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Inhibitory effect of 5-hydroxytryptamine on lipogenesis in rat adipose tissues

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5-Hydroxytryptamine (5-HT) inhibited the incorporation of ^{14}C from ^{14}C-labelled glucose, pyruvate, citrate and acetate into fatty acids but it did not inhibit the conversion of ^{14}C from citrate and acetate into CO_2, and the citrate conversion into glyceride-glycerol in epididymal and mesenteric adipose tissue from 24h-fasted rats. 5-HT stimulated the formation of lactate from glucose and pyruvate, and increased the ratio of lactate produced/pyruvate taken up. This ratio was similar to the NADH:NAD ratio. These results indicate that 5-HT inhibits fatty acid synthesis in rat white adipose tissue by mechanisms similar to those of the catecholamines.

5-Hydroxytryptamine (5-HT) stimulates the release of free fatty acids (FFA) from rat adipose tissues (in mesenteric adipose tissue, Itaya & Ui 1964; in brown adipose tissue, Yoshimura et al 1969; Steiner 1973; Itaya 1978a,b). Catecholamines inhibit fatty acid synthesis from glucose, pyruvate and other substrates although they also stimulate FFA release from adipose tissue. I have examined whether 5-HT has some influence on fatty acid synthesis in rat white adipose tissues.

MATERIALS AND METHODS

Male rats, Wistar-derived, 200 to 250 g, maintained on a chow diet were fasted for 24 h. The epididymal and mesenteric fat pads were removed, rinsed with saline and spread on moistened filter paper and then divided into two or four pieces (each about 50 mg). These were then incubated in 1 ml of medium (20 mg of bovine serum albumin per 100 ml of Krebs-Ringer bicarbonate buffer with or without 5-HT 250 $\mu g\ ml^{-1}$ and gassed with 6% CO_2 in oxygen) in 10 ml Erlenmeyer flasks at 37 °C for 3 h. Moreover unlabelled and ^{14}C-labelled-glucose, -pyruvate, -citrate or -acetate were added as indicated in each Table.

After the 3 h incubation, 0·2 ml of Hyamine[p-(diisobutylcresoxy-ethoxyethyl) dimethyl benzylamine] was injected through the rubber stopper into the hanging well and 0·2 ml of 0·5 M H_2SO_4 was injected into the medium and then the set of incubation vials were further incubated for 15 min at 37 °C. The production of $^{14}CO_2$ was determined by the method of Rodbell (1964). Each piece of pad was then removed from the flask, blotted, rinsed in saline and homogenized separately in chloroform-methanol (2:1 v/v). The lipids, extracted by the method of Folch et al (1957), were dried under nitrogen and saponified by the method of Rodbell (1964). The quantity of carbon from each substrate converted to CO_2, and taken up in the glyceride-glycerol and fatty acids (FFA) formed were then calculated from the initial specific activity of each substrate added to the medium and the quantity of radioactivity in the products. Results are expressed as micromoles of substrate converted by 1 g of wet tissue in 3 h.

In another experiment, glucose and lactate in the medium were determined by the method of Bergmeyer & Bernt (1963) and Barker & Summerson (1941), respectively. Glycogen content in adipose tissue and the FFA in the medium were determined colorimetrically by the method of Seifter et al (1950) and Itaya (1977), respectively, pyruvate by the method of Passonneau & Lowry (1974), NADH and NAD, by the method of Klingenberg (1974), and ATP, by the application of bioluminescent reaction (Strehler 1974). The incorporation of ^{32}P into the ATP fraction was determined according to Tokumitsu & Ui (1973).

RESULTS

Effect of 5-HT on glucose-U-^{14}C conversion to fatty acids. Some glucose-U-^{14}C was incorporated into fatty acids in the presence of 5-HT. The major recovery of the label was in the carbon dioxide fraction (Table 1). The extent of ^{14}C incorporation into glyceride-glycerol was about the same as in the control for fatty acids. However, the degree of incorporation of ^{14}C into fatty acids was diminished by 5-HT to less than one third of the control value.

In another experiment, 5-HT slightly but significantly ($P < 0.05$) stimulated glycogenolysis in

Table 1. Effect of 5-HT on glucose-U-^{14}C conversion to fatty acids, CO_2 or glyceride-glycerol. Pieces of epididymal and mesenteric adipose tissues were incubated in control and 5-HT containing medium (250 μg ml^{-1}), to which glucose-U-^{14}C (0·2 μCi ml^{-1}) was added, in the presence of 3mM glucose for 3 h at 37 °C. Each value represents μmol of glucose carbon converted to each fraction in 3 h g^{-1} of wet tissue. The number of experiments is in parentheses. NS, not significant.

Control	5-HT	Effect*	P
	CO_2 (μmol g^{-1} in 3 h)		
Epididymal 9·05	6·65	−2·40 ± 0·646 (6)	<0·025
Mesenteric 21·63	16·00	−5·63 ± 1·021 (6)	<0·005
	Glyceride-glycerol (μmol g^{-1} in 3 h)		
Epididymal 1·91	1·49	−0·42 ± 0·092 (6)	<0·01
Mesenteric 3·62	3·71	−0·09 ± 0·111 (6)	NS
	Fatty acid (μmol g^{-1} in 3 h)		
Epididymal 1·37	0·42	−0·95 ± 0·241 (6)	<0·025
Mesenteric 3·86	1·11	−2·75 ± 0·741 (6)	<0·025

* Each value for 'Effect' represents the mean ± s.e.m.

epididymal tissue only. Glycogen contents after 3 h incubation were 0·36 μmol as glucose g^{-1} of control tissue, and 0·21 μmol g^{-1} of 5-HT-treated tissue. Although glucose uptake was not inhibited by 5-HT, the lactate production from glucose for the 3 h incubation was stimulated from 1·19 to 1·77 mg g^{-1} of epididymal and from 1·35 to 2·45 mg g^{-1} of mesenteric adipose tissue.

These results suggest that the net intermediates for fatty acid synthesis from glucose were decreased by 5-HT.

Effect of 5-HT on conversion of ^{14}C from glucose-1-^{14}C and -6-^{14}C to fatty acids. The effects of 5-HT were compared on glucose-1-^{14}C and -6-^{14}C. A higher inhibition of ^{14}C onversion to fatty acids was seen with the -6-^{14}C (Table 2) but in the glyceride-glycerol conversion, there was little difference in the effect of 5-HT in epididymal tissue, and in mesenteric fat, 5-HT did not inhibit the incorporation of ^{14}C from either glucose-1-^{14}C or -6-^{14}C.

Effect of 5-HT on pyruvate conversion to fatty acids. 5-HT inhibited the uptake of ^{14}C from pyruvate-[U-^{14}C] into the three fractions. The extent of inhibition was highest in fatty acid fraction (Table 3). In another experiment using unlabelled pyruvate,

Table 2. Effect of 5-HT on glucose-1- or -6-^{14}C conversion to fatty acids, CO_2 or glyceride-glycerol. For experimental conditions except for glucose-1- or -6-^{14}C (0·1 μCi ml^{-1}) and 3 mM glucose, see legend to Table 1.

Glucose-1- or -6-^{14}C	Control	5-HT	Effect	P
	CO_2 (μmol g^{-1} in 3 h)			
Epididymal				
1	2·12	1·65	−0·47 ± 0·136 (6)	<0·025
6	1·34	1·00	−0·34 ± 0·052 (6)	<0·005
Mesenteric				
1	7·42	5·00	−2·42 ± 0·531 (6)	<0·01
6	2·57	2·15	−0·42 ± 0·124 (6)	<0·025
	Glyceride-glycerol (μmol g^{-1} in 3 h)			
Epididymal				
1	0·58	0·41	−0·17 ± 0·047 (6)	<0·025
6	0·75	0·57	−0·18 ± 0·027 (6)	<0·01
Mesenteric				
1	0·81	0·87	+0·06 ± 0·044 (6)	N.S.
6	1·29	1·45	+0·16 ± 0·091 (6)	N.S.
	Fatty acid (μmol g^{-1} in 3 h)			
Epididymal				
1	0·39	0·13	−0·26 ± 0·101 (6)	<0·05
6	0·96	0·25	−0·71 ± 0·053 (6)	<0·001
Mesenteric				
1	0·99	0·62	−0·37 ± 0·139 (6)	<0·05
6	2·84	1·14	−1·70 ± 0·158 (6)	<0·001

Table 3. Effect of 5-HT on pyruvate-U-^{14}C conversion to fatty acids, CO_2 or glyceride-glycerol. For experimental conditions except for pyruvate-U-^{14}C (0·1 μCi ml^{-1}) and 6 mM pyruvate, see legend to Table 1.

Control	5-HT	Effect	P
	CO_2 (μmol g^{-1} in 3 h)		
Epididymal 61·23	44·07	−17·15 ± 5·631 (6)	<0·05
Mesenteric 55·54	46·08	−9·46 ± 1·938 (6)	<0·005
	Glyceride-glycerol (μmol g^{-1} in 3 h)		
Epididymal 2·62	1·95	−0·67 ± 0·146 (6)	<0·01
Mesenteric 2·10	1·55	−0·55 ± 0·126 (6)	<0·01
	Fatty acid (μmol g^{-1} in 3 h)		
Epididymal 2·98	0·46	−2·52 ± 0·580 (6)	<0·01
Mesenteric 2·85	0·36	−2·49 ± 0·377 (6)	<0·005

5-HT stimulated lactate production from pyruvate from 0·73 to 0·83 in epididymal and from 0·74 to 1·10 mg g^{-1} in 3 h in mesenteric adipose tissue. NADH was increased in 5-HT-treated tissue while NAD was decreased. The ratio NADH/NAD in 5-HT-treated mesenteric adipose tissue (0·56) was higher than that in control tissue (0·26). The ratio of lactate produced: pyruvate taken up closely resembled the NADH: NAD ratio (0·60 in 5-HT-treated and 0·34 in control tissue).

Effect of 5-HT on citrate conversion to fatty acids. 5-HT inhibited the incorporation of ^{14}C from citrate

into fatty acids, but not into CO_2 or glyceride-glycerol (data not shown).

Effect of 5-HT on acetate conversion to fatty acids. 5-HT had no effect on $^{14}CO_2$ formation from acetate-U-^{14}C in either tissue, and incorporation of ^{14}C into glyceride-glycerol was slightly inhibited. However, 5-HT produces a significant decrease in lipid synthesis by 30 to 50% of control (Table 4).

Effect of 5-HT on ATP concentration in adipose tissue and incorporation of ^{32}P into ATP. The ATP content of the tissues was decreased by 5-HT from 93 to 74 and from 141 to 128 nmol g^{-1} for epididymal and mesenteric tissue, respectively. Incorporation of ^{32}P into ATP was also inhibited about 20% by 5-HT.

DISCUSSION

Although fatty acid synthesis is depressed in adipose tissue from fasted rats (Rose & Shapiro 1955), I used tissues of starved animals to avoid the dilution effect of ^{14}C-labelled substrate with the carbon of intermediates formed from glycogen by glycogenolysis and glycolysis. Lactate production after 3 h incubation of adipose tissue from fasted rats was insignificant in the absence of substrates added to the medium. The glycogen content in adipose tissue from fasted rats is generally low (0·04 to 0·1 mg g^{-1}), in contrast to other tissues such as liver and muscle (2 to 7 mg g^{-1}), therefore, even if glycogen from such tissue is extensively converted to glucose, the amount of glucose formed is about 1/10 to 1/100 that of glucose taken up by adipose tissue from the incubation medium in the 3 h incubation. Moreover, because the degree of glycogenolysis stimulated by 5-HT was slight and seen only in epididymal tissue, the dilution of ^{14}C-labelled intermediates with substrates derived from glycogen is negligible.

So, although I did not measure the specific ^{14}C activity of each precursor of fatty acid, the decrease in ^{14}C conversion from substrates to fatty acids by 5-HT can be taken as an inhibition of fatty acid synthesis by 5-HT.

Fatty acid synthesis has been shown to depend on the reducing effect of NADPH (Martin et al 1961) generated partly by the enzymes of the pentose shunt. 5-HT caused a decrease in the rate of $^{14}CO_2$ formation from glucose-1-^{14}C and -6-^{14}C, the ratio 1-$^{14}CO_2$:6-$^{14}CO_2$ decreasing in the presence of 5-HT in mesenteric but not in epididymal tissue. This suggests that the inhibitory effect of 5-HT on fatty acid synthesis is partly due to a decrease in NADPH formation.

5-HT increases FFA in mesenteric adipose tissue (Itaya & Ui 1964). FFA accumulation in cytoplasm induces a fall in ATP (Angel et al 1971). The lowering of the intracellular ATP concentration could be a possible mechanism of reduction of fatty acid synthesis by catecholamines. 5-HT reduced ATP concentrations in both epididymal and mesenteric tissues which may be a mechanism by which 5-HT inhibits fatty acid synthesis in these tissues.

The production of $^{14}CO_2$ from acetate-U-^{14}C and citrate-1,5-^{14}C was not affected by 5-HT. This suggests that the activity of TCA cycle itself was not inhibited by 5-HT, and that there are other steps inhibited by 5-HT distal to TCA cycle in fatty acid synthesis. Citrate is cleaved into acetyl-CoA and oxaloacetate by the ATP-dependent citrate cleavage enzyme in the cytosol. Although 5-HT did not inhibit the enzyme activity directly in vitro (data not shown), the rate of reaction catalysed by this cleavage enzyme must be reduced in the cytosol of the intact cells, because 5-HT decreased the concentration of ATP in this tissue.

The extent of inhibition by 5-HT of fatty acid synthesis from acetate or citrate was less than that from pyruvate. This suggests that the conversion of pyruvate to acetyl-CoA catalysed by pyruvate dehydrogenase may be decreased by incubation with 5-HT. This enzyme is regulated by a cyclic nucleotide-independent phosphorylation-dephosphorylation cycle (Coore et al 1971), but even in the active (dephosphorylated) form, the enzyme is inhibited by NADH (Batenburg & Olson 1976). This inhibition seems to occur in adipose tissue incubated with 5-HT,

Table 4. Effect of 5-HT on acetate-U-^{14}C conversion to fatty acids, CO_2 or glyceride-glycerol. Adipose tissues were incubated in the presence of 0·6 or 6 mM glucose. For other conditions except for acetate-U-^{14}C (0·1 μCi ml^{-1}) and 15 mM acetate, see legend to Table 1.

Glucose (mM)	Control	5-HT	Effect	P
	CO_2 (μmol g^{-1} in 3 h)			
Epididymal				
0·6	17·49	14·84	−2·65 ± 0·972 (6)	NS
6·0	15·53	10·73	−4·75 ± 1·382 (6)	NS
Mesenteric				
0·6	18·30	19·20	+0·90 ± 1·052 (6)	NS
6·0	17·23	17·45	+0·22 ± 0·854 (6)	NS
	Glyceride-glycerol (μmol g^{-1} in 3 h)			
Epididymal				
0·6	0·93	0·65	−0·28 ± 0·052 (6)	<0·005
6·0	0·90	0·71	−0·19 ± 0·054 (6)	<0·025
Mesenteric				
0·6	0·93	0·69	−0·24 ± 0·026 (6)	<0·001
6·0	1·04	0·64	−0·40 ± 0·094 (6)	<0·01
	Fatty acid (μmol g^{-1} in 3 h)			
Epididymal				
0·6	1·08	0·39	−0·69 ± 0·097 (6)	<0·001
6·0	2·44	1·47	−0·97 ± 0·167 (6)	<0·005
Mesenteric				
0·6	1·88	0·84	−1·04 ± 0·104 (6)	<0·001
6·0	4·39	2·05	−2·34 ± 0·460 (6)	<0·005

because the concentration of NADH in the tissue was increased by 5-HT.

The conversion of pyruvate or acetate into glyceride-glycerol was inhibited by 5-HT in both tissues (Tables 3, 4). Glucose conversion into the same fraction was inhibited by 5-HT in epididymal, but not in mesenteric tissue (Tables 1, 2). This shows the difference in metabolic pathway from glucose to α-glycerophosphate and from smaller intermediates such as pyruvate or acetate to α-glycerophosphate that may occur between epididymal and mesenteric adipose tissue. Earlier findings (Itaya & Ui 1964; Itaya 1978b) suggest this to be likely.

Thus 5-HT inhibits the fatty acid synthesis in rat white adipose tissues in a manner similar to catecholamines (Cahill et al 1959). However, whether the inhibitory action of 5-HT on fatty acid synthesis is mediated by noradrenaline contained in nerve endings in rat white adipose tissue like as the lipolytic action of 5-HT in brown adipose tissue (Steiner & Evans 1976) has yet to be ascertained.

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