*Journal of Physiology* (1988), **398**, *pp.* 361–370
*With 6 text-figures*
*Printed in Great Britain*



# SYMPATHETIC ACTIVATION OF LIPID SYNTHESIS IN BROWN ADIPOSE TISSUE IN THE RAT

BY YASUHIKO MINOKOSHI, MASAYUKI SAITO AND TAKASHI SHIMAZU

*From the Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu, Ehime 791-02, Japan*

(*Received 25 August 1987*)

SUMMARY

1. The roles of the sympathetic nerves in regulating lipid synthesis in brown adipose tissue (BAT) were studied by measuring incorporation of $^3H$ from $^3H_2O$ into glyceride glycerol and glyceride fatty acids in the interscapular BAT in anaesthetized rats.

2. When noradrenaline was infused intravenously at a total dose of 1–8 $\mu$g/100 g body weight over 30 min, $^3H$ incorporation into glyceride glycerol increased whereas $^3H$ incorporation into fatty acids did not change. Similar responses were found when the sympathetic nerves entering the interscapular BAT were stimulated continuously at 10 Hz. However, when electrical stimuli consisting of a much shorter train (2 s) were applied to the nerves at 3 min intervals at 10 Hz (stimulation in bursts), $^3H$ incorporation into both glyceride glycerol and fatty acids was enhanced. Stimulation in bursts elicited more pronounced lipogenic responses than other patterns that were employed, and involved the delivery of precisely the same number of impulses over the whole period of stimulation. The lipogenic responses to nerve stimulation in bursts were increased by increasing the stimulus frequency over the range 4–40 Hz.

3. Simultaneous administration of propranolol and phenoxybenzamine had little effect on either the fatty acid or the glyceride glycerol response to nerve stimulation. In contrast, these blocking agents almost completely eliminated the responses to noradrenaline infusion.

4. Pre-treatment with guanethidine effectively abolished the lipogenic response to nerve stimulation but potentiated the response to noradrenaline infusion.

5. It is concluded that lipid synthesis in BAT is enhanced by direct electrical stimulation of the sympathetic nerves only when they are stimulated in bursts. Sympathetic activation of lipogenesis in BAT is not solely attributable to the action of noradrenaline but involves some non-adrenergic mechanism.

## INTRODUCTION

Brown adipose tissue (BAT) has been identified as the major site for sympathetically controlled thermogenesis during cold acclimation and spontaneous overfeeding (Foster & Frydmann, 1978; Rothwell & Stock, 1981). It is generally

accepted that the first step in the sequence of cellular events associated with sympathetic activation of BAT thermogenesis consists of the binding of noradrenaline secreted from the sympathetic nerve endings to a specific adrenergic receptor. This is accompanied by the activation of adenylate cyclase, increased hydrolysis of triglyceride, and an increase in mitochondrial oxygen consumption, i.e. heat production (for a review, see Nicholls & Locke, 1984). Thus, the sympathetic nerves entering BAT directly control lipolysis in this tissue via this adrenergic mechanism.

In addition to the breakdown of triglyceride, its synthesis in BAT may also be under the direct control of the sympathetic nervous system. This was first suggested by studies of hypothalamic effects on lipid synthesis, in which electrical stimulation of the ventromedial hypothalamus in rats was found to elicit a marked increase in lipogenesis in BAT, but not in white adipose tissues or the liver (Shimazu & Takahashi, 1980). Subsequently, it was demonstrated that the stimulatory effect of the ventromedial hypothalamus on BAT lipogenesis was almost completely abolished by surgical sympathetic denervation of the BAT (Minokoshi, Saito & Shimazu, 1986). These results, together with other observations indicating the existence of a functional link between this region of the brain and the sympathetic nerves in BAT (Perkins, Rothwell, Stock & Stone, 1981; Saito, Minokoshi & Shimazu, 1987), are suggestive of direct sympathetic control of BAT lipogenesis.

Accordingly, we have examined the effects of electrical stimulation of the sympathetic nerves entering the interscapular BAT on lipogenesis in this tissue *in vivo* and compared the results with those obtained when noradrenaline was infused. The results show that sympathetic activation of lipogenesis in BAT is not solely attributable to the action of noradrenaline but involves some non-adrenergic mechanism.

## METHODS

### *Animals*

Female Sprague–Dawley rats weighing 200–250 g were obtained from Nihon Clea (Tokyo). They were housed in plastic cages at 22 ± 1 °C with a 12 h light–dark cycle (lights on at 06.00–18.00 h) and given free access to laboratory chow and water.

### *Experimental procedures*

The rats were anaesthetized with pentobartitone sodium (40 mg/kg, I.P.) and given atropine sulphate (0·3 mg/kg, I.P.). A tracheal cannula was inserted to facilitate respiration. A cannula was also placed in the right jugular vein for infusion of noradrenaline.

A small incision was made above the scapulae and the interscapular BAT was partially separated from the muscle below. Four branches of the nerves to the left side of the BAT were then isolated and sectioned. Segments of the distal portions of these nerves were placed on a bipolar electrode, which was embedded into a tiny plastic plate. The incision was then sutured to secure the electrode. The animals were transferred to a box kept at 30·0 ± 0·3 °C, and the electrode was connected to an electronic stimulator (Nihon Kohden, Tokyo, model SEN-3201).

Forty to sixty minutes later, the rats were injected intraperitoneally with 5 mCi of $^3H_2O$ (New England Nuclear, Boston, MA, U.S.A.) in 0·3 ml isotonic saline and electrical stimuli, consisting of a 2 s train of monophasic square pulses (2 ms, 4–40 Hz, 20 V), were applied to the nerves once every 3 min (stimulation in bursts). After stimulating for 30 min, the animal was killed and the left side of the BAT was quickly removed and weighed. In some experiments, the nerves were stimulated continuously at either 0·22 or 10 Hz (continuous stimulation), or intermittently for 20 s once every minute at 0·67 Hz (intermittent stimulation). In another series of experiments, noradrenaline ((−)-

arterenol hydrochloride, Sigma, St Louis, MO, U.S.A.) was infused into the jugular vein continuously at a final dose of 1–8 $\mu$g/100 g body weight over 30 min. Control rats which were neither stimulated nor infused were set up similarly with an electrode or jugular vein catheter.

When required, propranolol (DL-propranolol hydrochloride, Wako, Osaka) and phenoxybenzamine (phenoxybenzamine hydrochloride, Nakarai Chemicals, Kyoto) were given intraperitoneally at doses of 20 and 5 mg/kg body weight, respectively, 20 min before the start of nerve stimulation or noradrenaline infusion. Guanethidine (guanethidine sulphate, Tokyo Kasei, Tokyo) was administered by a single subcutaneous injection at a dose of 100 mg/kg body weight, 12 h prior to the experiments.

*Measurement of the rate of lipid synthesis*

Lipogenic activity was assessed by measuring the incorporation of $^3H$ from $^3H_2O$ into the fatty acid and glycerol moieties of triglyceride by the following procedures. The interscapular BAT was homogenized with chloroform:methanol (2:1) and the lipid extract was washed 3 times by the procedure of Folch, Lees & Sloane-Stanley (1957). For isolation of radioactive fatty acids, an aliquot of the extract was saponified with ethanolic KOH at 80 °C for 2 h, acidified with sulphuric acid, and extracted with petroleum ether, as described previously (Takahashi & Shimazu, 1982; Saito, Minokoshi & Shimazu, 1985). The $^3H$ radioactivities of total lipids and fatty acids isolated were measured using a Packard Tri-Carb liquid scintillation counter (Hewlett–Packard, I11, Model 300C). The radioactivity of glyceride glycerol was determined from the difference between the amounts of radioactivity incorporated into total lipids and fatty acids. The amount of $^3H$ incorporation was expressed as disintegrations per minute (d.p.m.) per milligram tissue in 30 min.

*Data analysis*

All values were presented as means ± S.E.M. Effects of sympathetic nerve stimulation and noradrenaline infusions were evaluated by one-way analysis of variance. Effects of drugs were also evaluated by two-way analysis of variance. These data were analysed after logarithmic conversion. When a significant effect was found, these results were further compared by the Newman–Keuls' multiple range test. The difference was considered to be significant if $P < 0.05$.

## RESULTS

*Effects of noradrenaline infusion on lipid synthesis in brown adipose tissue*

Figure 1 shows the amount of $^3H$ incorporated into glyceride glycerol and fatty acid moieties of BAT lipid when various doses of noradrenaline were given intravenously over a 30 min period. The $^3H$ incorporation into glyceride glycerol was substantially increased by increasing the dose of noradrenaline ($P < 0.01$ at all of the doses tested), and at 8 $\mu$g 100 $g^{-1}$ 30 $min^{-1}$ noradrenaline it was about 10 times greater than that in the controls. In contrast, $^3H$ incorporation into glyceride fatty acids remained at low levels, being unaffected by the noradrenaline infusion at any of the doses tested.

*Effects of sympathetic nerve stimulation on lipid synthesis in brown adipose tissue*

Effects of sympathetic nerve stimulation on $^3H$ incorporation into glyceride glycerol and fatty acids in BAT were examined using various patterns of stimulation. First, the sympathetic nerves entering the interscapular BAT were stimulated either continuously at 10 Hz, or for 2 s once every 3 min at the same frequency. As shown in Fig. 2, continuous stimulation of the nerves over a 30 min period resulted in a 4-fold increase in $^3H$ incorporation into glyceride glycerol ($P < 0.01$), but a slight and statistically insignificant increase of $^3H$ incorporation into fatty acids. On the other hand, nerve stimulation delivered in 2 s bursts at 3 min intervals produced not only

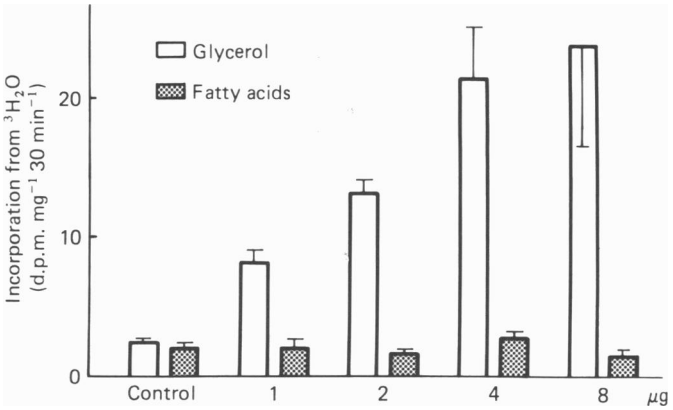


Fig. 1. Incorporation of $^3H$ into glyceride glycerol and fatty acids in response to noradrenaline infusion. Noradrenaline was infused through a jugular vein catheter continuously throughout a 30 min period at a total dose of 1–8 $\mu$g/100 g body weight over 30 min. Values are means ± S.E.M. for five rats.

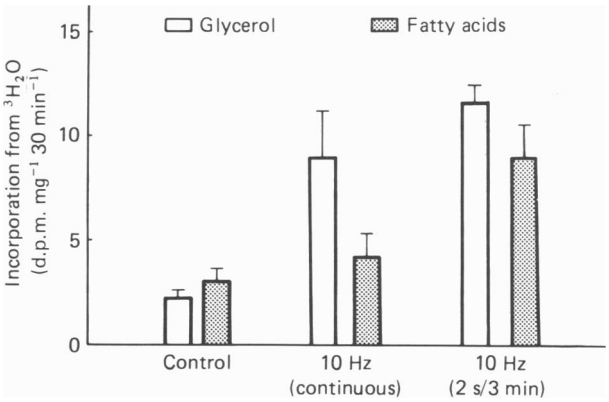


Fig. 2. Incorporation of $^3H$ into glyceride glycerol and fatty acids in the response to nerve stimulation. The sympathetic nerves entering the interscapular BAT were electrically stimulated either at 10 Hz continuously or for 2 s once every 3 min thoughout a 30 min period. Values are means ± S.E.M. for eight or nine rats.

a 5-fold increase of $^3H$ incorporation into glyceride glycerol ($P < 0.01$) but also a substantial (3-fold) increase of $^3H$ incorporation into fatty acids ($P < 0.05$).

The effect of the pattern of nerve stimulation was examined by delivering the same number of impulses over a 30 min period in three different patterns: the nerves were stimulated continuously at 0·22 Hz, or for 20 s once every minute at 0·67 Hz, or for 2 s once every 3 min at 20 Hz (Fig. 3). Stimulation in 2 s bursts produced a large increase in $^3H$ incorporation into fatty acids ($P < 0.01$) as well as into glyceride glycerol ($P < 0.01$). Continuous stimulation also increased the $^3H$ incorporation into these significantly (glycerol: $P < 0.01$; fatty acids: $P < 0.05$), but to a lesser extent than that obtained during stimulation in bursts (glycerol: $P < 0.01$; fatty acids: $P < 0.05$). Intermittent stimulation for 20 s once every minute produced a

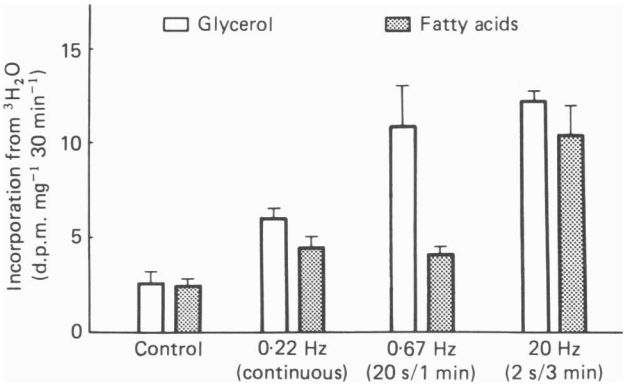


Fig. 3. Effects of pattern of stimulation of lipogenic responses to nerve stimulation delivering the same number of impulses. The sympathetic nerves were stimulated continuously at 0·22 Hz, for 20 s once every minute at 0·67 Hz or for 2 s once every 3 min at 20 Hz throughout a 30 min period. Values are means ± S.E.M. for five rats.

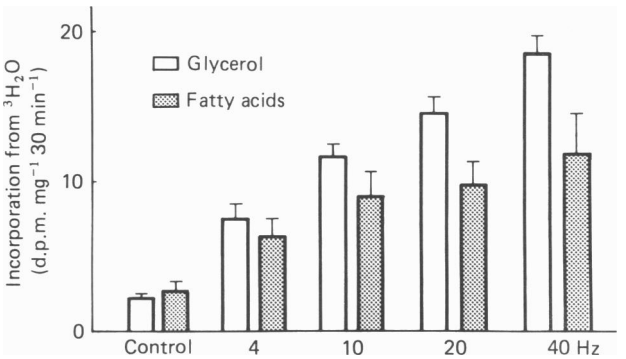


Fig. 4. Effects of stimulus frequency on lipogenic responses to nerve stimulation. The nerves were stimulated for 2 s once every 3 min at the stimulus frequencies of 4–40 Hz throughout a 30 min period. Values are means ± S.E.M. for eight or nine rats.

substantial increase in $^3H$ incorporation into glyceride glycerol ($P < 0·01$) but a much smaller effect on fatty acids ($P < 0·05$). Thus, $^3H$ incorporation into fatty acids was effectively increased only when the nerves were stimulated in bursts.

Incorporation of $^3H$ in response to nerve stimulation in bursts was dependent on the stimulus frequency, being increased by increasing the stimulus frequency (glycerol: $P < 0·01$; fatty acids: $P < 0·05$ at all of the frequencies tested over the range 4–40 Hz); at 40 Hz $^3H$ incorporation into fatty acids and glyceride glycerol was about 5 and 8 times the control values respectively (Fig. 4).

*Effects of α- and β-blockers on lipogenic responses to nerve stimulation and noradrenaline infusion*

As described above, sympathetic nerve stimulation in bursts had a quite different effect on BAT lipogenesis from noradrenaline infusion. Accordingly, the effects of α-

and $\beta$-adrenergic blockers on $^3H$ incorporation after nerve stimulation were examined, and compared with those after noradrenaline infusion.

As shown in Fig. 5, when a $\beta$-blocker (propranolol) and an $\alpha$-blocker (phenoxybenzamine) were given simultaneously, $^3H$ incorporation into glyceride glycerol after noradrenaline infusion was substantially diminished (by about 80%, $P < 0.01$), although it was still greater than the value in the absence of noradrenaline ($P < 0.05$). Incorporation of $^3H$ into fatty acids was not influenced by pre-treatment

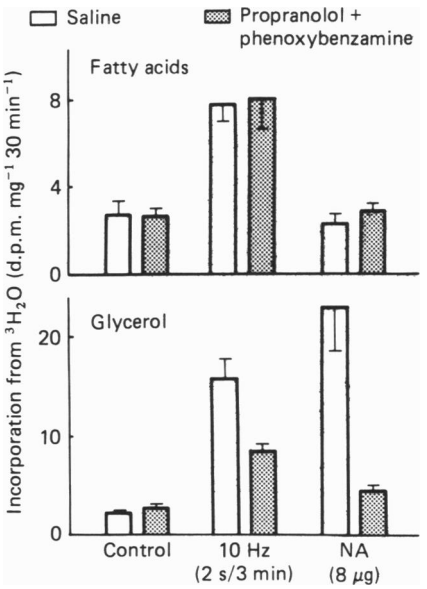


Fig. 5. Effects of adrenergic blockers on lipogenic responses to nerve stimulation and noradrenaline infusion. Propranolol (20 mg/kg) and phenoxybenzamine (5 mg/kg) were injected intraperitoneally, and 20 min later the nerves were stimulated for 2 s once every 3 min at 10 Hz or noradrenaline (NA, 8 $\mu$g 100 g body weight$^{-1}$ 30 min$^{-1}$) was infused. Values are means ± S.E.M. for eight or nine rats.

with these blocking agents. In contrast, $^3H$ incorporation into glyceride glycerol after the nerve stimulation in bursts was decreased by only 40% after combined $\alpha$- and $\beta$-adrenoceptor blockade (Fig. 5). Furthermore, pre-treatment with these blocking agents had no effect on the increased $^3H$ incorporation into fatty acids after nerve stimulation.

Thus, adrenergic blockade suppressed most of the stimulatory effect of noradrenaline on BAT lipogenesis, but it did not influence, or only partially decreased, the lipogenic responses to sympathetic nerve stimulation.

*Effects of guanethidine pre-treatment on the lipogenic responses to the nerve stimulation and noradrenaline infusion*

In order to confirm that the responses of the BAT to nerve stimulation in bursts are of sympathetic origin, the effects of pre-treatment with guanethidine (100 mg/kg) were examined (Fig. 6). This greatly reduced both $^3H$ incorporation

into fatty acids and glyceride glycerol after nerve stimultion in bursts, reducing both to the control levels (glycerol: $P < 0{\cdot}01$; fatty acids: $P < 0{\cdot}01$). In contrast, $^3H$ incorporation into glyceride glycerol during noradrenaline infusion was exaggerated by guanethidine pre-treatment ($P < 0{\cdot}01$), while that into fatty acids was not affected.

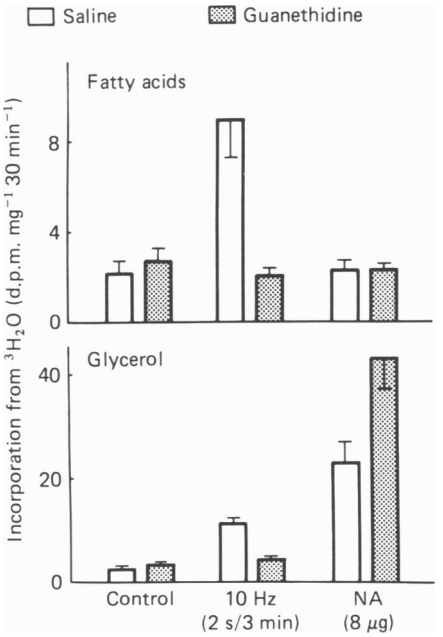


Fig. 6. Effects of guanethidine treatment on lipogenic responses. Guanethidine (100 mg/kg) or saline was administered by a single subcutaneous injection. Twelve hours later, $^3H$ incorporation into glyceride glycerol and fatty acids was measured after nerve stimulation in 2 s bursts at 10 Hz or noradrenaline (NA) infusion (8 $\mu$g 100 g body weight$^{-1}$ 30 min$^{-1}$). Values are means ± S.E.M. for five rats.

## DISCUSSION

In order to examine lipid synthesis in BAT, we used $^3H_2O$ as a radioactive precursor and measured the radioactivity of $^3H$ incorporated into fatty acid and glycerol moieties of lipids extracted from the tissue. This is the most reliable method for assessing lipogenic activity *in vivo*, and in particular for measuring the rate of fatty acid synthesis, because it is independent of the carbon sources. The present study showed that direct electrical stimulation of the sympathetic nerves entering the interscapular BAT caused synthesis of lipid in this tissue and that the effect was not solely attributable to actions of noradrenaline.

A continuous infusion of noradrenaline elicited a marked increase in the $^3H$ incorporation into glyceride glycerol, without any noticeable effect on the $^3H$ incorporation into glyceride fatty acids. It is possible that $^3H$ incorporation into glyceride glycerol is enhanced as a result of increased lipolysis, because $^3H_2O$ can be incorporated into the glycerol moiety if hydrolysis of triglyceride proceeds only to the level of mono- and diglycerides (Ma & Foster, 1986). However, it has been

reported that isotope incorporation from [$^{14}$C]glucose into glyceride glycerol is also increased by noradrenaline *in vitro* (Knight & Myant, 1970). Furthermore, there is evidence indicating that noradrenaline increases glucose utilization in BAT, probably by stimulating glucose transport and activation of certain glycolytic enzymes (Cooney, Caterson & Newsholme, 1985; Gibbins, Denton & McCormack, 1985; Ma & Foster, 1986). Thus, it seems more likely that the increased $^{3}$H incorporation into glyceride glycerol reflected increased synthesis of glycerol-3-phosphate from an intermediate of glycolysis, dihydroxy acetone phosphate.

A quite similar lipogenic response in BAT was observed when the sympathetic nerves were stimulated continuously: i.e. stimulation of the nerves caused an increase in $^{3}$H incorporation into glyceride glycerol, but not significantly into fatty acids. This lipogenic response is, however, qualitatively different from that seen after electrical stimulation of the ventromedial hypothalamus, which results in a concomitant increase in $^{3}$H incorporation into both glyceride glycerol and fatty acids (Minokoshi *et al.* 1986). Thus, neither continuous infusion of noradrenaline nor the continuous nerve stimulation mimics the sympathetically mediated effects of the ventromedial hypothalamus on BAT lipogenesis. This was, however, achieved by stimulation in bursts, which not only increased $^{3}$H incorporation into glyceride glycerol to nearly the same extent as that after continuous stimulation, but also invariably evoked a considerable increase in $^{3}$H incorporation into glyceride fatty acids. The lipogenic responses to nerve stimulation in bursts were also increased by increasing the stimulus frequency over the range 4–40 Hz. It should be noted that stimulation in bursts elicited more pronounced responses than continuous and intermittent stimulation during which precisely the same number of impulses were delivered over the whole period of stimulation (30 min). These findings suggest that the pattern of stimulation is an important determinant of the lipogenic response of BAT, conceivably more so than the absolute number of impulses. This view is compatible with recent reports that responses of various physiological functions to nerve stimulation can be substantially altered merely by changing the pattern of stimulation. For example, whereas continuous stimulation of the pelvic nerves evoked both muscular contraction and vasodilatation in the colon of the cat, stimulation at high frequency delivered for 1 s at 10 s intervals (stimulation in bursts) favours vasodilatation but is ineffective in eliciting colonic contraction (Andersson, Bloom & Järhult, 1983).

Direct sympathetic stimulation thus causes activation of lipid synthesis in BAT. The finding that the lipogenic response to nerve stimulation was qualitatively different from those to noradrenaline suggests that some non-adrenergic mechanism may contribute to sympathetic control of BAT. In support of this, we found that $\alpha$- and $\beta$-adrenergic blocking agents had little effect on the response of fatty acids to nerve stimulation. Similarly, these agents only suppressed a small part of the glyceride glycerol response. This is in contrast to the observation that the same agents almost completely eliminated the responses to noradrenaline infusion. These effects of $\alpha$- and $\beta$-blocking agents are apparently different from those of guanethidine, which is also known to block adrenergic neurones. Pre-treatment with guanethidine effectively abolished the lipogenic response to nerve stimulation, whereas it potentiated the response to noradrenaline. The latter effect could be due

to denervation supersensitivity and the former supports the involvement of a non-adrenergic mechanism, since it has been reported that guanethidine suppresses the release of a neuropeptide as well as that of noradrenaline from sympathetic nerve terminals in the spleen (Lundberg, Änggård, Theodorsson-Norheim & Pernow, 1984).

Thus, it seems likely that some factor or transmitter other than noradrenaline is responsible for sympathetic activation of fatty acid synthesis in BAT. At present, little information is available to suggest what this factor might be, but there are several obvious candidates. One of these is adenosine, which is reported to be released from canine subcutaneous adipose tissue during nerve stimulation and to play a critical role in lipid metabolism (Fredholm & Sollevi, 1981). Alternatively, a neuropeptide could well be involved. It is now widely accepted that some neuropeptides may function as neurotransmitters in certain autonomic effectors (for a review, see Lundberg & Hökfelt, 1986). Vasoactive intestinal polypeptide in parasympathetic neurones innervating the submandibular gland and neuropeptide Y in sympathetic nerves innervating blood vessels provide two obvious examples, and in both cases their release is potentiated when electrical stimuli are delivered in high-frequency bursts rather than continuously at the same average frequency (Andersson, Bloom, Edwards & Järhult, 1982; Allen, Bircham, Bloom & Edwards, 1984). This is entirely compatible with the present finding that non-adrenergic elevation of lipogenic activity in BAT is more pronounced when the nerves are stimulated in a bursting pattern. Furthermore, Cannon, Nedergaard, Lundberg, Hökfelt, Terenius & Goldstein (1986) demonstrated the existence of neuropeptide Y in rat BAT. Although metabolic effects of these neuropeptides on BAT have not yet been established, putative peptidergic control of BAT function is clearly demanding of further investigation.

This study was supported by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture of Japan.

## REFERENCES

ALLEN, J. M., BIRCHAM, P. M. M., BLOOM, S. R. & EDWARDS, A. V. (1984). Release of neuropeptide Y in response to splanchnic nerve stimulation in the conscious calf. *Journal of Physiology* **357**, 401–408.

ANDERSSON, P.-O, BLOOM, S. R., EDWARDS, A. V. & JÄRHULT, J. (1982). Effects of stimulation of the chorda tympani in bursts on submaxillary responses in the cat. *Journal of Physiology* **322**, 469–483.

ANDERSSON, P.-O., BLOOM, S. R. & JÄRHULT, J. (1983). Colonic motor and vascular responses to pelvic nerve stimulation and their relation to local peptide release in the cat. *Journal of Physiology* **334**, 293–307.

CANNON, B., NEDERGAARD, J., LUNDBERG, J. M., HÖKFELT, T., TERENIUS, L. & GOLDSTEIN, M. (1986). 'Neuropeptide tyrosine' (NPY) is co-stored with noradrenaline in vascular but not in parenchymal sympathetic nerves of brown adipose tissue. *Experimental Cell Research* **164**, 546–550.

COONEY, G. J., CATERSON, I. D. & NEWSHOLME, E. A. (1985). The effect of insulin and noradrenaline on the uptake of 2-[1-$^{14}$C]deoxyglucose *in vivo* by brown adipose tissue and other glucose utilizing tissues of the mouse. *FEBS Letters* **188**, 257–261.

FOLCH, J., LEES, M. & SLOANE-STANLEY, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

FOSTER, D. O. & FRYDMAN, M. L. (1978). Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. *Canadian Journal of Physiology and Pharmacology* **56**, 110–122.

FREDHOLM, B. B. & SOLLEVI, A. (1981). The release of adenosine and inosine from canine subcutaneous adipose tissue by nerve stimulation and noradrenaline. *Journal of Physiology* **313**, 351–367.

GIBBINS, J. M., DENTON, R. M. & MCCORMACK, J. G. (1985). Evidence that noradrenaline increases pyruvate dehydrogenase activity and decreases acetyl-CoA carboxylase activity in rat interscapular brown adipose tissue *in vivo*. *Biochemical Journal* **228**, 751–755.

KNIGHT, B. L. & MYANT, N. B. (1970). A comparison between the effects of cold exposure *in vivo* and of noradrenaline *in vitro* on the metabolism of the brown fat of new-born rabbits. *Biochemical Journal* **119**, 103–111.

LUNDBERG, J. M. & HÖKFELT, T. (1986). Multiple co-existence of peptides and classical transmitters in peripheral autonomic and sensory neurons – functional and pharmacological implications. In *Coexistence of Neuronal Messengers: a New Principle in Chemical Transmission*, ed. HÖKFELT, T., FUXE, K. & PERNOW, B., pp. 241–262. Amsterdam: Elsevier.

LUNDBERG, J. M., ÄNGGÅRD, A., THEODORSSON-NORHEIM, E. & PERNOW, J. (1984). Guanethidine-sensitive release of neuropeptide Y-like immunoreactivity in the cat spleen by sympathetic nerve stimulation. *Neuroscience Letters* **52**, 175–180.

MA, S. W. Y. & FOSTER, D. O. (1986). Uptake of glucose and release of fatty acids and glycerol by rat brown adipose tissue *in vivo*. *Canadian Journal of Physiology and Pharmacology* **64**, 609–614.

MINOKOSHI, Y., SAITO, M. & SHIMAZU, T. (1986). Sympathetic denervation impairs responses of brown adipose tissue to ventromedial hypothalamic stimulation. *American Journal of Physiology* **251**, R1005–1008.

NICHOLLS, D. G. & LOCKE, R. M. (1984). Thermogenic mechansims in brown fat. *Physiological Reviews* **64**, 1–64.

PERKINS, M. N., ROTHWELL, N. J., STOCK, M. J. & STONE, T. W. (1981). Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. *Nature* **289**, 401–402.

ROTHWELL, N. J. & STOCK, M. J. (1981). Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis. *Pflügers Archiv* **389**, 237–242.

SAITO, M., MINOKOSHI, Y. & SHIMAZU, T. (1985). Brown adipose tissue after ventromedial hypothalamic lesions in rats. *American Journal of Physiology* **248**, E20–25.

SAITO, M., MINOKOSHI, Y. & SHIMAZU, T. (1987). Ventromedial hypothalamic stimulation accelerates norepinephrine turnover in brown adipose tissue of rats. *Life Sciences* **41**, 193–197.

SHIMAZU, T. & TAKAHASHI, A. (1980). Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose tissue. *Nature* **284**, 62–63.

TAKAHASHI, A. & SHIMAZU, T. (1982). Hypothalamic regulation of lipid metabolism in the rat: effect of hypothalamic stimulation on lipogenesis. *Journal of the Autonomic Nervous System* **6**, 225–235.