Acyloin production from aldehydes in the perfused rat heart: the potential role of pyruvate dehydrogenase

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Aldehydes represent an important class of cytotoxic products derived from free radical-induced lipid peroxidation which may contribute to reperfusion injury following myocardial infarct. Metabolism of aldehydes in the heart has not been well characterized aside from conjugation of unsaturated aldehydes with glutathione. However, aliphatic aldehydes like hexanal do not form stable glutathione conjugates. We have recently demonstrated *in vitro* that pig heart pyruvate dehydrogenase catalyses a reaction between pyruvate and saturated aldehydes to produce acyloins (3-hydroxyalkan-2-ones). In the present study, rat hearts were perfused with various aldehydes and pyruvate. Acyloins were generated from saturated aldehydes (butanal, hexanal or nonanal), but not from 2-hexenal (an unsaturated aldehyde) or malondialdehyde. Hearts perfused with 2 mM pyruvate and 10–100 μ M hexanal rapidly took up hexanal in a dose-related manner (140–850 nmol/min), and released

3-hydroxyoctan-2-one (0.7–30 nmol/min), 2,3-octanediol (0–12 nmol/min) and hexanol (10–200 nmol/min). Small quantities of hexanoic acid (about 10 nmol/min) were also released. The rate of release of acyloin metabolites rose with increased concentration of hexanal, whereas hexanol release attained a plateau when hexanal infusion concentrations rose above 50 μ M. Up to 50% of hexanal uptake could be accounted for by metabolite release. Less than 0.5% of hexanal uptake was found to be bound to acid-precipitable macromolecules. When hearts perfused with 50 μ M hexanal and 2 mM pyruvate were subjected to a 15 min ischaemic period, the rates of release of 2,3-octanediol, 3-hydroxyoctan-2-one, hexanol and hexanoate during the reperfusion period were not significantly different from those in the pre-ischaemic period. Our results indicate that saturated aldehydes can be metabolically converted by the heart into stable diffusible compounds.

INTRODUCTION

Reactive oxygen species generated upon reperfusion of the ischaemic myocardium may induce lipid peroxidation which could exacerbate the ischaemic injury (for review see Yellon and Jennings, 1992). Peroxidation of polyunsaturated fatty acids in cellular membranes could alter membrane permeability and interfere with cellular function. Lipid peroxidation generates reactive aldehydes, which are considered as 'cytotoxic second messengers' (Schauenstein et al., 1977; Esterbauer, 1981; Esterbauer et al., 1990). The capacity of these aldehydes to diffuse to adjacent tissues may widen the original lesion. Malondialdehyde and hexanal are the most abundant aldehydes generated by lipid peroxidation. 4-Hydroxynon-2-enal, while generated in small amounts, is highly cytotoxic, presumably because it reacts with numerous functional groups (for review, see Esterbauer et al., 1991).

Detoxification of unsaturated aldehydes such as 4-hydroxynon-2-enal in the heart occurs, at least in part, by conjugation with glutathione (Ishikawa et al., 1986). There is very little information available on the heart's capacity to metabolize aliphatic aldehydes which do not form stable glutathione conjugates. Enzymes with broad specificity that convert aldehydes into carboxylic acids or alcohols are found in many tissues, including the heart (Holmes, 1978; Sladek et al., 1989; Brophy and Barrett, 1990; Bounds and Winston, 1991). These enzymes include NAD(P)⁺-linked aldehyde dehydrogenase, NAD⁺-linked alcohol dehydrogenase, NAD(P)⁺-linked aldehyde reductase, aldehyde oxidase and xanthine oxidase. Their importance in detoxifying aldehydes in the heart is not known.

Recently we have demonstrated that pig heart pyruvate dehydrogenase (PDH) catalyses the condensation of various saturated aldehydes with hydroxyethylthiamine pyrophosphate *in vitro* to form a series of acyloins (3-hydroxyalkan-2-ones) (Montgomery et al., 1992a). While this reaction (Figure 1) has been known for years (Green et al., 1942), it has not been considered as a mechanism for the detoxification of aldehydes *in vivo*. In this paper we report the conversion of reactive aldehydes to 3-hydroxyalkan-2-ones and their corresponding alcohols in the perfused rat heart.

Part of this work has been presented in abstract form (Montgomery et al., 1992b,c).

MATERIALS AND METHODS

Chemicals

Butanal, hexanal, *trans*-2-hexenal, nonanal, tetraethoxypropane and 2,3-hexanedione were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Sulphosalicylic acid, sodium borohydride (NaBH₄) and diethyl ether were from Fisher Scientific (Montreal, Quebec, Canada). *N*-Methyl-*N*-(*t*-butyldimethylsilyl)-trifluoroacetamide was obtained from Regis Chemical Co. (Morton Grove, IL, U.S.A.) and Tri-Sil/BSA was from Pierce Chemical Co. (Rockford, IL, U.S.A.). Anhydrous ammonia gas (99.99% minimum purity) was obtained from Matheson Gas Products Canada (Montreal, Quebec, Canada). Sodium borodeuteride (NaB²H₄), sodium [1,2,3-¹³C₃]pyruvate and *n*-[²H₁₃]hexanol were supplied by Merck Sharpe & Dohme Isotopes (Pointe-Claire, Quebec, Canada). All aqueous solutions were made with water purified by a Milli-Q system (Millipore, St.

Abbreviations used: g.c.–m.s., gas chromatography–mass spectrometry; PDH, pyruvate dehydrogenase; TBDMS, *tert*-butyldimethylsilyl; TBARS, thiobarbituric acid reactive substances; TMS, trimethylsilyl; acyloin corresponds to 3-hydroxyalkan-2-one.

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Figure 1 General mechanism of acyloin formation by PDH

Pyruvate is decarboxylated by PDH to form hydroxyethylthiamine pyrophosphate (HETPP), which in turn condenses with saturated aldehydes (C_n) to produce C_{n+2} 3-hydroxyalkan-2-ones.

Laurent, Quebec, Canada), and all other reagents were of analytical grade. Baker-10 SPE 3 ml quaternary aminesilane bonded silica gel columns (J. T. Baker Chemical Co., Phillipsburg, NY, U.S.A.) were used to remove traces of hexanoic acid from solutions of 100 mM hexanal in ethanol. Hexanal was eluted in 2 ml of ethanol and the concentration was adjusted to 1 mM with water for the heart infusion experiments. Malondialdehyde was prepared and purified as previously described (Des Rosiers et al., 1993). The internal standard for 2,3-hexanediol analyses was $[2,3\text{-}^2\text{H}_2]2,3\text{-hexanediol}$ prepared by the NaB^2H_4 reduction of 2,3-hexanedione. The internal standard for 2,3-octanediol analyses was $[1,2\text{-}^{13}\text{C}_2,2\text{-}^2\text{H}]2,3\text{-octanediol}$, prepared by incubating hexanal and $[1,2,3\text{-}^{13}\text{C}_3]\text{pyruvate}$ with PDH (Montgomery et al., 1992a) to form $[1,2\text{-}^{13}\text{C}_2]3\text{-hydroxyoctan-2-one}$, which was reduced with NaB^2H_4 to form the labelled 2,3-octanediol. Pig heart PDH was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and was used without further purification. Its specific activity was 0.8 unit/mg of protein and the preparation contained less than 15% 2-oxoglutarate dehydrogenase.

Rat heart perfusions

Hearts isolated from fed male Sprague-Dawley rats (300–400 g, Charles River) were perfused according to the Langendorff technique at a constant pressure of 80 mmHg with a non-recirculating modified Krebs–Ringer bicarbonate buffer (11 mM glucose, 1.3 mM Ca^{2+} , pH 7.41–7.45, 38 °C), equilibrated with 5% $\text{CO}_2/95\%$ O_2 . Coronary flow (Carolina Medical Electronics Inc., King, NC, U.S.A.), heart rate (Micro-Med Inc., Louisville, KY, U.S.A.) and perfusion temperature were continuously monitored. In some experiments the pulmonary artery was cannulated for $p\text{O}_2$ measurements using a Clark-type oxygen electrode (Instech Laboratories, Inc., Plymouth Meetings, PA, U.S.A.). Experiments commenced when physiological parameters had stabilized (normally after 20–30 min). Hearts that did not fulfil the following criteria were discarded: spontaneous heart rate above 200 beats/min and increase of coronary flow on release of a 30 s period of inflow occlusion (reactive hyperaemia). The hearts were allowed to beat spontaneously throughout the experiments.

Where indicated, 0.2 or 2 mM pyruvate was added directly to the perfusate and aldehydes in aqueous solution were infused into the perfusion stream. Perfusate aldehyde concentrations were kept constant despite variations in coronary flow using a

computer program developed for feedback control of the syringe pump (Harvard Instruments). Samples of influent and effluent perfusate were collected on ice at intervals and were immediately treated with NaB^2H_4 to convert aldehydes and oxoacids to their corresponding $[^2\text{H}]\text{alcohols}$ and $[^2\text{H}]\text{hydroxyacids}$. Samples were kept frozen prior to extraction. At the end of the experiment, hearts were freeze-clamped (with Wollenberger tongs) and stored in liquid nitrogen.

Perfusion protocols

Effect of hexanal concentration

Four hearts were perfused with modified Krebs–Ringer bicarbonate buffer containing 11 mM glucose and 2 mM pyruvate. Hexanal was infused during four successive 15 min periods at concentrations of 10, 25, 50 and 100 μM . Two hearts were freeze-clamped at the end of the 15 min infusion of 100 μM hexanal, and the other two were perfused for an additional 20 min after halting the hexanal infusion. Hearts were then freeze-clamped.

Effect of other aldehydes

Hearts were perfused with modified Krebs–Ringer bicarbonate buffer containing 11 mM glucose and 2 mM pyruvate, in the presence of 50 μM butanal, nonanal, 2-hexenal or malondialdehyde. The butanal, nonanal and 2-hexenal solutions were infused, while malondialdehyde was dissolved directly in the perfusion buffer. In one perfusion, butanal was also infused during seven successive 10 min periods at concentrations of 0, 10, 50, 100, 250, 500 and 1000 μM .

Effect of ischaemia–reperfusion

Three hearts were initially perfused with modified Krebs–Ringer bicarbonate buffer containing 11 mM glucose, and hexanal (50 μM) infusion into the influent perfusion stream was started. Following the stabilization period, pyruvate was dissolved in the buffer (concentration 0.2 mM; time 0–15 min) and then the pyruvate concentration was increased to 2 mM (time 15–30 min). Global ischaemia was induced by aortic clamping between 30 and 45 min. Hearts were then reperfused for 15 min (45–60 min) with 2 mM pyruvate and 50 μM hexanal, followed by infusion of 2 mM pyruvate alone for an additional 10 min (60–70 min). Hearts were then freeze-clamped.

Sample preparation for analysis by gas chromatography–mass spectrometry (g.c.–m.s.)

Acyloins and alkanediols

Acyloins and alkanediols were prepared and analysed using modifications of a previously described method (Montgomery et al., 1990). Internal standards (^2H - and/or ^{13}C -labelled alkanediols) were added to aliquots (2–5 ml) of effluent perfusates. The samples were acidified, saturated with NaCl and extracted three times with 5 ml of diethyl ether. The combined ether phases were dried with anhydrous sodium sulphate and gently evaporated under a nitrogen stream. The extract residues were converted to trimethylsilyl (TMS) derivatives using Tri-Sil/BSA.

Aldehydes, alcohols and fatty acids

Aldehydes and alcohols in aliquots of influent (1–2 ml) and effluent (2–3 ml) perfusate and in heart homogenates (2 ml;

80 mg wet weight/ml) spiked with an internal standard of perdeuterated alcohols were extracted and assayed as the *tert*-butyldimethylsilyl (TBDMS) derivatives as previously described (Des Rosiers et al., 1993). For fatty acids, the perdeuterated alcohols served as external standards and quantification was achieved using a standard curve (see calculation below). Briefly, for assay of tissue free plus bound hexanal, hearts were homogenized in ice-cold nitrogen-equilibrated 25 mM Hepes/145 mM KCl buffer, pH 7.4, containing butylated hydroxytoluene (0.04%). Free hexanal was assayed in the supernatant following deproteinization of the heart homogenate with sulphosalicylic acid (250 μ l of a saturated solution). For assay of free plus bound hexanal, heart homogenates were adjusted to pH 13 with NaOH and were heated for 30 min at 65 °C to hydrolyse hexanal bound as Schiff bases (Lee and Csallany, 1987), prior to deproteinization with sulphosalicylic acid. Recovery of hexanal was determined by processing parallel samples of control heart homogenates spiked with 25 nmol of hexanal ($62 \pm 12\%$ recovery). Procedures for extraction and analysis of the supernatant were as previously described (Des Rosiers et al., 1993).

G.c.–m.s. operating conditions

A Hewlett–Packard 5988 g.c.–m.s. was operated in electron impact mode (70 eV; source temperature 200 °C) or under positive-ion ammonia chemical ionization conditions [NH_3 pressure 133.3 Pa (1 Torr); source temperature 200 °C] using selected ion monitoring techniques. GC conditions were: 25 m $\times$ 0.2 mm HP-5 capillary column; helium flow 0.6–0.8 ml/min; split ratio 1:10; injector and transfer line at 250 °C.

2,3-Octanediol

The temperature program used was the following: 85 °C for 5 min; 5 °C/min to 110 °C; 10 °C/min to 180 °C; and 50 °C/min to 250 °C. The TMS derivatives of 2,3-alkanediols yielded two peaks on g.c. due to the presence of two chiral carbon atoms (Montgomery et al., 1990, 1992a). The retention times for the TMS derivatives of 2,3-octanediol diastereomers are 15.2 and 15.9 min. Using ammonia chemical ionization, the ions monitored are m/z 291, 292 and 294, corresponding to the $(M+H)^+$ ions for the bis-TMS derivatives of 2,3-octanediol (endogenously reduced acyloin), $[2\text{-}^2\text{H}]2,3\text{-octanediol}$ (from 3-hydroxyoctan-2-one reduction by NaB^2H_4) and $[1,2\text{-}^{13}\text{C}_2, 2\text{-}^2\text{H}]2,3\text{-octanediol}$ (internal standard).

2,3-Hexanediol and 2,3-undecanediol

The PDH-mediated condensation of hydroxyethylthiamine pyrophosphate with butanal and nonanal yields 3-hydroxyhexan-2-one and 3-hydroxyundecan-2-one (Montgomery et al., 1992a) respectively. Analytical conditions were the same as for 2,3-octanediol, except that ions monitored were m/z 263, 264 and 265, corresponding to the $(M+H)^+$ ions of the bis-TMS derivatives of 2,3-hexanediol, $[2\text{-}^2\text{H}]2,3\text{-hexanediol}$ (from 3-hydroxyhexan-2-one) and $[2,3\text{-}^2\text{H}_2]2,3\text{-hexanediol}$ (internal standard). The retention times for 2,3-hexanediol diastereomers were 12.0 and 12.9 min. For analysis of 2,3-undecanediol (retention times 19.4 and 19.8 min), ions at m/z 333 and 334 were monitored corresponding to the $(M+H)^+$ ions of endogenous 2,3-undecanediol and the NaB^2H_4 -reduced 3-hydroxyundecan-2-one. $[1,2\text{-}^{13}\text{C}_2, 2\text{-}^2\text{H}]2,3\text{-Octanediol}$ (m/z 294) was used as an external standard to provide an estimate of 2,3-undecanediol concentrations.

Aldehydes, alcohols and fatty acids

Samples were analysed by electron impact as previously described (Des Rosiers et al., 1993). The g.c. temperature programme was: 115 °C for 5 min; 4 °C/min for 165 °C; and then 50 °C/min to 250 °C. Elution times for the TBDMS derivatives were 5.8 min for $[^2\text{H}_{13}]$ hexanol, 6.1 min for hexanol and $[^2\text{H}]$ hexanol, and 8.5 min for hexanoic acid. Ions monitored were m/z 159, 160, 172 and 173, corresponding to the $M-57$ ions in the TBDMS derivatives of hexanol, $[^2\text{H}]$ hexanol, $[^2\text{H}_{13}]$ hexanol (internal standard) and hexanoic acid respectively.

Calculations

Peak areas, determined by computer integration, were corrected for naturally occurring heavy isotopes and for light isotopic impurities (Des Rosiers et al., 1988). Concentrations of aldehydes, alkanediols and alcohols in samples (Q_A) were calculated using the corrected area (CA) of the ion corresponding to the compound of interest (CA_A) compared with the corrected area of the internal standard (CA_{IS}), and the quantity of internal standard (Q_{IS}) added to each sample analysed:

$$Q_A = Q_{IS}(CA_A/CA_{IS})$$

$[^2\text{H}_{13}]$ Hexanol also served as an external standard for the assay of hexanoic acid. Quantification was achieved by using a response factor in the numerator of the above equation, determined experimentally with a standard curve (Des Rosiers et al., 1993).

Statistical analysis

Data are expressed as means $\pm$ S.E.M. They are compared using analysis of variance followed by a Bonferoni test, using $P = 0.05$ as the limit of significance.

RESULTS

Effect of hexanal concentration

In the first series of experiments, hearts perfused with 2 mM pyruvate and increasing concentrations of hexanal did not significantly change heart rate or coronary flow (results not shown). However, oxygen consumption was slightly, although not significantly, increased from 3.7 ± 0.6 $\mu\text{mol/min}$ (pre-hexanal) to 5.2 ± 0.8 , 4.9 ± 0.6 , 4.2 ± 0.3 and 4.0 ± 1.1 $\mu\text{mol/min}$ when hexanal was infused at 10, 25, 50 and 100 μM respectively.

Condensation of hexanal and pyruvate, presumably via PDH, yields 3-hydroxyoctan-2-one (Montgomery et al., 1992a) part of which can be endogenously reduced to 2,3-octanediol in the perfused heart. Because the samples were treated with NaB^2H_4 , both 2,3-octanediol (endogenously reduced) and 3-hydroxyoctan-2-one (chemically reduced to $[^2\text{H}]2,3\text{-octanediol}$) can be measured simultaneously. Both the alkanediol and acyloin release rates rose when the hexanal concentration was increased from 10 to 100 μM (Table 1), up to a maximum combined rate of 40 nmol/min, with the 3-hydroxyoctan-2-one rate more than double that of 2,3-octanediol. However, the quantitatively important metabolite produced by the heart was hexanol (Table 1). Its release increased up to 214 ± 40 nmol/min with infusion of 50 μM hexanal and then remained constant with 100 μM hexanal, although the uptake of hexanal continued to increase. When expressed as a percentage of the uptake of hexanal (Table 2), the combined release of 3-hydroxyoctan-2-one and 2,3-octanediol increased with each plateau of hexanal infused, while hexanol release rapidly attained a maximum (at 25 μM hexanal) and thereafter remained relatively constant. Together, these three

Table 1 Uptake of hexanal and release of the metabolites hexanol, 3-hydroxyoctan-2-one (3HO) and 2,3-octanediol (23OD) by isolated rat hearts perfused with 10–100 μ M hexanal

Values are the means $\pm$ S.E.M. of four perfusions and are expressed in nmol/min. nd, not detectable.

[Hexanal] (μ M)	Uptake/release (nmol/min)			
	Hexanal uptake	Hexanol release	3HO release	23OD release
10	143 $\pm$ 17	11 $\pm$ 4	0.7 $\pm$ 0.3	nd
25	312 $\pm$ 27	133 $\pm$ 33	1.6 $\pm$ 1.2	0.1 $\pm$ 0.1
50	474 $\pm$ 53	214 $\pm$ 40	16.3 $\pm$ 4.8	2.8 $\pm$ 1.7
100	852 $\pm$ 242	211 $\pm$ 59	28.8 $\pm$ 4.5	11.9 $\pm$ 5.4

Table 2 Release of 3-hydroxyoctan-2-one (3HO), 2,3-octanediol (23OD) and hexanol by isolated rat hearts perfused with 10–100 μ M hexanal

Results are expressed as percentages of hexanal uptake, and are means $\pm$ S.E.M.

[Hexanal] (μ M)	Release of metabolites (% of hexanal uptake)		
	23OD + 3HO	Hexanol	Total metabolites
10	0.6 $\pm$ 0.4	9 $\pm$ 4	9.4 $\pm$ 4
25	0.5 $\pm$ 0.4	44 $\pm$ 10	44 $\pm$ 10
50	3.8 $\pm$ 0.9	48 $\pm$ 10	51 $\pm$ 9
100	6.8 $\pm$ 1.2	32 $\pm$ 12	37 $\pm$ 13

metabolites accounted for up to 50% of the observed uptake (Table 2). A search for other metabolites revealed the presence of hexanoic acid which represented at most 1–2% of the hexanal uptake. In contrast to untreated hearts (Des Rosiers et al., 1993), hearts perfused with hexanal contained some hexanal both in the free and in the bound state. The bound hexanal was recovered with the acid-precipitable material. In hearts clamped immediately after infusion with 100 μ M hexanal, free and bound hexanal amounted to 18.6 $\pm$ 0.2 and 367 $\pm$ 39 nmol/g wet weight respectively. In hearts perfused with 2 mM pyruvate for 20 min after stopping the infusion of hexanal, the free hexanal content was similar (28 $\pm$ 6 nmol/g wet weight), but that of bound hexanal was reduced to 200 $\pm$ 8 nmol/g wet weight.

Effect of other aldehydes

The capacity of the heart to produce acyloins was also tested with other aldehydes: butanal or nonanal (saturated aldehydes), or 2-hexenal (an unsaturated aldehyde), or malondialdehyde. In agreement with results obtained *in vitro* (Montgomery et al., 1992a), acyloins were detected in the effluent from hearts perfused with 2 mM pyruvate and either 100 μ M butanal (3-hydroxyhexan-2-one; 84 nmol/min) or 50 μ M nonanal (3-hydroxyundecan-2-one; approx. 0.2 nmol/min), but not with 50 μ M 2-hexenal or malondialdehyde. To determine the aldehyde concentration at which acyloin production is saturated, the concentration of butanal, a moderately water-soluble aldehyde, was increased from 10 to 1000 μ M. At the highest butanal concentration, the rate of release of 3-hydroxyhexan-2-one was over 400 nmol/min, with no evidence of saturation. In PDH

Table 3 Effect of ischaemia–reperfusion on heart rate and coronary flow of isolated hearts perfused with hexanal and pyruvate

Hearts were perfused with 50 μ M hexanal for three sequential 15 min periods, first without added pyruvate (control), then with 0.2 mM pyruvate and then with 2 mM pyruvate. Hearts were then subjected to a 15 min period of global ischaemia, following which they were reperfused for 25 min, first with 50 μ M hexanal and 2 mM pyruvate (0–15 min), and then with 2 mM pyruvate alone (15–25 min). For each perfusion, values for the heart rate and coronary flow in the various time periods were averaged. Values reported are means $\pm$ S.E.M. of three heart perfusions. * $P < 0.01$ for pre-ischaemia versus reperfusion with 2 mM pyruvate.

Perfusion conditions	Heart rate (beats/min)	Coronary flow (ml/min)
Control	542 $\pm$ 88	16 $\pm$ 1
Pre-ischaemia:	626 $\pm$ 18	17 $\pm$ 1
50 μ M hexanal + 0.2 mM pyruvate		
Pre-ischaemia:	492 $\pm$ 83	20 $\pm$ 1
50 μ M hexanal + 2 mM pyruvate		
Reperfusion (0–5 min):	281 $\pm$ 71	13 $\pm$ 1*
50 μ M hexanal + 2 mM pyruvate		
Reperfusion (5–15 min):	432 $\pm$ 82	15 $\pm$ 1
50 μ M hexanal + 2 mM pyruvate		
Reperfusion (15–25 min):	538 $\pm$ 31	15 $\pm$ 1
2 mM pyruvate		

Table 4 Effect of pyruvate concentration and ischaemia–reperfusion on the uptake of hexanal and the release of the metabolites hexanol, 3-hydroxyoctan-2-one (3HO), 2,3-octanediol (23OD) and hexanoate

Rates are means $\pm$ S.E.M. for three perfusions, and are expressed in nmol/min.

Perfusion conditions	Uptake/release (nmol/min)				
	Hexanal uptake	Hexanol release	3HO release	23OD release	Hexanoate release
Pre-ischaemia (0.2 mM pyruvate)	672 $\pm$ 119	190 $\pm$ 46	0.6 $\pm$ 0.5	1.8 $\pm$ 0.6	9.7 $\pm$ 0.9
Pre-ischaemia (2.0 mM pyruvate)	821 $\pm$ 13	246 $\pm$ 38	5.4 $\pm$ 1.3	17.5 $\pm$ 3.8	13.0 $\pm$ 1.5
Reperfusion (2.0 mM pyruvate)	626 $\pm$ 70	214 $\pm$ 36	16.1 $\pm$ 1.9	20.8 $\pm$ 3.7	6.7 $\pm$ 3.5

incubations *in vitro* with pyruvate (10 mM) and butanal (0–5 mM), we also observed no sign of aldehyde saturation as there was a proportional increase in the production of 3-hydroxyhexan-2-one (results not shown).

Effect of ischaemia–reperfusion

In the second series of experiments, hearts were perfused with 50 μ M hexanal in the presence of 0.2 and 2 mM pyruvate (0–15 min and 15–30 min respectively) and were then subjected to 15 min of global ischaemia followed by a 25 min reperfusion period. The heart rate decreased slightly (not significantly) during the first 5 min of reperfusion and returned to pre-ischaemic levels within 20 min (Table 3). The coronary flow rate was significantly lower in the initial 5 min reperfusion period than in the pre-ischaemia perfusion period with 2 mM pyruvate (Table 3). Increasing the pyruvate concentration from 0.2 to 2 mM resulted in a 10-fold increase in the rate of release of 3-hydroxyoctan-2-one and 2,3-octanediol (Table 4). During reperfusion, the rate of release of 2,3-octanediol was comparable with that before ischaemia, but 3-hydroxyoctan-2-one release displayed a further 3-fold increase. As in the previous set of perfusions, hexanal was

Table 5 Effect of pyruvate concentration and ischaemia–reperfusion on the release of 3-hydroxyoctan-2-one (3HO), 2,3-octanediol (23OD), hexanol and hexanoic acid by hearts perfused with 50 μ M hexanal

Results are expressed as percentages of hexanal uptake, and are means $\pm$ S.E.M.

Perfusion conditions	Release of metabolites (% of hexanal uptake)			Total metabolites
	23OD + 3HO	Hexanol	Hexanoate	
Pre-ischaemia (0.2 mM pyruvate)	0.4 $\pm$ 0.2	27.8 $\pm$ 1.8	1.5 $\pm$ 0.3	29.7 $\pm$ 1.5
Pre-ischaemia (2 mM pyruvate)	2.8 $\pm$ 0.5	30.0 $\pm$ 4.9	1.6 $\pm$ 0.2	34.4 $\pm$ 5.3
Reperfusion (2 mM pyruvate)	6.3 $\pm$ 1.6	34.1 $\pm$ 3.0	1.1 $\pm$ 0.5	41.4 $\pm$ 3.9

the quantitatively important metabolite (Table 4). However, its rate of release did not vary with pyruvate concentration or pre- or post-ischaemia, nor did it vary significantly when expressed as a function of hexanal uptake (Table 5). Hexanoic acid exhibited a constant minor release into the effluent perfusate (approx. 10 nmol/min). Hexanal was detected in heart tissue both in the free form (23 ± 10 nmol/g wet weight) and in the bound form (156 ± 6 nmol/g wet weight), even when hexanal infusion was stopped and hearts were perfused for an additional 10 min with 2 mM pyruvate before being clamped.

DISCUSSION

The present study demonstrates that the rat heart can produce acyloins and 2,3-alkanediols in the presence of exogenous saturated aldehydes, representing products of lipid peroxidation, such as hexanal, butanal and nonanal. Unsaturated aldehydes did not result in acyloin formation. Based on the fact that the production of these metabolites is dependent on the pyruvate concentration, and on our recent *in vitro* study (Montgomery et al., 1992a), we suggest that in the rat heart, PDH is responsible for the condensation of saturated aldehydes to form acyloins, which can then be reduced endogenously to the corresponding 2,3-alkanediols. Since acyloins and 2,3-alkanediols are stable and freely diffusible, PDH could thus provide a means to an alternative and ubiquitous detoxification of saturated aldehydes produced by lipid peroxidation. It is of interest to note that in a review of enzymes of detoxification, a number of oxidases and dehydrogenases are included. General characteristics of these enzymes are a preference for lipophilic substrates and formation of products that are less reactive and more readily excreted (Jakoby and Ziegler, 1990). In this context, the production of 3-hydroxyalkan-2-ones, presumably via PDH, can be viewed as an enzymic detoxification of reactive aldehydes. Spontaneous formation of acyloins in the presence of pyruvate and aldehydes does not occur (Montgomery et al., 1992a).

Recognized metabolic pathways for aldehydes include reduction to alcohols and oxidation to carboxylic acids (Sladek et al., 1989). Unsaturated aldehydes, such as 2-alkenals and 4-hydroxyalk-2-enals, can also react with glutathione, either spontaneously or catalysed by glutathione transferases (Schauenstein et al., 1977; Esterbauer, 1981; Witz, 1989; Esterbauer et al., 1990, 1991), to form conjugates at the C-3 of the aldehyde. Metabolism of aldehydes to acids or alcohols leads to compounds which no longer contain a reactive double bond. In contrast, α,β -unsaturated aldehyde conjugation with thiol

groups in glutathione or cysteine, important in *in vivo* detoxification pathways, may still retain some toxic activity (Witz, 1989). A potential consequence of conjugation with glutathione of aldehydes is the depletion of the pool of cellular glutathione, which is required for the glutathione peroxidase-mediated inactivation of H_2O_2 and peroxyradical species, precursors to lipid peroxidation.

All enzymes known to catalyse conversion of aldehydes to alcohols or acids have been detected in heart homogenates (Holmes, 1978). In the present study, hearts perfused with hexanal produced 3-hydroxyoctan-2-one and 2,3-octanediol (up to 45 nmol/min), along with greater amounts of hexanol and lesser quantities of hexanoic acid. Maximal rates of hexanol release (200–250 nmol/min) by the heart were almost 20 times greater than the reported release rate of a glutathione-4-hydroxynon-2-enal conjugate (12 nmol/min per g wet weight) from rat heart perfused with 50 μ M 4-hydroxynon-2-enal (Ishikawa et al., 1986). Oxidation of aldehydes to acids has been recognized as the principal metabolic fate of aldehydes (Sladek et al., 1989), although their reduction to alcohols was also reported in some systems (Esterbauer et al., 1985; Brophy and Barrett, 1990; Grune et al., 1991). The low quantity of hexanoic acid recovered in the heart effluent implies that either (i) the oxidation pathway is a low-capacity process, or more likely, (ii) only a small proportion of the hexanoic acid produced by the heart is released into the effluent, the majority being oxidized to CO_2 and H_2O . The heart has a high capacity to oxidize fatty acids, including medium- and short-chain fatty acids. In the isolated heart, the reported oxidation rate of 0.1 mM octanoic acid, a C_8 fatty acid similar to hexanoic acid, is about 2 μ mol/min per g dry weight (Forsey et al., 1987) in the absence or presence of 5 mM glucose or pyruvate. Based on a wet weight/dry weight ratio of 5.5 (Taegtmeyer et al., 1980), this is equivalent to 360 nmol/min per g wet weight. Maximal measured rates of hexanal uptake were 800 nmol/min, of which up to 40–50% could be accounted for by metabolites recovered in the effluent or in the tissue extract (3-hydroxyoctan-2-one, 2,3-octanediol, hexanol, hexanoic acid and hexanal bound to proteins). Based on the rate of octanoic acid oxidation, formation of hexanoic acid and its oxidation to CO_2 and H_2O could account for most of the remaining hexanal uptake, although this remains to be experimentally determined. Some evaporative loss of hexanal from the surface of the heart cannot be excluded.

Quantitatively, the production of 3-hydroxyoctan-2-one and 2,3-octanediol from hexanal represents up to 15% of the hexanol release, but it nevertheless exceeds by 3–4-fold the maximal rate of release of the glutathione-hydroxynonenal conjugate (Ishikawa et al., 1986). Aside from the effect of pyruvate, acyloin formation does not appear to be subject to the same regulatory mechanisms as the PDH reaction. The presence of either inhibitors (fatty acids such as octanoate) or activators (dichloroacetate) of PDH (Olson et al., 1978) were without effect on the rate of combined release of 3-hydroxyoctan-2-one and 2,3-octanediol (results not shown). Phosphorylation of PDH is known to inhibit acetoin (3-hydroxybutan-2-one) formation (Alkonyi et al., 1976). However, ischaemia, which increases the proportion of phosphorylated PDH (Kobayashi and Neely, 1983), did not decrease total 3-hydroxyoctan-2-one and 2,3-octanediol formation. In all these perfusions pyruvate was present at a relatively high concentration (2 mM) and since pyruvate is known to keep PDH in the activated state (Cooper et al., 1974; Kobayashi and Neely, 1983), phosphorylation of PDH induced by ischaemia is likely to be counterbalanced by the presence of pyruvate. Interestingly, the 2,3-octanediol/3-hydroxyoctan-2-one ratio, which should depend on the redox state of the cell, was

lower in the first series of experiments (effect of aldehyde concentration) than in the second series of experiments (effect of ischaemia-reperfusion). This may be due to the fact that, in the first series of perfusions, pyruvate was present in the perfusate at a concentration of 2 mM from the beginning of the perfusion, even during the equilibration period (usually 20 min before aldehyde infusion), whereas in the second series it was added 15 min after the equilibration period, first at a concentration of 0.2 mM and then at a concentration of 2 mM. Perfusion of the heart with pyruvate results in a lower NADH/NAD⁺ ratio (Kobayashi and Neely, 1983; Bünger et al., 1986), which would be reflected in the lower 2,3-octanediol/3-hydroxyoctan-2-one ratio observed in the first series.

In the heart, the role of lipid peroxidation in the aetiology of ischaemia-reperfusion injury is not clear. Based on our measured overall rate of metabolism of aldehydes by the heart (about 0.8 µmol/min per g wet weight with 100 µM hexanal), and the reported metabolism of aldehydes in the liver and intestinal cells (25 µmol/min per g wet weight; Esterbauer et al., 1985), the heart might be more susceptible to the cytotoxic action of aldehydes. We found hexanal bound to acid-precipitable material, presumably proteins, although no significant changes in heart rate, coronary flow or oxygen consumption were observed under normal aerobic conditions or during reperfusion after a brief ischaemic period. Whether the heart would deal with aldehydes as easily if more severely compromised remains to be determined. We have attempted to induce lipid peroxidation using two ischaemia-reperfusion models: either 15–30 minutes of global ischaemia or 60 min of low flow (1 ml/min) followed by reperfusion. Although we observed several-fold increases in lactate dehydrogenase and glutathione release upon reperfusion, along with concomitant decreases in tissue glutathione, we did not observe increased lipid peroxidation in either model (as measured by production of malondialdehyde, 4-hydroxynon-2-enal and hexanal). Neither 3-hydroxyoctan-2-one nor 2,3-octanediol are measurable under these conditions. The reason why lipid peroxidation does not occur in these models is not clear.

Evidence for increased lipid peroxidation occurring during reperfusion after ischaemia is conflicting, either supporting increased lipid peroxidation (Guarnieri et al., 1980; Rao et al., 1983; Gauduel and Duvelleroy, 1984; Romaschin et al., 1987; Miki et al., 1988; Koller and Bergman, 1989; Weisel et al., 1989; Ambrosio et al., 1991; Kohman and Veit, 1991; Di Pierro et al., 1992) or not (Brasch et al., 1989; Lenz et al., 1989; Ceconi et al., 1991; Ballagi-Pordany et al., 1991; Park et al., 1991). Indices of lipid peroxidation measured in the various models of cardiac ischaemia were malondialdehyde measured as thiobarbituric acid reactive substances (TBARS) (Guarnieri et al., 1980; Rao et al., 1983; Gauduel and Duvelleroy, 1984; Miki et al., 1988; Ambrosio et al., 1991; Ballagi-Pordany et al., 1991; Kohman and Veit, 1991; Park et al., 1991), malondialdehyde by a more specific h.p.l.c. assay (Koller and Bergmann, 1989; Ballagi-Pordany et al., 1991; Ceconi et al., 1991; Di Pierro et al., 1992), conjugated dienes (Romaschin et al., 1987; Brasch et al., 1989) or 11-, 12- and 15-hydroxyeicosatetraenoates (Lenz et al., 1989). Measurements of malondialdehyde by TBARS and h.p.l.c. gave particularly conflicting results, perhaps because of the lack of specificity of the classical TBARS test (Janero, 1990).

Accumulation of aldehydes other than malondialdehyde in models of ischaemia-reperfusion in the heart has not been measured. As some aldehydes are more reactive and toxic, they may not be excreted as such by the heart. Measurement of their metabolites may represent a better index of their production. In this respect, identification of adducts and metabolites of

aldehydes is a first step in elucidating mechanisms of toxicity involving lipid peroxidation. Since hexanal is known to be a quantitatively important product, 3-hydroxyoctan-2-one and 2,3-octanediol may be good candidates as markers of lipid peroxidation. Also, if aldehydes are produced in the ischaemic-reperfused heart, and providing that they do act as toxic intermediates (Schauenstein et al., 1977; Esterbauer, 1981; Esterbauer et al., 1990) contributing to the ischaemia-reperfusion injury, then the PDH-mediated reaction offers another mechanism for the beneficial effect of pyruvate in the heart (Wikman-Coffelt et al., 1991; Bünger et al., 1989; Camacho et al., 1988). The exact mechanism underlying this cardioprotective effect is not known, but it is proposed to be due either to a metabolic effect (Bünger et al., 1986, 1989) and/or to an antioxidative effect (O'Donnel-Tormey, 1987; Salahudeen et al., 1991).

Stable, diffusible acyloins and their corresponding 2,3-alkanediols may prove to be useful as circulating markers of lipid peroxidation, although this remains to be proven. In contrast, alcohols or acids produced from aldehydes would not be expected to be useful *in vivo* markers because alcohols are likely to be reoxidized to acids in other organs, such as liver, with further oxidation to CO₂ and H₂O. The diagnostic value of acyloins and alkanediols, presumably formed via PDH, has already been demonstrated in a number of cases of disrupted metabolism, particularly those associated with increased aldehyde formation such as succinic semialdehyde accumulation in succinic semialdehyde dehydrogenase deficiency resulting in the excretion of 4,5-dihydroxyhexanoic acid (Brown et al., 1987), or acetaldehyde production during ethanol metabolism resulting in 2,3-butanediol formation (Mamer et al., 1978; Rutstein et al., 1983; Montgomery et al., 1990).

In conclusion, the ubiquitous nature of PDH may provide a mechanism to form acyloins from aldehydes derived from lipid peroxidation. In the perfused heart, acyloin formation is equally dependent on aldehyde and pyruvate concentrations, suggesting that PDH is responsible for the condensation. Acyloins can be endogenously reduced to the corresponding 2,3-alkanediols. These metabolites are stable and diffusible compounds and could represent the products of detoxification pathways for reactive aldehydes produced from lipid peroxidation.

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