Previous work from our laboratory[6,7] has shown that a high platelet aggregation value is generally associated with certain stress conditions. The rate was high, 15–18, in myocardial infarction, moderately high, 13–18, in cerebrovascular disease, and slightly elevated, 11–14, in cases of diabetes mellitus.

The finding that bromelain significantly reduces high platelet aggregation values of individuals has important clinical implications. Our previous studies[4] indicated that bromelain (administered as Ananase®) did not significantly affect any of the normally measured clinical parameters. Also in its clinical use over a period of more than 10 years, there have been no reports of significant undesirable side effects. This drug would then seem to be worthy of testing as a long term maintenance drug for those individuals who have problems with enhanced platelet aggregation rates.

Zusammenfassung. Die orale Verabreichung von Bromelain in Form von 2 Ananase®-100-Tabletten an freiwillige Versuchspersonen verminderte bei diesen die Empfänglichkeit für die durch ADP induzierte Aggregation der Blutplättchen.

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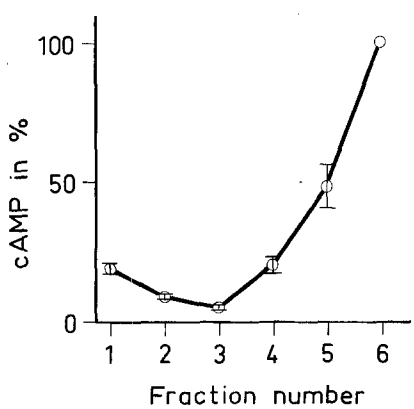
Cyclic AMP of Blood Platelets: Accumulation in Organelles Storing 5-Hydroxytryptamine and ATP

Cyclic 3′,5′-adenosine monophosphate (cAMP) and prostaglandin-activated adenyl cyclase occur in human blood platelets, but their subcellular localization is not known[1–4]. In platelets of rabbits, guinea-pigs, and probably man, non-cyclic phosphonucleotides (e.g. adenosine-5′-triphosphate, ATP) have recently been shown to accumulate in subcellular organelles which also store 5-hydroxytryptamine (5HT)[5–7]. It was therefore of interest to investigate whether cAMP shows a similar subcellular localization as its precursor ATP.

Materials and methods. Blood platelets of rabbits and guinea-pigs were homogenized by ultrasonication, and thereafter the particulate matter (mitochondria, α-granules, 5HT-nucleotide storage organelles, etc.) was subjected to ultracentrifugation in a continuous Urografin® density gradient as previously described[5]. The liquid content of the centrifugation tube was divided into 5 equal parts. Fractions 3 and 4 consisted mainly of mitochondria and α-granules respectively. The fraction containing the organelles storing 5HT and nucleotides was localized as a fine film at the bottom of the tube (fraction 6). It showed virtually no contamination with other subcellular particles as judged by electron microscopy[7]. The level of cAMP in the homogenates of whole platelets, in the isolated storage organelles as well as in the other subcellular fractions (of rabbit platelets only) was determined by a saturation method[8] and by a radioimmuno-assay[9] and related to the amounts of protein[10]. In some experiments, the total nucleotide extract of storage organelles was submitted to chromatography on Dowex AG 50 WX 4, 200–400 mesh. The cAMP of the eluate was assayed[8] before and after incubation at 37°C for 15 min with 3′,5′-cyclic nucleotide phosphodiesterase isolated from rat brain[11].

Results and discussion. The cAMP in the various subcellular fractions of rabbit platelets showed a similar distribution to that previously reported for ATP and 5HT[7,12]. The concentration of cAMP (in *p*moles/mg protein) in the pure 5HT-nucleotide storage organelles markedly exceeded that in the other subcellular fractions (Figure). It was more than 100 times higher than in homogenates of whole platelets (Table). In guinea-pigs,



Subcellular distribution of cyclic AMP (cAMP) in the particulate matter of blood platelets of rabbits determined by the saturation method. The values indicate the cAMP content in *p*moles/mg protein and are expressed in percentages of the values found in fraction 6, i.e. the pure storage organelles (= 100). Averages with S.E. of 8 experiments.

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too, the concentration of cAMP measured with both methods in the isolated storage organelles exceeded that in the whole platelets by more than 100 times.

The identity of the cAMP in the storage organelles was substantiated by the findings that a) the values determined with the saturation method were not significantly different ($p > 0.05$) from those obtained with the radioimmune-assay (Table) and b) no cAMP could be detected by either method after incubation of the nucleotide extracts with phosphodiesterase. Furthermore, the cAMP was probably not formed from ATP during the precipitation procedure (with $ZnSO_4$ + Ba $(OH)_2$) used for the saturation method[8,13]. Thus, the cAMP values showed no difference whether the ATP had been removed by precipitation or by absorption on Dowex AG50 WX4. A reduction by the ultrasonication of the extragranular cAMP is unlikely since platelets homogenized by freezing and thawing exhibited the same cAMP content as those subjected to ultrasonication.

These results indicate that cAMP accumulates together with 5'-phosphonucleotides like ATP and a biogenic amine (5HT) in subcellular storage organelles. Thereby, the concentration of the cyclic nucleotide is about 4000 times lower than that of ATP (8.5×10^6 *p*moles/mg protein)[5]. According to preliminary results with rabbit platelets, no measurable adenyl cyclase activity (using α-^{32}P-ATP as a substrate)[14] could be detected in the isolated storage organelles or in their membranes, even in the presence of NaF or prostaglandin E_1[15]. In the isolated cytoplasmatic membranes, however, adenyl cyclase activity was present and markedly activated by NaF as well as prostaglandin E_1. Therefore, the site of formation of the cAMP found in the storage organelles is not yet clear.

cAMP possibly has an influence on platelet function, e.g. in platelet aggregation, but its physiological and pathophysiological role is still unsettled[4,16-20]. Storage in subcellular organelles might result in biological inactivation of the cyclic nucleotide. Therefore, the organelles, due to their capacity of accumulating and possibly releasing cAMP, may be part of a system controlling the biological activity of the cyclic nucleotide in the platelets. It remains to be elucidated whether other types of organelles, e.g. the vesicles storing ATP and catecholamines in sympathetic nerve endings or brain, behave like platelet organelles with regard to cAMP, and if so, whether the presynaptically stored cAMP has a function in neurohumoral transmission.

Zusammenfassung. Die subzellulären Organellen, welche in Blutplättchen von Ratten und Meerschweinchen 5-Hydroxytryptamin und Adenosin-5'-triphosphat speichern, enthalten bis über 100 Mal mehr cyclisches Adenosin-5'-monophosphat (cAMP) als die ganzen Plättchen und die übrigen subzellulären Fraktionen. Durch die Speicherung in subzellulären Organellen kommt es möglicherweise zu einer biologischen Inaktivivierung von cAMP in den Plättchen.

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Content of cyclic AMP in isolated blood platelets and storage organelles of rabbits

Method	Blood platelets	Storage organelles
Saturation assay	24 ± 5 (10)	2501 ± 640 (8)
Immunological assay	9 ± 1 (8)	1975 ± 293 (7)

The values represent averages with S.E. and are expressed in *p*moles/mg protein. Number of experiments in parentheses.

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Cyclic AMP: its Effect on an Estrogen-Sensitive Antigen in Organ Cultures of the Cervicovaginal Epithelium from Neonatal Mice[1]

Cyclic AMP is known to regulate important activities in several different cells and to be essential in the action of many hormones. Its role for the expression of the sex steroid effects has recently been dealt with[2,3].

Immunofluorescence studies have shown that the cervicovaginal epithelium of neonatal and adult mice contains antigenic material(s) (CVA) specific for this epithelium. Antibodies against CVA do not cross-react with any antigen in several tissues tested (uterus, kidney, intestine, submaxillary gland etc.)[4]. In estradiol injected animals, the amount of CVA is considerably increased but still restricted to the cervicovaginal epithelium. When the uterine cervix from neonatal mice was cultured in vitro in a medium containing cyclic AMP, the amount of CVA was likewise increased compared with the controls[5].

In organ cultures of the neonatal uterine cervix, the cyclic AMP induced CVA was localized to the most apical part of the epithelial cells and to the lumen. This prompted an ultrastuctural study on the relation between CVA and the cell surface.

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