated and stimulated by the circulating hormone, adrenaline ($beta_H$-receptors).

How then do the observations on white and brown adipocyte sensitivity relate to their receptor classification and density of innervation? At first sight it would appear that the brown adipocyte beta-adrenoceptor behaves like typical $beta_1$-adrenoceptors—they are innervated and display the expected supersensitivity. The failure of white adipocytes to exhibit supersensitivity and the apparently sparse innervation suggests that it should be a $beta_2$-adrenoceptor. Examination of the literature, however, shows that the beta-adrenoceptor classification of rat brown and white adipocytes is equivocal compared with cardiac and smooth muscle.

According to potency orders of adrenaline and noradrenaline [1, 12] and radioligand binding [9–11], the beta-adrenoceptors mediating lipolysis in rat white and brown adipocytes are of the $beta_1$-subtype. However, as described in the introduction, a number of studies on the lipolytic responses to synthetic agonists and their antagonism have led to the suggestion that the receptor mediating lipolysis is of a similar atypical type in both white and brown adipocytes [13–16, 19–21]. One group has proposed that there is a heterogeneous population of functional $beta_1$- and $beta_2$-adrenoceptors in rat brown adipose tissue [43], but this is not the case with the functional receptors in white adipocytes, since selective $beta_1$- and $beta_2$-antagonists give identical pA_2 values with selective antagonists [16–18]. It is worth noting, however, that the characteristics of the typical $beta_1$-adrenoceptor of white adipocytes are only revealed when endogenous catecholamine agonists are used. It is possible that these adrenoceptors have evolved through mutations such that the normal interactions with catecholamines are retained, but synthetic agonists and antagonists combine with the receptor in an abnormal fashion [44].

Thus standard pharmacological approaches to receptor characterization do not place the beta-adrenoceptor of rat white and brown adipocytes in to either the $beta_1$ or $beta_2$ subtypes. The observation that reserpine-induced supersensitivity occurs for responses mediated via $beta_1$- but not $beta_2$-adrenoceptors, when applied to lipolysis, also fails to clarify the receptor classification. The results do permit the conclusion that white adipocytes of rats are poorly innervated whereas brown adipocytes are probably densely innervated.

Acknowledgements—This work was supported by a Science Research Council Co-operative Award in Science and Engineering. We are grateful to Duphar and Ciba-Geigy for gifts of drugs.

REFERENCES

1. A. M. Lands, A. Arnold, J. P. McAuliff, F. P. Luduena and T. G. Brown, Jr, *Nature, Lond.* **214**, 597 (1967).
2. E. J. Ariëns and A. M. Simonis, in *Beta-Adrenoceptor Blocking Agents, the Pharmacological Basis of Clinical Use* (Eds P. R. Saxena and R. P. Forsyth), p. 3. North-Holland, Amsterdam (1976).
3. E. J. Ariëns and A. M. Simonis, *Biochem. Pharmac.* **32**, 1539 (1983).
4. R. F. Furchgott, in *Handbook of Experimental Pharmacology* (Eds H. Blaschko and E. Muscholl), p. 283. Springer Verlag, Berlin (1972).
5. W. W. Fleming, J. J. McPhillips and D. P. Westfall, *Ergeb. Physiol.* **68**, 55 (1984).
6. M. H. Hawthorn and K. J. Broadley, *Eur. J. Pharmac.* **105**, 245 (1984).
7. K. J. Broadley, R. G. Chess-Williams and P. F. Grassby, *J. Physiol.* **373**, 367 (1986).
8. P. F. Grassby and K. J. Broadley, *J. Pharmac. exp. Ther.* **237**, 950 (1986).
9. L. T. Williams, L. Jarrett and R. J. Lefkowitz, *J. biol. Chem.* **251**, 3096 (1976).
10. D. Bojanic and S. R. Nahorski, *Eur. J. Pharmac.* **93**, 235 (1983).
11. L. Bukowiecki, N. Follea, J. Vallieres and J. Leblanc, *Eur. J. Biochem.* **92**, 189 (1978).
12. L. Bukowiecki, N. Follea, A. Paradis and A. Collet, *Am. J. Physiol.* **238**, E552 (1980).
13. S. R. Jolly, J. J. Lech and L. A. Menahan, *Biochem. Pharmac.* **27**, 1885 (1978).
14. S. Tan and P. B. Curtis-Prior, *Int. J. Obesity* **7**, 409 (1983).
15. H. H. Haarms, J. Zaagsma and B. Van de Wal, *Eur. J. Pharmac,* **25**, 87 (1974).
16. J. De Vente, A. Bast, L. Van Bree and J. Zaagsma, *Eur. J. Pharmac.* **63**, 73 (1980).
17. E. Belfrage and B. B. Fredholm, Abstract 7th International Congress of Pharmacology, IUPHAR, Paris p. 553 (1978).
18. D. Bojanic, J. D. Jansen, S. R. Nahorski and J. Zaagsma, *Br. J. Pharmac.* **84**, 131 (1985).
19. H. H. Harms, J. Zaagsma and J. De Vente, *Life Sci.* **21**, 123 (1977).
20. C. Wilson, S. Wilson, V. Piercy, M. V. Sennitt and J. R. S. Arch, *Eur. J. Pharamc.* **100**, 309 (1984).
21. J. R. S. Arch, A. T. Ainsworth, M. A. Cawthorne, V. Piercy, M. V. Sennitt, V. E. Thody, C. Wilson and S. Wilson, *Nature, Lond.* **309**, 163 (1984).
22. M. Rodbell, *J. biol. Chem.* **239**, 375 (1964).
23. P. B. Garland and P. J. Randle, *Nature, Lond.* **196**, 987 (1962).
24. K. J. Broadley and P. Lumley, *Br. J. Pharmac.* **59**, 51 (1977).
25. T. E. Tenner Jr., J. H. McNeill and O. Carrier Jr., *Eur. J. Pharmac.* **50**, 359 (1978).
26. D. K. Meisheri, T. E. Tenner Jr. and J. H. McNeill, *Life Sci.* **24**, 473 (1979).
27. T. E. Tenner, Jr, A. Mukherjee and R. K. Hester, *Eur. J. Pharmac.* **77**, 61 (1982).
28. C. Wirsen and B. Hamberger, *Nature. Lond.* **214**, 625 (1967).
29. W. J. Cottle, in *Brown Adipose Tissue* (Ed. O. Lindberg), p. 155. American Elsevier, New York (1970).
30. M. K. Cottle and W. H. Cottle, *J. Histochem. Cytochem.* **18**, 116 (1970).
31. B. B. Fredholm, in *New Perspectives in Adipose Tissue: Structure, Function and Development* (Eds A. Cryer and R. L. R. Van), p. 45. Butterworths, London (1985).
32. C. Wirsen, *Nature, Lond.* **202**, 913 (1964).
33. B. Ballantyne and A. T. Raftery, *J. Physiol.* **205**, 31P (1969).
34. S. Rosell and E. Belfrage, *Physiol. Rev.* **59**, 1078 (1979).
35. H. Daniel and D. M. Derry, *Can. J. Physiol. Pharmac.* **47**, 941 (1969).

36. I. Diculescu and M. Stoica, *J. Neuro-Visc. Relat.* **32**, 25 (1970).
37. B. G. Slavin and K. W. Ballard, *Anat. Res.* **191**, 377 (1978).
38. J. W. Correll, *Science* **140**, 387 (1963).
39. S. Rosell and E. Belfrage, *Nature, Lond.* **253**, 738 (1975).
40. M. K. Meier, M. E. Alig, M. E. Bürgi-Saville and M. Müller, *Int. J. Obesity* **8**, Suppl 1, 215 (1984).
41. C. Wilson and C. Lincoln, *J. Cardiovasc. Pharmac.* **6**, 1216 (1984).
42. E. Krstew, E. Malta and C. Raper, *J. Pharmac. Meth.* **8**, 279 (1982).
43. N. J. Rothwell, M. J. Stock and D. K. Sudera, *Am. J. Physiol.* **248**, E397 (1985).
44. H. H. Harms, J. De Vente and J. Zaagsma, *Br. J. clin. Pharmac.* **13**, 181S (1982).