Pergamon

Biochemical Pharmacology, Vol. 50, No. 5, pp. 601–608, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00185-9

SELECTIVE ACTIVATION OF BROWN ADIPOCYTE HORMONE-SENSITIVE LIPASE AND cAMP PRODUCTION IN THE MOUSE BY β_3-ADRENOCEPTOR AGONISTS

MEI-FEN SHIH AND PETER V. TABERNER*

Department of Pharmacology, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, U.K.

(*Received* 16 *August* 1994; *accepted* 13 *April* 1995)

Abstract—Acute injection of either noradrenaline or isoprenaline in mice activated both brown (BAT) and white (WAT) adipose tissue hormone-sensitive lipase activity (HSL). Dose–response studies indicated that isoprenaline (0.05–0.15 mg/kg) produced a dose-dependent activation of HSL in both BAT and WAT, whereas SR 58611A produced no change in HSL in WAT over a dose range (1–5 mg/kg) which, at the same time, dose-dependently increased HSL activity in BAT. The other β_3-adrenoceptor agonists, ZD 7114 (10 mg/kg) and BRL 35135 A (5 mg/kg) also selectively increased HSL activity in BAT, these doses having previously been shown to stimulate lipogenesis *in vivo*. Higher doses of ZD 7114 and BRL 35135 produced no further increase in HSL activity and, in the case of BRL 35135, provoked symptoms of non-selective β-adrenoceptor activation. The increase in HSL activity could be prevented by pretreating the mice with propranolol, 10 mg/kg, i.p., 30 min prior to the agonist. The activation of HSL activity by the β_3-adrenoceptor agonists was associated with an increase in tissue cAMP production which was also prevented by pretreatment with propranolol. The degree of cAMP accumulation was least with BRL 35135 and greatest with ZD 7114. We conclude that, in the mouse adipocyte, the atypical β-adrenoceptor (β_3) is present in BAT, but is not present or functional in WAT.

Key words: β_3-adrenoceptor; cAMP; hormone-sensitive lipase

BAT† and WAT are now recognized as being anatomically and functionally distinct. In addition to lipogenesis and lipolysis, BAT has the specific function of thermogenesis, in which the uncoupled oxidation of lipids, derived from intracellular stores of triglyceride, leads directly to heat production. BAT thermogenesis can be activated indirectly by feeding or exposure to cold (non-shivering thermogenesis), or directly by the action of catecholamines acting on adipocyte adrenoceptors [1]. Although all three β-adrenoceptor subtypes had originally been identified in the adipose tissue of various species, as a result of both binding and functional studies [2, 3], the increasing availability of selective β_3-adrenoceptor agonists has suggested that the lipolytic response to noradrenaline in the rat is mediated specifically via β_3 receptors on BAT [3–5].

Whereas β_3-adrenoceptor agonists such as BRL 35135 and BRL 37344 [4], SR 58611A [6, 7] and ZD 7114 [7, 8] have all been shown to activate BAT *in vivo*, the cellular response at the enzyme level to β-adrenoceptor activation has not been fully investigated. However, the cellular signalling process by which β-agonists stimulate adipocyte lipolysis is well documented. β-Adrenoceptors, linked to G_S proteins, activate adenylate cyclase to increase cAMP formation, which activates a cAMP-dependent kinase which, in turn, phosphorylates and activates hormone-sensitive lipase [9]; HSL (EC 3.1.1.34) being the key enzyme in the regulation of lipolysis. Murphy *et al.* [10], using BRL 37344, have recently shown that lipolysis in rat white adipose tissue can be directly correlated with the activation of cAMP-dependent protein kinase. However, a number of species-specific and tissue-specific differences in β_3 receptor distribution and function have been reported [2, 11, 12]. Also, the relationship between cAMP production and lipolysis in white adipocytes appears to vary between species [13]; adipocytes from the rat showing a much steeper dose response relationship for isoprenaline than human tissue. The cloning of the atypical β_3-adrenoceptor has tended to confirm its identity with the atypical receptor of adipose tissue [11, 14]. Isolation of the gene which specifically encodes the mouse β_3-adrenoceptor has demonstrated an 82% homology with the human atypical (β_3) adrenoceptor, as well as a pharmacological profile which closely resembles the human receptor [14]. We have therefore compared the effects of three different β_3-agonists *in vivo* on HSL activity and cAMP production in both BAT and WAT of the mouse in order to determine the relative functional significance of the β_3-adrenoceptors in these tissues. A preliminary account

* Corresponding author. Tel. 0117 928 7637; FAX 0117 925 0168.

† Abbreviations: BAT, brown adipose tissue; cAMP, adenosine 3′:5′-cyclic phosphate; HSL, hormone-sensitive lipase; WAT, white adipose tissue; BRL 35135 A, R*, R* - (±) - methyl - 4 - (2 - (2 - hydroxy - 2 - (3 - chlorophenyl) - ethylamino) - propyl) - phenoxyacetate HBr; ZD 7114, (*S*) - 4 - (2 - hydroxy - 3 - phenoxypropylaminoethoxy) - *N* - (2 - methoxyethyl)phenoxyacetamide HCl; SR 58611 A, ethyl - ((7*S*) - 7 - ((2*R*) - 2 - (3 - chlorophenyl) - 2 - hydroxyethylamino)5,6,7,8-tetrahydronaphthalen-2-yloxy)acetate HCl.

of some of these findings has been published in abstract form [15].

MATERIALS AND METHODS

Materials. Triolein, oleic acid, phosphatidylcholine, leupeptin, hemisulphate salt, pepstatin A, DL-dithiothreitol, collagenase (Type I), cAMP, noradrenaline, isoprenaline and propranolol were all obtained from the Sigma Chemical Company (Poole, Dorset, U.K.). Glycerol tri[9,10 (*n*)-^{3}H]-oleate, [1-^{14}C] oleic acid and [8-^{3}H]cAMP, ammonium salt were obtained from Amersham International (Aylesbury, Bucks, U.K.). BRL 35135 A, was a gift from SmithKline Beecham Pharmaceuticals (Epsom, Surrey, U.K.); ZD7114 was a gift from Zeneca Pharmaceuticals (Alderley Park, Cheshire, U.K.); SR 58611 A was a gift from Sanofi Midy S.P.A. (Milan, Italy). Binding protein was kindly provided by Dr E Kelly at the University of Bristol.

Animals. Male CBA/Ca mice, bred within the University of Bristol Medical School, were 4–5 months old with body weight 30–34 g. They were housed at 20 ± 1° with 12 hr light cycle (9 a.m. to 9 p.m.).

Drug treatments. Drugs were made up freshly in physiological saline (0.9%, w/v) for i.p. injection. Ascorbate (10^{-4} M) was added as a preservative for noradrenaline and isoprenaline in solution. A single dose of noradrenaline (0.6 mg/kg), isoprenaline (0.1 mg/kg), BRL 35135 A (5 mg/kg), ICI D7114 (10 mg/kg), or SR 58611 A (2 mg/kg) was administered 1 hr prior to the enzyme or cAMP assays. This time period was selected since it corresponds to the observed time course for the acute thermogenic effects of the β-agonists *in vivo* [7, 16]. Control mice received an equivalent volume of saline. Propranolol (10 mg/kg) was given 30 min prior to the agonist. All treatments were carried out between 9.30 and 11.30 a.m.

Enzyme preparation. Interscapular BAT was dissected out, and the connective tissue removed. The clean BAT was minced, then homogenized in 10 volumes of pH 7.0 medium containing 0.25 M sucrose, 1 mM EDTA, 4 μg/mL leupeptin, 1 μg/mL pepstatin A, and 1 mM dithiothreitol. This was centrifuged at 105 000 *g* for 45 min at 4°. The top 'fat cake' was removed and the clear infranatant

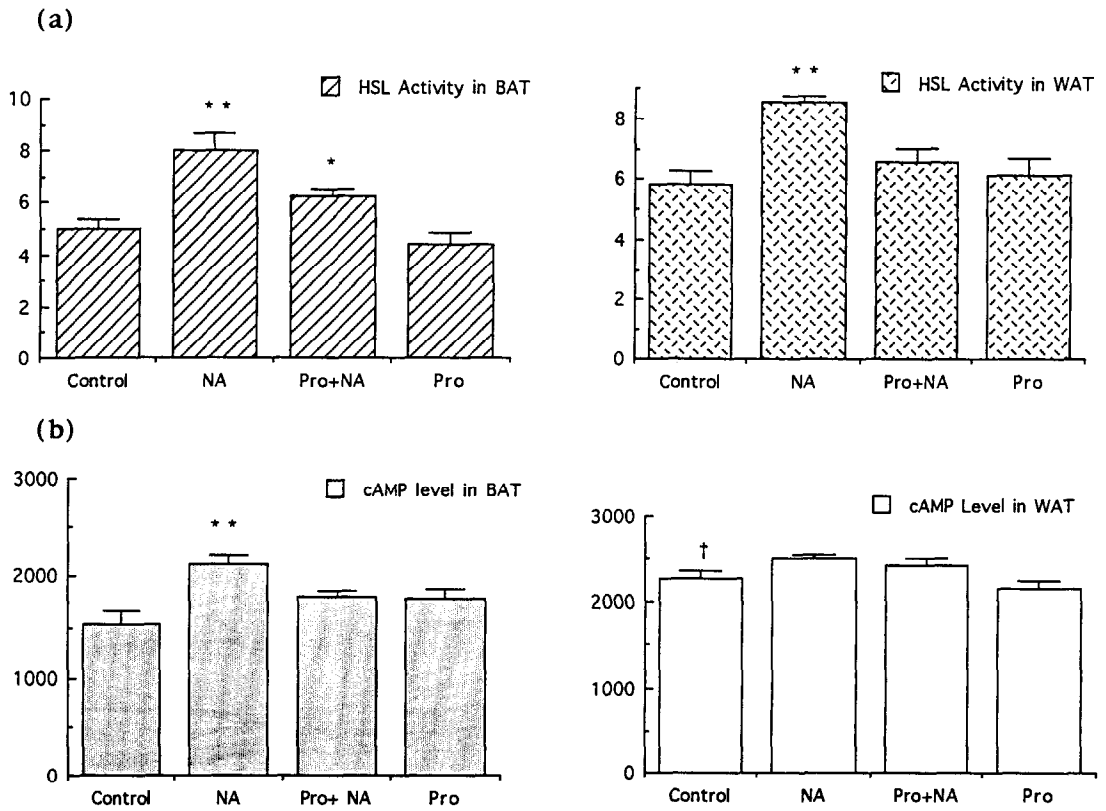

Fig. 1. Effects of noradrenaline (NA, 0.6 mg/kg) and propranolol (Pro, 10 mg/kg) on: (a) hormone sensitive lipase activity (expressed as nmol free fatty acid released/min/mg protein); (b) cAMP accumulation (expressed as pmol/mg protein) in brown and white adipose tissue. Propranolol was administered 30 min prior to the noradrenaline (Pro + NA). Data are the means ± SEM of 6–7 independent observations performed in triplicate. * Pro + NA < NA, $P < 0.05$; ** NA > Control, $P < 0.01$; † cAMP in WAT > cAMP in BAT, $P < 0.01$.

fraction decanted and used for the assay of HSL activity [17]. Epididymal white fat pads (WAT) were removed and prepared as for BAT, except that 2 vols of homogenization medium were used.

HSL activity assay. This was based on the standard assay procedure of Nilsson-Ehle and Schotz [18]. The assay substrate was prepared immediately before use to give a final concentration of each reagent in the 200 μL assay volume as follows: 100 mmol/L Tris HCl, pH 7.0; 5 g/L of BSA (fatty acid-free, fraction V), and 4.58 mmol/L triolein as substrate. (For the detailed preparation of triolein emulsion see ref. [18]). The omission of apolipoprotein CII from the assay medium and the addition of NaCl (250 mmol/L) ensured that no lipoprotein lipase activity would be present.

The assay tubes were incubated at 37° with 100 cycles/min shaking, and the reaction begun by adding 100 μL of the assay substrate. After 15 min incubation, the reaction was stopped by adding 3.25 mL of methanol/chloroform/heptane (1.41:1.25:1), 50 μL of [^{14}C]oleic acid (2 mM, 0.33 μCi/mL) as internal standard for estimating recovery [19], and 1 mL of extraction buffer (K_2CO_3 buffer, pH 10.5). The assay tubes were then vortexed for 30 sec and centrifuged at 3000 rpm for 10 min at room temperature. A 0.5 mL aliquot of the aqueous upper phase was taken for double-label liquid scintillation spectrometry in LSC cocktail (Packard, Groningen, The Netherlands). Protein concentrations were determined by the Coomassie Blue method of Bradford [20]. The results (medians of triplicate determinations) were expressed as nmol FFA released/min/mg protein.

cAMP accumulation. Adipocytes were prepared from BAT and WAT tissue according to Rodbell [21] but using Krebs–Ringer phosphate buffer, pH 7.4, containing 128 mM NaCl, 1.4 mM $CaCl_2$, 1.4 mM $MgSO_4$, 5.2 mM KCl, and 10 mM Na_2HPO_4. The concentration of collagenase was 3 mg/mL for BAT and 1 mg/mL for WAT and the incubation time was reduced to 30 min [22]. The suspended adipocytes were washed three times with collagenase-free Krebs–Ringer phosphate buffer containing 4% BSA. HCl (20 μL of 2M) was added to a 200 μL volume of suspended cells. The mixture was vortexed and then centrifuged at 3000 *g* for 5 min at 4°. Finally, 150 μL of the supernatant was collected and frozen at −20° for the cAMP measurement.

cAMP determination. A standard competitive binding assay was used to assay tissue cAMP [23]. Briefly, standard cAMP or sample cAMP plus [^{3}H]-

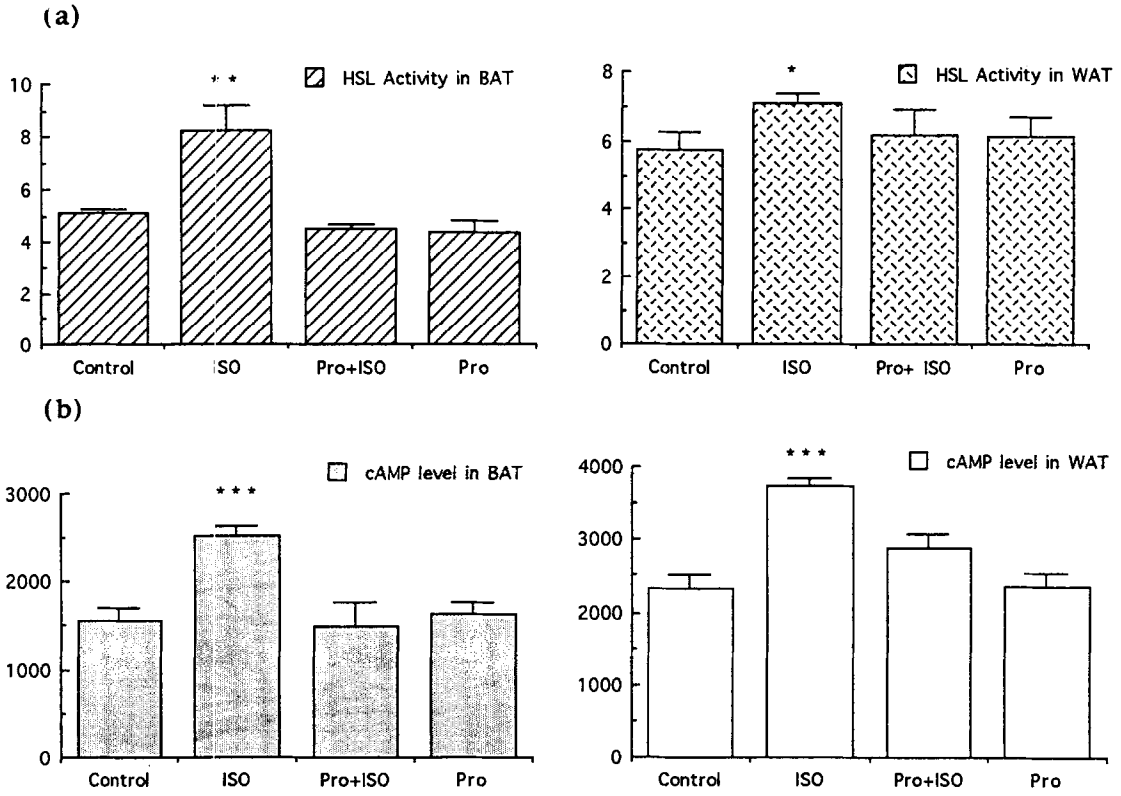

Fig. 2. Effects of isoprenaline (ISO, 0.1 mg/kg) and propranolol (Pro, 10 mg/kg) on: (a) hormone sensitive lipase activity (expressed as nmol free fatty acid released/min/mg protein); (b) cAMP accumulation (expressed as pmol/mg protein) in brown and white adipose tissue. Propranolol (10 mg/kg) was administered 30 min prior to the isoprenaline (Pro + ISO). Data are the means ± SEM of 6–7 independent observations performed in triplicate. Isoprenaline > control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

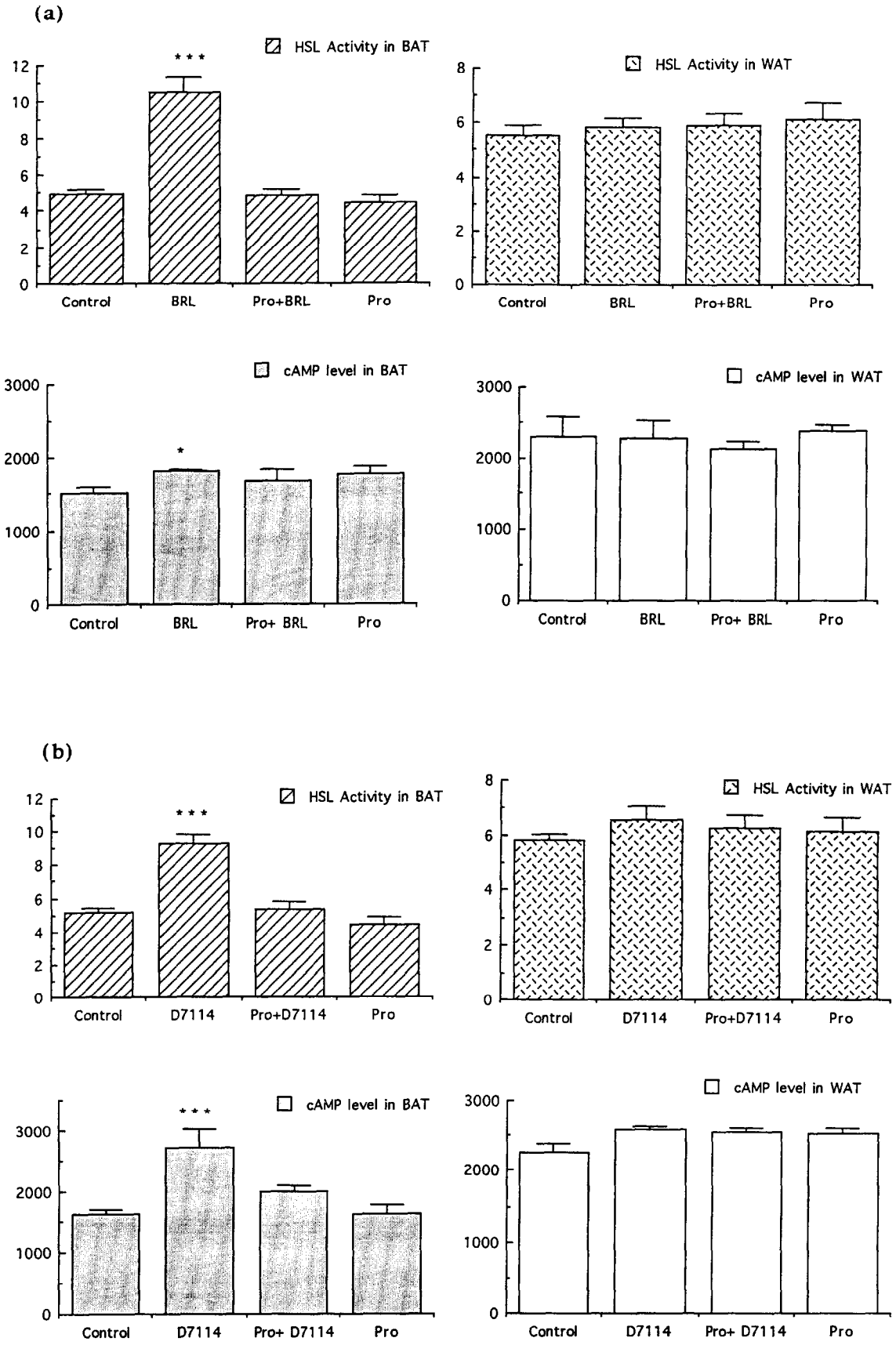
(a)
HSL Activity in BAT
***
12
10
8
6
4
2
0
Control
BRL
Pro+BRL
Pro
HSL Activity in WAT
8
6
4
2
0
Control
BRL
Pro+BRL
Pro
cAMP level in BAT
3000
2000
1000
0
*
Control
BRL
Pro+ BRL
Pro
cAMP level in WAT
3000
2000
1000
0
Control
BRL
Pro+ BRL
Pro
(b)
HSL Activity in BAT
***
12
10
8
6
4
2
0
Control
D7114
Pro+D7114
Pro
HSL Activity in WAT
8
6
4
2
0
Control
D7114
Pro+D7114
Pro
cAMP level in BAT
***
3000
2000
1000
0
Control
D7114
Pro+ D7114
Pro
cAMP level in WAT
3000
2000
1000
0
Control
D7114
Pro+ D7114
Pro

Fig. 3(a) and (b).

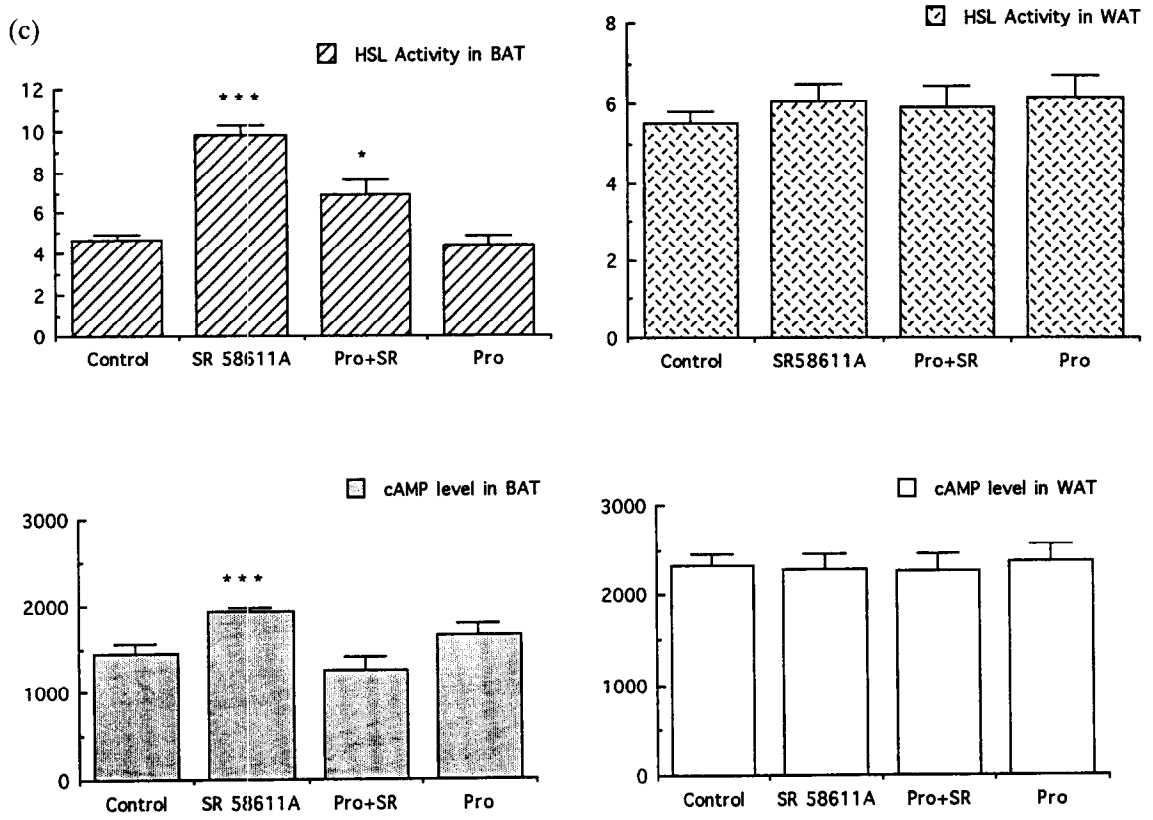

Fig. 3. Effects of selective β_3 adrenoceptor agonists on hormone sensitive lipase activity (expressed as nmol free fatty acid released/min/mg protein), and cAMP accumulation (expressed as pmol/mg protein) in brown and white adipose tissue. (a) BRL 35135 (BRL, 5 mg/kg), (b) ICI D7114 (D7114, 10 mg/kg), (c) SR 58611A (SR, 2 mg/kg). Propranolol (Pro, 10 mg/kg) was administered 30 min prior to the agonists. Data are the means ± SEM of 6–7 independent observations performed in triplicate. Drug treatment > control: * $P < 0.05$; *** $P < 0.005$.

cAMP (40 nCi) in Tris-HCl/EDTA buffer (50 mM/4 mM, pH 7.4) were pipetted into assay tubes and the binding reaction started by adding 100 μL of diluted binding protein (prepared from bovine adrenal cortex) into the assay tubes. After 90 min incubation at 4°, 200 μL of 'charcoal' (0.25 g charcoal plus 0.1 g BSA in 50 mL of TE buffer) was added and then incubated for further 20 min at 4°. The tubes were centrifuged at 3000 × g for 15 min at 4° and the supernatant decanted into scintillation vials for counting. Each assay was performed in triplicate and standard curve data were fitted to a logistic expression. The protein content of cell suspensions was determined as described above, and the results expressed as pmol of cAMP/mg protein.

Statistical analysis. Results are shown as means ± SEM of N = 6–7 combined from two or three different experimental days. The statistical significances of the differences between groups were analysed by Student's t-test for independent samples.

RESULTS

Noradrenaline and isoprenaline stimulation of HSL

HSL activity in both brown and white adipose tissue was significantly increased following an acute dose of NA (Fig. 1a). At the same time there was an equivalent increase in the cAMP level in BAT and a smaller, non-significant increase in WAT (Fig. 1b). Although the levels of HSL activity in BAT and WAT were very similar, the accumulation of cAMP under control conditions was significantly greater (by 50%) in WAT compared to BAT. This suggests that the link between HSL activity and cAMP level may be different in the two tissues. The increases in HSL activity and BAT cAMP level were blocked by pretreating the mice with propranolol 30 min prior to the NA. Propranolol alone at the same dose had no effect on HSL activity or cAMP accumulation in either tissue.

A single acute dose of isoprenaline produced equivalent parallel increases in HSL (Fig. 2a) and cAMP accumulation (Fig. 2b) in both brown and white adipose tissue. Although the increase in HSL activity in WAT was similar in magnitude to that produced by noradrenaline, there was, in contrast, a highly significant increase in cAMP (by 40%). These increases were also blocked by pretreatment with the same dose of propranolol that had blocked the stimulation produced by NA.

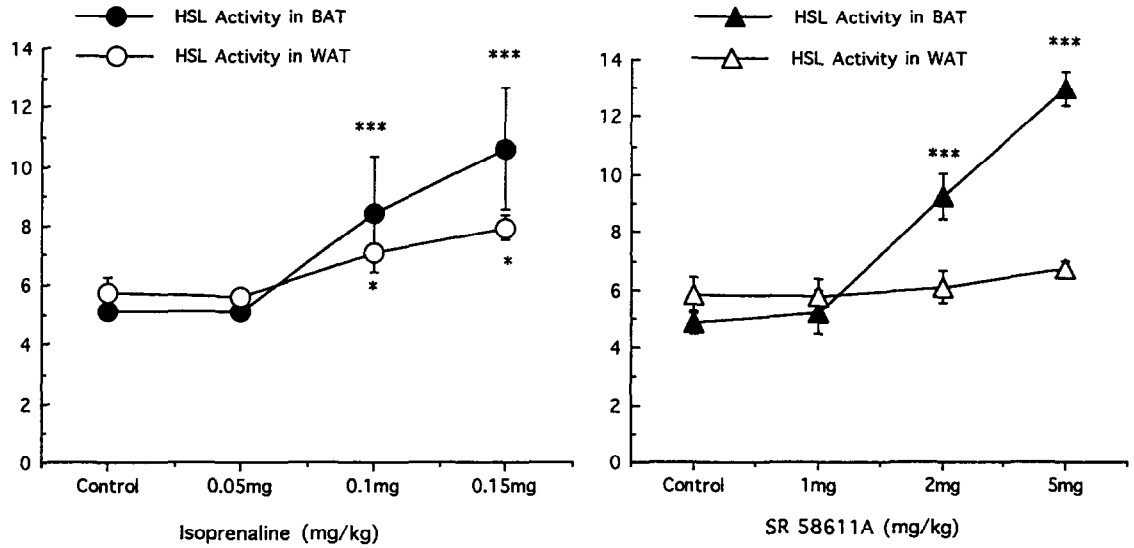

Fig. 4. Dose–response effect of isoprenaline and SR 58611A on hormone-sensitive lipase activity (expressed as nmol free fatty acid released/min/mg protein) in brown and white adipose tissue. Drug treatment < control: * $P < 0.05$; *** $P < 0.005$.

β_3-Agonist stimulation of HSL

The same experiment was repeated with the three selective β_3 agonists BRL 35135A, ICI D7114, and SR 58611A at doses which had previously been found to maximally stimulate adipose tissue fatty acid synthesis *in vivo* [6, 7]. All three drugs produced significant increases in HSL activity and cAMP accumulation in BAT (Fig. 3). It is worth noting that, although the degree of stimulation of BAT HSL activity was very similar for the three drugs at the doses used, the concomitant increase in cAMP varied widely, being lowest for BRL 35135 and highest for ICI D7114. In WAT, in contrast to the responses to noradrenaline and isoprenaline, no stimulation of HSL activity was observed. The doses of both BRL 35135 (5 mg/kg) and ICI D7114 (10 mg/kg) were already the maximum compatible with a selective β_3-agonist effect. Higher doses (BRL 35135: 7.5 mg/kg and ICI D7114: 15 mg/kg) produced no further increase in HSL activity in BAT and no activation of HSL in WAT. cAMP accumulation was not measured at these higher doses. However, in order to eliminate the possibility that the difference in the tissue responses to the selective β_3-agonists was dose-related, the dose–response relationships for isoprenaline and SR 58611A on HSL activity in BAT and WAT were compared (Fig. 4). It is evident that, whereas isoprenaline produced dose-dependent increases in HSL activity in both tissues, BAT showing a larger increase than WAT, SR 58611A was completely selective towards BAT over a five-fold dose range and had no effect on WAT HSL activity.

DISCUSSION

Previous studies of the correlation of β-adrenoceptor activation, cAMP production and lipolysis have tended to use isolated white adipocytes or cultured cells *in vitro* (for example, refs [2, 10, 13, 22, 24, 25]). However, since one important therapeutic application of the β_3-agonists would be to reduce obesity, presumably through the activation of lipolysis and thermogenesis in WAT and BAT, respectively, the *in vivo* effects of these drugs on lipolytic activity is also of importance. This is the first study which has undertaken concomitant measurement of HSL activation in BAT and WAT following the *in vivo* administration of a number of β-agonists in the mouse, and in which cAMP accumulation has been measured over the same time period. We have already demonstrated, using a functional assay, that the selective β_3-agonists can activate BAT and increase lipogenesis *in vivo* in the mouse [6, 8]. The present findings indicate that the same drugs, under identical experimental conditions, simultaneously increase cAMP accumulation and HSL activity in BAT, and that this action can be blocked by the non-selective β-antagonist propranolol. (At present there are no selective β_3-antagonists generally available). It would therefore appear that fatty acid synthesis and lipolysis and, by inference, thermogenesis can be stimulated by β_3-adrenoceptor activation in BAT, and that this response occurs through cAMP-dependent activation of HSL.

The role of the β_3-adrenoceptor in rodent adipocyte lipolysis and thermogenesis is well documented [10–12]. Under normal circumstances, adipose tissue lipid metabolism is principally under the control of circulating catecholamines and insulin, although BAT is unusual in that it is densely innervated by the sympathetic system. It has been shown that surgical sympathectomy of the interscapular BAT in the rat leads to a reduction in sucrose-induced

lipogenesis and a specific increase in β_3-receptor mRNA levels in this tissue [26, 27]. Also, injections of β-adrenoceptor agonists eventually lead to down-regulation of β_3 mRNA after 8 hr. These and other direct neurophysiological findings [28] suggest that sympathetic innervation of BAT is important in controlling thermogenesis.

Other workers have suggested that although the β_3-receptor on white adipocytes is largely responsible for lipolysis, the β_1-subtype also has a role [29]. In this context it is interesting that, whereas exogenously administered NA in our present studies stimulated HSL is both brown and white adipose tissue, the selective β_3-agonists at the same time had no effect on HSL activity in WAT. This would suggest that β_3-adrenoceptor-mediated stimulation of lipolysis in the mouse occurs selectively in BAT and, since β_2-adrenoceptors appear to be absent in the WAT of rodents [3], the adrenoceptors responsible for stimulating lipolysis in WAT in the mouse are β_1. Our present results, based on the *in vivo* action of the selective adrenoceptor agonists are in contrast to the findings of Murphy *et al.* [10] who used isolated rat adipocytes. In their model, stimulation of WAT lipolysis consequent upon cAMP production was clearly mediated by β_3-adrenoceptor activation. In addition, Langin *et al.* [2] have demonstrated that isoprenaline and BRL 37344 (the active metabolite of BRL 35135) are approximately equipotent at stimulating lipolysis *in vitro* in white adipocytes. Their cross-species investigation of WAT β-adrenoceptor subtypes included rat, hamster, rabbit, dog and human tissue, but not the mouse. However, they also found that the same concentrations of selective β_3-agonists did not stimulate lipolysis in human white fat cells. It would appear that WAT in the mouse differs from the rat and may be more similar to the human in terms of functional β-adrenoceptor subtype. Lafontan and Berlan [11] in their recent review of the control of white and brown fat cell function, have emphasized this inter-species variation in the distribution and function of adrenoceptor subtypes in adipocytes.

Nevertheless, there is now direct molecular genetic evidence for β_3-adrenoceptor mRNA expression in human white fat cells [30] and the same authors have also found that BRL 37344 acts as a partial agonist compared to isoprenaline in an assay of lipolysis in human omental and subcutaneous fat cells, although the relative potencies were different in the two tissues [31]. The observation that glucagon injected *in vivo* in the rat suppresses β-adrenoceptor mRNA in BAT but not WAT [27] suggests that cAMP-mediated control of the expression of adipocyte β_3 adrenoceptors may also differ between brown and white fat even within the same species.

Our finding that similar levels of HSL activation in BAT were associated with differing levels of cAMP accumulation following stimulation by the different agonists support the earlier findings by Hollenga *et al.* [13] who demonstrated that the relationship between adenylyl cyclase activation and lipolysis in rat adipocytes was different for isoprenaline and BRL 37344. They suggested that compartmentalization of cAMP within the adipocyte could be responsible, which would also explain the discrepancy between adenylyl cyclase activation and lipolysis in white fat from different species. An alternative *in vivo* pharmacokinetic explanation for this variation, which we also observed in the case of NA activation of HSL in WAT (Fig. 1), is that the time course of the adrenoceptor-mediated cAMP accumulation in the two tissues could well be different for the different drugs, whereas the activity of HSL was measured at a single point at which time the cAMP level may have passed its peak. It is an unavoidable constraint of *in vivo* studies of this type that only one time point can be studied in each animal.

Although clinical trials have indicated the potential of selective β_3-agonists as anti-obesity agents in obese human subjects [32, 33], the drugs have not proved as effective as earlier studies in rodent models of obesity would suggest [4, 5] and there is still some doubt concerning the physiological significance of BAT in humans. Our present findings clearly indicate that there are within-species differences in adrenoceptor-mediated stimulation of lipolysis between BAT and WAT in terms of the receptor subtype. The physiological significance of this difference in adrenoceptor function in the mouse is still unclear, although it may reflect the different roles of the two tissues in controlling overall metabolic efficiency and energy balance in a small animal in which BAT thermogenesis is relatively important.

REFERENCES

1. Rothwell NJ and Stock MJ, Brown adipose tissue and diet-induced thermogenesis. In: *Brown Adipose Tissue* (Eds. Trayhurn P and Nicholls G), pp. 269–298. Edward Arnold, London, 1986.
2. Langin D, Portillo MP, Saulnier-Blache J-S and Lafontan M, Coexistence of three β-adrenoceptor subtypes in white fat cells of various mammalian species. *Eur J Pharmacol* **199**: 291–301, 1991.
3. Arner P, Adrenergic receptor function in fat cells. *Am J Clin Nutr* **55**: 228S–236S, 1992.
4. Arch JRS, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE, Wilson C and Wilson S, Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature* **309**: 163–165, 1984.
5. Astrup A, Toubro S, Christensen NJ and Quaade F, Pharmacology of thermogenic drugs. *Am J Clin Nutr* **55**: 246S–248S, 1992.
6. Al-Qatari M and Taberner PV, Effect of SR 58611A, a novel atypical β-adrenoceptor (β_3) agonist, on BAT lipogenesis. *Br J Pharmacol* **106**: 65P, 1992.
7. Holloway BR, Howe R, Rao BS, Stribling D, Mayers RM, Briscoe MG and Jackson JM, ICI D7114 a novel selective β-adrenoceptor agonist selectively stimulates brown fat and increases whole-body oxygen consumption. *Br J Pharmacol* **104**: 97–104, 1991.
8. Al Qatari M and Taberner PV, Effect of ICI D7114, a selective β_3-adrenoceptor agonist, on BAT lipogenesis in CBA/Ca obese mice. *J Physiol* **452**: 176P, 1992.
9. Stralfors P, Bjorgell P and Belfrage P, Hormone regulation of hormone-sensitive lipase in intact adipocytes: Identification of phosphorylated site and effects on the phosphorylation by lipolytic hormones and insulin. *Proc Natl Acad Sci USA* **81**: 3317–3321, 1984.
10. Murphy GJ, Kirkham DM, Cawthorne MA and Young P, Correlation of β_3-adrenoceptor-induced activation

of cAMP-dependent protein kinase with activation of lipolysis in rat white adipocytes. *Biochem Pharmacol* **46**: 575–581, 1993.
11. Lafontan M and Berlan M, Fat cell adrenergic receptors and the control of white and brown fat cell function. *J Lipid Res* **34**: 1057–1091, 1993.
12. Zaagsma J and Nahorski SR, Is the adipocyte β-adrenoceptor a prototype for the recently cloned atypical 'β_3 adrenoceptor'?, *Trends Pharmacol Sci* **11**: 3–7, 1990.
13. Hollenga C, Brouwer F and Zaagsma J, Differences in functional cyclic AMP compartments mediating lipolysis by isoprenaline and BRL 37344 in four adipocyte types. *Eur J Pharmacol* **200**: 325–330, 1991.
14. Nahmias C, Blin N, Elalouf J-M, Mattel MG, Strosberg AD and Emorine LJ, Molecular characterization of the mouse β_3-adrenergic receptor: relationship with the atypical receptor of adipocytes. *EMBO J* **10**: 3721–3727, 1991.
15. Shih M-F and Taberner PV, Activation of adipocyte hormone-sensitive lipase in mice by β_3 adrenoceptor agonists. *Br J Pharmacol* **112**: 599P, 1994.
16. Arch JRS, Cawthorne MA, Coney KA, Gusterson BA, Piercy V, Sennitt MV, Smith SA, Wallace J and Wilson S. β-Adrenoceptor-mediated control of thermogenesis, body composition and glucose homeostasis. In: *Obesity and Cachexia* (Eds. Rothwell NJ and Stock MJ), pp. 241–268. John Wiley and Sons, Chichester, 1991.
17. Huttunen JK, Ellingboe J, Pittman RC and Steinberg D, Partial purification and characterization of hormone sensitive lipase from rat adipose tissue. *Biochim Biophys Acta* **218**: 333–346, 1970.
18. Nilsson-Ehle P and Schotz MC, A stable, radioactive substrate emulsion for assay of lipoprotein lipase. *J Lipid Res* **17**: 536–545, 1976.
19. Belfrage P and Vaughan M, Simple liquid–liquid partion system for isolation of labled oleic acid from mixtures with glycerides. *J Lipid Res* **10**: 341–344, 1969.
20. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* **72**: 248–254, 1976.
21. Rodbell M, Metabolism of isolated fat cells. *J Biol Chem* **239**: 375–380, 1964.
22. Shepherd RE, Malbon CC, Smith CJ and Fain JN, Lipolysis and adenosine 3′5′-monophosphate metabolism in isolated white fat cells from genetically obese-hyperglycaemic mice (ob/ob) *J Biol Chem* **252**: 7243–7248, 1977.
23. Farndale RW, Allan LM and Martin BR, Adenylate cyclase and cAMP. In: *Signal Transduction, a Practical Approach* (Ed. Milligan G), pp. 75–86. IRL Press, Oxford, 1985.
24. Allen DO and Quesenberry JT, Quantitative differences in the cyclic AMP-lipolysis relationships for isoproterenol and forskolin. *J Pharmacol Exper Ther* **244**: 852–858, 1988.
25. Feve B, Emorine LJ, Lasnier F, Blin N, Baude B, Nahmias C, Strosberg AD and Pairault J, Atypical β-adrenergic receptor in 3T3-F422A adipocytes. *J Biol Chem* **266**: 20329–20336, 1991.
26. Granneman JG and Campbell RG, Effects of sucrose feeding and denervation on lipogenesis in brown adipose tissue. *Metabolism* **33**: 257–261, 1984.
27. Granneman JG and Lahners KN, Differential adrenergic regulation of β_1- and β_3-adrenoceptor messenger ribonucleic acids in adipose tissues. *Endocrinology* **130**: 109–114, 1992.
28. Holt SJ and York DA, Interaction of intracerebroventricular insulin and glucose in the regulation of the activity of sympathetic efferent nerves to brown adipose tissue in lean and obese Zucker rats. *Brain Res* **500**: 384–388, 1989.
29. Hollenga C and Zaagsma J, Direct evidence for the atypical nature of functional β-adrenoceptors in rat adipocytes. *Br J Pharmacol* **98**: 1420–1424, 1989.
30. Krief S, Lonnqvist F, Raimbault S, Baude B, Van Sprousen A, Arner P, Strosberg AD, Ricquier D and Emorine LJ, Tissue distribution of β_3-adrenergic receptor mRNA in man. *J Clin Invest* **91**: 344–349, 1993.
31. Lonnqvist F, Krief S, Strosberg AD, Nyberg B, Emorine LJ and Arner P, Evidence for a functional β_3-adrenoceptor in man. *Br J Pharmacol* **110**: 929–936, 1993.
32. Smith SA, Sennitt MV and Cawthorne MA, BRL 35135: an orally active anti-hyperglycaemic agent with weight reducing effects. In: *New Anti-diabetic Drugs* (Eds. Bailey CJ and Flatt PR), pp. 177–189. Smith-Gordon, London, 1990.
33. Cawthorne MA, Sennitt MV, Arch JRS and Smith SA, BRL 35135, a potent and selective atypical β-adrenoceptor agonist. *Am J Clin Nutr* **55**: 252S–257S, 1992.