Br. J. Pharmac. (1983), **80**, 81–83

The actions of Paf-acether (platelet-activating factor) on guinea-pig isolated heart preparations

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1 Paf-acether (platelet-activating factor) is a phospholipid capable of stimulating platelets to release their granular contents and cause platelet aggregation. When Paf-acether was administered to isolated heart preparations from normal guinea-pigs there was a significant concentration-dependent reduction in coronary flow and contractile force. The high concentration of Paf-acether was equally effective in reducing these cardiac parameters in the presence of atropine.

2 The non-acetylated Paf-acether analogue, 2-lyso Paf-acether, the enantiomer, and a closely related phospholipid 1,α-lysophosphatidylcholine palmitoyl, did not affect coronary flow and contractile force, indicating the specificity of Paf-acether.

3 These data demonstrate a potent effect of Paf-acether on cardiac function. Whether or not these effects are direct or mediated through generation of endogenous mediators remains to be established.

Introduction

For the past several years, investigations of Paf-acether (platelet-activating factor) have centred on its ability to stimulate cells at the nmolar level. This phospholipid mediator, now identified as 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (Demopoulos, Pinckard & Hanahan, 1979; Benveniste, Tencé, Varenne, Bidault, Boullet & Polonsky, 1979), causes aggregation of platelets and provokes the release of their vasoactive amines (Benveniste, Henson & Cochrane, 1972; Henson, 1977; Chignard, Le Couedic, Tencé, Vargaftig & Benveniste, 1979; Cazenave, Benveniste & Mustard, 1979). It also stimulates neutrophils to aggregate and release their proinflammatory content (reviewed by Henson, 1981). Few studies have made use of isolated organ preparations to examine the possible direct effects of this phospholipid. The present work was undertaken to assess the action of Paf-acether on coronary flow, heart rate and contractile force of isolated hearts of guinea-pigs and to investigate its effects on various other heart preparations.

Methods

Male Hartley guinea-pigs (498 ± 11 g; $n = 43$) were injected with heparin (2500 μ/kg, i.p.) and killed by a blow on the head. The heart was removed.

Perfused heart preparations

Whole hearts were perfused (10 ml min^{-1}) through the aorta according to the Langendorff technique using Chenoweth's solution prewarmed to 37°C and gassed with 5% CO_2 in O_2. A load of 1.5 g was applied to the heart by attaching a cotton thread from the apex of the left ventricle to an isometric force transducer (Narco F 60) (Houston, TX, USA). Each heart was allowed to equilibrate for 45 min and received only one injection (vehicle or phospholipids). The spontaneous rate and contractile force were monitored by means of a Narco physiograph. The coronary flow was measured by placing a fraction collector (Narco) below the heart so that the perfusion fluid could be collected every 30 s for 20 min after injection. Paf-acether, 1, α-lysophosphatidylcholine palmitoyl, 2-lyso Paf-acether or Paf-acether enantiomer were injected at the concentrations indicated. In three experiments we injected Paf-acether (1 nM) in the presence of atropine (0.5 nM). This concentration of atropine suppressed cardiac responses to 2 μM acetylcholine. In two experiments perfusion fluid from Paf-acether-injected

hearts was added to a guinea-pig ileum to detect the presence of histamine.

Data analysis

The results are shown as percentage reduction of control values (mean ± s.e.mean). Data were compared statistically by Student's t test for unpaired variates.

Drugs

Histamine dihydrochloride and 1, α-lysophosphatidylcholine palmitoyl were from Sigma (St-Louis, MO, USA). Paf-acether (1-*O*-octadecyl-2-acetyl- *sn* -glyceryl-3-phosphorylcholine), 2-lyso Paf-acether and Paf-acether enantiomer, were provided by Prof. J.J. Godfroid (Université Paris VII). The phospholipids were initially dissolved as a stock solution containing 60% ethanol. Dilutions were made daily in 60% ethanol and the injection solution (0.2 ml) was diluted in Chenoweth's medium such as to contain only 1% ethanol. The control injections (vehicle) were given as 0.2 ml of a 1% ethanol solution.

Results and Discussion

The wet weight of the heart preparations was 2.4 ± 0.1 g ($n = 43$). The force (spontaneous contraction) at the end of the equilibration period was 0.33 ± 0.02 mg ($n = 14$). The heart rate at the end of the 45 min equilibration period was 146.0 ± 12.2 beats min^{-1} ($n = 14$). Twenty min after vehicle injection the heart rate was 130.0 ± 12.2 (n = 8). The mean coronary flow was 3.1 ± 0.2 ml min^{-1} ($n = 14$).

One, 3, 6 and 20 min after injection of various concentrations of Paf-acether the heart rate was not significantly different from controls. By contrast the coronary flow and the contractile force, assessed by measured amplitude of contraction, of perfused hearts were decreased following injection of increasing concentrations of Paf-acether (Table 1). Reduction of the two parameters persisted at 20 min at the same level as 6 min (results not shown). Paf-acether (1 nM; $n = 2$) exhibited the same effects in the presence of atropine. No histamine was detected in fluids from Paf-acether-injected hearts ($n = 2$). The coronary flow and contractile force were not changed by injection of 1, α-lysophosphatidylcholine palmitoyl (29 μM; $n = 2$). In addition, 2-lyso Paf-acether (4.5 nM; $n = 3$) did not affect coronary flow at 1, 6 and 20 min after injection (% reduction: 7.7 ± 1.4; 0.0 ± 0.0 and 0.0 ± 0.0, respectively). Similarly it had no effect on contractile force at 1, 6 and 20 min subsequent to the injection (% reduction: 11.8 ± 2.2; 12.7 ± 4.5; 0.9 ± 0.9; respectively). Paf-acether enantiomer (2.6 μM; $n = 3$) did not induce any measurable change in coronary flow and contractile force.

The effects of Paf-acether on coronary flow and contractile force that we observed support the observation of Burke, Levi, Hanahan & Pinckard (1982) who, in a preliminary study, reported that acetylglyceryl ether phosphorylcholine (Paf-acether) reduced left ventricular contractile force as well as coronary flow in isolated hearts. In our hands, the effect of Paf-acether on the isolated heart was highly specific, since lyso-lecithin, 2-lyso Paf-acether, and the optical isomer of Paf-acether were not effective in changing these parameters. These data are in keeping with those of Bessin, Bonnet, Apffel, Soulard, Desgroux, Pelas & Benveniste (1983) who observed 56% reduction of coronary flow with concomitant electrocardiogram changes in anaesthetized dogs following intravenous injection of Paf-acether.

The present results indicate that Paf-acether pos-

Table 1 Effects of Paf-acether on guinea-pig isolated perfused hearts

		Coronary flow (% reduction)			*Contractile force* (% reduction)		
Time (min)		1	3	6	1	3	6
Controls*	(14)	4.6 ± 2.5	4.3 ± 2.9	9.7 ± 2.3	1.4 ± 2.9†	0.9 ± 1.5	4.2 ± 1.7
Paf-acether	10 pM (3)	16.8 ± 13.5[a]	32.6 ± 9.3	35.5 ± 4.1	35.0 ± 4.9†	8.5 ± 1.3	23.2 ± 5.3
	100 pM (3)	32.0 ± 13.8	54.5 ± 8.2	60.0 ± 5.0	7.6 ± 4.1[b]	32.5 ± 20.0	38.0 ± 5.0
	1 nM (3)	54.5 ± 7.4	75.5 ± 11.1	66.0 ± 1.4	13.5 ± 7.2	41.5 ± 20.5	63.0 ± 12.5
	1 nM (2)[c]	46.0 ± 14.3	72.0 ± 8.2	52.0 ± 5.6	19.5 ± 5.4	52.5 ± 11.6	58.0 ± 8.0

Number of experiments is shown in parentheses.
* Isolated heart preparations which were injected with vehicle.
† Indicates % increase in response. All results are expressed as means ± s.e.mean.
[a] Values which were significantly different from vehicle-injected controls ($0.05 > P > 0.01$), except when indicated (b).
[c] Experiments done in the presence of 0.5 nM atropine. No statistical analysis was performed.

sesses other biological properties than the well-known platelet- and neutrophil-activating effects. The cardiac actions of Paf-acether may be platelet-independent since it is unlikely that a significant number of these cells persist in the cardiac vascular bed after 45 min perfusion with an artificial buffer and no histamine was released in the perfusion fluid, indicating absence of platelet activation. The failure of atropine to influence the cardiac effects of Paf-acether shows that this phospholipid alters both coronary flow and contractile force independently from the release of endogenous acetylcholine. However, until a careful pharmacological study utilizing various blocking agents is undertaken, the exact nature of the effect of Paf-acether on isolated heart preparations remains to be elucidated.

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(*Received February 7, 1983.*
Revised May 6, 1983.)