Reduction of Serum Prostacyclin Stability in Ischemic Stroke

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SUMMARY Prostacyclin is a powerful vasodilator and inhibitor of platelet aggregation that has been implicated to play a role in cerebrovascular disease. Prostacyclin is unstable in aqueous solution and stabilized in serum by binding to an unidentified serum protein as measured by gel filtration. In 15 patients with ischemic stroke we measured the serum prostacyclin binding capacity and the rate of degradation of exogenously added prostacyclin. There was a significant reduction in serum prostacyclin binding capacity and a significant increase in rate of degradation in the patients with ischemic stroke as a whole compared to controls, and in patients with persistent deficits compared to those with transient deficits. Decreased serum prostacyclin binding capacity and accelerated rate of prostacyclin degradation in vitro, may reflect an accelerated rate in vivo of prostacyclin degradation, thereby increasing susceptibility to stroke. Since only a small number of patients were investigated, the findings are of a preliminary nature and must be confirmed by further studies with large numbers of patients and appropriate patient controls.

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ALTHOUGH MUCH IS KNOWN about the biochemistry of prostaglandins and their metabolites,¹ the role of these substances in cerebrovascular disease remains speculative. Prostacyclin (PBI₂), an arachidonate metabolite, is a powerful vasodilator and inhibitor of platelet aggregation which has been implicated to play a potential role in defense against thrombosis.² Prostacyclin is unstable in aqueous solution and stabilized in serum by binding to an unidentified serum protein as measured by gel filtration. In 15 patients with ischemic stroke we measured the serum prostacyclin binding capacity and the rate of degradation of exogenously added prostacyclin. There was a significant reduction in serum prostacyclin binding capacity and a significant increase in rate of degradation in the patients with ischemic stroke as a whole compared to controls, and in patients with persistent deficits compared to those with transient deficits. Decreased serum prostacyclin binding capacity and accelerated rate of prostacyclin degradation in vitro, may reflect an accelerated rate in vivo of prostacyclin degradation, thereby increasing susceptibility to stroke. Since only a small number of patients were investigated, the findings are of a preliminary nature and must be confirmed by further studies with large numbers of patients and appropriate patient controls.

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influence of age on prostacyclin binding and degradation was unclear, we divided the normal subjects into 2 groups according to age, i.e. age 21-44 and age 45 and above. Normal subjects did not take aspirin containing drugs. Three individuals took medications regularly (an oral hypoglycemic agent for adult onset diabetes, a diuretic for hypertension and a thyroid replacement for thyroidism).

**Preparation Of Serum**

Blood was withdrawn from an antecubital vein into glass tubes and incubated at 37°C for 2 hours to allow blood clotting. The blood was centrifuged at 2000g for 20 minutes to prepare serum. Occasionally, blood was drawn at night and kept at 4°C overnight. There was no apparent difference in PGI₂ degradation rate or binding between 2 ways of serum preparation. Nor was there any difference between freshly prepared samples or samples stored at -70°C.

**Prostacyclin Binding Capacity**

³H-Prostacyclin methylester (12 ci/m mol) was obtained from New England Nuclear, Boston, MA, reduced to ³H-PGI₂ sodium and purified by thin layer chromatography. ³H-PGI₂ was incubated with 0.35 ml of serum at room temperature for 3 minutes and applied to a sephadex G25 column. Fractions of the gel filtrate were collected and the radioactivity was counted. The PGI₂, eluted in 2 peaks. The bound PGI₂ was eluted in the first peak, and the free PGI₂, eluted in the second peak. The PGI₂ binding capacity was expressed as the percentage of radioactivity in the binding peak.

**Serum PGI₂ Degradation**

Reduction of PGI₂ activity as a function of time was determined by a modification of the procedure described previously. In each experiment, a PGI₂ concentration which inhibited 75-95% of ADP-induced platelet aggregation was chosen to be incubated with normal or patient serum at 37°C. An aliquot (50 µl) of the mixture was removed at 1, 5, 15 minute, etc., and its PGI₂ activity determined by relating its anti-aggregatory activity to the calibration curve. The serum half life of PGI₂ was determined as the time interval at which 50% of the added PGI₂ activity had disappeared.

**Results**

There was no difference in the serum PGI₂ binding percentage between the two age-control groups (fig. 1). The binding was significantly reduced for all patients with stroke (p < 0.01) and for the subgroups of patients with persistent deficit (p < 0.01) and transient deficit (p < 0.02) when compared with the control. The persistent group had a significantly lower binding value than the transitory group (p = 0.03). The serum PGI₂ half life was not influenced by age in the control subjects. The PGI₂ half life was significantly reduced in all patients with ischemic stroke (p < 0.01), those with persistent deficits (p = 0.01) and those with transient deficits (p = 0.01) when compared to the control group (fig. 2). There was no difference in the rate of PGI₂ degradation between the persistent and the transitory groups.

Of the 4 patients with ischemic stroke due to cardiac emboli, one had a very low binding value and short half life. In addition, one patient with lacune infarct had a low binding value.

**Discussion**

Our data indicate that a heterogenous group of patients with ischemic stroke have a decreased serum
PGI₂ binding and an increased rate of PGI₂ degradation as compared to age and sex matched normal controls. Accelerated PGI₂ degradation may lead to insufficiency of PGI₂ activity at vascular damage sites resulting in a perturbation of a balance between PGI₂ and TXA₂, which has been considered by Vane, Moncada and Associates to play an important role in maintenance of normal hemostasis. PGI₂ is generally regarded to function as an autacoid rather than a circulating hormone because normally only a miniscule quantity is detectable in the circulation. Its insufficiency at the damaged vascular site may cause an excessive accumulation of thrombi and may account in part for the platelet hyperaggregability reported to occur in patients with ischemic stroke.

In the four patients with the lowest binding capacities, there was one cardiac embolus, one lacune, and two thromboembolic infarctions. It is unclear at this time whether there is any definite relationship between binding capacity and mechanism of stroke. Prostacyclin binding capacity also does not appear to be related to age as there was no correlation between binding capacity and age in any group.

Despite the intriguing observations, our findings should be regarded as of a preliminary nature because of the small number of patients included in this study. It remains unclear whether the abnormal PGI₂ degradation is a specific phenomenon for stroke or it may represent an epiphenomenon not related to the pathogenesis of stroke. Work is now in progress to study a large number of patients with thrombotic stroke and other thromboembolic disorders, non-thrombotic conditions as patient controls in order to ascertain the clinical importance of this observation.

References

Reduction of serum prostacyclin stability in ischemic stroke.
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