Oxidation of ^{14}C-1 and ^{14}C-6-Glucose by Hormone Synthesizing and Hormone Secreting Portions of Neurohypophysial Neurons

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(Received September 3, 1965)

SUMMARY

The conversion of ^{14}C-1 of glucose to $^{14}CO_2$ *in vitro* by bovine posterior pituitary slices was 13–20 times as great as that of ^{14}C-6; this finding indicates an active hexose monophosphate (HMP) shunt as reported for other endocrine tissues. Nerve endings isolated by centrifugation from the posterior pituitary gave a similar ratio. Tissue obtained from the paraventricular nuclei (PVN) of bovine hypothalamus gave a C-1:C-6 oxidation ratio of 1.1, similar to ratios found in other areas of the brain including the hypothalamus and indicative of glucose oxidation primarily through the Embden-Meyerhof and Krebs cycles. Anterior pituitary slices gave C-1:C-6 oxidation ratios similar to those from posterior lobe, but total glucose oxidation was only 5% to 10% as great. Total glucose oxidation by posterior pituitary tissue was significantly higher than that of brain tissue. Epinephrine, 10^{-4} M, stimulated C-1, but not C-6, oxidation in PVN and in anterior and posterior pituitary. The percentage increase in C-1 conversion to CO_2 produced by epinephrine was similar for posterior pituitary slices and for isolated nerve endings. Assuming that the neurohypophysis is representative of endocrine organs in general, it appears that the HMP pathway is concerned with hormone storage and/or secretion rather than with hormone synthesis.

INTRODUCTION

The main route of glucose catabolism in endocrine glands is apparently the hexose monophosphate (HMP) shunt, as indicated by the observation that carbon of the 1-position (C-1) is more readily converted to CO_2 in these tissues than is carbon of the 6-position (C-6). C-1:C-6 oxidation ratios ranging from 20 to 50 have been reported, for example, for thyroid (1) as well as for testis, ovary, anterior pituitary, parathyroid, and adrenal (2). On the other hand, ratios close to unity have been reported for kidney and skeletal muscle (3) and brain (4, 5). Although complications such as recycling of the triose phosphates into the HMP shunt and the existence of alternative pathways for glucose-6-phosphate metabolism limit the interpretation of data obtained from the use of ^{14}C-1- and ^{14}C-6-glucose, it is generally accepted that a C-1:C-6 oxidation ratio greater than unity indicates an operative HMP pathway (6, 7). Haynes *et al.* (8) have implicated the HMP shunt in the regulation of steroidogenesis in the adrenal, and Fields *et al.* (2) have suggested that this pathway may be concerned with hormone synthesis by the endocrines in general.

The neurosecretory cells of the neurohypophysis are functional nerve cells embryologically related to brain neurons, but in addition they serve an endocrine func-

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tion. In these cells the components which synthesize the neurohypophysial peptide hormones are anatomically distinct from those portions which store the secretory products to release them upon appropriate stimulation. This organ would appear to be ideal for examining glucose metabolism in each of the functionally distinct parts of the same cell, i.e., the perikaryon within specific hypothalamic nuclei and the axon terminations within the posterior lobe of the pituitary gland. The C-1:C-6 oxidation ratio has been reported for anterior (2, 9, 10), but not for posterior, pituitary tissue.

In our laboratory we have been investigating storage and release of anterior and posterior pituitary hormones, as well as the enzymic and metabolic properties of the respective glands. The conversion of ^{14}C-1-labeled and ^{14}C-6-labeled glucose to $^{14}CO_2$ has been examined *in vitro* in tissue slices from both lobes of bovine pituitary gland, in isolated neurohypophysial nerve terminals, and in slices from various areas of bovine brain including the region of the paraventricular nuclei.

METHODS AND MATERIALS

Tissues. Bovine pituitary glands and brains were obtained at a nearby slaughterhouse[2] approximately 30 min after death of the animals. The tissues were transported to the laboratory in vessels surrounded by ice, the trip taking about 20 min. Each lobe of the pituitary was isolated, freed of connective tissue and fat, and blotted on filter paper to remove blood. The brain was dissected to reveal the ventricle, and the two adjacent paraventricular nuclei, visibly demarcated in the fresh brain, were carefully dissected out with a razor blade as free of surrounding tissue as possible. Isolated nerve endings were prepared by centrifuging homogenates of posterior pituitary at 4200 g in 15 min as previously described (11, 12).

^{14}C-labeled glucose. Crystalline glucose-^{14}C-1 and glucose-^{14}C-6 (14 μC/mg) were obtained from New England Nuclear Corporation. The glucose was dissolved in 0.9% NaCl and frozen in a concentration of 2.5 μC/ml. The immediate addition of H_2SO_4 (the reagent employed to terminate the tissue incubation reaction and drive off $^{14}CO_2$) to a solution containing ^{14}C-glucose, but no tissue, resulted in the evolution of a labile ^{14}C-labeled substance. The absolute amount of radioactivity which contaminates $^{14}CO_2$ evolved during incubation with tissue varies in different experiments from 200 to 1000 dpm/0.5 μC. To determine whether a radioactive impurity was present in the glucose, thin layer chromatography in two different solvent systems resolved only a single detectable radioactive spot corresponding to glucose. The labile ^{14}C-labeled contaminant has been found in every batch of ^{14}C-1 or ^{14}C-6-glucose purchased from the above supplier. This background activity can be subtracted and does not interfere insofar as counts due to $^{14}CO_2$ produced by tissue slices are significantly higher. This labile contaminant has been noted also by Hoskin (13) and could seriously interfere with studies in which $^{14}CO_2$ activity is of a low order.

Determination of $^{14}CO_2$ produced during incubation of tissue slices or subcellular fractions. Tissues were sliced with a razor blade and diced into 0.2-mm squares with a McIlwain Tissue Chopper. The slices were preincubated at 37° for 15 min under an atmosphere of 95% O_2/5% CO_2 in a single large flask with Krebs-bicarbonate medium pH 7.4 containing 2 mg glucose/100 ml. The medium was decanted and 50-mg aliquots of the blotted tissue placed into 25-ml serum bottles containing medium and test substances to a final volume of 5.0 ml. To each bottle was added 0.5 μC ^{14}C-glucose in 0.2 ml. Subcellular fractions were suspended in Krebs-bicarbonate so that the desired amount of fraction was contained in 2.0 ml. Trapping and counting of $^{14}CO_2$ was carried out in the apparatus described by Cuppy and Crevasse (14). The bottles were gassed with 95% O_2/5% CO_2 and quickly sealed with rubber stoppers from which were suspended stainless-steel vial mounts containing glass center-wells. The bottles were incubated at 37° for 30 min in a Dubnoff shaker, 0.3 ml 6 N H_2SO_4 was injected through the stopper to

[2] Canada Packers Ltd., St. Boniface, Manitoba.

stop the reaction and evolve $^{14}CO_2$, and 0.3 ml hyamine hydroxide was injected through the stopper into the center well. The bottles were shaken for 60 min at room temperature, the center wells were removed and placed into counting vials containing 0.4% PPO and 0.01% POPOP in toluene, and radioactivity was measured in a Packard scintillation spectrometer.

RESULTS

On a weight basis, posterior pituitary slices metabolized C-1 of glucose to CO_2 more readily than did tissue from anterior pituitary (Table 1, Expt. 1), cerebral cortex (Table 1, Expt. 1), and hypothalamus (Table 1, Expt. 3). High oxidation ratios of C-1:C-6 occurred for both anterior and posterior pituitary tissue, whereas ratios close to 1 were found for brain. Thus, brain oxidized C-6 of glucose to CO_2 much more actively than either lobe of the pituitary. Tissue from the paraventricular nucleus (PVN) gave almost identical ratios as did other areas of the hypothalamus and cortex (Table 1, Expts. 4 and 5), although metabolizing exogenous glucose at a higher rate. Hypothalamic tissue was obtained several centimeters from the PVN (Table 1, Expt. 3), and in two experiments (Expts. 4 and 5) only a few millimeters away. The C-1:C-6 ratios were very similar irrespective of the area of the hypothalamus chosen.

Isolated nerve endings from the posterior pituitary gave C-1:C-6 ratios similar to those obtained with slices of this gland (Table 2). The progressively decreasing C-1:C-6 oxidation ratios determined in the more slowly sedimenting fractions (Table 2, Expt. 2) apparently reflects the greater proportion of free mitochondria to nerve endings, the former particles being much more active with respect to C-6 oxidation. Considerable day-to-day variability in the metabolic activity of the tissues was encountered, but this has been noted previously by others (9).

Epinephrine stimulated the metabolism of C-1 of glucose, but not of C-6, in all three tissues (Table 3). The percentage stimula-

TABLE 1

$^{14}CO_2$ production from ^{14}C-1- and ^{14}C-6-glucose in vitro by anterior pituitary, posterior pituitary, and brain.

Expt. no.	Tissue[c]	$^{14}CO_2$ (CPM/g in 30 min)[a,b]		
		^{14}C-1	^{14}C-6	^{14}C-1:^{14}C-6
1	A.P.	6,500	600	11
	P.P.	91,900	6,600	14
	Cortex	67,700	53,000	1.3
2	A.P.	12,700	620	20
	A.P. + Epi.	35,900 (+183%)	—	
3	P.P.	290,000	13,500	21
	P.P. + Epi.	413,000 (+42%)	—	
	Hypothal.	95,400	81,900	1.2
	Hypothal. + Epi.	321,000 (+236%)	—	
4	PVN	316,000	279,000	1.1
	Hypothal.	253,000	235,000	1.1
5	PVN	165,000	152,000	1.1
	Hypothal.	143,000	104,000	1.4

[a] Each value is the mean of duplicates.

[b] Values in parentheses represent percentage increase in counts with epinephrine.

[c] A.P., anterior pituitary; P.P., posterior pituitary; Hypothal., hypothalamus; PVN, paraventricular nucleus; Epi., epinephrine, 10^{-4} M.

TABLE 2
$^{14}CO_2$ production from ^{14}C-1- and ^{14}C-6-glucose in vitro by subcellular fractions from posterior pituitary gland

Expt. no.	Fraction	$^{14}CO_2$ (CPM/mg protein in 30 min)[a] ^{14}C-1	^{14}C-6	^{14}C-1: ^{14}C-6
1	Nerve endings	47,900	3,500	14
2	Nerve endings	13,300	1,100	12
	Nerve endings + Epi.[c]	17,600 (+32%)[b]	—	—
	Heavy mitochondria	8,100	1,100	7
	Light mitochondria	1,900	312	6

[a] Each value is the mean of duplicates.
[b] Value in parentheses represents percentage increase in counts with epinephrine.
[c] Epi., epinephrine, 10^{-4} M.

TABLE 3
Effect of epinephrine on $^{14}CO_2$ production from ^{14}C-1- and ^{14}C-6-glucose in vitro by anterior pituitary, posterior pituitary, and hypothalamus

Tissue	Epinephrine (M)	$^{14}CO_2$ (CPM/g in 30 min)[a] ^{14}C-1	^{14}C-6	^{14}C-1: ^{14}C-6
A.P.	0	15,800	860	18
	10^{-6}	15,700	820	19
	10^{-5}	19,000	690	28
	10^{-4}	27,800 (+76%)	860	32
P.P.	0	381,000	9,780	39
	10^{-4}	777,000 (+104%)	10,600	74
PVN	0	219,000	209,000	1.1
	10^{-4}	379,000 (+73%)	205,000	1.8

[a] Each value is the mean of duplicates.
Values in parentheses represent percentage increase in counts with 10^{-4} M epinephrine.

tion of C-1 oxidation by epinephrine was similar for posterior pituitary slices (Table 1, Expt. 3) and for isolated nerve endings from that gland (Table 2, Expt. 2), when these two preparations were tested at the same time. In another experiment, however, oxidation of C-1 by posterior pituitary slices was stimulated to a much higher degree by the same concentration of epinephrine (Table 3).

DISCUSSION

The posterior pituitary gland, although comprised of cells embryologically related to other neurons within the central nervous system, possesses, in common with other endocrine glands, an active HMP pathway as indicated by our studies with ^{14}C-glucose. In fact, the C-1:C-6 oxidation ratios which range from 12 to 21 in different experiments are among the highest reported. Confirmation that the measured metabolic activity of the posterior pituitary is due to the neuronal terminals in the gland, not to supporting elements, was provided by similar findings on isolated nerve endings. Furthermore, the degree of stimulation of oxidation induced by epinephrine was similar for slices and isolated endings from the posterior lobe.

On the other hand, the cell bodies of the neurohypophysial neurons which reside in the hypothalamus, primarily in the supraoptic and paraventricular nuclei, apparently metabolize glucose almost exclusively through the Embden-Meyerhof and Krebs cycles. This conclusion was reached through the observation that the C-1:C-6 oxidation ratio determined on isolated PVN was close to unity, conforming to results obtained with all other areas of the brain which we have studied or which have been reported. Even if significant contamination by non-neurosecretory cells existed in the isolated PVN tissue, the C-1:C-6 oxidation ratio would presumably have been altered, at least to some extent, by the neurosecretory cell bodies if they possess as active an HMP shunt as the nerve endings. It has been recently reported (15) that in the isolated, arterially perfused bovine brain high C-1:C-6 oxidation ratios were ob-

tained with labeled glucose. This observation must await corroboration, since it contradicts previous findings of others (16) which agree with all reported *in vitro* data.

A variety of experimental approaches have established that the neurohypophysial peptide hormones are synthesized in the cell bodies of the neurosecretory cells and are transported bound to a carrier protein to the axonal terminations located in the posterior pituitary gland. Histological studies have shown the axonal flow of neurosecretion (17), and Sachs (18) has provided evidence by the use of isotopic sulfur that vasopressin is synthesized in the cell bodies, although some additional synthesis takes place in the axoplasm and to a slight extent in the terminals. The posterior pituitary serves as a storage depot for vasopressin and oxytocin and secretes the hormones upon appropriate stimulation. Our findings with the neurohypophysis would tend to support the hypothesis that an active HMP pathway in endocrine tissues is concerned with secretory activity, rather than with supplying NADPH in the synthesis of hormone as proposed by some workers (2). Harding and Nelson (19), for example, pointed out that no evidence is available to indicate that the concentration of NADPH is directly related to the rate of synthesis of adrenal steroids.

Barondes *et al.* (9) have reported that epinephrine and other neuroamines added to anterior pituitary tissue *in vitro* stimulate oxidation of C-1, but not of C-6, of glucose to CO_2, and we have confirmed these findings. They suggested that a functional relationship exists between this metabolic pathway and hormone secretion, since the active amines are reported to stimulate adenohypophysial hormone secretion, also. However, they failed to achieve any enhanced oxidation of glucose with posterior pituitary hormones which are known to enhance release of anterior pituitary hormones (20, 21). Furthermore, the concentrations of amines which were required in Barondes' studies to stimulate glucose metabolism render extrapolation of the *in vitro* findings to physiological situations doubtful. We have been able to stimulate the release of adenohypophysial hormones *in vitro* with low concentrations of epinephrine and other agents which do not affect the oxidation of labeled glucose (unpublished observations). Therefore, there is no *immediate,* direct relationship between glucose-^{14}C-1 oxidation and trophic hormone secretion.

Actively secreting cells may require NADPH or some other substance generated by the HMP shunt for synthesis of components which are rapidly turning over. For example, this reduced coenzyme is required for the synthesis of lipids, substances which may comprise sites that are constantly being degraded and renewed on the membranes of secretory cells. The failure to find any immediate correlation between HMP shunt activity and hormone production and/or secretion (9, 19) may be explained on the basis that this metabolic pathway is concerned with long-term renewal of secretion-linked constituents of endocrine tissue cells. Alternatively, HMP shunt activity may be concerned with storage mechanisms of endocrine cells, perhaps to provide substrates for secretory granule or granule-membrane formation and/or maintenance. This last hypothesis has support in the conclusions of Hokin and Hokin (22), who have recently reviewed their investigations. They believe that phospholipid synthesis which is accelerated during active zymogen secretion by pancreatic cells is concerned, not with the extrusion process per se, but with some step in the segregation of proteins in preparation for zymogen granule formation.

The hypothesis presented in the present report may explain certain apparent anomalies or exceptions to the rule that nonendocrine tissues have relatively low C-1: C-6 oxidation ratios. The reported high ratio for actively phagocytosing leukocytes (23) may reflect lipid reconstitution associated with active membrane phenomena. An active HMP shunt is to be expected in adipose tissue (24) which is continuously synthesizing lipids and in mammary tissue (25) whose secretion is rich in lipids. On the other hand, the active HMP shunt of red blood cells (26) cannot apparently be

explained on this basis, thus suggesting that HMP shunt activity subserves processes in some tissues other than those concerned with lipid and/or membrane synthesis.

ACKNOWLEDGMENTS

We are indebted to Mr. Elliott Bindler for the preparation of subcellular fractions used in this investigation.

This study was supported by USPHS Grant AM-05896, Medical Research Council of Canada, and the American Heart Association.

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