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CHRONIC INHIBITION OF GABA TRANSAMINASE RESULTS IN ACTIVATION OF THERMOGENESIS AND BROWN FAT IN THE RAT

R. HORTON, N. J. ROTHWELL* and M. J. STOCK
Department of Pharmacology and Department of Physiology, St George's Hospital Medical School, Tooting, London SW17 0RE, U.K.
[*Tel*: (01) 672-1255]

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Abstract—1. Oral administration of the GABA transaminase inhibitor ethanolamine-*O*-sulphate (EOS, 5 mg/ml in drinking water) to rats for 14 days suppressed food intake by 24%, but reduced weight gain by over 35%.

2. Thus, feed efficiency (g gain/MJ eaten) was decreased by over 15% in EOS-treated rats, suggesting that there had been an increase in metabolic rate.

3. The thermogenic response (rise in oxygen consumption, VO_2) to injection of noradrenaline was enhanced by 50% and the thermogenic activity of brown adipose tissue (BAT, assessed from mitochondrial GDP-binding) was increased by 38% in EOS-treated rats.

4. Injection of baclofen (a $GABA_B$ agonist, 0.5 mg/kg s.c.) stimulated VO_2 in both groups, with a significantly greater response in EOS treated rats, and this was enhanced by bicuculline ($GABA_A$ antagonist, 0.5 g/kg s.c.) in control rats and attenuated by muscimol ($GABA_A$ agonist, 0.5 mg/kg s.c.) in control and EOS-treated rats.

5. The data indicate that increasing brain GABA concentrations with EOS results in lower levels of metabolic efficiency and increases in thermogenesis.

There is now considerable evidence to suggest that brain gamma-aminobutyric acid (GABA) may be involved in the regulation of food intake and body weight. Addition of GABA to the diet of rats or mice suppresses food intake and body weight and these effects are greater when the diet is deficient in protein (Tews *et al.*, 1980; Tews, 1981). The relatively high levels of GABA which must be added to the diet (2–5% to induce these effects, suggest that its actions may be central. Increasing central GABA concentrations by systemic or central injections of GABA transaminase inhibitors, such as ethanolamine-*O*-sulphate (EOS), also inhibits food intake and suppresses body weight gain (Cooper *et al.*, 1980; Howard *et al.*, 1980; Coscina and Nobrega, 1984; Sykes *et al.*, 1984).

We have previously observed that peripheral or central administration of the $GABA_B$ agonist, baclofen causes stimulation of metabolic rate and reductions in body weight in normal rats (Addae *et al.*, 1986; Rothwell and Stock, 1986). These effects of baclofen appear to result from sympathetic activation of heat production in brown adipose tissue (BAT; Addae *et al.*, 1986; Rothwell and Stock, 1986) due to uncoupling of oxidative phosphorylation via the proton conductance pathway (see Nicholls and Locke, 1984 for review). Hypothalamic injection of less than 1 μg of baclofen results in activation of brown fat thermogenesis, but this effect is not mimicked by central injection of GABA (Addae *et al.*, 1986). Since GABA does not readily cross the blood–brain barrier we have attempted to investigate the role of GABA in animals treated chronically with EOS to raise endogenous brain GABA concentrations (Sykes *et al.*, 1984), and have followed the effects of treatment on thermogenic capacity and BAT activity. In addition, we have studied the effects of selective $GABA_A$ and $GABA_B$ agonists on thermogenesis in EOS-treated animals.

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

METHODS

Two experiments were performed on male, Sprague–Dawley SPF rats (aged 7 weeks, approx. 130–150 g wt from Charles River, Kent, U.K.). The animals were housed in pairs at 24°C with free access to pelleted stock diet (PRD, Christopher Hill Group Ltd, Dorset, U.K.). Half the animals received water containing EOS (5 mg/ml). Food intake and body weight were recorded daily. Energy intake was calculated from the metabolizable energy density of the diet (12 kJ/g).

In the first experiment, resting oxygen consumption (VO_2) was measured in closed-circuit respirometers (Stock, 1975) at 29°C for 2 hr before and 2 hr after injection of noradrenaline (0.25 mg/kg, s.c.) in eight control and eight EOS-treated rats after 10 days treatment. On day 14, all rats were killed by cervical dislocation, the interscapular BAT depot was dissected, weighed and homogenized in 0.2 M sucrose. Mitochondria were isolated and the activity of the proton conductance pathway was assessed from the binding of [^{3}H]guanosine diphosphate (GDP, 2 Ci/mmol, Amersham International, Bucks, U.K.) to mitochondria (Brooks *et al.*, 1982). Tissue and mitochondrial protein contents were

assessed using a dye-reagent method (Bio-Rad, Watford, U.K.).

The second study was performed on 16 control and 16 EOS-treated rats. Resting VO_2 was measured on three occasions in each animal at least 2 days apart in respirometers as described above. VO_2 was measured for 2 hr before and for up to 3 hr after a subcutaneous injection of baclofen (0.5 mg/kg). The response to baclofen was also measured after the animals had been pre-treated with two injections [one before (−2 hr) and one with baclofen] of either bicuculline ($GABA_A$ anatagonist, 0.5 mg/kg s.c.), or muscimol ($GABA_A$ agonist, 0.5 mg/kg s.c.). All animals were killed on day 14, and GDP-binding to mitochondria isolated from BAT was measured as above.

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

RESULTS

In the first experiment, metabolisable energy intake was suppressed by 24% in EOS-treated rats over the 14 day experiment, but body weight gain was reduced by 35% (Table 1). Thus, feed efficiency (weight gain per unit energy intake, g/MJ) was significantly lower (15%) in animals given EOS.

Resting VO_2 measured at thermoneutrality (29°C) did not differ significantly between groups. Noradrenaline injection elicited a significant rise in VO_2 (peak 40–70 min) in both, but the response to noradrenaline (increment of % increase) was significantly enhanced in EOS-treated rats (Table 1). The mass and protein content of interscapular BAT was similar for both groups, but specific mitochondrial GDP-binding was 38% greater in the rats given EOS (Table 1).

In the second experiment, energy intake was reduced by 23% and body weight gain by 39% in EOS-treated rats over the 14 days of treatment. Feed efficiency was also significantly lower (21%) in EOS-treated rats (21.2 ± 0.7 g/MJ) than controls (26.7 ± 0.4, $P < 0.001$).

Resting VO_2 in the absence of any additional drug treatment was slightly, but not significantly, increased in EOS-treated rats (Table 2) and was not affected by pretreatment with muscimol or bicuculline (data not shown). Baclofen produced a significant increase in VO_2 in both groups (peak response at 60–90 min) which was greater in EOS-treated rats (Table 2). The response to baclofen was significantly enhanced by bicuculline in control rats but not in the EOS group, whereas pretreatment with muscimol attenuated the response to baclofen in both groups, particularly in those given EOS. Measurements made when the animals were killed revealed a significant ($P < 0.05$) increase in BAT mitochondrial GDP-binding in EOS-treated rats (control 61 ± 4, EOS 81 ± 7 pmol/mg protein).

Table 1. Effect of chronic EOS (ethanolamine-o-sulphate) treatment on body weight, oxygen consumption and BAT activity

	Control	EOS
Feed Efficiency		
Final body wt (g)	236 ± 5	199 ± 8**
Wt gain (g)	108 ± 3	70 ± 6***
Energy intake (kJ)	3890 ± 140	2970 ± 80**
Wt gain/MJ eaten	27.7 ± 0.5	23.4 ± 1.0*
Resting VO_2 (ml/min/kg$^{0.75}$)		
Before noradrenaline	17.0 ± 0.2	16.2 ± 0.6
After noradrenaline	23.5 ± 0.7	25.1 ± 1.7
Increment	5.8 ± 0.2	8.9 + 0.2*
% Increase	34 ± 3	55 ± 1**
Interscapular BAT		
Mass (mg)	250 ± 21	230 ± 15
Protein (mg)	25.0 ± 0.2	24.2 ± 0.1
Specific mitochondrial GDP-binding (pmol/mg protein)	37 ± 3	51 ± 3*

Mean values ± SEM; $n = 8$.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to control.

Table 2. Resting VO_2 (ml/min/kg$^{0.75}$) of control and EOS treated rats in response to peripheral treatments

	Control	EOS
Resting VO_2 pretreatment	13.8 ± 0.5	14.2 ± 0.5
Response to baclofen (0.5)		
Increment	1.7 ± 1.2	3.5 ± 0.6**
% Increase	12 ± 1	24 ± 3**
Response to baclofen plus bicuculline (0.5)		
Increment	2.4 ± 0.2†	2.8 ± 0.2
% Increase	17 ± 1†	21 ± 1
Response to baclofen plus muscimol (0.5)		
Increment	1.1 ± 0.2	1.0 ± 0.3††
% Increase	8 ± 1	7 ± 2†††

( ) all doses in mg/kg (subcut).
Mean values ± SEM; $n = 8$.
†$P < 0.01$ compared to controls; ††$P < 0.01$, †††$P < 0.001$ compared to baclofen alone.

DISCUSSION

Previous studies using the same dose and route of administration of ethanolamine-*O*-sulphate have shown that it produces large increases in brain GABA concentrations but also causes a paradoxical increase in the density of both $GABA_A$ and $GABA_B$ receptors (Skyes *et al.*, 1984). Addition of EOS to the drinking water did not influence fluid intake. The depression of food intake in EOS-treated rats was sustained throughout the experiment and was consistent with previous studies using this drug (Cooper *et al.*, 1980; Howard *et al.*, 1980; Coscina and Nobrega, 1984). However, weight gain was suppressed to a greater extent than food intake so that feed efficiency was reduced by EOS. This finding contrasts with the normal effect of experimental food restriction, which usually induces an increase in feed efficiency and suggests that EOS may have stimulated metabolic rate—i.e. it has thermogenic and anorectic effects.

Thermogenesis associated with overeating (diet-induced thermogenesis) or cold-exposure (non-shivering thermogenesis) is largely dependent on sympathetic activation of BAT (see Rothwell and Stock, 1984 for review). Hyperphagic or cold-adapted animals show increases in energy expenditure (although these differences are not always revealed by short term measurements of VO_2 at thermoneutrality), enhanced thermogenic capacity and increased activity of BAT (Nicholls and Locke, 1984; Rothwell and Stock, 1984). The data obtained in the present study indicate that increasing brain GABA concentrations with EOS also enhances thermogenic capacity (i.e. increased responses to noradrenaline) and increases the activity of the thermogenic mitochondrial proton

conductance pathway in brown adipose tissue (i.e. increased mitochondrial GDP-binding).

These effects of EOS are comparable to those of baclofen. Acute central or peripheral administration of this $GABA_B$ agonist stimulates metabolic rate, body temperature and BAT activity in the rat (Addae *et al.*, 1986, unpublished data). In addition, chronic peripheral injections of baclofen suppress weight gain with little or no effect on food intake, and stimulate BAT activity, probably by enhancing sympathetic outflow (Addae *et al.*, 1986; Rothwell and Stock, 1986). Although we have been unable to mimic these responses with GABA alone, recent studies indicate that this may be due to the coexistence of central $GABA_A$ receptors which inhibit brown fat thermogenesis and $GABA_B$ receptors which stimulate thermogenesis (R. Horton, N. J. Rothwell and M. J. Stock, unpublished data).

The results presented in Table 2 on the effects of the $GABA_A$ agonist and antagonist support the suggestion that the $GABA_A$ sites may oppose the effects $GABA_B$-site stimulation on metabolic rate. The thermogenic response to baclofen in controls was enhanced by injection of the $GABA_A$ antagonist bicuculline and attenuated by the $GABA_A$ agonist muscimol. A submaximal peripheral dose of baclofen produced a greater rise in VO_2 in EOS-treated rats than controls and this may reflect the increased density of central GABA receptors previously observed in EOS-treated rats (Sykes *et al.*, 1984). Muscimol was also effective in reducing the baclofen response in EOS-treated rats, but bicuculline did not enhance the response. One possible explanation for this, is that in EOS-treated rats the response to baclofen was equivalent to that seen with a maximal dose (1 mg/kg) and further enhancement of its thermogenic action was not possible.

These findings support our previous suggestions (Addae *et al.*, 1986; Rothwell and Stock, 1986), that central stimulation of $GABA_B$ sites activates sympathetically mediated thermogenesis, but also indicate that $GABA_A$ receptors may oppose this effect and inhibit thermogenesis. The physiological significance of these findings and the precise location of the $GABA_A$ and $GABA_B$ sites are as yet unknown, but the results demonstrate that metabolic rate, and hence energy balance, can be modified by treatments which alter central GABA concentrations. This could be relevant to the development of novel pharmacological approaches to the treatment of obesity.

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