Radioimmunoassay at alkaline pH: A method for the quantitative determination of prostacyclin

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Quantitative determination of prostacyclin in biological fluids has been achieved by relatively non-specific bioassay techniques. The most widely used is that which utilizes its ability to inhibit platelet aggregation (Moncada et al., 1977). More accurate physicochemical methods of analysis such as radioimmunoassay and g.l.c.-mass spectrometry (Claeys et al., 1980; Morris et al., 1981) have relied on quantitative determination of the hydrolysis product of prostacyclin, 6-oxoprostaglandin F$_{1\alpha}$. We have modified a radioimmunoassay for 6-oxoprostaglandin F$_{1\alpha}$ so that specific determination of prostacyclin can be performed. The specificity of the method relies on the ability of base and albumin to stabilize any prostacyclin present in a sample. Quantitative determination by radioimmunoassay of the 6-oxoprostaglandin F$_{1\alpha}$ present in this sample, together with that in a duplicate that has undergone mild acid hydrolysis allows the original prostacyclin content to be calculated.

Prostacyclin, 6-oxoprostaglandin F$_{1\alpha}$, or diluted biological sample in 100µl of 50mm-Tris/HCl buffer, pH 8.5 (Tris buffer), was cooled on ice in polystyrene tubes. 6-Oxol$^3$H$^3$prostaglandin F$_{1\alpha}$ (5Ci) was added in Tris buffer (100µl) containing 3% (w/v) human serum albumin (essentially fatty acid-free). This concentration was chosen to minimize any effect of endogenous albumin present in the samples. Antiserum (1:4500 dilution) to 6-oxoprostaglandin F$_{1\alpha}$ (Hensby et al., 1981) in 100µl of Tris buffer was then added. The contents of the tubes were vortex-mixed and incubated on ice for 3h. Then 2.5mg of bovine γ-globulin in Tris buffer (100µl) was added, followed by saturated aqueous (NH$_4$)$_2$SO$_4$ solution (400µl). The precipitate was sedimented by centrifugation (1000g for 15 min at 4°C), the supernatant was decanted and the pellet was washed once with 800µl of saturated aqueous (NH$_4$)$_2$SO$_4$ solution/water (1:1, v/v). The washed pellet was dissolved in distilled water (1.3ml), and the bound radioactivity was counted in Instagel (10ml) in a Packard 2650 scintillation counter.

Rat aortic rings were prepared by a modification of the method described by Moncada et al. (1977), and incubated in citrated platelet-poor plasma. At given times 100µl of plasma was removed, and the prostacyclin was stabilized by the immediate addition of 2.5µl of 2M-NaOH and stored at -20°C overnight. The prostacyclin content of these plasma samples was assayed against standard prostacyclin on ADP-induced platelet aggregation and against standard 6-oxoprostaglandin F$_{1\alpha}$ by radioimmunoassay as the procedure described below.

A portion of the sample (30µl) was diluted 20- or 100-fold with Tris buffer on ice. A 250µl portion was hydrolysed (addition of 5µl of 2M-HCl); after 15 min at room temperature the pH was adjusted to 8.5 (addition of 5µl of 2M-NaOH), and the mixture was returned to ice. A second 250µl portion treated with 1M-NaCl (10µl) provided the non-hydrolysed sample. Duplicates (2 x 100µl) of these hydrolysed and non-hydrolysed samples were then analysed by radioimmunoassay for 6-oxoprostaglandin F$_{1\alpha}$. The prostacyclin concentration was calculated as the difference in 6-oxoprostaglandin F$_{1\alpha}$ content between the hydrolysed and non-hydrolysed samples.

Under the conditions of the assay 10% displacement of 6-oxo-$^3$H$^3$prostaglandin F$_{1\alpha}$ occurred with 15 pg of 6-oxo-prostaglandin F$_{1\alpha}$; this was taken to be the limit of sensitivity. Inter-assay variation (10 x 200pg) and intra-assay variation (10 x 200pg) were 14% and 7% respectively. The cross-reactivity of prostacyclin in the assay was determined from separate incubations of hydrolysed and non-hydrolysed prostacyclin samples. At 50% displacement of 6-oxo-$^3$H$^3$prostaglandin F$_{1\alpha}$ 7.9% cross-reaction was observed. This was considered to be acceptable, and probably represented the contamination of the prostacyclin with 6-oxoprostaglandin F$_{1\alpha}$, and the adventitious hydrolysis of prostacyclin during the assay.

The analysis by bioassay of the prostacyclin concentration of plasma samples incubated with aortic rings (Ritter et al., 1982) was correlated with that obtained by the radioimmunoassay described above. A correlation coefficient of 0.92 was obtained. It has been proposed that a factor that stimulates prostacyclin production is elevated in plasma from uralic patients (Remuzzi et al., 1980). We have employed the assay described in the present communication to determine the effect of plasma from patients in renal failure on the synthesis of prostacyclin by rat aortic rings. Aortic rings incubated with plasma from patients in renal failure for 30min produced 0.69 ± 0.15 ng of prostacyclin/mg wet wt. of tissue ($n = 8$). Rings incubated for 30min in plasma from age- and sex-matched controls with normal renal function produced 1.16 ± 0.35 ng of prostacyclin/mg wet wt. of tissue ($n = 8$). There was no significant difference between the two groups.


Fatty acid specificity of human platelet-membrane phospholipase A$_2$ and diacylglycerol lipase

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Platelet activation by agents such as thrombin or collagen is accompanied by the oxygenation of arachidonic acid into prostaglandins and related compounds (Samuelson, 1977). Platelet arachidonic acid is mainly located in membrane glycoporphospholipids and has to be released before its oxygenation by the prostaglandin synthetase complex and the lipoxygenase (Marcus, 1978). The liberation of arachidonic acid from platelet phospholipids can occur either through phospholipase A$_2$ activity acting principally on phosphatidylcholine and ethanolamine (Broekman et al., 1980; McKean et al., 1981), or through the phospholipase C diacylglycerol lipase cascade (Mauco et al., 1978; Rittenhouse-Simmons, 1979; Bell et al., 1980).