by bromocriptine in smooth muscle (Gibson & others, 1977) and within the cns (Lew, Hata & others, 1977) may explain some of the other diverse actions of this drug.

The effect of bromocriptine on the responses to vasopressin was investigated to check the specificity of any antagonism of noradrenaline. The enhancement of the responses to vasopressin in the presence of bromocriptine is interesting but as yet unexplained. It does not appear to be related to α-blockade since the effect was not observed with phentolamine. At low concentrations bromocriptine exhibited no agonist activity on the preparation, but at high concentrations ($>10^{-4}$ M) a small rise in perfusion pressure was observed. Thus it seems that in addition to α-adrenoceptor blockade bromocriptine exerts a second effect on the blood vessels which potentiates responses to vasopressin.

M.S. is supported by a grant from the University Azarabadeghan, and the Ministry of Science, Iran. The authors thank Sandoz A.G. for the gift of bromocriptine. November 25, 1977

REFERENCES

GIBSON, A., JAMES, T., SHAW, N. & TRACEY, E. (1977). *Br. J. Pharmac.*, **61**, 471–472P.

GREENACRE, J. K., TEYCHENNE, P. F., PETRIE, A., CALNE, D. B., LEIGH, P. N. & REID, J. L. (1976). *Br. J. clin. Pharmac.*, **3**, 571–574.

LEW, J. Y., HATA, F., OSHASHI, T. & GOLDSTEIN, M. (1977). *J. Neural Trans.*, **41**, 109–121.

MACGREGOR, D. D. (1965). *J. Physiol., Lond.*, **177**, 21–30.

STUMPE, K. O., KOLLOCH, R., HIGUCHI, M., KRUCK, F. & VETTER, H. (1977). *Lancet*, **2**, 211–214.

THORNER, M. O. (1975). *Ibid.*, **1**, 662–665.

VAN DEN BRINK, F. G. & LIEN, E. J. (1977). *Eur. J. Pharmac.*, **44**, 251–270.

Need for Ca ions in the lipolytic action of 5-hydroxytryptamine in rat brown adipose tissue

KOICHI ITAYA, *Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan*

Although there are reports of the lipolytic effect of 5-hydroxytryptamine (5-HT) on rat epididymal adipose tissue, the effect has not been resolved. Vaughan (1961) reported 5-HT to stimulate glucose uptake at 0·4 μmol ml^{-1}, but it did not significantly alter free fatty acid (FFA) release by epididymal fat pads. Later, we showed that 5-HT did not alter FFA release by epididymal fat pads but it significantly stimulated release by mesenteric adipose tissue (Itaya & Ui, 1964). Furthermore Bieck, Stack & Westerman (1966) showed the lack of lipolytic activity of 5-HT to be mainly due to its rapid inactivation by monoamine oxidase when incubated with rat epididymal adipose tissue.

In contrast, the lipolytic effect of 5-HT on brown adipose tissue has been described recently (Yoshimura, Hiroshige & Itoh, 1969; Fain, Jacobs & Clement-Cormier, 1973; Steiner, 1973). More recently Steiner & Evans (1976) found that the noradrenaline-like action of 5-HT might be indirect and could be mediated by the release of noradrenaline from neurons within the brown fat-pad. On the other hand, Woolley (1958a) had demonstrated that there was need for Ca ions in the contractions of the rat uterus by 5-HT. Ca ions are required for noradrenaline secretion and hence omission of Ca ions would be expected to result in loss of 5-HT effect if this effect is mediated through the stimulation of noradrenaline secretion.

The present study was designed to examine whether 5-HT promotes lipolysis in the interscapular brown adipose tissue in the absence of Ca ions and the effect of 5-HT was compared with that of noradrenaline in tissue from cold-acclimatized rats.

Interscapular brown adipose tissues were obtained from male Wistar strain rats, 200 to 300 g, maintained on a standard pellet diet (Oriental). Animals were allowed free access to food and water. Some rats were placed into a cold room (4°) for 12 days to test the effect of cold acclimation. After decapitation, tissues were rapidly excised, freed of other white fat and muscle, cut into two or four pieces, and added to the flasks containing 1 ml of Krebs-Ringer bicarbonate buffered solution (pH 7·3) with 20 mg bovine serum albumin (Armour). In experiments testing the requirement for Ca ions, Ca ions were omitted from the incubation medium and EDTA was added to 10^{-3} M. Flasks were gassed with 6% CO_2 in oxygen and incubated for 3 h at 37° in a metabolic shaker. One of each pair of tissues obtained from each rat served as a control. Samples of medium before and after incubation were taken for determination of FFA and glycerol by the colorimetric methods of Itaya & Ui (1965) and Burton (1957) respectively.

Dose response relations are shown in Fig. 1. 5-HT at $2·5 \times 10^{-5}$ M markedly promoted lipolysis of brown

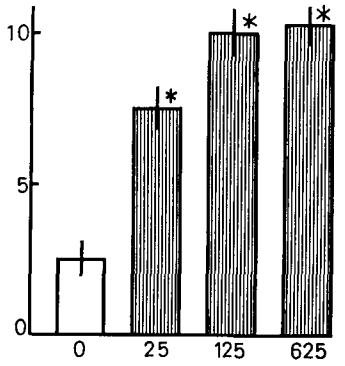

FIG. 1. Dose response relation on tissue from rats of 280 to 300 g. Experimental conditions as in Table 1, except for the Ca^{2+} containing solution. 5-HT was used at the concentrations indicated. Each bar represents the mean ± s.e.m. of six observations. * Indicates a statistically significant difference when compared with the values of control in all figures (*, $P < 0.01$; **, $P < 0.005$). Ordinate: Glycerol release (μmol g^{-1} per 3 h). Abscissa: 5-HT (μM).

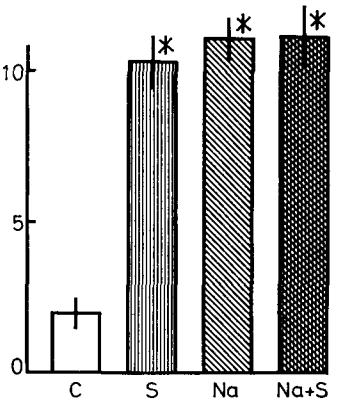

FIG. 2. Effect of the combined addition of 5-HT and noradrenaline on tissue from rats of 270 to 290 g. Experimental conditions as in Table 1, except for the Ca^{2+} containing solution. Each hormone used at 60 μM. C, control; S, 5-HT; Na, noradrenaline. Ordinate: Glycerol release (μmol g^{-1} per 3 h).

adipose tissue. Maximal stimulation of glycerol release was seen with a concentration higher than 1.25×10^{-4} M.

The effect of 5-HT was compared with that of noradrenaline, a natural stimulator in brown adipose tissue (Joel, 1966), at 6×10^{-5} M which is thought to show the maximal stimulation of lipolysis in the fat pads, since catecholamines are usually used at 10^{-5} to 10^{-8} M *in vitro*. The enhancement of lipolysis obtained by 5-HT at the same concentration (60 μM) was nearly as great as that stimulated by noradrenaline (Fig. 2). No additive effect of noradrenaline was observed in the response to 5-HT at which nearly maximal enhancement was observed, suggesting that a system of lipolysis, including adenylate cyclase and triglyceride lipase stimulated by 5-HT, is the same as that enhanced by noradrenaline. Analogous results using ACTH, adrenaline, and glucagon were reported by Birnbaumer & Rodbell (1969) in a plasma membrane-rich fraction of rat epididymal fat cells, 'ghosts'.

Are Ca ions required for the increase in glycerol release by 5-HT as are required for the 5-HT effect on rat uterus contraction reported by Woolley (1958a)? In accordance with his work, Ca ions were required in the incubation medium to obtain the enhancement in glycerol release by 5-HT, but not by noradrenaline (Table 1). Where in the present study Ca ions have been omitted, a chelating substance (EDTA) was added at 10^{-3} M to remove Ca ions completely. The incubation of the tissue in the medium from which Ca ions were omitted and EDTA added, enhanced basal lipolysis, suggesting that Ca ions may be a regulator of basal lipolysis with the capacity to inhibit a lipase activity or the contact of the enzyme to substrates, or EDTA itself may stimulate lipolysis in brown adipose tissue (Appleman & Sevilla, 1970). It was also reported that the Ca ion is required for the enhancement of FFA and glycerol release by catecholamine and in basal release in human adipose tissue (Efendić, Alm & Löw, 1970).

Woolley (1958b) demonstrated that, in the absence of Ca ions, 5-HT could not be extracted by organic solvents containing its specific receptor substance. This suggests that in 5-HT actions, Ca ions may be needed for a complex with the receptor to be formed. Recently it was also reported that 5-HT action on brown fat is mediated by noradrenaline-containing stromal cells (Steiner & Evans, 1976). 5-HT stimulated the release of noradrenaline from the cells. This suggests that Ca ions are essential for 5-HT to release noradrenaline from these cells. On the other hand, in brown adipose tissue of cold-acclimatized rats, the effect of 5-HT on FFA and glycerol release was small in comparison with that

Table 1. *Effect of Ca ions on 5-HT effect.* Rats weighing 220 to 240 g. Samples of brown adipose slices were incubated in 1 ml of Ca^{2+} free Krebs-Ringer bicarbonate buffer pH 7·3, containing 2% bovine serum albumin at 37° for 3 h in a metabolic shaker (120 rev min^{-1}). 5-HT or noradrenaline was added at the concentration of 60 μM. Each value for 'Effect' represents the mean ± s.e.m. The numbers of experiments given in parentheses. NS, not significance.

		Glycerol release (μmol g^{-1} per 3 h)			
Addition	Ca^{2+}	Control	Hormone	Effect	*P*
5-HT	+	3·27	12·23	+8·96 ±1·371 (6)	<0·005
	−	7·89	7·42	−0·47 ±0·715 (6)	NS
Noradrenaline	+	3·13	13·20	+10·07 ±1·366 (6)	<0·001
	−	7·64	13·27	+5·63 ±1·181 (6)	<0·01

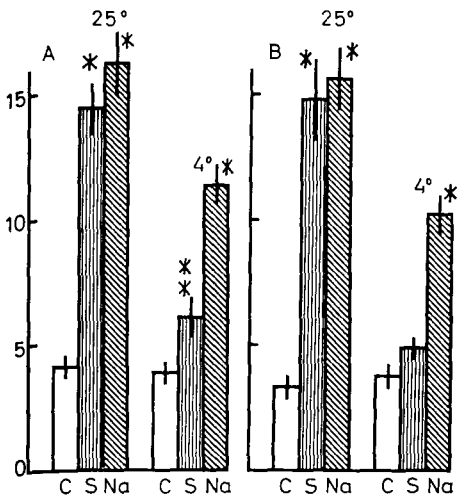

FIG. 3. Lipolytic effect of 5-HT and noradrenaline in brown adipose tissue of rats, 200 to 220 g acclimatized at 4° for 12 days; A, glycerol release; B, FFA release. Normal rats of 240 to 250g were also used. Experimental conditions as in Table 1, except for the Ca^{2+} containing solution. Ordinate: Release (μ mol g^{-1} per 3 h).

in the tissue of normal rats (Fig. 3A and B). The spontaneous lipolysis remains almost unchanged even after cold adaptation, and, although reduced, a certain degree of stimulatory effect of exogenous noradrenaline is present in the same tissue.

Reduced lipolytic response similar to that herein described has been reported with catecholamines, ACTH, and AMP and an enhanced lipolytic response with NaF has also been seen (Dorigo, Maragno & others, 1971; Muirhead & Himms-Hagen, 1971). However, I have found that the brown adipose tissue from cold acclimatized rats is responsive to exogenous noradrenaline, whereas the effect of 5-HT was almost completely abolished. This shows that the lipolytic action of 5-HT is not direct as reported by Steiner & Evans (1976). There may be a shortage of endogenous noradrenaline responsible for the stimulation by 5-HT, or a loss of action of Ca ions with 5-HT or receptors in the brown adipose tissue from the cold-acclimatized rats. Another possibility is that the new cells formed by cell division induced by stress of cold acclimation may not be the same as their mother cells with respect to responsiveness of receptors for several hormones, because receptor production would not keep pace with the cell growth (Kono, 1969; Manganiello & Vaughan, 1972).

These results show the need for Ca ions in the lipolytic action of 5-HT and the indirectness of this action in rat interscapular brown adipose tissue.

I am greateful to Dr M. Ui for his helpful advice.

November 30, 1977

REFERENCES

APPLEMAN, M. M. & SEVILLA, C. L. (1970). *Role of Cyclic AMP in Cell Function*, pp 209–216. Editors: Greengard, P. and Costa, E., New York: Raven Press.

BIECK, P., STACK, K. & WESTERMAN, E. O. (1966). *Life Sci.*, **5**, 2157–2163.

BIRNBAUMER, L. & RODBELL, M. (1969). *J. biol. Chem.*, **244**, 3477–3482.

BURTON, R. M. (1957). *Methods in Enzymolozy*, Vol. 3, p. 246–249. Editors: Colowick, S. P. and Kaplan, N. O. New York: Academic Press.

DORIGO, P., MARAGNO, I., BRESSA, A. & FASSINA, G. (1971). *Biochem. Pharmac.*, **20**, 1201–1211.

EFENDIĆ, S., ALM, B. and LÖW, H. (1970). *Horm. Metab. Res.*, **2**, 287–291.

FAIN, J. N., JACOBS, M. D. & CLEMENT-CORMIER, Y. C. (1973). *Am. J. Physiol.*, **224**, 346–351.

ITAYA, K. & UI, M. (1964). *Biochim. biophys. Acta*, **84**, 604–606.

ITAYA, K. & UI, M. (1965). *J. Lipid Res.*, **6**, 16–20.

JOEL, C. D. (1966). *J. biol. Chem.*, **241**, 814–821.

KONO, T. (1969). *Ibid.*, **244**, 5777–5784.

MANGANIELLO, V. & VAUGHAN, M. (1972). *J. Lipid Res.*, **13**, 12–16.

MUIRHEAD, M. & HIMMS-HAGEN, J. (1971). *Can. J. Biochem.*, **49**, 802–810.

STEINER, G. (1973). *The Pharmacology of Thermoregulation*, p. 42–56. Editors: Lomax, P. and Schönbaum, E. Basel: Karger.

STEINER, G. & EVANS, S. (1976). *Am. J. Physiol.*, **231**, 34–39.

VAUGHAN, M. (1961). *J. biol. Chem.*, **236**, 2196–2199.

WOOLLEY, D. W. (1958a). *Proc. natn. Acad. Sci. U.S.A.*, **44**, 197–201.

WOOLLEY, D. W. (1958b). *Ibid.*, **44**, 1202–1210.

YOSHIMURA, K., HIROSHIGE, T. & ITOH, S. (1969). *Jap. J. Physiol.*, **19**, 176–186.