A Saturation Assay Method for Adenosine 3':5'-Cyclic Monophosphate in Plasma and its Use in Studies of the Action of Bovine Parathyroid Hormone

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In previous studies of factors affecting cyclic AMP concentrations investigators have found it necessary to extract the plasma and to subsequently purify the cyclic AMP before it can be assayed. We have devised a modification of the protein binding assay method of Brown et al. (1971) that avoids the need for extraction and purification, and has enabled us to assay plasma samples directly, in large numbers.

A major problem associated with the direct measurement of cyclic AMP in plasma is the presence of phosphodiesterase, which is active even in frozen samples. Unless theophylline was added to plasma the concentration of cyclic AMP was found to fall by 26-56% of the initial value after storage at -20°C for 24 h and by 37-100% after storage at -20°C for a month. In contrast, the cyclic AMP content of theophylline-containing samples was found to remain the same even after prolonged storage.

Degradation of cyclic AMP in plasma is rapid, with a half-life of 2.6 h at 37°C and 16.3 h at 4°C (Fig. 1). It is therefore important to ensure that losses are minimal during the collection and separation of plasma. In the present investigation blood was collected in cooled heparinized tubes and centrifuged within 2 min. The resulting plasma was immediately diluted with an equal volume of buffer [50 mM-Tris-HCl buffer, pH 7.4, containing theophylline (8 mM) and 2-mercaptoethanol (6 mM)] and the samples were frozen and stored at -20°C until assayed.

Plasma samples that had been stored at room temperature for 24 h were found to have lost all measurable cyclic AMP, and this observation was exploited in order to prepare plasma free of cyclic AMP. The effect of cyclic AMP-free plasma on the assay response curves is shown in Fig. 2. It is clear that these assay curves were not identical, showing that constituents in the plasma of different subjects altered the curve to different extents.

Plasma containing high concentrations of endogenous cyclic AMP gave a dilution curve in cyclic AMP-free plasma from the same subject that was indistinguishable from that of exogenous cyclic AMP. Therefore allowance can be made for the non-specific effects of a given plasma by adding a portion of that plasma (freed of endogenous cyclic AMP) to each sample used for the standard response curve.

In the assay, 50 μl of the diluted plasma sample or the standard was added to each assay tube in a total incubation volume of 300 μl. In other respects the conditions of the assay and details of the separation technique were identical with those already reported.

The average concentration of cyclic AMP in plasma samples taken randomly from 12 normal subjects was found to be 15.1 ± 3.3 pmol/ml (mean ± S.D.). This is similar to the range reported by Broadus et al. (1970), who used an enzymic method of assay. Variation in a single subject was studied in eight samples taken from a normal female volunteer on different days; the mean concentration ± S.D. was 17.1 ± 3.3 pmol/ml.

The assay method was found to be highly reproducible, with an inter-assay coefficient...
Fig. 1. Disappearance of endogenous cyclic AMP from plasma

Plasma was incubated at two different temperatures: (a) 4°C; (b) 37°C. Samples were taken at intervals and further degradation was inhibited by the addition of an equal volume of theophylline-containing buffer. The samples were stored at -20°C until assayed. The half-life of disappearance was found to be 2.6 h at 27°C and 16.3 h at 4°C.

We have used this assay method in studies of the effects of parathyroid hormone on circulating cyclic AMP in man. Bovine parathyroid hormone has been shown to stimulate the adenylate cyclase of rat kidney (Marcus & Aurbach, 1969) and to increase urinary excretion of cyclic AMP in man (Chase & Aurbach, 1967). When infused in small doses over about 1 h it has also been shown to cause a moderate increase in plasma cyclic AMP in man (Kaminsky et al., 1970).

In the present study highly purified bovine parathyroid hormone (200 units) was infused in four normal subjects over a period of 15 min. A remarkably rapid response was found. Within 3 min all subjects showed an increase in circulating cyclic AMP, which ultimately reached a maximum of 4–6 times the pre-infusion concentration. The concentration of cyclic AMP fell rapidly when the infusion was stopped, with a half-life of 14–16 min.

We have in addition obtained direct evidence for the renal origin of parathyroid-hormone-induced increases in plasma cyclic AMP by simultaneously measuring the concentration in plasma from the renal and antecubital veins after injection of bovine
Fig. 2. Effect of plasma on the cyclic AMP response curve

Three assays were set up with the same reagents. 0, Assay curve in buffer; △ and □, assays supplemented with cyclic AMP-free plasma (25μl) from different subjects, in place of an equal volume of buffer.

parathyroid hormone. Cyclic AMP in renal-vein plasma was found to increase more rapidly than in the peripheral circulation, to a much higher maximum value. Likewise the subsequent decline was quicker, and occurred earlier than in peripheral plasma.