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SHORT COMMUNICATIONS

Age-dependent changes in the adenylate cyclase and phosphodiesterase activity of rat erythrocytes

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STUDIES of the changing concentrations of various enzymes during postnatal development of the rat have been pursued by many investigators and have included the membrane-bound enzyme, adenylate cyclase. Bär and Hahn[1] demonstrated peak adenosine 3′,5′-monophosphate (cyclic AMP) production at 2–10 days old in the presence or absence of stimulators of hepatic adenylate cyclase such as adrenaline (0·1 mM), NaF (10 mM) and glucagon (5·7 μM). Lower levels of production achieved at 35 days of age remained low up to 90 days. In terms of per cent stimulation over basal, it would appear that these values increased with age. Bitensky *et al.*[2] showed an age-dependent decrease in adenylate cyclase activity of hepatic cell membranes. With homogenates of rat cerebrum, cerebellum and brain stem, Weiss[3] found that NaF-stimulated cyclic AMP production was highest at 20, 30 and 10 days, respectively, and decreased slightly up to 60 days of age. Using slices of rabbit brain regions Schmidt and Robison[4] showed that the norepinephrine-stimulated production of cyclic AMP was maximum in 9–14 days of age and then, with the exception of the cerebellum, fell to low levels in the mature rat. Schmidt *et al.*,[5] using chopped rat brain, showed maximum norepinephrine and NaF response in 9 days, which remained elevated over a 22-day period. The finding of Fleisch *et al.*[6] that β-receptor activity of rat aorta decreased with age suggests that one may be dealing with a generalized phenomenon of an age-dependent loss of hormone responsiveness.

Since the presence of a catecholamine-stimulated adenylate cyclase has been demonstrated in the rat erythrocyte ghost, it was of interest to determine if its activity altered with age.

Female Sprague–Dawley rats obtained from Zivic Miller, Laboratories, Inc., Allison Park, Pa., were given ether and blood was drawn by heart puncture into heparinized syringes. The preparation of the ghosts, incubation for 30 min with ATP-8-^{14}C and unlabeled cyclic AMP, and the paper chromatographic isolation of the products of the reaction were performed as described elsewhere.[7] The phosphodiesterase activity was determined by incubating the ghost-free hemolysate with cyclic AMP-8-^{3}H and separating the products by paper chromatography.[8]

The ATP-8-^{14}C tetrasodium salt (35–50 mCi/m-mole) and cyclic AMP-8-^{3}H (12·7 Ci/m-mole) were obtained from Schwarz BioResearch; D(−)isoproterenol from Winthrop Laboratories; 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone was synthesized by Dr. M. Hoffer, Hoffmann-La Roche, Inc., Nutley, N.J.

Previous studies from this laboratory have used the erythrocytes of mature animals of various ages and the results with several of these have been quite comparable to that of the 74-day-old rat in Fig. 1. In this case, the stimulation by NaF and isoproterenol was relatively small. The increase over basal production, was approximately 50 and 100 per cent for stimulation with isoproterenol (50 μM) and NaF (1 mM) respectively. The cyclase from animals which were over 1 year of age (372 days) did not differ significantly, except that the isoproterenol stimulation tended to be greater. The 11- and 25-day-old animals, however, had a significantly greater capacity to produce cyclic AMP under all conditions. Basal production was highest in the ghosts from the 11-day-old rat. The increased cyclic AMP production in the presence of isoproterenol was significantly greater in the 11-day-old as compared to the mature animals, but was highest in the erythrocyte ghosts of the 25-day-old animals. Cyclic AMP production in the presence of fluoride achieved equally high values in the 11- and 25-day-old rats, and these values were significantly greater than those of the mature animals. Even in terms of the per cent stimulation over basal values, fluoride stimulation was greater in the immature animals. With isoproterenol, however, per cent stimulation was greater than in the 372-day-old animals only in the 25-day-old rat.

Phosphohydrolase activity was also elevated in the younger animals, as evidenced by the increased adenosine production (Table 1), and showed the usual inhibition by NaF.

In conformance with the greater cyclase activity obtained with erythrocyte ghosts of the younger animals were the increased phosphodiesterase activities seen in Table 2. The hemolysate of the erythrocytes from both the 11- and 25-day-old rats had almost 3 times the capacity to hydrolyze

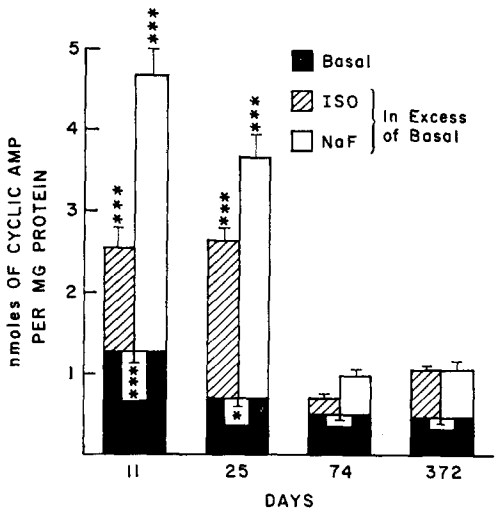

FIG. 1. Production of cyclic AMP by ghosts of erythrocytes from rats of various ages. Each incubation contained from 0·3 to 0·65 mg protein from ghosts washed once with 1 mM Tris–200 mg/100 ml of glucose buffer, pH 7·4. The final concentrations of D(−)-isoproterenol and NaF were 50 μM and 1 mM, respectively, in an incubation volume of 0·5 ml of 40 mM Tris–200 mg/100 ml of glucose buffer (pH 7·4). The 11-day-old rats were still suckling, and the values represent the mean of three pairs of pups run in triplicate. The values for remaining groups of animals represent the means of five, five and four animals run in triplicate for the 25-, 74- and 372-day-old rats. The "T" associated with each bar represents the standard error of the mean. P values compared with the 74-day-old rats are indicated by a single asterisk ($< 0{\cdot}05$) or a triple asterisk ($< 0{\cdot}001$).

TABLE 1. ADENOSINE PRODUCTION BY ERYTHROCYTE GHOSTS FROM RATS OF VARIOUS AGES

Age (days)	Basal (nmoles adenosine/mg protein ±S.E.)	NaF
11	4·45 ± 0·56	2·85 ± 0·28
25	4·28 ± 0·53	2·25 ± 0·21
74	2·05 ± 0·18	1·35 ± 0·07
372	2·28 ± 0·15	1·41 ± 0·14

TABLE 2. PHOSPHODIESTERASE ACTIVITY OF HEMOLYSATES OF ERYTHROCYTES FROM RATS OF DIFFERENT AGES AND ITS INHIBITION BY 10^{-7} M 4-(3-BUTOXY-4-METHOXYBENZYL)-2-IMIDAZOLIDINONE

Age (days)	Hydrolysis (%/mg protein)	Inhibition (%)
11	36·5	47·2
25	33·2	51·5
74	12·1	47·6
372	12·4	52·5

cyclic AMP as those from mature animals. Inhibition by 10^{-7} M 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone was not significantly different among the hemolysates from rats of different ages.

It is clear from these results that the adenylate cyclase activity of the rat erythrocyte and its stimulation by fluoride and hormones were greater in the immature as compared to the mature rat. It is difficult to compare the time course of decline with other reports in the literature,[1-5] since the sampling was done at different ages. However, even when sampling time was identical, the cyclase response was not necessarily the same for all tissues.[3,4] In all of these studies there was a decrease in basal, fluoride and hormone stimulation with time. This suggests that the membrane composition is very significantly altered during the maturation process. Either the amount of cyclase in the membrane is reduced or its activity is in some way masked. The recent implication of phospholipids in the activity of adenylate cyclase[9,10] prompted us to examine the lipid composition of these membranes. Preliminary examination of these lipids by thin-layer chromatography failed to disclose any readily distinguishable alterations in lipid patterns.

The finding that the age-dependent alteration in the cyclase activity of the rat erythrocyte resembles that of such different tissues as the brain and liver suggests that one is dealing with some very fundamental aspects of the maturation process. The observation that phosphohydrolase activity of the membrane was also decreased points to an alteration of the membrane that is generalized rather than specific in its effects.

The reduction in the soluble enzyme, phosphodiesterase, indicates that age-dependent changes may be reflected in more than just membrane alteration. One is forced to consider the possibility that in the case of the erythrocytes the age of the circulating cell may be of importance. Evidence has been presented that older erythrocytes tend to have reduced levels of a number of enzymes,[11] and it is probable that a younger animal has a younger population of erythrocytes.

In any event, it is apparent that rat erythrocytes can serve as a useful indicator of the maturing process, including changes which might be occurring in other adrenergically stimulated adenylate cyclase systems, and that these measurements may be made without the necessity of sacrificing the animal.

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