Adenyl Cyclase Activity in Non-Nucleated Red Blood Cells: Evidence for its Localization in the Reticulocytes

In contrast to earlier findings[1–5], SHEPPARD and BURGHARDT[6,7] have shown that adenyl cyclase is present not only in nucleated but also in non-nucleated erythrocytes; the enzyme activity they measured in ghost membranes from rats and mice was almost exclusively stimulated by β-sympathomimetic agents.

The significance of the presence of adenyl cyclase in the erythrocyte membrane is yet unknown. It is well established, however, that various enzymes found in red blood cells are not evenly distributed throughout the whole erythrocyte population but lose their activities partially[8–11] or even completely[8,9] during the maturation process. It seemed to be of interest, therefore, to find out whether this would be true for adenyl cyclase activity also: if adenyl cyclase activity were present only during distinct stages of the red cell maturation, e.g. in the reticulocytes, then this might provide an indication for the functional role of this enzyme in the red cell.

Therefore, an attempt was made to measure adenyl cyclase activities in blood samples from rats with different degrees of reticulocytoses. According to GANZONI[12], a pronounced reticulocytosis occurs during the early period of rapid growth. This reticulocytosis indicates an enhanced erythropoietic response due to the relatively high plasma volume, and disappears slowly with increasing age. In the experiments described below, we therefore used 5 groups of male Wistar rats with body weights of 30–40, 80–90, 120–130, 170–180, and 220–240 g.

Methods. From heparinized blood pooled from 20 to 50 Wistar rats, ghosts were prepared as described by SHEPPARD and BURGHARDT[6] and by DODGE et al.[13] Freeze-dried ghosts were used in all experiments.

Assays of adenyl cyclase activity were performed essentially according to SHEPPARD and BURGHARDT[6]; as a major modification, an ATP-regenerating system was added. ^{14}C-labeled cyclic AMP formed from 8-^{14}C-ATP was separated from other nucleotides by the method of KRISHNA et al.[14] Reticulocytes were counted in blood smears after staining with brillant cresyl blue. Protein was determined using the method of LOWRY et al.[15].

Results. As can be seen from Figure 1, adenyl cyclase activity was stimulated more than 10-fold by NaF and about 4- to 6-fold by D(-)-isoproterenol in all groups of animals, irrespectively of body weight or age. With

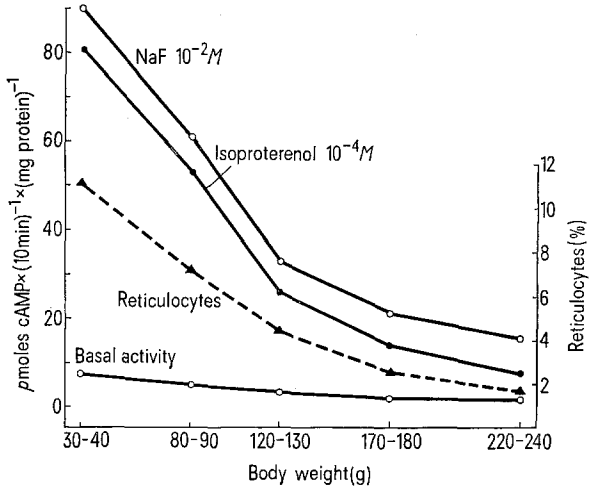

Fig. 1. Decrease of adenyl cyclase activities (basal, NaF- and D(-)-isoproterenol-stimulated; left ordinate) in erythrocyte ghost preparations, and decrease of reticulocyte counts (% of erythrocytes; right ordinate) in blood samples from growing rats.

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Adenyl cyclase in the erythrocyte membrane

Rat			*Man*		
Reticulocytes [%]	Adenyl cyclase activity[a] Basal	NaF $10^{-2}M$	Reticulocytes [%]	Adenyl cyclase activity Basal	NaF $10^{-2}M$
Untreated controls (180–220 g, $n = 7$)			Healthy male volunteers ($n = 5$)		
2.2 ± 0.4	2.7 ± 1.0	49 ± 10	1.2 ± 0.2	0.5 ± 0.2	7.6 ± 0.6
APH-treated[b] ($n = 8$)			Patients with pernicious anaemia[c] ($n = 3$)		
65 ± 6	11 ± 2	200 ± 16	24 ± 4	1.6 ± 0.7	20 ± 2

[a] Expressed as pmoles cAMP formed/10 min/mg protein ± SEM. [b] Treatment with 3 × 60 mg/kg i.m. 1-acetyl-2-phenyl hydrazine on 3 consecutive days, 7 days after 1st injection. [c] 7 days after beginning of treatment with cyanocobalamine.

increasing age, a continuous decrease in per cent reticulocytes occurred with a final value of less than 2% reached in adult (220–240 g) rats. Corresponding with the decrease in reticulocyte counts, there was a pronounced decrease in adenyl cyclase activities; in 220–240 g animals, NaF- and isoproterenol-stimulated enzyme activities were only 10 to 15% of those found in the 30 to 40 g group.

The obvious correspondence between per cent reticulocytes and adenyl cyclase activities can be clearly seen in Figure 2 where the adenyl cyclase activities are plotted against the respective reticulocyte counts: enzyme activities and per cent reticulocytes were linearly correlated, the correlation coefficients amounting to 0.99 for the basal and the NaF-stimulated, and to 0.98 for the isoproterenol-stimulated activities ($n = 5$). Moreover, it can be concluded from Figure 2 that mature erythrocytes contain only negligible amounts of adenyl cyclase activity: the respective regression lines tend to cross the origin.

Discussion. The results presented lead to the conclusion that adenyl cyclase activity in the non-nucleated red blood cells from rats is localized in the reticulocytes. Support for this view comes also from preliminary experiments[16] with rats which were treated with 1-acetyl-2-phenyl hydrazine: these animals show marked enhancement of both reticulocyte counts and adenyl cyclase activities (Table).

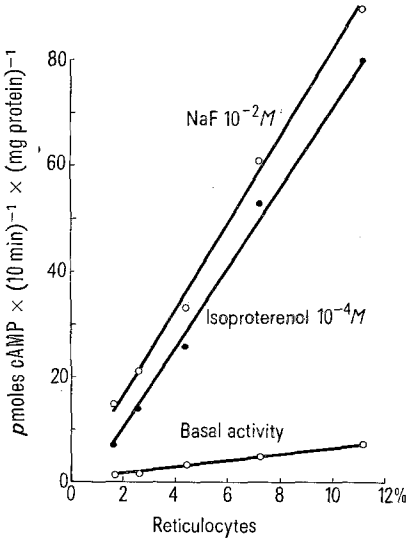

Fig. 2. Linear correlation between adenyl cyclase activities (basal, NaF- and D(-)-isoproterenol-stimulated) in erythrocyte ghost preparations, and reticulocyte counts in the respective blood samples (rat).

Comparison of adenyl cyclase activities in red blood cells from different species seems also to support the assumption that adenyl cyclase is localized in the reticulocytes. In our investigations (Table) adenyl cyclase activities in erythrocyte ghosts from 180–220 g rats were found to be several times higher than in ghost preparations from man. Likewise, the respective mean reticulocyte counts amounted to 2–3% in rat and about 1% in human blood samples. In addition, several cases of pernicious anaemia were investigated in which the reticulocyte crisis occurring after treatment with cyanocobalamine was accompanied by a marked increase in the adenyl cyclase activities measured in red cell ghost preparations (see Table).

If – according to our results – adenyl cyclase is confined to the reticulocytes, then this enzyme is not necessarily localized in the plasma membrane: it is known that reticulocytes contain – besides mitochondria – also ribosomes which cell constituents are, in part, firmly bound to the plasma membrane[17].

Concerning the physiological significance of adenyl cyclase in red blood cells, it seems that the enzyme has a functional role only in very young erythrocyte forms or, perhaps, in the erythrocyte precursors. This view is supported by recent experimental results[18,19] from which a functional role of cyclic AMP and of an adrenergic β-receptor system in the regulation of erythropoiesis may be assumed.

Zusammenfassung. Die durch Natriumfluorid und Isoproterenol stimulierbare Adenylcyclase-Aktivität in Erythrocyten-Schatten von 30–240 g schweren Ratten ist direkt proportional dem prozentualen Retikulocytengehalt des Blutes. Hinweise darauf, dass die Adenylcyclase-Aktivität kernloser Erythrocyten nahezu ausschliesslich in den Retikulocyten lokalisiert ist, konnten auch an menschlichem Blut erhalten werden.

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A New Peripheral Monoamine Oxidase Inhibitor: 2,9-Dimethyl-β-carbolinium Iodide

The origin of the hypotensive action of certain drugs and perhaps hypertension itself may be determined by utilizing a specific peripheral monoamine oxidase (MAO) inhibitor. Furthermore, the mechanism of reversal of reserpine-induced sedation by this type of compound and ultimately the site of reserpine action could also be ascertained. In addition, a specific peripheral MAO inhibitor would be valuable in the therapy of angina pectoris because of the absence of central behavioral effects.

We have achieved selective inhibition of peripheral MAO with the use of 2,9-dimethyl-β-carbolinium iodide (DMCI). In the in vitro studies using tryptamine as substrate, DMCI exerts greater inhibition of MAO from human liver mitochondria than that from rat and bovine liver[1]. This report describes the selective inhibition of peripheral MAO in vivo.

Materials and methods. Male Sprague-Dawley rats (200–250 g) were injected i.v. via the tail vein with