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SYMPATHETIC MECHANISMS IN DIET-INDUCED THERMOGENESIS: MODIFICATION BY CICLAZINDOL AND ANORECTIC DRUGS

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1 The sympathetic noradrenergic activation of brown adipose tissue and the biochemical mechanisms involved in diet-induced thermogenesis were studied in rats.

2 A close correlation was found between brown adipose tissue Na^+, K^+-adenosinetriphosphatase (Na^+, K^+-ATPase) activity *in vitro* and *in vivo* measurements of resting oxygen consumption (VO_2). The effects of noradrenaline on *in vitro* Na^+, K^+-ATPase activity in brown adipose tissue and *in vivo* VO_2 could be mimicked by a variety of agents. These included β-adrenoceptor agonists and agents known to induce the release of noradrenaline or inhibit the noradrenaline uptake process. The pharmacological evidence suggests that dopaminergic mechanisms may also be involved in the control of thermogenesis.

3 Amphetamine did not increase VO_2 in rats without causing associated increases in locomotor activity. Ciclazindol at doses of 3–30 mg/kg intraperitoneally increased VO_2 but did not appear to increase locomotor activity or evoke any other signs of CNS stimulation including lengthening of time to sleep onset or stereotypy. Separation of metabolic and CNS effects occurred only at the lowest dose of mazindol used (0.3 mg/kg i.p.). These results are probably a reflection of (a) the relative abilities of these drugs to inhibit brain and brown adipose noradrenaline uptake processes and (b) the relatively high accumulation of ciclazindol in brown adipose.

4 Of the drugs tested, only ciclazindol was a more potent inhibitor of the noradrenaline uptake system in brown adipose tissue (BAT) than in brain. Kinetic analysis also revealed that the actions of ciclazindol on the NA uptake system and Na^+, K^+-ATPase in BAT differed from those of mazindol.

5 These findings suggest that ciclazindol may produce an energy wasting effect in rodents without causing overt CNS stimulation; the implications of these findings in terms of human obesity are discussed.

Introduction

Research into the regulation of energy balance and the development of obesity in man is often hampered by the limitations placed on human experimentation and many studies have therefore concentrated on various animal models of obesity (see Festing, 1979). A number of biochemical and physiological defects have been suggested to explain the obesity of these animals but in simple thermodynamic terms it is obvious that excess fat deposition must result from either an increased energy intake, a reduced expenditure or both. Although many obese animals show increased food intake, this is usually accompanied by a greater efficiency of energy utilization, whilst in normal lean rats hyperphagia does not always result in excessive weight gain. Rats fed a highly palatable 'cafeteria' diet consume up to 80% more energy than stock fed controls but this is often accompanied by a large increase in metabolic rate, or diet-induced thermogenesis (DIT), which reduces the rate of fat deposition. Diet-induced thermogenesis involves similar mechanisms to the non-shivering thermogenesis seen in cold-adapted animals since both are due to sympathetic stimulation of brown adipose tissue (BAT) (Rothwell & Stock, 1979; 1980). However, the mechanism by which catecholamines activate heat production in brown adipose tissue is unclear (for review see Himms-Hagen, 1976).

The study of energy balance and thermogenesis in normal lean animals can provide a useful approach to the understanding and later treatment of obesity and in this paper we describe results relating to the biochemical pharmacology of noradrenergic synaptic events in brown adipose tissue associated with heat production. The thermogenic action of ciclazindol (Wy 23409), a noradrenaline uptake inhibitor (Beckett, Southgate & Sugden, 1973) is also discussed in relation to its pharmacological action at the synapse. A comparison is also made with appetite suppressant (anorectic) agents traditionally used in the treatment of obesity and the use of thermogenic

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agents as an alternative approach is discussed. An account of some of this work has been presented to the British Pharmacological Society (Latham, Rothwell, Stock, White & Wyllie, 1981).

Methods

Adult male Sprague-Dawley rats (150–200 g from Charles River) were allowed free access to water and a standard pelleted diet (PRD, Christopher Hill Group). Some of the animals were also maintained on a cafeteria diet as described by Rothwell & Stock (1979) for 10–12 days before the experiment. The food used in the experiment are listed in Table 1.

Table 1 List of food items presented to cafeteria rats during the experiment

Chopped ham & pork	Battenburg cake
Corned beef	Fruit cake
Liver & bacon paté	Trifle sponges
Luncheon meat	'Toblerone'
Pork sausages	Chocolate marshmallow
Beef sausages	Plain marshmallows
Lean bacon	Milk chocolate
Shortcake	'Mars' bars
Chocolate wafers	'Crunchie' bars
Digestive biscuits	Lasagna
Coconut crunch cake	Popcorn
Chocolate roll	Chocolate rice crisps
Swiss roll	Cheese
Chocolate miniroll	

Rats had continuous access to the stock diet and received four different palatable food items each day. Two foods were presented on the morning and the other two were added in the evening. The food intake on the respective diets was in the range (kJ/$W^{0.75}$ daily) 650 ± 10, control; 1140 ± 30, cafeteria.

The following analyses were undertaken. Resting oxygen consumption (VO_2) was measured in a closed-circuit respirometer (Stock, 1975) during the day at a temperature of 29 ± 1°C for 2 h before and after dosing with the test compounds or vehicle. Animal behaviour was monitored by casual observation.

Homogenates of brown adipose tissue were prepared by homogenizing interscapular brown adipose tissue in a Potter-S homogenizer (2000 rev/min, 7 strokes). A 10% w/v homogenate in 0.32 M sucrose was prepared at 0–4°C. Microsomal material was separated by centrifugation of the brown adipose tissue homogenate at 18000 g_{av} for 10 min and the resultant supernatant was then centrifuged at 50000 g_{av} for 30 min. The material designated as ultrasonicated was prepared by ultrasonication for 30 s with a Brinkman polytron, and collection of particulate material at 50000 g_{av} for 30 min. The pellet was then washed and recentrifuged under the same conditions.

Fluorimetric analysis of noradrenaline (NA) before and after addition of α-methyl-*p*-tyrosine (250 mg/kg i.p.), to obtain a measure of turnover, was carried out as described by Gilbert & Wyllie (1980). Uptake of NA into brown adipose tissue was measured in a medium containing (mM): NaCl 136, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 10, ascorbate 1 and Tris 20, which was adjusted to pH 7.4 with HCl and gassed with pure oxygen. The uptake experiments were carried out using radiolabelled noradrenaline ([^{3}H]-(−)-NA) at a concentration of 10^{-7} M using the method of Wood & Wyllie (1981) for brain slices and modified by using brown adipose tissue as the starting material for uptake studies into brown adipose tissue homogenates. $Uptake_1$ was taken as that component completely inhibited by imipramine at a concentration of 10^{-4} M and $uptake_2$ as the component of uptake which was inhibited by corticosterone at a concentration of 5×10^{-6} M.

Adenyl cyclase activity was assessed by measuring the conversion of adenosine triphosphate (ATP) to cyclic adenosine 3′,5′-monophosphate (cyclic AMP) as described by Kebabian, Petzold & Greengard (1975). Adenosinetriphosphatase activities were measured by monitoring the release of inorganic phosphate (Pi) from ATP as described by Gilbert & Wyllie (1975). Sodium, potassium-activated, magnesium-dependent adenosinetriphosphatase (EC 3.6.1.3.) (Na^+, K^+-ATPase) was calculated as the difference between total and sodium ATPase. Tissue levels of ciclazindol were measured by determining the level of [^{14}C]-ciclazindol in dried tissues, 2 h after oral administration (3 mg/kg). Total radioactivity was corrected by toluene extraction to correct for metabolites (Swaisland, Franklin & Southgate, 1975) and radioactivity measurements were converted to concentrations by assessing the ratio of tissue wet weight to dry weight and assuming a uniform distribution through the total tissue water volume. The following drugs were used: ciclazindol HCl (10-(*m*-chlorphenyl)-2,3,4, 10-tetrahydropyrimido [1,2α] indol-10-ol) guanabenz and mazindol HCl (Wyeth), (+)-amphetamine sulphate (SKF), fenfluramine (Servier), phentolamine mesylate (Ciba), haloperidol (Janssen), pimozide (Janssen), naloxone HCl (Endo), (±)-propranolol (ICI), prazosin (Pfizer), salbutamol (Allen and Hanburys), (−)-[7,8-^{3}H] noradrenaline (20–40 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, [^{14}C]-ciclazindol was synthesized by Wyeth Laboratories.

Results

Brown adipose tissue biochemistry

Assays of Na^+, K^+-ATPase activity in brown adipose tissue homogenates revealed a large difference between control and cafeteria-fed animals (control 0.23 ± 0.01, cafeteria 0.32 ± 0.02 μmol Pi mg^{-1} protein h^{-1}, $P < 0.001$). In addition, a remarkably high correlation ($r = 0.968$, $P < 0.001$) existed between these *in vitro* values for Na^+, K^+-ATPase and the *in vivo* measurements VO_2 made when the animals were eating their respective diets. Noradrenaline caused a marked stimulation of enzyme activity and the maximal response in cafeteria rats was more than twice that of control rats. The concentration of NA required to produce half-maximal stimulation was 5×10^{-6} and 1×10^{-6} M in control and cafeteria rats respectively. This was a significant increase in sensitivity to NA of the latter group.

There were no differences in levels of noradrenaline or extraneuronal or intraneuronal uptake of NA into brown adipose tissue homogenate prepared from control or cafeteria-fed animals. The absolute adenyl cyclase activity and the stimulation of this enzyme by NA was also similar in both groups. However, cafeteria-fed animals displayed a statistically significantly higher turnover of NA in brown adipose tissue homogenates than did controls. Another difference between the two groups was that the homogenate Na^+, K^+-ATPase specific activity was greater in cafeteria than control rats. This difference in absolute enzyme activities, however, was not apparent in sonicated homogenates or microsomal preparations (Table 2). In all fractions studied, homogenate, ultrasonicated homogenate and microsomal fractions, Na^+, K^+-ATPase activity was stimulated by NA. The degree of stimulation of the enzyme was dependent on the amine concentrations (in the range 10^{-9}–10^{-4} M) and was always greater in cafeteria-fed animals. Ciclazindol stimulated Na^+, K^+-ATPase activity only in homogenates.

The effects of drugs on brown adipose tissue Na^+, K^+-ATPase

The degree of stimulation of Na^+, K^+-ATPase by NA was similar to that observed with isoprenaline or salbutamol but greater than that with guanabenz, phenylephrine or dopamine (Table 3). The rank order of potency of these agonists in stimulating microsomal Na^+, K^+-ATPase from control rats was similar to that in cafeteria-fed rats.

The stimulation observed with NA was partially antagonized by propranolol and to a lesser extent by phentolamine or prazosin (not shown). Complete blockade could only be achieved in the presence of a β-adrenoceptor antagonist and either haloperidol (Figure 1) or pimozide (not shown). The concentration of propranolol required to reduce noradrenaline stimulation by 50% was $7.2 \times 10^{-8} \pm 0.3$ M (6). Even at 10^{-4} M the other antagonists did not reduce the stimulation by 50%.

Agents known to increase NA release (e.g. amphetamine) or decrease neuronal re-uptake (e.g. imipramine, mazindol, amitriptyline or ciclazindol) had variable effects (Table 3 and Figure 2). Amphetamine and isoprenaline had similar stimulating profiles to NA. There was a brief marked increase in Na^+, K^+-ATPase activity but the effect of amphetamine was more transient than that observed with the addition of NA or isoprenaline to the incubation medium. Mazindol resulted in a slight delayed increase in Na^+, K^+-ATPase activity which was also not sustained. Fenfluramine was without effect on the enzyme whereas there was enzyme inhibition in the presence of imipramine. Ciclazindol resulted in a large increase in Na^+, K^+-ATPase activity which,

Table 2 Brown adipose Na^+, K^+-ATPase activity

Animal type	*Drug addition* (M)	*Na^+, K^+-ATPase activity* (μmol Pi mg^{-1} protein h^{-1}) *Homogenate*	*Disrupted homogenate*	*Microsomal fraction*
Control	None	0.23 ± 0.01 (4)	0.18 ± 0.01 (4)	1.32 ± 0.04 (4)
Cafeteria	None	0.32 ± 0.02 (6)††	0.19 ± 0.01 (6)	1.30 ± 0.06 (6)
Control	NA 10^{-4}	*0.29 ± 0.02 (4)	*0.23 ± 0.01 (4)	**1.64 ± 0.06 (4)
Cafeteria	NA 10^{-4}	***0.47 ± 0.03 (6)†††	***0.30 ± 0.02 (6)†	***2.17 ± 0.04 (6)†††
Control	Ciclazindol 10^{-4}	**0.27 ± 0.01 (4)	0.18 ± 0.03 (4)	1.31 ± 0.04 (4)
Cafeteria	Ciclazindol 10^{-4}	**0.40 ± 0.02 (3)†††	0.20 ± 0.03 (4)	1.28 ± 0.06 (6)

Activity significantly higher than in control animals: †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$.
Significant stimulation of Na^+, K^+-ATPase by the drug (relative to the activity in the drug's absence): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 Stimulation of brown adipose tissue microsomal Na^+, K^+-ATPase by noradrenergic agents

Compound	Na^+, K^+-ATPase activity (μmol Pi mg^{-1} protein h^{-1}) Cafeteria	Control	Concentration to produce 50% max stimulation† (μM)
None	1.30 ± 0.05 (6)	1.32 ± 0.04 (4)	
Noradrenaline	2.17 ± 0.4 (4)***	1.64 ± 0.06 (4)***	1.1 ± 0.3 (4)
Isoprenaline	2.33 ± 0.03 (6)***	1.70 ± 0.05 (4)***	0.7 ± 0.2 (3)
Salbutamol		1.68 ± 0.03 (4)	1.8 ± 0.2 (4)
Phenylephrine	1.57 ± 0.04 (4)**	1.45 ± 0.03 (4)	$>10^{-4}$
Guanabenz		1.46 ± 0.05 (4)	$>10^{-4}$
Dopamine	1.65 ± 0.08 (3)*	1.49 ± 0.03 (3)*	$>10^{-4}$
Ciclazindol	1.31 ± 0.04 (3)	1.28 ± 0.06 (6)	
Mazindol	1.32 ± 0.05 (3)	1.36 ± 0.04 (3)	
Dexamphetamine	1.33 ± 0.04 (3)	1.34 ± 0.03 (3)	

All drugs were present at a final concentration of 10^{-4} M and pre-incubated with the enzyme for 30 min before starting the reaction.
Values are the mean ± s.e.mean. Student's t test was used to determine statistical significance: *$P<0.05$; **$P<0.01$; ***$P<0.001$.
†Maximum stimulation was taken as that observed with NA (10^{-4} M) in control animals.

although slow in onset, was maintained over a long period. The action of amitriptyline resembled that of mazindol although there was an initial inhibition of the enzyme.

Uptake of noradrenaline into brown adipose tissue and brain

The effects of drugs on the uptake of NA into brown adipose tissue homogenates are shown in Figure 3. The drug having the greatest action in inhibiting the uptake of NA was ciclazindol. Mazindol and amphetamine, although having effects at low concentrations, were not as active and their maximal effects were lower than that of ciclazindol. Imipramine, desmethylimipramine (DMI) and amitriptyline (not

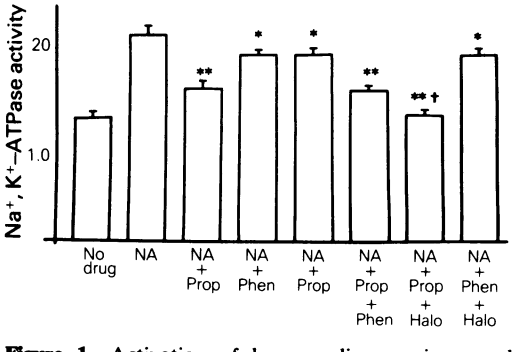

Figure 1 Activation of brown adipose microsomal Na^+, K^+-ATPase by noradrenaline (NA) (10^{-4} M) in the presence of antagonists (10^{-4} M). The time of incubation was 30 min and histograms represent the mean of 4–6 experiments, vertical lines show s.e.mean. Antagonists had no direct action on control Na^+, K^+-ATPase activity in the absence of noradrenaline. Units of activity μmol Pi mg^{-1} protein h^{-1}. Prop = Propranolol; Phen = Phentolamine; Hal = Haloperidol.
*$P<0.01$; **$P<0.001$ relative to NA alone.
†$P<0.01$ relative to NA + propranolol.

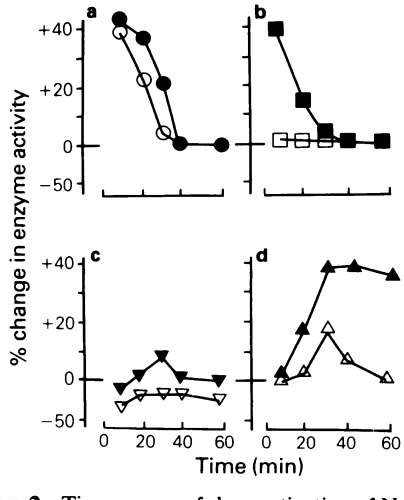

Figure 2 Time course of drug activation of Na^+, K^+-ATPase. Drugs were added to the incubation medium at times shown on the abscissa scale before the start of the reaction. The results are plotted as the percentage change in the absence of drug. The symbols represent the mean of 5 or 6 experiments. S.e.mean (not shown) were all less than 5% of the appropriate mean. All drugs were present at a final concentration of 10^{-5} M. (a) Noradrenaline (●) and isoprenaline (○); (b) amphetamine (■) and fenfluramine (□); (c) imipramine (▽) and amitriptyline (▼); (d) ciclazindol (▲) and mazindol (△).

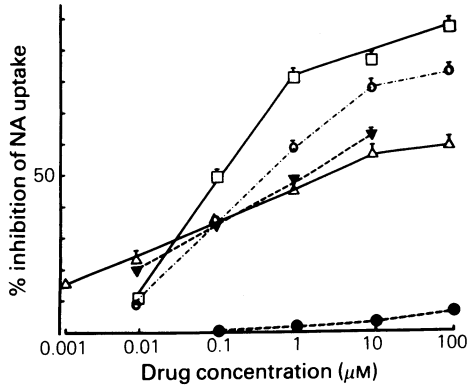

Figure 3 Effects of drugs on the uptake of [^{3}H]-noradrenaline ([^{3}H]-NA) (10^{-7} M) into brown adipose tissue. All values are the mean of 4 experiments; vertical lines show s.e.mean. Ciclazindol (□); imipramine (○); dexamphetamine (▼); mazindol (△); fenfluramine (●). *The curves for amitriptyline and desmethylimipramine were similar to imipramine (IC_{50} values see Table 4).

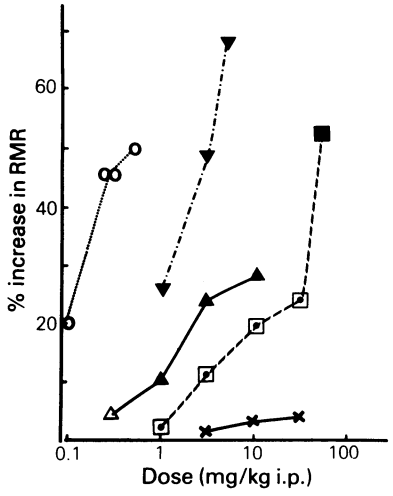

Figure 4 Effects of noradrenergic agents on resting metabolic rate in rats: noradrenaline (○); dexamphetamine (▼); mazindol (△) (▲); ciclazindol (□) (■); fenfluramine (X). Open symbols represent changes in resting metabolic rate (RMR) in rats without associated CNS stimulation, closed symbols represent changes with associated stimulation. All values are the mean of 6 experiments.

shown) were less active at the highest concentration used (100 μM) and the concentrations required to produce 50% inhibition were greater than the concentrations required of ciclazindol. With the exception of ciclazindol, all these compounds, especially DMI and mazindol, possessed a lower IC_{50} value for inhibiting brain uptake systems than brown adipose tissue systems (Table 4). Fenfluramine inhibited uptake only at high concentrations.

Although all compounds except fenfluramine resulted in complete inhibition of the uptake of NA in brain slices, only ciclazindol blocked NA uptake into brown adipose tissue by more than 90% (Figure 3).

The pharmacology of diet-induced thermogenesis

The effects of a number of pharmacologically active agents on VO_2 are shown in Table 5. In all cases prior to drug treatment the resting metabolic rates of cafeteria-fed rats were significantly higher than that of control animals. The elevated VO_2 of cafeteria-fed rats was significantly reduced by the β-adrenoceptor antagonist, propranolol (lowest effective dose 0.3 mg/kg i.p.), the dopamine antagonist pimozide, or the ganglion blocker hexamethonium. Phentolamine, pentobarbitone, morphine and naloxone were without effect.

The effects of noradrenergic agents on resting metabolic rate are shown in Figure 4. NA injection (i.p.) resulted in an increase in resting metabolic rate. This effect could be mimicked by ciclazindol, amphetamine or mazindol but not by fenfluramine. The effect of amphetamine at all doses tested was accom-

Table 4 Relative potencies for the inhibition of noradrenaline (NA) uptake in brown adipose tissue (BAT) and brain

Compound	IC_{50} (M) *BAT*	*Brain**
Mazindol	$2.8 \pm 0.1 \times 10^{-6}$	$3.6 \pm 0.5 \times 10^{-9}$
Fenfluramine	$> 10^{-4}$	$> 10^{-4}$
Amitriptyline	$1.5 \pm 0.1 \times 10^{-6}$	$2.3 \pm 0.4 \times 10^{-8}$
Imipramine	$4.6 \pm 0.3 \times 10^{-7}$	$2.4 \pm 0.2 \times 10^{-7}$
DMI	$7.3 \pm 0.3 \times 10^{-7}$	$2.0 \pm 0.2 \times 10^{-8}$
Ciclazindol	$1.2 \pm 0.2 \times 10^{-7}$	$4.2 \pm 0.4 \times 10^{-7}$
Amphetamine	$1.3 \pm 0.1 \times 10^{-6}$	$2.7 \pm 0.4 \times 10^{-8}$

All values are the mean ± s.e.mean of 3–7 experiments
*All compounds with the exception of fenfluramine completely inhibited brain NA uptake.

Table 5 Effects of drugs on resting oxygen consumption (VO_2) in rats

Drug	*Dose* (mg/kg i.p.)	*RMR VO_2* (ml $min^{-1}w^{-0.75}$) *Control*	*Cafeteria*
Propranol	0	11.92 ± 0.28	14.20 ± 0.37**
	5	11.65 ± 0.18	11.95 ± 0.24†
Phentolamine	0	11.39 ± 0.31	13.47 ± 0.29***
	5	11.20 ± 0.41	12.84 ± 0.48*
Morphine	0	11.78 ± 0.30	14.11 ± 0.29***
	1	11.01 ± 0.32	13.31 ± 0.22***
Naloxone	0	11.75 ± 0.26	13.73 ± 0.28**
	1.25	12.06 ± 0.24	14.03 ± 0.31**
Pimozide	0	13.13 ± 0.45	16.07 ± 0.30***
	5	12.22 ± 0.58	12.58 ± 0.58†
Pentobarbitone	0	12.05 ± 0.20	13.99 ± 0.18**
	20	11.58 ± 0.21	13.32 ± 0.21**
Hexamethonium	0	13.08 ± 0.38	16.34 ± 0.64***
	5	12.73 ± 0.32	13.64 ± 0.33†

All values are the mean ± s.e.mean of 6–12 experiments
Significant difference between control and cafeteria-fed animals: *$P<0.05$; **$P<0.01$; ***$P<0.001$.
†$P<0.05$ significant inhibition by drug.
All animals were monitored 2 h before and up to 3 h post drug.

panied by increased time to sleep onset, increased locomotor activity and at the highest dose stereotyped behaviour (Fletcher, unpublished). Ciclazindol increased VO_2 at doses which did not cause any obvious increase in time to sleep onset or locomotor activity (3–30 mg/kg i.p.). However, the largest dose used (50 mg/kg) did increase locomotor activity and resulted in a further increase in resting metabolic rate. Mazindol superficially resembled ciclazindol except that only the lowest dose (0.3 mg/kg) produced an increase in VO_2 without increasing time to sleep onset or locomotor activity.

Table 6 Distribution of ciclazindol after oral administration of [^{14}C]-ciclazindol (3mg/kg orally)

Tissue	*Concentration*† (μM)
Plasma	0.19 ± 0.05
Brain	0.41 ± 0.07
Heart	3.7 ± 0.2
Liver	> 30
Kidney	> 30
Small intestine	> 30
Interscapular BAT	3.9 ± 0.3
*White adipose	0.15 ± 0.03
*Skeletal muscle	< 0.15
*Skin	< 0.15

*These were directly underlying or overlying the interscapular brown adipose tissue (BAT) depot.
†Assuming equilibration throughout extracellular and intracellular water volume. All values are the mean ± s.e.mean of 4 or 5 experiments.

Tissue distribution of ciclazindol

The highest levels of ciclazindol were found in tissues associated with the gastrointestinal tract and sites of metabolism (Table 6). Interestingly, the concentration of ciclazindol in brown adipose tissue was much higher than that in surrounding tissues, plasma or brain.

Discussion

There have been many theories developed to explain the physiological and biochemical origins of thermogenesis. There is now considerable evidence to link thermogenesis to brown adipose tissue and the ability of this tissue to respond to NA (Himms-Hagen, 1976; Foster & Frydman, 1978; Rothwell & Stock, 1979; Latham *et al.*, 1981). Increased food intake or cold exposure are both known to increase NA turnover in several tissues (Young & Landsberg, 1979) and the present study shows that it is also increased in the brown adipose tissue of cafeteria-fed rats. The greater turnover noted in the current investigation was not due to alterations in re-uptake of NA or NA metabolism, but was due to an augmented release of NA from the nerve terminals (Rothwell, Stock & Wyllie, 1981). This greater release of NA, in itself, could account for the increases in brown adipose tissue thermogenesis seen in cafeteria-fed rats (Himms-Hagen, 1976).

In addition to this presynaptic event there were also postsynaptic changes, including an increased

response to NA in cafeteria rats. In the microsomal fraction of brown adipose tissue from cafeteria rats NA stimulated Na^+, K^+-ATPase activity by approximately 70% whereas the maximum stimulation of this enzyme in control rats was only 30%. It has been shown that there is also a significant shift to the left of the concentration-response curve in cafeteria animals. This represents an increased NA sensitivity (Rothwell *et al.*, 1981). The pharmacological evidence (Figure 1, Table 3, Rothwell *et al.*, 1981) indicates noradrenaline activation of Na^+, K^+-ATPase as being predominantly linked to the β-adrenoceptor although there may also be a contribution from the α-adrenoceptor and the dopamine receptor. Cafeteria feeding did not result in a general increase in β-adrenoceptor density as the absolute activity and catecholamine sensitivity of adenyl cyclase, an enzyme generally considered to be linked to the β-adrenoceptor (Iversen, 1977), was unchanged.

The close relationship between Na^+, K^+-ATPase and diet-induced thermogenesis was further substantiated when comparing the data from Figure 1 and Table 5. Both the catecholamine activation of Na^+, K^+-ATPase and VO_2 were inhibited by β-adrenoceptor antagonists and to a lesser extent by α-adrenoceptor or dopamine antagonists. Hexamethonium presumably blocked sympathetic ganglia, reducing the sympathetic outflow to brown adipose tissue and thus reduced VO_2. Theoretically, therefore, any agent capable of increasing brown adipose tissue Na^+, K^+-ATPase should also increase VO_2 and *vice versa*. A similar association has been described between Na^+, K^+-ATPase activity and non-shivering thermogenesis (Horowitz & Eaton, 1975).

The data in Tables 2 and 3 and Figure 2 illustrate that drugs can affect Na^+, K^+-ATPase in a variety of ways. The first category includes adrenoceptor agonists (NA and isoprenaline) which stimulate the enzyme directly. NA, for example, was active in both types of homogenates and in microsomal preparations. The second category includes ciclazindol which was only active in homogenates retaining some degree of synaptic integrity and NA stores. This may explain why absolute Na^+, K^+-ATPase activity was greater only in homogenates prepared from cafeteria-fed animals than from control animals (Table 2). Their homogenates contained endogenous NA which was partially stimulating the enzyme, and as Na^+, K^+-ATPase from cafeteria-fed animals was stimulated by noradrenaline to a greater extent, the absolute activities appear different. On the other hand, sonicated preparations or microsomal fractions had no endogenous NA, so the enzyme was unstimulated and the absolute activity in the absence of added agonist was the same for both groups in these fractions.

The time scale of drug action on Na^+, K^+-ATPase can also be indicative of direct or indirect action. NA, when added *in vitro*, had an immediate effect (Figure 2) which was slowly reduced, presumably due to metabolism and re-uptake, whereas isoprenaline, which is not taken up intraneuronally (Iversen, 1975), had a longer duration of action. Amphetamine, a known NA-releasing agent, had an immediate effect but this was not maintained, presumably due to metabolism and intraneuronal and extraneuronal re-uptake of the released NA. Mazindol, a known NA-uptake inhibitor (Sugrue, Shaw & Charlton, 1977) had a very slow onset of action which might be due to a slow build-up of NA at the synapse. The rate of onset of ciclazindol's action was of the same order as mazindol's, though the degree of stimulation with ciclazindol was greater and was sustained over a longer period and this could be a reflection of its greater potency for the brown adipose tissue uptake system than mazindol (Figure 3). Since ciclazindol was more active than other agents tested in inhibiting the uptake of noradrenaline into brown adipose tissue (Figure 5) it is probable that in addition to inhibiting intraneuronal (U_1) uptake, ciclazindol also inhibits extraneuronal uptake and further unpublished evidence (Wyllie) is compatible with this suggestion. Thus, in the presence of both an uptake$_1$ inhibitor (imipramine, 10^{-4} M) and an uptake$_2$ inhibitor (corticosterone, 5×10^{-6} M), the degree of inhibition (induced by ciclazindol) was the same as that achieved with ciclazindol alone. The sustained Na^+, K^+-ATPase stimulation in the presence of ciclazindol could be due to the fact that if all uptake sites were blocked, NA would not be rapidly removed from the synaptic cleft. Classical NA-uptake inhibitors such as imipramine might have been expected to stimulate the enzyme because they too increase synaptic NA levels, but tricyclics are known also to have a direct inhibitory action on the enzyme (Nag & Ghosh, 1975; Gilbert & Wyllie, 1980). Fenfluramine which has no well described action on NA uptake systems, had no effect on Na^+, K^+-ATPase. However, it should be noted that synaptic NA levels were not measured. The assumption is that indirectly acting agents could increase NA levels to an extent capable of stimulating the enzyme.

Some of the observed increase in VO_2 after drug injection may be due to locomotor activity. Casual observations of animal behaviour indicated that amphetamine had quite marked effects on physical activity as did the higher doses of mazindol. Ciclazindol had no apparent effects on behaviour or activity except at the highest dose. All of these agents which raised VO_2 also stimulated brown adipose tissue Na^+, K^+-ATPase. This finding together with the close correlation between Na^+, K^+-ATPase and VO_2 in stock and cafeteria-fed rats suggests that the activi-

ty of this enzyme is intimately involved with thermogenesis. A recent study on human erythrocyte Na^+, K^+-ATPase indicates that a defect in this enzyme system is associated with obesity (De Luise, Blackburn & Flier, 1980). In the treatment of obesity, this type of specific stimulation of VO_2 is an attractive alternative to the use of anorectic agents such as fenfluramine, mazindol and amphetamine which often have associated CNS stimulant properties. These agents may also influence VO_2 but their relative selectivity for the CNS (Table 4) could prove disadvantageous in man. However, ciclazindol is about three times more potent in inhibiting the brown adipose tissue NA uptake system and because of high brown adipose tissue accumulation at the time of peak plasma levels (Swaisland, Pierce & Franklin, 1977) (Table 6), it is possible to separate out the central effects and peripheral thermogenic actions. The described weight loss effect of ciclazindol in man (Greenbaum & Harry, 1980) could therefore be due to activation of thermogenesis.

Reprint requests to M.G.W., please.

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