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ACTIVATION OF THERMOGENESIS OF BROWN FAT IN RATS BY BACLOFEN

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Summary—Injection of baclofen (0.5–5 μg) into the ventromedial hypothalamus (VMH) of anaesthetized rats produced marked increases in the temperature (over 2°C) and thermogenic activity of brown adipose tissue (BAT). These effects were abolished by ganglionic or β-adrenergic blockade, or denervation of the tissue, but unaffected by hypophysectomy, adrenalectomy or vagotomy. Injections into the hypothalamus close to, but outside the ventromedial hypothalamus did not affect brown adipose tissue. Intravenous administration of baclofen produced similar increases in the temperature of brown adipose tissue, but at larger doses (50–1000 μg), and a subcutaneous injection stimulated the metabolic rate in conscious rats. Chronic treatment with baclofen suppressed weight gain and stimulated activity of brown adipose tissue without affecting food intake. These effects of baclofen, which were not mimicked by injections of γ-aminobutyric acid (GABA), indicate a novel action of baclofen in the ventromedial hypothalamus, leading to marked increases in metabolic rate and body temperature by stimulating sympathetic outflow to brown fat.

Key words: baclofen, brown fat, thermogenesis, adipose tissue, hypothalamus.

Production of heat in brown adipose tissue (BAT) results from physiological uncoupling of mitochondrial respiration and is controlled by noradrenaline released from a rich sympathetic innervation within the tissue (see Nicholls and Locke, 1983; Girardier, 1983 for reviews). This tissue is particularly active in small animals, especially rodents, where brown fat may represent only a few per cent of body weight but is capable of producing a doubling of the total metabolic rate. Brown adipose tissue is activated in response to low environmental temperatures, resulting in non-shivering thermogenesis (NST), or during hyperphagia when thermogenesis (DIT) induced by diet minimizes excess weight gains (Girardier, 1983; Rothwell and Stock, 1983, 1984). The tissue can also be stimulated pharmacologically and is particularly responsive to β-adrenergic agonists (see reviews by Landsberg and Young, 1983; Girardier, 1983).

Little is known about the central control of temperature of brown fat observed in response to baclofen, resulted from passive heating from other tissue in response to feeding or fluctuations in temperature originates within the hypothalamus. Several years ago it was observed that electrical stimulation of the ventromedial hypothalamus (VMH) of the rat caused activation of brown adipose tissue (Perkins, Rothwell, Stock and Stone, 1981), suggesting that this area may be involved in the control of thermogenesis in addition to its well-documented effects on feeding, body weight and glucose homeostasis (see Jeanrenaud, 1978; Bray and York, 1979 for reviews).

Many anorectic agents, used in the treatment of obesity, are assumed to act on the hypothalamic areas controlling intake of food, but often produce other behavioural side-effects. However, several groups (see Coscina and Nobrega, 1984 for reviews) have reported that baclofen, a compound which is generally known as a $GABA_B$-agonist (Bowery, Doble, Hill, Hudson, Shaw and Turnbull, 1980) and is used clinically in the treatment of spasticity, depresses body weight in rodents with only minimal effects on food intake. This implies a thermogenic effect of the drug, and in the studies reported here, it was observed that intravenous administration of baclofen stimulated brown adipose tissue but with a long delay, indicating a possible central action. Subsequent experiments revealed potent effects of this drug on brown adipose tissue when administered into the ventromedial hypothalamus.

METHODS

Male, Sprague–Dawley rats (Charles River, Kent, U.K., 210–300 g body weight) were used in these studies.

Intravenous and hypothalamic injections of baclofen

Rats were anaesthetized with urethane (0.15 g/100 g body weight) and thermocouples were placed beneath the interscapular of brown adipose tissue (close to Sulzer's vein) and 5 cm into the rectum, to allow continuous recording of temperatures. In some cases arterial blood pressure was also recorded from the carotid artery. Animals were not artifically heated but room temperature was above 23°C and rectal temperatures were always between 35.5 and 38°C at

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the start of the experiment. Intravenous injections of ± baclofen or 0.9% saline were made through a cannula inserted into the femoral vein. One dose of baclofen and one injection of noradrenaline (maximum dose of 25 μg) were tested in each rat, and temperatures were recorded for at least 40 min. Hypothalamic injections (1 μl) were stereotaxically located in or around the ventromedial hypothalamus (co-ordinates from Pellegrino, Pellegrino and Cushman, 1981: AP 0.8, L 0.5, V 8.8 mm), and subsequently confirmed by histological examination of the brains. Ventilation rates were recorded before and after injections.

Temperature responses were also measured after intravenous or hypothalamic injections of baclofen in hypophysectomized rats ($n = 4$, obtained from Charles River), adrenalectomized (bilateral, $n = 1$), vagotomized (bilateral subdiaphragmatic, $n = 3$) and animals in which the sympathetic nerves supplying the interscapular depot of brown adipose tissue had been severed (five nerves supplying each lobe). All these procedures were performed under halothane anaesthesia at least 7 days prior to the experiments. In addition, the responses to baclofen were tested in animals pretreated with propranolol (10 mg/kg, s.c.) or hexamethonium (10 mg/kg s.c.), and the effects of central (25 μg) and peripheral administration (5 mg) of GABA were also tested.

Mitochondrial measurements in brown adipose tissue

In separate animals, the two lobes of the interscapular depot and the perirenal and para-aortic depots of brown adipose tissue were removed separately 15 min after hypothalamic, or 30 min after intravenous injections of saline or baclofen (when a peak temperature response to baclofen had been achieved). The tissue was homogenized in 0.2 M sucrose and mitochondria prepared (Slinde, Pederson and Flatmark, 1975) for assessment of the activity of the thermogenic proton conductance pathway from the binding of [^{3}H]guanosine diphosphate (GDP, Amersham International, Bucks, U.K.; 10 Ci/mmol) using 200 μM unlabelled nucleotide to assess non-specific binding (NSB). Mitochondrial protein was measured using a dye reagent method (Bio-Rad, Herts, U.K.).

Studies on conscious animals

In an acute experiment, the resting oxygen consumption (VO_2; Stock, 1975) and rectal temperatures were measured at 29°C in conscious Sprague–Dawley rats pretreated with saline or propranolol (10 mg/kg, s.c.), before and after subcutaneous injection of baclofen (2 mg/kg). The effect of a single dose of baclofen (2 mg/kg, s.c.) on oxygen consumption was also tested in lean (+/?) and genetically obese (fa/fa) Zucker rats which are known to have defective thermogenesis (see Trayhurn and James, 1983 for review).

A chronic trial involved injecting Sprague–Dawley rats twice daily for 15 days with either saline or baclofen (2 mg/kg, s.c.) and recording food intake and body weight. Resting oxygen consumption was measured on day 10, before and after a single injection of noradrenaline (25 μg/100 g body weight, s.c.) and at the end of the experiment, the binding of [^{3}H]GDP to mitochondria isolated from the interscapular depot of brown adipose tissue was measured.

Statistics

Values have been presented as means ± SEM. Significant differences were assessed using a two-tailed Student's t-test for matched (within the same animal) or unmatched (comparisons between groups) data.

RESULTS

Intravenous injection of baclofen caused significant increases in the temperature of brown adipose tissue (threshold 50–60 μg) with a delay of 7.8 ± 1.6 min from injection (Fig. 1). The peak response occurred after 20–30 min and was usually sustained for a further 5–10 min. Rectal temperature also increased, but the delay was slightly greater and the peak response smaller than for brown adipose tissue. The largest increase in temperature, which occurred at doses greater than 500 μg, was of comparable magnitude to the increase following injection of a maximal thermogenic dose (25 μg) of noradrenaline (Fig. 1).

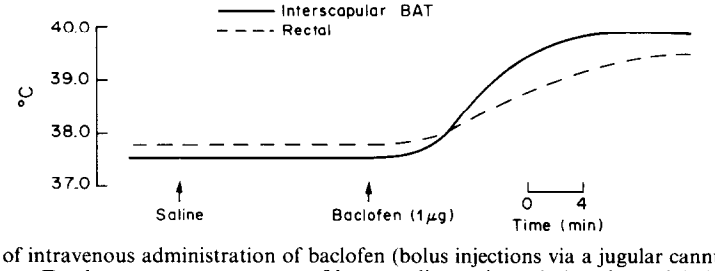

Fig. 1. Effect of intravenous administration of baclofen (bolus injections via a jugular cannula) to anaesthetized Sprague–Dawley rats on temperature of brown adipose tissue (●) and rectal (○) temperature. Values represent peak responses (usually achieved 25–30 min after administration of the drug) and are means of 3–7 animals at each point. The effects of vehicle and 1–2 doses of baclofen were tested on each animal. *Maximal response to noradrenaline (20–25 μg) tested on 4 animals. Bars denote SEM.

Table 1. Effects of central injection of baclofen on the temperature of brown adipose tissue (BAT) and rectum of anaesthetized rats (220–300 g body weight)

Dose (μg)	n	Brown adipose tissue	Rectum
0.2–0.3	2	0	0
0.5	2	0.3–0.7	0.1–0.4
1.0	12	1.73 ± 0.18	1.49 ± 0.21
2.0	3	1.78 ± 0.11	1.41 ± 0.43
5.0	3	2.50 ± 0.84	1.77 ± 1.00

Mean values $\pm$ SEM. n = number of separate animals, one injection tested on each rat.

Injections of baclofen into the hypothalamus were very much more potent and administration of less than 1 μg into the ventromedial hypothalamus produced a significant increase in the temperature of brown adipose tissue (Table 1). This increase was initiated within 8 min of injection (average delay 4.2 ± 0.6 min) with a peak at 20.9 ± 4.2 min (Fig. 2), and rectal temperature again showed a smaller and more delayed response (2–4 min after brown adipose tissue; Table 1, Fig. 2). At a dose of 5 μg baclofen (Table 1), the temperature of brown adipose tissue often exceeded 40°C and remained elevated for up to 2 hr. Injections into the hypothalamus which were greater than 1 mm from the ventromedial hypothalamus failed to elicit a significant change in temperature, and intravenous or central injections of saline were also without effect. Baclofen usually caused a slight slowing of respiration from 120 to 105 breaths/min, but did not affect mean arterial blood pressure (values ranged from 100 to 160 mmHg).

Temperature responses to baclofen were almost identical in hypophysectomized, adrenalectomized and vagotomized rats, but were abolished by propranolol (Table 2). Responses to central and intravenous injection were also prevented by surgical denervation of the tissue or injection of hexamethonium (Table 2). Injection of GABA, by either route, caused small and variable reductions in temperature and failed to produce an increase even when injected into the ventromedial hypothalamus in combination with nipecotic acid (25 μg) to suppress uptake.

Table 2. Effects of hypophysectomy, adrenalectomy, subdiaphragmatic vagotomy, surgical denervation of brown adipose tissue or injections of propranolol or hexamethonium on the temperature responses of brown adipose tissue to injections of baclofen into the ventromedial hypothalamus

Treatment	n	Dose of baclofen (μg)	Response (Δ°C)
Control	12	1	1.2
Hypophysectomy	4	1	1.08
Adrenalectomy	1	1	1.0
Vagotomy	3	1	0.98
Denervation	2	1	0.4
	2	5	0.2
Propranolol	2	1	0.1
	2	5	0.2
Hexamethonium	2	1	0
	2	5	0.5

Specific mitochondrial binding of GDP in interscapular brown adipose tissue was increased following intravenous injection of baclofen (control $= 32 \pm 3$, baclofen $= 51 \pm 3$ pmol/mg protein, $P < 0.001$). Central injection of baclofen caused a 44% increase in binding of GDP (average of three depots of brown adipose tissue $= 93 \pm 5$: control $= 64 \pm 4$ pmol/mg protein $P < 0.001$). The binding of GDP was increased more in the lobe of the interscapular depot, ipsilateral to the stimulation of the ventromedial hypothalamus ($100 \pm 11\%$ above control) than in the contralateral lobe ($54 \pm 7\%$), and other depots of brown adipose tissue were activated to a lesser extent ($22 \pm 4\%$).

Resting oxygen consumption of conscious Sprague–Dawley rats was significantly increased (peak at 40–70 min) by injections of baclofen (Table 3) and rectal temperature was also elevated ($+0.81 \pm 0.2$°C) above pre-injection value), but the oxygen consumption response (24% increase) was diminished by propranolol (9% increase). Baclofen also produced similar increases in resting oxygen consumption in both lean and genetically-obese Zucker rates (Table 3).

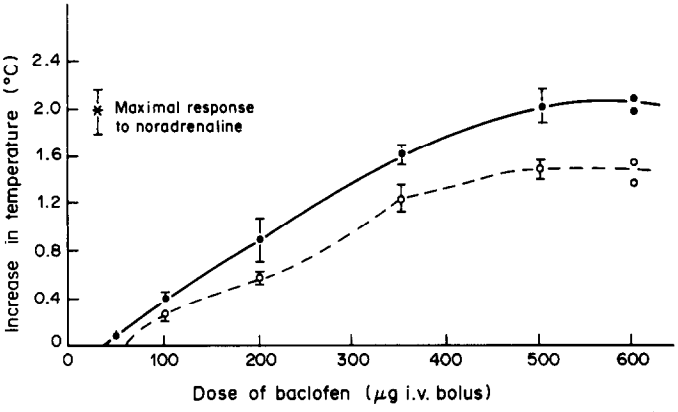

Fig. 2. Recordings of interscapular brown adipose tissue (BAT) and rectal temperature in an anaesthetized male rat (240 g) before and after injection of either saline (1 μl), or baclofen (1 μg/1 μl) into the ventromedial hypothalamus.

Table 3. Effect of subcutaneous injection of baclofen on oxygen consumption (VO_2) in Sprague–Dawley and Zucker rats

Sprague–Dawley rats	Before baclogen	After	Percentage increase
VO_2 (ml/min per $W^{0.75}$)	10.18 ± 0.37	12.58* ± 0.78	23.6 ± 2.8
With propranolol	10.04 ± 0.43	10.92 ± 0.33	8.8 ± 0.3
Zucker			
Lean	10.78 ± 0.23	13.33* ± 0.47	21.9 ± 5.1
Obese	8.13 ± 0.22	9.90* ± 0.78	24.1 ± 5.6

Mean values ± SEM ($n = 8$). *$P < 0.01$ compared to pre-injection value.

Chronic treatment of Sprague–Dawley rats with baclofen suppressed the gain in body weight without significantly affecting food intake (Table 4). The thermogenic response to noradrenaline (increase in oxygen consumption) and the protein content of brown adipose tissue and mitochondrial binding of GDP were also elevated in rats treated with baclofen.

DISCUSSION

Measurements of the temperature of brown adipose tissue are an indirect, but nevertheless reliable and routine method of assessing thermogenesis in the tissue. It is unlikely that the large increases in the temperature of brown fat observed in response to baclofen, resulted from passive heating from other tissues, since the temperatures of rectal and skeletal muscle showed slower and smaller increases (data not shown). The temperature of brown fat, although usually lower than core temperature at the start of the study, always rose well above the rectal temperature after the injection of baclofen. Furthermore, the activity of the proton conductance pathway (assessed from binding of GDP) was dramatically increased after injection of baclofen and this is thought to be the primary mechanism responsible for production of heat in the tissue (Nicholls, 1979). It is impossible to assess heat production from temperature of brown adipose tissue alone since activation of brown fat is associated with simultaneous and dramatic increases in blood flow which tend to dissipate the heat produced. However, it is likely that the hypothalamic and intravenous injections of baclofen may have caused up to a two-fold increase in the whole body metabolic rate since the temperature responses were comparable to a maximal thermogenic dose of noradrenaline (Fig. 1), which can double the total heat production.

Table 4. Chronic effects of baclofen on body weight and thermogenesis

	Control	Baclofen
Body weight gain (g)	120 ± 3	105 ± 2*
Energy intake (kJ)	4255 ± 80	4095 ± 150
Oxygen consumption in response to noradrenaline (% increase)	48 ± 4	70 ± 10*
Interscapular brown adipose tissue		
Mass (g)	237 ± 13	240 ± 15
Protein content (mg)	19.3 ± 0.5	23.2 ± 0.9*
Mitochondrial binding of GDP (pmol/mg protein)	54 ± 4	76 ± 2*

Mean values ± SEM ($n = 8$). *$P < 0.01$ vs control.

It seems likely that stimulation of brown adipose tissue by baclofen is mediated by actions on the hypothalamus to increase sympathetic outflow. Baclofen was least potent when administered subcutaneously and most potent when injected directly into the ventromedial hypothalamus. This locus seems to be quite specific since injections into the hypothalamus only 1 mm from the ventromedial hypothalamus failed to elicit a response, although other sites of action within or outside the hypothalamus cannot be excluded. The ventromedial hypothalamus was once thought of as a "satiety" centre because of its inhibitory effects on food intake. However, it also seems to be involved in the control of energy expenditure since electrical stimulation of the ventromedial hypothalamus causes increases in lipid metabolism (Shimazu and Takahashi, 1980) and temperature of brown adipose tissue (Perkins *et al.*, 1981). In spite of the close anatomical and functional relationship with the pituitary and its connections via the hypophyseal portal system, the effects of injection of baclofen into the hypothalamus were unaffected by hypophysectomy. The rather long delay after intravenous injection of baclofen may have partly reflected time to diffuse across the blood–brain barrier, but there was also some delay (4 min) after hypothalamic administration when compared to the response to electrical stimulation of the ventromedial hypothalamus (2 min, Perkins *et al*, 1981).

The dependence of the effect of baclofen on brown adipose tissue on the sympathetic nervous system was clearly demonstrated since its actions were abolished by a ganglionic antagonist (hexamethonium), a β-adrenergic antagonist (propranolol) and by sympathetic denervation of the tissue. Adrenalectomy or vagotomy had no effect on the response to baclofen. Brown adipose tissue has a very rich sympathetic innervation and is activated mainly by noradrenaline, with circulating catecholamines probably exerting only minor effects (Girardier, 1983). It seems that hypothalamic injection of baclofen caused fairly discrete stimulation of sympathetic outflow since the blood pressure was unaffected, and the activity of brown adipose tissue was most affected in the ipsilateral lobe of the interscapular depot, with only modest increases in the activity of other depots. This also suggests that different sites of brown adipose tissue may be controlled by distinct areas within the brain. It is unlikely that baclofen has any direct effects on brown fat since it failed to stimulate denervated tissue and no changes were found in respiration of isolated brown adipocytes after addition of baclofen (Rothwell, Stock and Sudera, unpublished data).

The mechanism of action of baclofen is unknown although many of its other effects have been ascribed to actions at $GABA_B$-receptors, which are also responsive to GABA itself but insensitive to bicuculline

(Bowery *et al.*, 1980). However, it was not possible to mimic any of the effects of baclofen on brown adipose tissue with five-fold greater doses of GABA plus nipecotic acid, suggesting another mechanism of action. Nevertheless, baclofen is also known to interfere with peptide function (Kudo, Ajoka and Fukuda, 1981; Saito, Konshi and Otsaka, 1975; Sawynok and Labella, 1981), and may also have a direct postjunctional action on central neurones, causing hyperpolarization (Newberry and Nicholl, 1985; Inoue, Matsuo and Ogata, 1985).

Apart from their relevance to the central control of thermogenesis of brown fat, these data may also reveal a novel approach to the pharmacological modification of body weight and energy balance. Although less potent than the central effects, peripheral administration of baclofen stimulated metabolic rate, even in genetically-obese rodents where thermogenesis is impaired. Chronic treatment with baclofen depressed the body weight in lean (Table 4) and genetically-obese rodents (see Coscina and Nobrega, 1984 for review), and in view of the present data, this is probably due to activation of thermogenesis in brown adipose tissue. It also seems likely that these peripheral effects are mediated by the ventromedial hypothalamus since so far it has not been possible to stimulate metabolic rate in conscious animals which have received electrolytic lesions of the ventromedial hypothalamus (unpublished data).

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