Vol. 100, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1981 Pages 291-296

MECHANISM OF ACTION OF OXFENICINE ON MUSCLE METABOLISM

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Received March 2,1981

Summary

In isolated rat diaphragms, the stimulation of pyruvate decarboxylation by oxfenicine but not by its metabolite, 4-hydroxyphenylglyoxylate (HPG), was blocked by aminooxyacetate. However, when administered intravenously to fat-fed rats, HPG was only poorly transported into the heart and did not activate cardiac pyruvate dehydrogenase. Oxfenicine (2mM) was a more potent inhibitor of the oxidation of palmitate (52%) by rat diaphragm than of palmitylcarnitine (17%) or octanoate (20%). In mitochondria incubated with palmitate, CoA, ATP and [^{14}C]carnitine, oxfenicine did not inhibit palmityl-[^{14}C]carnitine formation. However, HPG was a potent inhibitor in heart mitochondria (46% at 50μM, 61% at 1mM) but not in liver mitochondria (24% at 5mM). We conclude that oxfenicine (via HPG) stimulates myocardial carbohydrate oxidation by cardioselective inhibition of long-chain fatty acid oxidation at the level of fatty acyl-CoA synthetase or carnitine acyltransferase.

Introduction

We have previously shown that oxfenicine (S-4-hydroxyphenylglycine) attenuates cell damage in working rat hearts perfused with high concentrations of fatty acids and subjected to brief global ischaemia and reperfusion (1). Furthermore, oxfenicine reduces enzyme release from ischaemic dog hearts (2) and increases the pacing time to angina in patients with obstructive coronary artery disease (3). These therapeutic actions are believed to accrue from causing the heart to oxidise carbohydrate rather than fatty acids as its major fuel (1), and in this paper we present evidence indicating the mechanism by which oxfenicine achieves this switch in myocardial metabolism.

Materials and Methods

Oxidation of radiolabelled substrates (The Radiochemical Centre, Amersham and New England Nuclear) to $^{14}CO_2$ by isolated rat diaphragms was measured as described previously (1). When lipid substrates (palmitate, palmitylcarnitine and octanoate) were used, bovine plasma albumin (Fraction

*Abbreviations used : HPG, 4-hydroxyphenylglyoxylate ; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

0006-291X/81/090291-06$01.00/0

V, Armour Pharmaceutical Co.) was added to the incubation medium (6mg/ml). Active and total pyruvate dehydrogenase activities were determined in extracts of hearts from fat-fed rats by decarboxylation of [1-^{14}C] pyruvate (1). HPG* was extracted into ether from powdered heart tissue, separated by high-performance liquid chromatography, and measured by its absorbance at 290nm.

Mitochondria were prepared from rat liver or rat hearts by conventional centrifugation techniques in Hepes*-buffered sucrose (4) after gentle homogenisation with a Polytron PT10 tissue processor (1). Mitochondrial respiration rates were measured by the polarographic technique of Estabrook (5). Palmityl-[^{14}C]carnitine formation was determined by minor modifications of the method of Bremer (6). Preliminary experiments confirmed that the rate of palmityl-[^{14}C]carnitine formation was linear over the incubation period.

Results were analysed by Student's t-test with correction, where appropriate, for unequal variances.

Results

Evidence that HPG is the active metabolite of oxfenicine. Pyruvate oxidation in diaphragms from fat-fed rats was stimulated to similar extents by oxfenicine and by its metabolite, HPG (Table 1). No stimulation was obtained with the enantiomer, R-4-hydroxyphenylglycine (data not shown). This led us to suspect that metabolism of oxfenicine might be a prerequisite for the drug's action. Thus, inhibition of transaminase activity with aminooxyacetate abolished the activity of the amino acid, but not the keto acid (HPG). The slight enhancement of the effect of HPG by aminooxyacetate

TABLE 1

EFFECTS OF OXFENICINE AND HPG (IN THE PRESENCE AND ABSENCE OF AMINO-OXYACETATE) ON PYRUVATE OXIDATION BY ISOLATED DIAPHRAGMS FROM FAT-FED RATS

Additions	Pyruvate oxidation (μmol/g.h)	
	Control	Aminooxyacetate (2mM)
None	4.49±0.19 (37)	4.32±0.37 N.S.
2mM Oxfenicine	7.61±0.28 **	5.60±0.21 ††
1mM Oxfenicine	6.54±0.59 *	4.43±0.24 †
2mM HPG	7.11±0.54 **	9.13±0.39 †
1mM HPG	6.21±0.35 **	6.31±0.44 N.S.

Oxidation of [2-^{14}C] pyruvate (4mM, 6.25μCi/mmol) by diaphragm tissue from fat fed rats was measured as described previously (1). Results are means ± SEM of 11 observations except where indicated. $^{*}P < 0.01$, $^{**}P < 0.001$ vs. untreated controls. $^{†}P < 0.01$, $^{††}P < 0.001$, N.S. $P > 0.05$ vs. corresponding group without aminooxyacetate.

TABLE 2

CARDIAC PDH ACTIVITY AND HPG LEVELS AFTER TREATMENT OF FAT-FED RATS WITH OXFENICINE OR HPG

Treatment	PDH activity (% of total)	HPG concentration (nmol/g)
Saline	24.1±2.6(8)	1.7±1.1(8)
Oxfenicine (0.3mmol/kg)	73.4±5.6(6)	55.4±2.2(6)
HPG (0.3mmol/kg)	22.8±6.5(3)	2.3±0.3(3)
HPG (0.6mmol/kg)	8.9±1.8(3)	9.7±4.7(3)

Fat-fed rats were injected with saline, oxfenicine or HPG via the tail vein. 1h later they were sacrificed and the hearts rapidly removed into liquid nitrogen. Cardiac PDH activity and HPG levels were measured as described in Methods. Total PDH was measured after activating an aliquot of heart homogenate with Ca^{2+} + Mg^{2+}. Results are means ± SEM of no. of observations in brackets.

could have resulted from inhibition of its reverse transamination to the inactive precursor, oxfenicine.

Pretreatment of fat-fed rats with oxfenicine resulted in marked activation of cardiac pyruvate dehydrogenase (PDH) and this coincided with substantial accumulation of HPG in the myocardium (Table 2). However HPG itself was very poorly transported into the heart and thus produced no effect when administered intravenously.

Inhibition of long-chain fatty acid oxidation. Quarter diaphragms from normal (41B)-fed rats were incubated with various [1-^{14}C] lipid substrates in the presence and absence of oxfenicine to determine the relative potency of the drug at inhibiting oxidation of long and medium-chain fatty acids and of long-chain acylcarnitine. Oxfenicine (2mM) was considerably more potent (Table 3) at inhibiting oxidation of palmitate (52%) than of octanoate (20%) or of palmitylcarnitine (17%).

In isolated rat heart mitochondria incubated with 50μM palmitylcarnitine (complexed to 10mg/ml bovine plasma albumin) and 0.2mM malate, state 3

TABLE 3

INHIBITION OF OXIDATION OF PALMITATE, OCTANOATE AND PALMITYLCARNITINE BY OXFENICINE IN ISOLATED RAT DIAPHRAGM

Oxfenicine concentration	Oxidation rate (nmol/g.h)		
	0	0.5mM	2mM
[1-^{14}C] Palmitate(0.1mM)	20.4±1.2	13.5±1.1**	9.7±1.2**
[1-^{14}C] Octanoate(0.5mM)	1211±49	1066±59	973±30 **
[1-^{14}C] Palmityl-Carnitine(0.1mM)	12.0±0.7	12.0±0.7	10.0±0.6*

Specific activity of substrates was 0.1 (octanoate) or 0.5μCi/μmol. Results are means ± SEM of 9 or 10 observations.
*P < 0.05, **P < 0.005 vs. control.

respiration was induced by adding 1mM ADP. Addition of 1mM oxfenicine or 1mM HPG did not significantly alter this respiration rate (-6% and +3% respectively) thus indicating that neither compound was a direct inhibitor of β-oxidation.

Effects on palmitylcarnitine formation. Palmityl-[^{14}C]carnitine formation was measured in rat liver and rat heart mitochondria incubated with palmitate, CoA, ATP and [^{14}C]carnitine. The rate of palmityl-[^{14}C]carnitine formation was thus dependent on the activity of two enzymes - palmityl-CoA synthetase and carnitine-palmityltransferase.

Even at 8mM, oxfenicine itself did not significantly inhibit palmitylcarnitine formation in mitochondria from either rat liver (7%) or rat heart (4%). In contrast, its metabolite, HPG, was a potent inhibitor in heart mitochondria (46% at 50μM, 61% at 1mM; see Fig. 1). However, HPG was much less potent in liver mitochondria (Fig. 1), producing only 24% inhibition at 5mM.

Discussion

The ability of HPG, but not oxfenicine, to inhibit palmitylcarnitine formation in heart mitochondria indicates that oxfenicine must first be

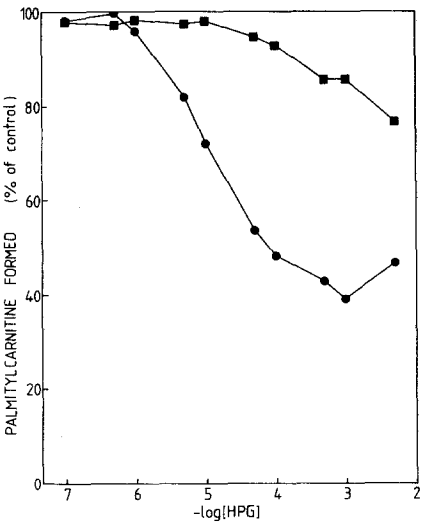

Fig. 1. Inhibition by HPG of palmitylcarnitine formation in isolated mitochondria. Palmityl-[^{14}C]carnitine formation by mitochondria from rat heart (●) or rat liver (■) was measured as described in Methods. Results are the means of 2 or 3 separate experiments. Control rates of palmitylcarnitine formation were 1.26 and 2.65nmol/min.mg protein for heart and liver mitochondria respectively.

metabolised to its keto-acid derivative for it to be biologically active. Furthermore, the ability of aminooxyacetate to inhibit the action of oxfenicine, but not HPG, in isolated rat diaphragm suggests that an intracellular transaminase is responsible for this conversion. As HPG itself was very poorly transported into the myocardium, we conclude that the amino acid, oxfenicine, acts as a pro-drug for its active metabolite, HPG.

The relative inactivity of HPG in liver mitochondria compared to heart mitochondria lends support to the notion that the metabolic effects of oxfenicine in vivo are largely confined to the heart. Thus, unlike other drugs affecting lipid or carbohydrate metabolism, e.g. dichloroacetate (7), 2-bromopalmitate (8) or 2-tetradecylglycidic acid (9), no significant alterations in arterial glucose, lactate or fatty acid concentrations have been observed following oxfenicine administration to intact animals (10).

Because of the close coupling between rates of lipid and carbohydrate oxidation in muscle (11), previous studies (1) did not indicate whether the

primary action of oxfenicine was inhibition of fatty acid oxidation or direct stimulation of carbohydrate oxidation. The present finding that HPG inhibits palmitylcarnitine formation in isolated mitochondria is reinforced by oxfenicine's greater potency as an inhibitor of carnitine-dependent (palmitate) rather than carnitine-independent (octanoate or palmitylcarnitine) fatty acid oxidation. These results, together with the absence of a direct inhibition of β-oxidation, suggest either fatty acyl-CoA synthetase or carnitine acyltransferase as the primary site of action of the drug. Elucidation of the exact site and nature of this inhibition is currently under investigation.

Acknowledgements

We are grateful to Dr. D.A. Stopher and Mr. A.P. Beresford for HPG measurements, and to Messrs. M.S. Robinson, T.A. Briggs and S.F. Tickner for skilful technical assistance.

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