Effects of Melatonin and Serotonin on ^{14}C-Glucose Metabolism in Rabbit Adipose Tissue

Recently, melatonin has been identified in blood using mass spectroscopy[1]. Evidence of peripheral action of melatonin on gonads and certain blood constituents have also been described previously[2,3]. The current study was undertaken to investigate the effects of melatonin and serotonin on glucose metabolism in rabbit adipose tissue in vitro[4].

Materials and methods. The melatonin[5] and serotonin were obtained from Sigma Chemical Co., St. Louis, Mo. The experiments were performed on rabbit perirenal fat pads using the method of TANAKA et al.[6] with the following modifications: uniformly labeled ^{14}C-glucose was used as the tracer in these experiments; $^{14}CO_2$ was collected in center wells using hyamine hydroxide. The fat pads and medium were analyzed together. Lipids were extracted by the method of DOLE and MEINERTZ[7]. One portion of the heptane layer was brought to dryness in a counting vial and the lipid content was determined gravimetrically before adding scintillator to the counting vial to determine total lipid radioactivity. A second heptane aliquot was dried and then saponified with the 5 ml of 0.05 *M* KOH in 85% ethanol for 90 min at 75 °C. Fatty acids were then extracted by the method of BÖRGSTROM[8] using N-heptane as solvent. A portion of the heptane extract was evaporated, weighed gravimetrically, and counted. The glyceride-glycerol incorporation was then determined as the difference between the total lipid radioactivity and the fatty acid radioactivity.

All counting was done using liquid scintillation spectrometry in a Nuclear Chicago Isocap 300 System The scintillator was prepared with 3% PPO and 0.1% POPOP in toluene.

DPM were calculated using an external standard ratio with the aid of an Olivetti 602 Computer System. The various experiments were compared taking the controls as 100%, and other effects were determined as percentages of the control values. Statistical analysis was performed using the analysis of variance and the Student's *t*-test[9].

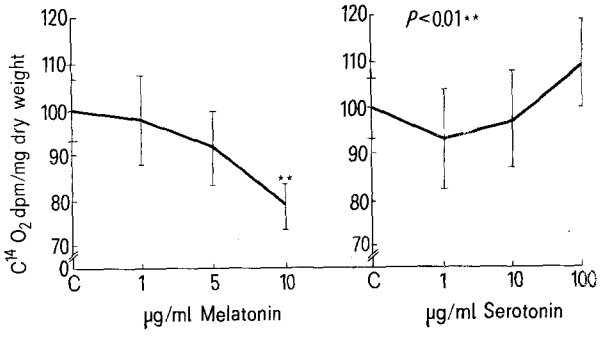

Fig. 1. Effect of serotonin and melatonin on ^{14}C-glucose oxidation in rabbit adipose tissue. Statistically significant effect ($p < 0.01$) were noted at 10 µg/ml, while no effects were seen with serotonin even at higher concentrations. Each point represents mean values of 6 experimental samples expressed as percentages of the control, arbitrarily fixed at 100%. The vertical lines indicate ± S.E.M. ** $P < 0.01$. The values for significance are based on the difference between the experimental and control results.

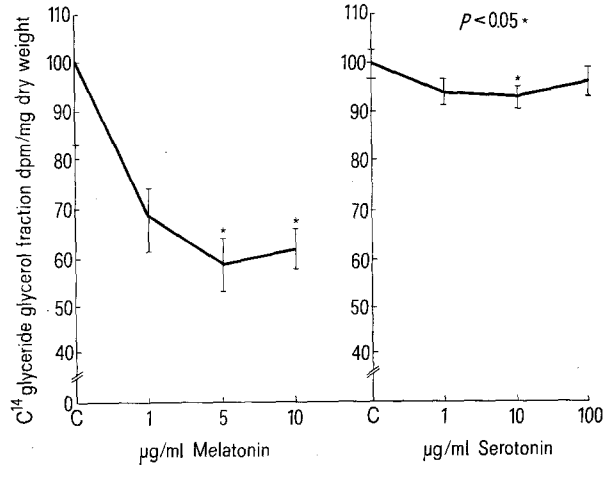

Fig. 2. Effects of melatonin and serotonin on ^{14}C-glyceride-glycerol synthesis. Both melatonin and serotonin decreased the incorporation of ^{14}C-glucose into glyceride-glycerol fractions. Significant *p*-values are indicated. Each point represents mean values of 6 experimental samples expressed as percentages of the control, arbitrarily fixed at 100%. The vertical lines indicate ± S.E.M. * $p < 0.05$. The values for significance are based on the difference between experimental and control results.

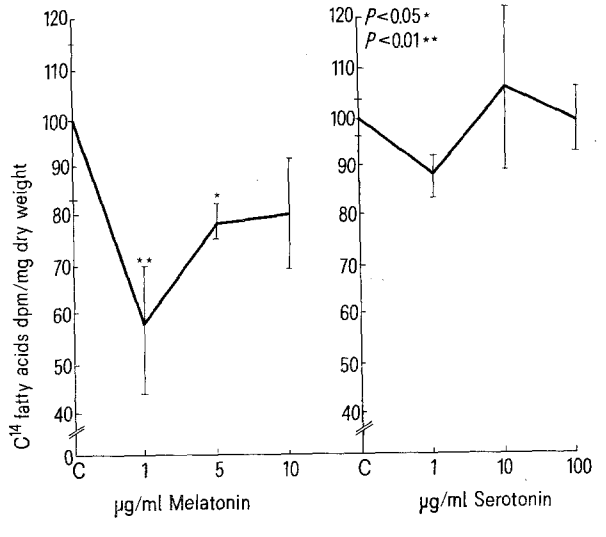

Fig. 3. Effect of melatonin and serotonin on ^{14}C-fatty acid synthesis from ^{14}C-glucose. Melatonin inhibited ^{14}C-fatty acid synthesis while serotonin did not show similar effects. Each value represents mean values of 6 experimental samples expressed as percentages of control, arbitrarily fixed at 100%. The vertical lines indicate ± S.E.M. * $P < 0.05$; ** $p < 0.01$. The values for significance are based on the difference between the experimental and control results.

[1] R. W. PELHAM, Biochem. biophys. Res. Commun. *46*, 1236 (1972).
[2] L. DEBELJUK, Endocrinology *89*, 1117 (1971).
[3] J. K. BURNS, J. Physiol., Lond. *226*, 106 (1972).
[4] G. G. MURTHY and R. R. MODESTO, Clin. Res. *21*, 980 (1973).
[5] The concentrations of melatonin in the rabbit blood at present are unknown. The doses used (1–10 µg/ml) are arbitrary and are in accordance with those used in similar experiments[12]. The effects noted at these levels may be physiological or pharmacological.
[6] A. TANAKA, B. T. PICKERING and C. H. LI, Arch. Biochem. Biophys. *99*, 294 (1962).
[7] V. P. DOLE and H. MEINERTZ, J. biol. Chem. *235*, 2595 (1960).
[8] G. BÖRGSTROM, Acta physiol. scand. *25*, 101 (1952).
[9] R. C. CAMPBELL, *Statistics for Biologists* (Cambridge University Press 1967).

Results. Effect of melatonin. Melatonin decreased ^{14}C-glucose oxidation to $^{14}CO_2$ at concentration of 10 µg/ml Simultaneously, it decreased its incorporation into glyceride-glycerol fraction at a level of 5 µg/ml and into fatty acids at concentration of 1 µg/ml (Figures 1–3).

Effect of serotonin. Serotonin did not have any statistically significant effect on glucose oxdiation or fatty acid synthesis at concentrations even higher than 10 µg/ml. A decrease in glucose incorporation into the glyceride-glycerol fraction was noted at the 10 µg level (Figures 1–3).

Discussion. Although melatonin and serotonin are structural analogues, their effects on glucose metabolism appear to be different. Melatonin decreased ^{14}C-glucose oxidation while serotonin was inactive. Melatonin decreased ^{14}C-glucose incorporation into glyceride-glycerol fractions and serotonin showed qualitatively similar effects. Melatonin decreased ^{14}C-fatty acid synthesis while serotonin did not show similar effects. These data indicate that melatonin may have peripheral effects, other than those described previously[2,3], and its peripheral inhibitory effects[10] may partly be mediated by its effects on glucose metabolism. The physiological significance of these observations in regulation of lipid metabolism in vivo is at present uncertain and remains to be elucidated[11].

Résumé. Nous avons étudié les effets de la mélatonine et de la sérotonine sur le métabolisme du ^{14}C-glucose dans le tissu adipeux du lapin. La mélatonine semble inhiber la synthèse des acides gras et l'oxidation du glucose alors que la sérotonine est sans action. La mélatonine et la sérotonine empêchent l'incorporation du ^{14}C-glucose dans la fraction glycéride-glycérol.

G. G. MURTHY and R. R. MODESTO

Research Building 61, Veterans Administration Hospital, Northport (New York 11768, USA), 15 August 1974.

[10] J. AXELROD, Science *184*, 1341 (1974).

[11] This research was supported by Veterans Administration Institutional Funds at the Veterans Administration Hospital, Northport, New York 11768, USA. The authors wish to acknowledge the secretarial assistance of Mrs. GRACE NAUGHTON of the Research Service, Veterans Administration Hospital, Northport, in the preparation of this paper.

[12] C. E. LEGRAND, Endocrinology *90*, 17 (1962).

Hormonal Imputs and Brain Tryptophan Metabolism: the Effect of Growth Hormone

A large body of experimental evidence in animals and in the human has accumulated in recent years for the participation of brain monoamines, norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in the neurohormonal control of anterior pituitary (AP) hormones[1].

Much attention has been given to the presence of serotoninergic pathway(s) mediating neuroendocrine regulation of gonadotropins and prolactin secretion. Intraventricular instillation of 5-HT in the rat enhanced prolactin concentration in the peripheral plasma and decreased plasma concentrations of gonadotropins[2]. Systemic administration of 5-HT precursors, l-tryptophan (TP) or 5-hydroxy-L-tryptophan (5-HTP) increased serum prolactin concentrations in both laboratory animal[3] and the human[4]; 5-HTP has also been implicated in the control of growth hormone (GH) release in the human[5], a finding not corroborated by other studies[6].

Studies on the effect of varying endocrine function on 5-HT metabolism have also been reported, though most have been concerned with the effects of adrenal steroids[7–10]. Here we report data on brain 5-HT metabolism in two experimental conditions which have in common a lack of pituitary GH and refer also to the effect of a GH replacement therapy.

Female Sprague-Dawley rats were obtained at weaning, fed a standard laboratory diet ad lib, maintained at 22 ± 2 °C and exposed to 14 h of light each day (06.00–20.00 h). Experiments were performed 15 days following hypophysectomy in 40-day-old rats. Age-matched intact rats were used as controls. On the day of the experiment animals were killed by decapitation between 08.30–09.00 h. Brain was removed, weighed and stored at −20 °C until assayed for TP, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA)[11,12]. Brain concentrations of both TP and 5-HIAA were markedly higher in hypophysectomized rats than in age-matched intact controls, while brain 5-HT levels were comparable in the two groups (Table I). It is generally recognized that the rate of 5-HT formation in the brain is regulated principally by the availability of the amino acid precursor TP, the normal concentration of which in tissues is below the *Km* for the first enzyme in 5-HT biosynthetic pathway, tryptophan hydroxylase[13]. The finding that hypophysectomized rats had elevated brain TP levels, and the reported inability of hypophysectomy to affect brain TP hydroxylase[14], suggest that pituitary ablation may be associated with an increased brain 5-HT metabolism. The observation that brain 5-HIAA concentrations were also increased by hypophysectomy further substantiates this hypothesis. However, an alteration of 5-HIAA efflux rate from the brain cannot be excluded at present.

The effect of hypophysectomy on brain 5-HT metabolism could be ascribed to the deficiency of one or more hormonal product(s) secreted by the AP itself or by a peripheral target gland under AP regulatory control.

[1] R. J. WURTMAN, Neurosciences Res. Prog. Bull. *9*, 171 (1970).
[2] I. A. KAMBERI, R. S. MICAL and J. C. PORTER, Endocrinology *88*, 1288 (1971).
[3] K. H. LU and J. MEITES, Endocrinology *93*, 152 (1973).
[4] J. H. MCINDOE and R. W. TURKINGTON, J. clin. Invest. *52*, 1972 (1973).
[5] H. IMURA, Y. NAKAI and T. YOSHIMI, J. clin. Endocr. Metab. *36*, 204 (1973).
[6] E. E. MÜLLER, F. BRAMBILLA, F. CAVAGNINI, M. PERACCHI and A. PANERAI, J. clin. Endocr. Metab. *39*, 1 (1974).
[7] E. C. AZMITIA and B. S. MCEWEN, Science *166*, 1274 (1969).
[8] W. LOWENBERG, Fifth Int. Pharmacol. Congr. (1972), p. 250.
[9] S. A. MILLARD, E. COSTA and E. M. GAL, Brain Res. *16*, 227 (1972).
[10] E. C. AZMITIA, S. ALGERI and E. COSTA, Science *169*, 201 (1970).
[11] P. F. SPANO, K. SZISKA, C. L. GALLI and A. RICCI, Pharmac. Res. Commun. *6*, 163 (1974).
[12] G. CURZON and A. R. GREEN, Br. J. Pharmac. *39*, 653 (1970).
[13] E. JEQUIER, W. LOVENBERG and SJOERDSMA, Molec. Pharmac. *3*, 274 (1967).
[14] T. DEGUCHI and J. BARCHAS, in *Serotonin and Behavior* (Eds. J. B. BARCHAS and E. USDIN; Academic Press, New York 1973), p. 33.