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PYOCYANIN AND METABOLIC PATHWAYS IN LIVER SLICES *IN VITRO*

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SUMMARY

The effect of pyocyanin, an oxidation–reduction mediator, on the metabolism of ^{14}C-labeled mannose, fructose, glycerol, pyruvate, acetate, propionate, butyrate, and CO_2 in rat-liver slices has been studied. In general, pyocyanin markedly increased the oxidation to CO_2 of those substrates expected to be metabolized significantly via the phosphogluconate pathway. However, while fructose and glycerol are presumed to enter the Embden–Meyerhof pathway at the triose level, glycerol but not fructose oxidation to CO_2 was increased by pyocyanin addition. Pyocyanin decreased the incorporation of the labeled substrates into glycogen and fatty acids and also the oxidation of pyruvate, acetate, propionate, and butyrate to CO_2. Pyocyanin thus appears to have effects on pathways in addition to the phosphogluconate pathway.

INTRODUCTION

Oxidation–reduction mediators can alter the metabolic pathways followed by glucose in various tissues[1-3]. CAHILL *et al.*[1] demonstrated that in liver slices the mediator pyocyanin preferentially increased the oxidation to CO_2 of C-1 of glucose relative to that of C-6. This was presumed to reflect an increase in the quantity of glucose metabolized by the phosphogluconate pathway as a consequence of an increase in the availability of oxidized NADP. Decreased incorporation of glucose carbon into glycogen and fatty acids was also observed in the presence of pyocyanin. A similar result was obtained with pyruvate as substrate and methylene blue as mediator.

The pattern of metabolism in liver slices of a number of substrates in the absence and presence of pyocyanin has now been determined in an effort to define better the mode of action of this mediator. The substrates studied include mannose, fructose, glycerol, pyruvate, acetate, propionate, and butyrate.

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MATERIALS AND METHODS

Male albino rats of the Wistar strain weighing about 200 g were used. They were maintained on purina chow *ad libitum* until the time of sacrifice by stunning and exsanguination.

As previously described[4,5] tissue slices were prepared and incubated in a medium of ionic composition: K^+, 110; Mg^{2+}, 20; Ca^{2+}, 10; HCO_3^-, 40; substrate, 20 or 40; and Cl^-, 130 or 90 mmoles per liter. All incubations were for 90 min. For each substrate paired flasks were used, identical in all respects, except that one contained pyocyanin (10^{-4} M)*. The labeled substrates used were D-[$^{14}C_6$]mannose (20 mM), D-[$^{14}C_6$]fructose (20 mM), [1,3-$^{14}C_2$]glycerol (40 mM), [2-^{14}C]pyruvate (40 mM), [1-^{14}C]acetate (40 mM), [1-^{14}C]propionate (40 mM), [1-^{14}C]butyrate (40 mM), and $NaH^{14}CO_3$**. The $NaH^{14}CO_3$ was added to the flask following equilibration of the incubation solutions with O_2–CO_2 (95:5), as previously reported[6].

Liver slices were analyzed for glycogen and the media for glucose, fructose, glycerol, pyruvate, and lactate[5-7]. Initial specific activities of mannose and fructose were determined as their phenylosazones, pyruvate was assayed as the 2,4-dinitrophenylhydrazone, glycerol was oxidized to formaldehyde and assayed as the formaldimedon, and acetate, propionate, and butyrate were assayed as their benzylthiouronium salts. Liver glycogen and fatty acids were assayed as previoulsy described. CO_2 was collected and counted as $BaCO_3$. The methods of isolation and assay of glucose and lactate, and the degradation of lactate have also been reported[5-7].

The following parameters have been calculated by standard methods in the absence and presence of pyocyanin: (1) initial and final liver slices glycogen concentrations and final medium glucose and lactate concentrations; (2) fructose, glycerol, and pyruvate uptake; (3) substrate oxidation to CO_2 and incorporation into glycogen and fatty acids; (4) pyruvate and glycerol incorporation into glucose; (5) pyruvate incorporation into lactate and the distribution of activity in the lactate; (6) CO_2 incorporation into glucose and lactate.

RESULTS

Tables I–III give the results, means and their standard errors, for the incubations of the several substrates in the absence (control) and presence of pyocyanin. Initial and final glycogen data are expressed as μmoles/g of liver. All other results are as μmoles/g of liver/90 min incubation. The standard errors of the means, except for initial glycogen have been obtained from an analysis of variance which was performed to reduce contributions from animal variation[8]. Each mean and standard error is based upon three observations except for uniformly labeled [^{14}C]mannose where 4 experiments were performed.

In all experiments the final glycogen content of the liver slices was less than the

* Pyocyanin (as pyocyanine chloride) was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

** Uniformly-labeled [^{14}C]mannose was obtained from Schwarz Laboratories, Inc., Mount Vernon, N.Y.; $NaH^{14}CO_3$ from New England Nuclear Corp., Boston, Mass.; [1,3-$^{14}C_2$]glycerol was provided by Dr. M. L. KARNOVSKY; and the remainder of the labeled substrates were obtained from Nuclear-Chicago Corp., Chicago, Ill.

initial glycogen content. This glycogen breakdown was greater in the presence than in the absence of pyocyanin. A correspondingly greater quantity of glucose was present in the medium containing pyocyanin at the end of the incubation and the quantity of glucose present in the medium was similar in magnitude to the decrease in liver glycogen. Final medium lactate concentrations, where measured, were unaffected by the presence of pyocyanin.

When uniformly labeled [^{14}C]mannose was substrate (Table I), pyocyanin addition resulted in an increase in mannose conversion to CO_2 and a decrease in its incorporation into glycogen and fatty acids. When uniformly labeled [^{14}C]fructose was

TABLE I

METABOLISM OF UNIFORMLY LABELED [^{14}C]MANNOSE (20 mM), UNIFORMLY LABELED [^{14}C]FRUCTOSE (20 mM), AND [1,3-$^{14}C_2$]GLYCEROL (40 mM), IN THE ABSENCE AND PRESENCE OF PYOCYANIN

Initial and final glycogen data are expressed as μmoles per gram of liver; all other results are expressed as μmoles per gram of liver per 90 min incubation.

Substrate	*Pyocyanin*	*Initial glycogen*	*Final glycogen*	*Medium glucose*	*Lactate formed*	*Substrate uptake*	*^{14}C-labeled substrate to*		
							CO_2	*Glycogen*	*Fatty acids*
Uniformly labeled [^{14}C]mannose	—	241	157	73	20		4.6	11.5	0.51
	+		127	80	23		7.8	5.0	0.12
	S.E.	47	3	2	1		0.5	0.5	0.03
Uniformly labeled [^{14}C]fructose	—	255	162	88	52	87	10.3	17.6	1.07
	+		136	92	64	86	12.4	8.7	0.21
	S.E.	1	6	1	5	3	0.8	0.6	0.16
[1,3-$^{14}C_2$]Glycerol	—	239	184	74	23	147	3.7	8.1*	0.01
	+		159	83	21	138	13.3	7.1	0.02
	S.E.	16	8	2	1	3	0.9	0.7	0.01

* Incorporation into glucose was 52.8 μmoles per gram of liver in the absence (—) and 48.8 in the presence (+) of pyocyanin (S.E., 2.8). Portions of the control data for glycerol have been presented previously[5].

substrate, in the presence of pyocyanin, there was no change in fructose uptake, a slight if any increase in oxidation of fructose to CO_2, and a decrease in its incorporation into glycogen and fatty acids. When [1,3-$^{14}C_2$]glycerol was substrate, uptake was unchanged, but in the presence of pyocyanin more than three times as much substrate was oxidized to $^{14}CO_2$, while incorporation into glycogen and glucose was questionably effected. There was negligible incorporation of ^{14}C into fatty acids in the control as well as the pyocyanin experiments.

In the experiments recorded in Table II, [2-^{14}C]pyruvate and $^{14}CO_2$ were incubated in the absence and presence of pyocyanin. In the presence of pyocyanin, the uptake of pyruvate and its oxidation were decreased. There was also a decrease in the incorporation of ^{14}C of the pyruvate into glycogen, glucose, and fatty acids. The lactate formed and its activity was not significantly different from the control experiments. Incubations identical to those with pyruvate were simultaneously performed except that the CO_2 was labeled with ^{14}C and not the pyruvate. There was a decrease in ^{14}C incorporation into glycogen and glucose in the presence of pyocyanin. Incorporation into fatty acids appeared to be decreased, but the quantity of CO_2

TABLE II

METABOLISM OF [2-^{14}C]PYRUVATE (40 mM) AND $^{14}CO_2$ IN THE ABSENCE AND PRESENCE OF PYOCYANIN*

Initial and final glycogen data are expressed as μmoles per gram of liver; all other results are expressed as μmoles per gram of liver per 90 min incubation.

Substrate	Pyocyanin	Initial glycogen	Final glycogen	Medium glucose	Lactate formed	Pyruvate uptake	^{14}C-labeled substrate to					
							CO_2	Glycogen	Glucose	Fatty acids	Lactate C-1	Lactate C-2,3
[2-^{14}C]-Pyruvate	—	312	191	93	110	239	44.6	15.8	16.9	3.5	8.1	92
	+		163	107	116	175	35.0	3.4	10.4	0.6	7.2	87
	S.E.	36	4	2	1	6	4.8	0.3	1.9	0.3	0.3	2
$^{14}CO_2$ + pyruvate	—		189	94	110	234		9.5	8.6	0.012	4.8	0.35
	+		156	102	122	176		2.1	5.1	0.005	9.8	0.15
	S.E.		6	4	5	1		1.3	0.4	0.003	1.3	0.06

* Portions of the control data have been presented previously[6].

incorporated into fatty acids was very small in the control experiments. There was fixation of CO_2 in the presence as well as in the absence of pyocyanin, as evidenced by the appearance of radioactivity in the carboxyl carbon (C-1) of lactate. About one-half as much ^{14}C from $^{14}CO_2$ as from [2-^{14}C]pyruvate was incorporated into glycogen and glucose in both the absence and presence of pyocyanin. The possible significance of the latter observation has been considered[6].

In the experiments recorded in Table III, [1-^{14}C]acetate, [1-^{14}C]propionate, and [1-^{14}C]butyrate were the substrates. Compared to the control experiments, the presence of pyocyanin resulted in a decrease in the oxidation of the ^{14}C of these substrates to CO_2, and their incorporation into glycogen as well as into fatty acids.

It should be noted (Table III) that in the control experiments the ratio of acetate oxidized to CO_2 (22.4 μmoles) to its incorporation into fatty acids (3.3 μmoles) was

TABLE III

METABOLISM OF [1-^{14}C]ACETATE, [1-^{14}C]PROPIONATE AND [1-^{14}C]BUTYRATE (40 mM) IN THE ABSENCE AND PRESENCE OF PYOCYANIN

Initial and final glycogen data are expressed as μmoles per gram of liver; all other results are expressed as μmoles per gram of liver per 90 min incubation.

Substrate	Pyocyanin	Initial glycogen	Final glycogen	Medium glucose	^{14}C-labeled substrate to		
					CO_2	Glycogen	Fatty acids
[1-^{14}C]Acetate	—	171	69	91	22.4	0.10	3.27
	+		52	98	17.7	0.02	0.60
	S.E.	33	2	2	1.3	0.01	0.07
[1-^{14}C]Propionate	—	220	111	95	18.6	0.49	0.25
	+		86	105	11.3	0.13	0.06
	S.E.		1	1	0.2	0.06	0.05
[1-^{14}C]Butyrate	—	302	158	90	25.4	0.12	1.29
	+		141	97	21.0	0.06	0.28
	S.E.	23	1	1	1.0	0.01	0.01

similar in magnitude (Table II) to the ratio of the oxidation of pyruvate to CO_2 (44.6 μmoles) to its incorporation into fatty acids (3.5 μmoles). However, the conversion of acetate to glycogen (0.10 μmole) was much less than the conversion of pyruvate to glycogen (15.8 μmoles).

DISCUSSION

Pyocyanin addition may permit an estimation of the degree of access of a substrate to the phosphogluconate pathway. Thus more uniformly labeled [^{14}C]mannose and [1,3-$^{14}C_2$]glycerol, but less [2-^{14}C]pyruvate, [1-^{14}C]acetate, [1-^{14}C]propionate and [1-^{14}C]butyrate, were oxidized to $^{14}CO_2$ in the presence than in the absence of pyocyanin. This probably occurred because of an increase in metabolism of mannose and glycerol, but not the other substrates via the phosphogluconate pathway. However, uniformly labeled [^{14}C]fructose, which is believed to enter metabolic pathways at the triose level in liver, as does glycerol, was not oxidized to CO_2 in significantly greater quantity in the presence of pyocyanin. Presumably much of the oxidation of fructose observed under control conditions occurred via the tricarboxylic acid cycle and much of the oxidation of glycerol via the oxidative pathway, despite the fact that significant quantities of both substrates appeared as glycogen. In accord with this dissimilarity in the pathways of oxidation to CO_2 was the much greater incorporation of fructose than glycerol into fatty acids and the greater quantity of lactate formed from fructose than from glycerol despite substrate uptakes of the same magnitude. The similarity of fructose and glycerol metabolism, when only their incorporation into glucose and glycogen is considered, has been noted[7].

Differences in fructose and glycerol patterns of metabolism at low as well as high substrate concentrations have also been reported[5]. Other observations also suggest that glycerol and fructose may not share common triose intermediates in the course of their metabolism. Thus, LANDAU AND MERLEVEDE[9,10] obtained significant quantities of 3,4-labeled glucose from liver glycogen when D-[3-^{14}C]glyceraldehyde was administered to rats. This labeling is in accord with the conversion of glyceraldehyde to glycerol prior to its conversion to triose phosphates. In contrast [6-^{14}C]fructose yielded only 1,6-labeled glucose despite its presumed metabolism in the liver via dihydroxyacetone phosphate and [3-^{14}C]glyceraldehyde. MUNTZ AND VANKO[11] on administering [^{14}C]fructose to rats frequently found liver glucose to have a higher specific activity than fructose 6-phosphate and glucose 6-phosphate which are generally assumed to be intermediates in the conversion of fructose to glucose. They have suggested that fructose metabolism may be by pathways other than those presently accepted.

Decreases in the incorporation of ^{14}C into fatty acids on pyocyanin addition may be attributable to the oxidation of the hydrogens of NADPH via the electron-transfer system with a consequent decrease in their availability for fatty acid synthesis. Decreases in incorporation of ^{14}C into glycogen may reflect increased breakdown of glycogen to glucose 6-phosphate with increased entrance of the latter into the phosphogluconate pathway. Considerations as to the mode of incorporation of radioactivity from [1-^{14}C]propionate into fatty acids have been made[6]. It is of interest that this incorporation is also decreased by pyocyanin.

[1-^{14}C]Acetate metabolism to CO_2 and fatty acids was similar to the metabolic pattern for [2-^{14}C]pyruvate. This might be expected since decarboxylation of the

pyruvate would result in [1-^{14}C]acetyl. The greater conversion of pyruvate to glycogen, than acetate to glycogen, is in keeping with the gluconeogenic pathways directly accessible to pyruvate but not acetate.

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