Discussion

The results show that theophylline inhibits rabbit cardiac adenylate cyclase in a dose-dependent manner. The non-competitive nature of the inhibition shows that theophylline is not directly competing with MgATP for binding at the active site. In sea-urchin sperm (Garbers 1977), however, theophylline was shown to inhibit the adenylate cyclase in a competitive manner, with respect to MnATP. Whether this difference reflects different sites of action, for MnATP and MgATP, or is due to species and/or tissue differences is not known.

The inhibitory effect of theophylline is seen under basal conditions and when adenylate cyclase is stimulated with NaCl. In neither of these cases are guanine nucleotides included in the assay medium. This action of theophylline does not, therefore, depend on an activation of the stimulatory guanine nucleotide binding protein (Ns-protein).

The non-competitive nature of the inhibition resembles that seen with P-site agonsits (Welton & Simko 1980). The lack of additivity of inhibition by theophylline with that due to 2′-deoxyadenosine 3′-monophosphate provides evidence that the two may be acting at the same site. As the effect is also seen with caffeine (Jakobs et al 1972) and methyl isopropylxanthine (Garbers 1977) as well as isobutylmethylxanthine, the effect is likely to be common to all methylxanthines. If methylxanthines can indeed inhibit the adenylate cyclase through the P-site, this could give some insight into the structure of this site. It also follows that phosphodiesterase inhibitors other than methylxanthines ought to be used in studies where the P-site may be of interest. Furthermore, the high concentrations (1–10 mM) of methylxanthines, particularly theophylline, which are used routinely to block the phosphodiesterase in most adenylate cyclase studies could be inadvertently reducing the activity of the enzyme.

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Baclofen is a potent activator of brown fat metabolism

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The injection of (±)-baclofen intravenously or directly into the ventromedial hypothalamus of urethane-anaesthetized rats, produced an activation of brown fat metabolism. This was seen as an increase of brown fat and rectal temperatures, and an increase of GDP binding in brown fat mitochondria. The activation was mediated by the sympathetic supply.

The thermogenic activity of brown adipose tissue (BAT) is a vital component in non-shivering and diet-induced thermogenesis (see Rothwell & Stock 1983 for reviews), but little is known of the central nervous control of this tissue. Electrical stimulation of the ventromedial hypothalamus will produce activation of BAT (Perkins et al 1981) but we have recently observed that injections of baclofen intravenously or directly into the hypothalamus will also cause a very marked stimulation of brown fat thermogenesis.

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Methods

Injections of (±)-baclofen were made both intravenously (femoral vein) and directly into the hypothalamus of rats anaesthetized with urethane (1·5 g kg^{-1} i.p.) while temperature of the interscapular BAT and core (rectal) temperatures were continuously monitored. Central injections were made into the ventromedial hypothalamus at stereotaxic coordinates AP-0·8, L 0·5, V 8·8 using the atlas of Pellegrino et al (1981). Injections were made in a volume of not more than 1 µl over 1 min and injection sites subsequently confirmed histologically.

Results

A dose of 1 μg of baclofen in 1 μl of 0·9% NaCl saline injected into the hypothalamus produced a large rise in BAT temperature of about 2 °C (Table 1), which was initiated 4·2 ± 0·6 min after the injection, and reached a peak at approximately 20 min (20·9 ± 4·2 min). Responses of up to 4 °C (mean 2·50 Table 1) were obtained at a dose of 5 μg of baclofen. Rectal temperature, which in normal anaesthetized rats is higher than BAT temperature, showed a delayed rise, slower than the increase of BAT temperature, and was eventually overtaken by it (Table 1). This is consistent with the concept that the elevation of BAT metabolism was responsible, at least in part, for the secondary change in rectal temperature.

Table 1. The effects of intrahypothalamic (±)-baclofen on BAT and rectal temperature in the anaesthetized rat.

Baclofen dose μg	Temperatures °C BAT Preinjection	Δ	Rectal Preinjection	Δ	n
0·5	37·1	0·5	37·3	0·25	2
1	36·82 ± 0·31	1·73 ± 0·18*	36·91 ± 0·21	1·49 ± 0·21	12
2	36·64 ± 0·21	1·78 ± 0·11	36·71 ± 0·22	1·41 ± 0 43	3
3	37·15 ± 0·19	2·50 ± 0·84	37·31 ± 0·19	1·77 ± 1·00	3

* Mean ± s.e.m.

The primary activation of BAT was confirmed by an examination of guanosine diphosphate (GDP) binding to isolated mitochondria from BAT to assess the activity of the thermogenic proton conductance pathway that uncouples oxidative phosphorylation. Brown fat was removed 15 min after an injection of baclofen when the rise of temperature was well established and the binding of GDP examined as described in previous publications (Brooks et al 1982). GDP binding in the mitochondria from activated tissue (93 ± 5 pmol mg^{-1} protein) was significantly greater than in control animals (64 ± 4 pmol (mg protein)$^{-1}$ [$P < 0·01$, t-test]). Intrahypothalamic injections of vehicle alone (saline) had no effect on BAT temperature and injection sites more than 1 mm away from the ventromedial nucleus in any direction, as revealed by subsequent histology, also were ineffective in elevating BAT temperature.

Intravenous injections of baclofen at doses of 50 μg per rat or greater also produced an elevation of BAT temperature (peak response +2·1 °C at a dose of 500 μg), and were associated with increases in GDP-binding of interscapular brown fat mitochondria.

Activation of BAT by baclofen (and any changes in rectal temperature) was prevented by propranolol (10 mg kg^{-1} s.c.), by hexamethonium (10 mg kg^{-1} s.c.) or by surgical denervation of the BAT depot indicating mediation by the sympathetic supply to the tissue. These latter two procedures did not prevent the activation of brown fat by direct intravenous injections of noradrenaline, indicating that the metabolic state of the tissue had not been changed. The results are, however, consistent with the view that injections of baclofen centrally or peripherally can activate sympathetic efferent fibres to the BAT depot.

The mechanism by which baclofen produces the activation of brown adipose tissue reported in this paper is still unclear, although preliminary experiments indicate that GABA does not share the same action. However, the effect of baclofen is an extremely potent one, in that the activation of BAT produced by an injection of 1 μg into the ventromedial hypothalamus is greater than the *maximal* response which can be produced by peripheral injections of noradrenaline. In view of the likely involvement of brown adipose tissue in thermogenesis and energy balance regulation, it is likely that baclofen might prove a useful substance in the further study of this inter-relationship. It may also be worth considering the possibility that baclofen or related compounds might prove useful as stimulators of BAT metabolism and thus as potential anti-obesity compounds in man.

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