Neuropharmacology Vol. 27, No. 4, pp. 363–366, 1988
Printed in Great Britain

0028-3908/88 $3.00 + 0.00
Pergamon Press plc

OPPOSING EFFECTS OF ACTIVATION OF CENTRAL $GABA_A$ AND $GABA_B$ RECEPTORS ON BROWN FAT THERMOGENESIS IN THE RAT

R. W. HORTON, R. A. LEFEUVRE*, N. J. ROTHWELL* and M. J. STOCK
Department of Pharmacology and Department of Physiology, St. George's Hospital Medical School, Tooting, London, SW17 0RE

(*Accepted* 7 *October* 1987)

Summary—Baclofen (a $GABA_B$ agonist) stimulates body temperature, metabolic rate and brown adipose tissue (BAT) in the rat through a central action, but no effects of γ-aminobutyric acid (GABA) itself on these parameters were observed. In the present study, it was found that the central effects of (±)baclofen (0.5–2.0 μg injection i.c.v.) on the temperature (1.2°C increase) and metabolic rate (44–76% increase) of brown adipose tissue were inhibited by previous treatment with the $GABA_A$ agonist, muscimol (0.05 μg). Injection of GABA alone (12 μg) did not significantly affect these parameters, but in the presence of the $GABA_A$ antagonist bicuculline (2.5 μg), GABA significantly increased the temperature (0.3°C) and oxygen consumption (22%) of brown fat. (−)Baclofen was found to be approximately 50-times more effective in stimulating the temperature of brown adipose tissue than (±)baclofen. The results indicate that activation of central $GABA_B$ receptors stimulates the activity and hence metabolic rate of brown adipose tissue. However, activation of the $GABA_A$ receptors opposes the effects of $GABA_B$ stimulation on the thermogenesis of brown fat.

Key words: GABA receptors, baclofen, brown fat, temperature, thermogenesis.

Addae, Rothwell, Stock and Stone (1986) have previously observed that the $GABA_B$ agonist baclofen (Bowery, Hill and Hudson, 1983) caused marked increases in body temperature, metabolic rate and the activity of brown adipose tissue in the rat. Brown adipose tissue is the effector tissue for non-shivering thermogenesis in animals exposed to cold and thermogenesis by diet during hyperphagia and can produce up to two-fold increases in metabolic rate in small rodents (see Foster, 1986; Himms-Hagen, 1986; Rothwell and Stock, 1986b for reviews). The remarkable thermogenic capacity of brown fat is due to a unique proton conductance pathway in the inner mitochondrial membrane which results in uncoupling of oxidative phosphorylation (see Nicholls, Cunningham and Rial, 1986, for review). The activity of this pathway (assessed from the binding of purine nucleotides to isolated mitochondria) is increased following acute or chronic administration of baclofen (Addae *et al.*, 1986; Rothwell and Stock, 1986a). Subcutaneous administration of 0.5–5 mg/kg baclofen produces acute increases in metabolic rate which are probably mediated centrally, since very small doses (0.5–5 μg) injected into the third ventricle or directly into the ventromedial hypothalamus (VMH), cause very large (over 2°C) increases in the temperature of brown adipose tissue. These effects appear to be due to activation of the sympathetic outflow since they are inhibited by surgical denervation of brown adipose tissue, injection of a ganglionic blocker (hexamethonium) or a β-adrenergic antagonist (propranolol) but are unaffected by hypophysectomy, adrenalectomy or vagotomy. Addition of γ-aminobutyric acid (GABA) to the diet (Tews, 1981) or increasing the concentration of GABA in brain by administration of inhibitors of GABA transaminase, suppresses appetite and weight gain (Coscina and Nobrega, 1984) and stimulates the metabolic rate and activity of brown adipose tissue (Horton, Rothwell and Stock, unpublished data) in the rat. However, it has not proved possible to mimic the effects of central or peripheral injections of baclofen on thermogenesis with large doses of GABA, even in the presence of the inhibitor of the reuptake of GABA, nipecotic acid (Addae *et al.*, 1986), suggesting that the effects may not be mediated by a GABA receptor. An alternative possibility which has been investigated in this study is that activation of $GABA_A$ receptors may inhibit thermogenesis, thus masking any stimulating effect of GABA on $GABA_B$ receptors. This problem has been particularly difficult to study in the absence of a selective antagonist for $GABA_B$ receptors and has had to rely on the use of agonists and antagonists for $GABA_A$ receptors. Nevertheless, the results obtained using this approach suggest that central $GABA_A$ receptors may act to inhibit the increases in thermogenesis and activity of brown adipose tissue induced by activation of $GABA_B$-receptors.

*Present address: Department of Physiological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT.

METHODS

Male Sprague–Dawley (Charles River, Kent, U.K.) or Wistar (St. George's Medical School) rats (200–300 g body weight) were used in these studies. All animals were maintained with free access to pelleted stock diet and housed at 24 ± 1°C.

Temperature responses

Rats were anaesthetised with urethane (0.15 g/100 g body weight, i.p.) and thermocouples were placed beneath the interscapular depot of brown adipose tissue (close to Sulzer's vein) and 5 cm into the rectum to allow continuous recording of temperatures. The animals were not artificially heated but the room temperature was maintained at above 24°C and rectal temperatures were always between 35.5 and 38°C at the start of the experiment. This procedure has been adopted for routine measurements of temperature responses of brown adipose tissue because it avoids artifactual changes caused by external heating devices. A standard intravenous injection of noradrenaline (5 μg) was given through an indwelling cannula in the jugular vein, in order to verify the position and function of the thermocouple beneath the interscapular brown adipose tissue. This usually produced a transient (peak within 5 min) rise in temperature of 0.2–0.5°C.

Oxygen consumption

Rats were anaesthetised with sodium pentobarbitone (50 mg/kg) and guide cannulae (23 gauge needle) were implanted into the third ventricle (co-ordinates from Pellegrino, Pellegrino and Cushman, 1981: AP + 0.2, V 8.8 mm) and fixed with dental cement. The skin around the cannula was stitched and the animals were allowed to recover. Each animal was studied on 2–3 occasions at least 3 days apart, but any rats showing signs of infection or reduced gain in weight were excluded. The positioning of the guide cannulae was later confirmed histologically.

Resting oxygen consumption (VO_2) was measured in closed circuit respirometers (Stock, 1975) at 29°C for 2 hr before and up to 3 hr after central injections (1 μl). The effects of central (intracerebroventricular, i.c.v.) injections of (±)baclofen (0.5–1 μg), (−)baclofen (0.5–1 μg), (+)baclofen (1–50 μg), GABA (12 μg) or vehicle (0.9% saline) were tested in the presence or absence of the β-adrenergic antagonist propranol (20 μg i.c.v. or 10 mg/kg, s.c.), the $GABA_A$ agonist muscimol (0.05 μg i.c.v.) or the $GABA_A$ antagonist bicuculline (2.5 μg i.c.v.), given at least 10 min previously.

Values are presented as means ± SEM. Significant differences were assessed by Student's *t*-test using two tailed probabilities.

RESULTS

A single injection of (±)baclofen (1 μg) into the third ventricle of rats anaesthetised with urethane produced a large (1.2°C) sustained (delay 6–8 min, peak 19 ± 3 min) increase in the temperature of the interscapular depot of brown adipose tissue (Table 1). The rectal temperature showed a smaller (0.9 ± 0.3°C) and delayed (up to 4 min after the brown adipose tissue) increase in temperature, but injection of vehicle alone had no effect on either temperature (data not shown). The two isomers of baclofen differed widely in their effects; (−)baclofen produced similar responses to (±)baclofen and although only limited supplies of (+)baclofen were available, no effect on the temperature of brown adipose tissue was seen with doses of up to 25 μg and a dose of 50 μg was required to elicit a response comparable to 1–3 μg of either (−)baclofen or (±)baclofen. Previous injection of the $GABA_A$ agonist muscimol had no effect on the temperature of brown adipose tissue or rectal temperature, but completely abolished the effect of baclofen (Table 1). γ-Aminobutyric acid or bicuculline had little or no effect on body temperature when given separately, but caused significant increases in the temperature of brown adipose tissue in 5 out of the 8 animals tested, when injected together (Table 1).

Table 1. Effects of central injections (i.c.v.) on temperature responses of brown adipose tissue (BAT) in anaesthetised rats

Treatment	(Dose)	Increase in temperature (°C) of BAT	No. of animals responding
(±)Baclofen	(1 μg)	1.2 ± 0.2	22/22
(±)Baclofen plus muscimol	(1 μg) (0.05 μg)	0	0/7
GABA	(12 μg)	0.1 ± 0.1	1/10
GABA plus bicuculline	(12 μg) (2.5 μg)	0.3 ± 0.1	5/8
Bicuculline	(2.5 μg)	0.12 ± 0.1	1/3
(−)Baclofen	(1 μg)	1.1 ± 0.3	6/6
(+)Baclofen	(1–25 μg)	0.1 ± 0.2	1/6
(+)Baclofen	(50 μg)	1.0	2/2

Mean values ± SEM.

In conscious rats with implanted guide cannulae, the values for resting oxygen consumption (adjusted for metabolic body size, $kg^{0.75}$) were very similar to those routinely obtained for normal animals and were not affected by central injections of saline (Table 2). A dose of 0.5 μg of (±)baclofen resulted in a 44% increase in VO_2 (peak at 30–70 min), whereas a dose of 2.0 μg caused a 76% response. Injection of muscimol did not significantly affect the resting VO_2, but completely abolished the effect of the smaller dose of baclofen (0.5 μg) on metabolic rate, and significantly attenuated the effect of 2.0 μg (Table 2). Injection of either GABA or bicuculline caused small, transient (peak at 20–30 min, duration 70–90 min) increases in VO_2, but when given in combination a slightly greater increase was observed and the values remained elevated for more than 120 min.

Peripheral injection of the β-adrenergic antagonist propranolol (10 mg/kg, s.c.) did not affect the resting VO_2, but significantly inhibited the effect of baclofen

Table 2. Effect of central injections in (i.c.v.) on resting oxygen consumption (ml/kg$^{0.75}$/min) in conscious rats

	Before treatment	After treatment	Response Increment	Response Percentage	(n)
Saline	15.9 ± 1.2	16.2 ± 0.6	0.3 ± 0.2	2 ± 1	(4)
Baclofen (0.5 μg)	15.2 ± 0.8	21.8 ± 1.0**	6.6 ± 0.9***	44 ± 8**	(4)
Baclofen (2.0 μg)	16.7 ± 0.3	29.0 ± 1.6***	12.3 ± 1.2**	76 ± 6***	(6)
Baclofen + muscimol (0.5 μg) (0.05 μg)	14.6 ± 0.6	15.6 ± 0.7††	1.0 ± 0.3†††	7 ± 2†††	(5)
(2.0 μg) (0.05 μg)	15.5 ± 0.7	22.9 ± 1.1†	7.5 ± 0.5†	48 ± 2†††	(4)
Muscimol (0.05 μg)	15.5 ± 0.4	16.3 ± 0.4	0.7 ± 0.4	5 ± 3	(3)
GABA (12 μg)	15.8 ± 0.5	17.7 ± 1.2	1.9 ± 06*	12 ± 5	(4)
Bicuculline (2.5 μg)	15.5 ± 1.1	17.8 ± 10	2.3 ± 0.2*	15 ± 3*	(4)
GABA + bicuculline (12 μg) (2.5 μg)	15.7 ± 0.5	19.2 ± 0.8	3.5 ± 0.4*	22 ± 4*	(6)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to saline.
†$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared to the same dose of baclofen, given alone.
Mean values ± SEM.

Table 3. Effect of propranolol on resting oxygen consumption (ml/min/$^{0.75}$) central injections (i.c.v.) of baclofen in conscious rats

	Before treatment	After treatment	Response Increment	Response Percentage	n
Baclofen (0.5 μg)	14.5 ± 0.4	21.1 ± 0.6	6.5 ± 0.4	45 ± 3	(5)
Baclofen + propranolol (0.5 μg) (10 mg/kg, s.c.)	15.1 ± 0.8	16.9 ± 0.3**	1.8 ± 0.3**	12 ± 2**	(4)
Baclofen + propranolol (0.5 μg) (20 μg i.c.v.)	14.1 ± 0.4	19.8 ± 0.6	5.7 ± 0.5	40 ± 4	(4)
Propranolol (20 μg i.c.v.)	14.9 ± 0.4	15.8 ± 0.2	0.9 ± 0.4	6 ± 1	(4)

**$P < 001$ compared to baclofen alone.
Mean values ± SEM.

(Table 3). However, the response to baclofen was unaffected by central injection of propranolol.

DISCUSSION

Measurements of the temperature of brown adipose tissue in response to baclofen confirmed the results of previous studies (Addae *et al.*, 1986) and the results for VO_2 indicated that these increases in temperature were accompanied by large, dose-dependent increases in metabolic rate which could be inhibited by subcutaneous injections of propranolol. The increases in metabolic rate were comparable to the maximum thermogenic responses to noradrenaline which are generally observed in rats of this age and were not associated with any observable change in physical activity. Baclofen is considered to be a selective agonist for the $GABA_B$ site (Bowery *et al.*, 1983), and the high stereoselectivity of these effects of baclofen on thermogenesis (i.e. (−)baclofen was at least 50-times more potent than (+)baclofen) supports the suggestion that they are mediated by central $GABA_B$ sites.

In this, as in the earlier experiments (Addae *et al.*, 1986), the injection of GABA itself failed to affect the temperature of brown adipose tissue in 9 out of 10 animals (Table 1) and caused only a small rise in VO_2 (Table 2). However, following blockade of the $GABA_A$ receptor with bicuculline, GABA then produced significant and sustained increases in both parameters. Bicuculline alone only produced small, transient effects and no effect of bicuculline on the thermogenic effects of baclofen was observed. Conversely, muscimol (a selective $GABA_A$ agonist, which has little or no direct effect on the $GABA_B$ site; Bowery *et al.*, 1983), inhibited the increases in temperature of brown adipose tissue and VO_2 which normally occurred after injection of baclofen. The effects of baclofen appear to involve the activation of sympathetic activity, since they were inhibited by peripheral injections of propranolol, which is consistent with previous findings (Addae *et al.*, 1986) showing similar effects of sympathectomy of brown adipose tissue and ganglionic blockage with hexamethonium. The thermogenic effects of baclofen did not seem to depend on central β-adrenoreceptors since they were unaffected by central injections of propranolol. This would also tend to rule out the possibility that baclofen was acting by potentiating central β-adrenergic responses of cyclic AMP (Hill and Dolphin, 1984; Karbon and Enna, 1985).

The present results strongly suggest that central $GABA_A$ and $GABA_B$ sites have opposing effects on the sympathetic outflow to brown adipose tissue, and hence thermogenesis. It is not clear if the inhibitory thermogenic effects of activation of $GABA_A$ receptors operate through direct inhibition of $GABA_B$-mediated responses, or at other sites. The precise location of the receptors mediating these effects is unknown, but the ventromedial hypothalamus is one possible site since previous studies (Addae *et al.*, 1986) have shown that this is particularly sensitive to baclofen, while adjacent areas are insensitive. The ventromedial hypothalamus has a well established role in the regulation of body weight and energy balance (see Bray and York, 1979; Jeanrenaud, 1978 for reviews) and electrical stimulation of the ventromedial hypothalamus activates thermogenesis of

brown adipose tissue (Perkins, Rothwell, Stock and Stone, 1981). Receptors for GABA have been identified in the ventromedial hypothalamus and the activity of glutamate decarboxylase, the enzyme responsible for the synthesis of GABA, is decreased in the brains of rats made obese by destruction of the ventromedial hypothalamus (see York, 1987 for review).

Recently, a selective $GABA_B$ antagonist, phaclofen (Kerr, Ong, Prager, Gynther and Curtis, 1987) has been described and should help confirm the subtype of receptor responsible for the thermogenic effects of baclofen. In separate studies, it has been observed (unpublished data) that chronic peripheral administration with ethanolamine-O-sulphate (a GABA transaminase inhibitor, known to raise central levels of GABA; Sykes, Prestwich and Horton, 1984) stimulated thermogenesis of brown adipose tissue and inhibited the gain in weight in rats. Thus, as well as demonstrating some novel effects of central activation of GABA receptors, these studies raise the possibility of producing a new class of drug for modifying energy balance and body weight in animal studies, or in the clinical treatment of obesity.

Acknowledgements—This work was supported by a grant from ICI plc. NJR is a Royal Society University Research Fellow. We are grateful to CIBA Geigy U.K. for the gifts of baclofen.

REFERENCES

Addae J. I., Rothwell N. J., Stock M. J. and Stone T. W. (1986) Activation of thermogenesis of brown fat in rats by baclofen. *Neuropharmacology* **25:** 627–631.

Bowery N. G., Hill D. K. and Hudson A. C. (1983) Characteristics of $GABA_B$ receptor binding sites in rat whole brain synaptic membranes. *Br. J. Pharmac.* **78:** 191–206.

Bray G. A. and York D. A. (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol. Rev.* **59:** 719–809.

Coscina D. V. and Nobrega J. N. (1984) Anorectic potency of inhibiting GABA transaminase in brain: studies of hypothalamic, dietary and genetic obesities. *Int. J. Obesity* **8:** Suppl. 191–200.

Foster D. O. (1986) Quantitative roles of brown adipose tissue in thermogenesis. In: *Brown Adipose Tissue* (Trayhurn P. and Nicholls D. G., Eds), pp. 31–52. Edward Arnold, London.

Hill D. R. and Dolphin A. C. (1984) Modulation of adenylate cyclase activity by $GABA_B$ receptors. *Neuropharmacology* **23:** 829–830.

Himms-Hagen J. (1986) Brown adipose tissue and cold acclimation. In: *Brown Adipose Tissue* (Trayhurn P. and Nicholls D. G., Eds), pp. 214–268. Edward Arnold, London.

Jeanrenaud B. (1978) An overview of experimental models of obesity. In: *Recent Advances in Obesity Research II* (Bray G. A. Ed.), pp. 111–122. Newman, London.

Karbon E. W. and Enna S. J. (1985) Characterisation of the relationship between γ-aminobutyric acid B agonists and transmitter-coupled cyclic nucleotide-generating systems in rat brain. *Molec. Pharmac.* **27:** 53–59.

Kerr D. I. B., Ong J., Prager R. H., Gynther B. D. and Curtis D. R. (1987) Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.* **405:** 150–154.

Nicholls D. G., Cunningham S. A. and Rial E. (1986) The bioenergetic mechanisms of brown adipose tissue mitochondria. In: *Brown Adipose Tissue* (Trayhurn P. and Nicholls D. G., Eds), pp. 52–85. Edward Arnold, London.

Pellegrino C. J., Pellegrino A. S. and Cushman A. J. (1981) *A Stereotaxic Atlas of the Rat Brain.* Plenum Press, New York.

Perkins M. N., Rothwell N. J., Stock M. J. and Stone T. W. (1981) Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. *Nature* **289:** 401–402.

Rothwell N. J. and Stock M. J. (1986a) Chronic effects of baclofen on energy balance and thermogenesis in the rat. *Proc. Nutr. Soc.* **45:** 112A.

Rothwell N. J. and Stock M. J. (1986b) Brown adipose tissue and diet-induced thermogenesis. In: *Brown Adipose Tissue* (Trayhurn P. and Nicholls D. G., Eds), pp. 269–298. Edward Arnold, London.

Stock M. J. (1975) An automatic, closed circuit oxygen consumption. Apparatus for small animals. *J. appl. Physiol.* **39:** 849–850.

Sykes C., Prestwich C. and Horton R. (1984) Chronic administration of the GABA transaminase inhibitor enthanolamine-O-sulphate leads to up-regulation of GABA binding sites. *Biochem. Pharmac.* **33:** 387–393.

Tews J. K. (1981) Dietary GABA decreases body weight of genetically obese mice. *Life. Sci.* **29:** 2535–2542.

York D. A. (1987) Neural activity in hypothalamic and genetic obesity. *Proc. Nutr. Soc.* **46:** 105–117.