Rapid Effects of Isoprenaline, Glucagon, Pacing and Potassium Arrest on Post-Heparin Lipoprotein Lipase Activity in the Perfused Rat Heart

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(Received 23 April 1979)

The amount of lipoprotein lipase activity released by heparin into the perfusion medium of isolated rat hearts could be increased within 60s by isoprenaline, glucagon or pacing. Potassium arrest and propranolol inhibited the effects of isoprenaline and pacing respectively.

Lipoprotein lipase (EC 3.1.1.34), the enzyme responsible for the production of fatty acids from chylomicron and very-low-density lipoprotein triacylglycerol, is functionally active at the luminal surface of capillary cell membranes (Borensztajn & Robinson, 1970). Heparin, a polyanion, is capable of interfering with the electrostatic forces holding lipoprotein lipase on to the membrane (Olivecrona *et al.*, 1977), and is administered in most studies involving lipoprotein lipase in order to solubilize the enzyme before assay. The present study shows that the amount of lipoprotein lipase activity released by heparin in the perfused rat heart can alter rapidly under several conditions.

Materials

Heparin (grade I, from porcine intestinal mucosa; 158 U.S.P. J-A units/mg, 182 WHO Std. III units/mg), adenosine and protamine sulphate (grade I) were obtained from the Sigma London Chemical Co., Kingston-upon-Thames, Surrey, U.K.; isoprenaline sulphate was from Macarthy's, Romford, Essex, U.K.; glucagon hydrochloride was from Eli Lilly and Co., Indianapolis, IN, U.S.A.; Inderal (propranolol hydrochloride in citric acid solution) was from ICI, Macclesfield, Cheshire, U.K.

Methods

Male Wistar rats (230–270g) were allowed free access to food (standard laboratory diet) and water until 13:00h, when they were anaesthetized with diethyl ether. Their hearts were removed into 500 μ M-NaHCO₃/142 mM-NaCl at 4°C, and trimmed of non-muscular material. The aorta was cannulated, and myocardial tissue perfused via the coronary arteries at 8 kPa (80 cmH₂O), at 37°C, in a non-

recirculatory perfusion apparatus. The perfusion medium, gassed with O₂/CO₂ (19:1), consisted of NaCl (118.5 mM), NaHCO₃ (25 mM), KCl (4.7 mM), KH₂PO₄ (1.2 mM), MgSO₄ (0.6 mM) and CaCl₂ (1.2 mM), unless cardiac arrest was maintained by raising the K⁺ concentration to 32 mM (NaCl concentration decreased proportionately). At times described in the Results and Discussion section, heparin, adenosine, isoprenaline sulphate, glucagon hydrochloride and propranolol hydrochloride were infused into the aortic cannula at constant rates. The perfusate concentrations of these substances may be calculated from the infusion rates and coronary flow rates given in the Results and Discussion section (if infusion rate is x mol/min and flow rate is y ml/min, then perfusate concentration is x mol/ y ml). Hearts were paced in some experiments with a modified Catheter Pacemaker (type B; Devices Ltd., Welwyn Garden City, Herts., U.K.). The indifferent electrode was attached to the metallic aortic cannula, and the catheter electrode to a silver wire touching the ventricular muscle. Heart rate was recorded within the last 20s of each minute on a Mingograf 12B electrocardiograph (Elema-Schonander, Stockholm, Sweden) by using the same electrode attachment points as described for pacing. Perfusate effluent was collected at 1 min intervals in glass vials kept in ice, and 2 ml portions were assayed immediately for lipoprotein lipase by the method of Riley & Robinson (1974). All lipolytic activities described were shown to be lipoprotein lipase activity, since they were inhibited by 85% or more by protamine sulphate (1 g/l, final assay concentration), by omission of assay medium serum or by NaCl (500 mM). The results are expressed in units of lipoprotein lipase activity released/min of perfusion per g wet wt. of heart, where 1 unit represents the production of 1 μ mol of fatty acid/h at 37°C in the assay medium.

Results and Discussion

When heparin was introduced into the perfusion medium of isolated beating rat hearts, a relatively large amount of lipoprotein lipase activity was released into the perfusate within 60s (Fig. 1). The activity so released is regarded as being the fraction of tissue activity involved *in vivo* in the uptake of fatty acids from circulating triacylglycerol (Borensztajn & Robinson, 1970). The amount of this heparin-releasable activity varied greatly between experiments, probably as a result of the various

Fig. 1. Effects of heparin and isoprenaline on the release of lipoprotein lipase activity into the perfusion medium of isolated rat hearts

△, Experiment in which isoprenaline was infused into the perfusion medium at 2.7 nmol/min from the 10th min. ○, Experiment in which heparin was infused into the perfusion medium at 350 U.S.P. J-A units/min during the 4th and 5th min, and isoprenaline was infused at 2.7 nmol/min from the 10th min. Results are means $\pm$ S.E.M.

nutritional states of individual (nocturnal-feeding) rats at 13:00h, the time at which experiments commenced (see Borensztajn *et al.*, 1970). From the second minute of administration of heparin, a sustained release of small amounts of activity could be observed, which continued for the duration of the perfusion, even if isoprenaline (see below) was not administered (Simpson, 1977). After withdrawal of heparin, as in Fig. 1, this release was probably sustained by heparin trapped within the heart's vascular system (Robinson & Jennings, 1965; Simpson, 1977). The physiological function of this fraction of tissue lipoprotein lipase activity is unknown (Rogers & Robinson, 1974). When isoprenaline was administered, a rapid and transient increase in release of lipase activity was observed, but only if heparin had been administered beforehand (Fig. 1). Such an increase could be achieved by isoprenaline at any time during the perfusion after, or concurrent with, heparin administration (Simpson, 1977), and was unaffected by a decrease in the infusion rate of heparin from 350 to 40 U.S.P. J-A units/min (Table 1). In 40% of experiments, more lipase activity was released by post-heparin isoprenaline than by heparin alone, although on average (ten experiments), the increment in activity in the perfusate during the first 2 min of isoprenaline administration was 61% of the activity released during the first minute of heparin administration.

Isoprenaline also induced increases in coronary flow rate and heart rate within the first 1–2 min of administration (Tables 1 and 2), therefore the influence of these haemodynamic variables on the release of lipoprotein lipase activity was investigated. Coronary flow rates could be greatly increased with adenosine, a vasodilator, but no significant increase in the release of lipolytic activity occurred (Table 1). A slight increase in the release of activity was observed when heart rates were increased by pacing,

Table 1. Comparison between the effects of isoprenaline, adenosine and pacing on the post-heparin release of lipoprotein lipase activity from perfused rat hearts

Heparin was infused into the perfusion medium at 40 U.S.P. J-A units/min during the 4th and 5th min of perfusion in each of the four series of experiments tabulated. In the first series, isoprenaline was infused at 2.7 nmol/min during the 10th min. In the second series, adenosine was infused at 150 nmol/min during the 10th min. In the third and fourth series, hearts were paced at 480 beats/min during the 10th min. In the fourth series, propranolol hydrochloride was infused at 20 nmol/min from the 2nd min. Results are means $\pm$ S.E.M. for the numbers of hearts indicated in parentheses. S, 10 min value is significantly different ($P < 0.02$; paired *t* test) from 9 min value; NS, 10 min value is not significantly different ($P > 0.10$; paired *t* test) from 9 min value.

Procedure	Perfusate lipoprotein lipase activity (units/min per g)		Coronary flow rate (ml/min)		Heart rate (beats/min)	
	9 min	10 min	9 min	10 min	9 min	10 min
Isoprenaline	1.9 $\pm$ 0.3 (3)	4.4 $\pm$ 0.4 (3)	9.9 $\pm$ 0.5 (3)	10.7 $\pm$ 0.2 (3)	336 $\pm$ 3 (3)	400 $\pm$ 8 (3)
Adenosine	1.3 $\pm$ 0.3 (5)	1.9 $\pm$ 0.4 (5) NS	9.4 $\pm$ 0.5 (5)	13.0 $\pm$ 0.5 (5) S	331 $\pm$ 28 (3)	308 $\pm$ 30 (3)
Pacing	1.4 $\pm$ 0.2 (6)	2.5 $\pm$ 0.5 (6) S	9.5 $\pm$ 0.4 (6)	9.5 $\pm$ 0.3 (6) NS	300 (1)	480
Pacing+propranolol	2.1 $\pm$ 0.4 (7)	2.5 $\pm$ 0.4 (7) NS	9.0 $\pm$ 0.4 (7)	9.0 $\pm$ 0.3 (7) NS	343 $\pm$ 9 (4)	480

Table 2. *Effects of glucagon and potassium arrest on the release of lipoprotein lipase activity in heparin-perfused isolated rat hearts*

Heparin was infused into the perfusion medium at 40 U.S.P. J-A units/min during the 4th and 5th min in each experiment. Glucagon series: the results of six experiments have been combined. Glucagon hydrochloride was infused at rates varying from 1.39 to 15.3 µg/min from the 10th min. Potassium-arrest series: the perfusate concentration of potassium was 32 mM throughout. Isoprenaline was infused at 2.7 nmol/min from the 10th min (four experiments). Perfusate lipoprotein lipase activity (LPLA) is expressed in units/min per g, and coronary flow rates in ml/min. Results are means ± S.E.M. *Significantly greater ($P < 0.02$; paired *t* test) than 8 min or 9 min value.

Procedure	Time (min)	...	3	4	8	9	10	11	12
Glucagon	LPLA		-0.2 ± 0.2	14.3 ± 4.7	1.7 ± 0.6	1.2 ± 0.5	2.3 ± 0.3	2.8 ± 0.6*	1.8 ± 0.4
	Flow		12.6 ± 0.7	11.7 ± 0.6	9.6 ± 0.3	9.5 ± 0.3	9.2 ± 0.3	11.1 ± 0.5*	11.4 ± 0.6
K ⁺ arrest+isoprenaline	LPLA		0.1 ± 0.1	5.5 ± 1.5	0.6 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.4 ± 0.4
	Flow		6.6 ± 1.1	5.8 ± 1.1	5.3 ± 0.8	5.4 ± 0.7	6.2 ± 0.8	6.6 ± 0.8	6.8 ± 0.8

but the effect was smaller than that induced by isoprenaline and could be blocked by propranolol, a β -adrenergic blocker (Table 1). It seems likely that pacing caused a liberation of endogenous catecholamines from myocardial storage vesicles (see Robson *et al.*, 1974), which influenced the release of lipoprotein lipase activity in a similar manner to isoprenaline, and it was not the increase in heart rate itself that stimulated the release of enzyme activity.

Other workers have suggested that cyclic AMP is involved in the regulation of lipoprotein lipase activity (Rault *et al.*, 1974; Robinson & Wing, 1970), therefore the effect of glucagon was examined, since glucagon, like catecholamines, causes an increase in tissue cyclic AMP concentration (Butcher, 1970). The results shown in Table 2 demonstrate that glucagon did stimulate the release of lipoprotein lipase activity, although the effect was less marked than that of isoprenaline. An involvement of cyclic AMP was also suggested by the results of experiments with potassium-arrested hearts (Table 2). In these experiments, the effect of isoprenaline on lipoprotein lipase activity was abolished, and it has previously been shown that potassium arrest diminishes catecholamine-induced increases in tissue cyclic AMP concentrations (Namm *et al.*, 1968).

Isoprenaline and glucagon may increase the release of lipoprotein lipase activity by stimulating the transportation of the enzyme to the capillary luminal surface, by unmasking enzyme already present or by activating a previously inactive form of the enzyme; but, whatever the case, the effect is too rapid to involve protein synthesis. Other catecholamine-induced and glucagon-induced alterations in lipoprotein lipase activity reported previously probably do involve enzyme synthesis (Borensztajn *et al.*, 1973; Mallov & Cerra, 1967).

In vivo, the process stimulated by isoprenaline and glucagon may function to increase rapidly the lipoprotein lipase activity at the capillary luminal surface under conditions of stress, when blood catecholamine and glucagon concentrations are

elevated. This would quickly increase triacylglycerol fatty acid uptake by the myocardium to meet the increased energy demand, and would be particularly important to fed animals whose capillary lipoprotein lipase activity is normally low (Borensztajn & Robinson, 1970).

I am grateful for grants from the Wellcome Trust and the Medical Research Council, and to Professor M. F. Oliver, Royal Infirmary, Edinburgh, who provided laboratory facilities for this work.

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