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ACETYLSALICYLIC ACID ANTAGONIZES THE BLEEDING-INDUCED CHANGES IN HEMOGLOBIN PROPORTIONS IN NORMAL ADULT RATS

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Summary: Normal adult Sprague-Dawley rats were made anemic by repeated phlebotomy. Ion-exchange chromatography of anemic blood showed newborn like hemoglobin proportions, involving the same six hemoglobin components as is found when newborn and adult blood are compared. However, acetylsalicylic acid intake during anemia failed to demonstrate the changes in hemoglobin proportions, either totally or partially, depending upon the doses. Since acetylsalicylic acid inhibits prostaglandin synthesis, the data suggest that one or more prostaglandins may be involved in the process of reverse switching of hemoglobin in adult rat erythroid cells during erythropoietic stress.

Postnatal changes in hemoglobin proportions occur in many animals. Humans have both fetal and adult hemoglobins at birth, but almost solely adult hemoglobins beyond one year of age (1). Rat has no specifically fetal hemoglobin like humans. However, we have reported that a significant beta chain switching phenomenon is seen between newborn and adult rat red cells (2).

Prostaglandins including PGE_2 have been implicated as modulators of erythropoiesis, both in vivo (3) and in vitro (4-6). Our observations in man demonstrate that in addition to an overall increase in Hb synthesis, PGE_2 preferentially stimulates Hb F synthesis in BFU-E derived colonies from blood of normal adults and patients with sickle cell anemia (7). Furthermore, fetal calf serum used for BFU-E cultures contain significant amount of E-prostaglandins (8), and a

Abbreviations: ASA, acetylsalicylic acid; PGE_2, prostaglandin E_2; Hb F, fetal hemoglobin; Hb, hemoglobin; BFU-E, burst forming unit-erythroid.

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dialyzable fetal calf serum factor has been reported recently to promote increased Hb F synthesis in normal adult BFU-E (9).

Bleeding-induced erythropoietic stress in man and baboons has been shown to stimulate fetal Hb synthesis in adult life (10, 11), and the mechanisms suggested for these changes are mostly speculative. This study was designed using rat as an experimental animal to elucidate: (i) whether adult rats under erythropoietic stress are capable of changing Hb proportions toward newborn values, and if so; (ii) is ASA, an inhibitor of prostaglandin synthesis (12), effective in blocking the changes in Hb proportions due to bleeding-induced anemia?

MATERIALS AND METHODS

Sprague-Dawley rats from Southern Animal Farms (Prattville, AL) were used to develop colonies with genotype BB. Normal adult rats weighing 450 gm to 600 gm were bled by cardiac puncture under ether anesthesia every other day for 10 days by which time they had developed severe anemia as revealed by several hematologic parameters, e.g., reticulocyte counts and hematocrit values.

For monitoring the effect of ASA intake during bleeding-induced anemia, several rats were given ASA-mixed water to establish 5, 20, 40, 60 and 75 mg per day ASA intake groups respectively per 500 g body weight.

Baseline (unbled) and experimental samples including age-matched control bled samples were lysed with a lysing buffer at pH 8.6 to avoid Hb precipitation, then dialysed against the same buffer and finally applied to DEAE-cellulose columns for separation of Hb components using a gradient of NaCl dissloved in a glycine-KCN buffer as detailed earlier (2). Eluted fractions were read at 415 nm for optical density. Quantitation of individual Hb component was calculated as percent of total Hb recovered from the column, computed after applying a small correction for rising background.

Since we have established before that genetically altered rats affect the changing proportions of IIIβ chains during development (13), rats of one genetic background (BB) were used in this study to avoid complications in the results.

RESULTS AND DISCUSSION

Figure 1 shows results of chromatographic separation of the hemoglobins from an adult BB rat made anemic by bleeding and compares the percent Hb components with the corresponding initial sample values (bled vs unbled). The most prominent differences are in peaks I, II, IV, V, VI and VIII. Components I, II and IV are higher in the last bled

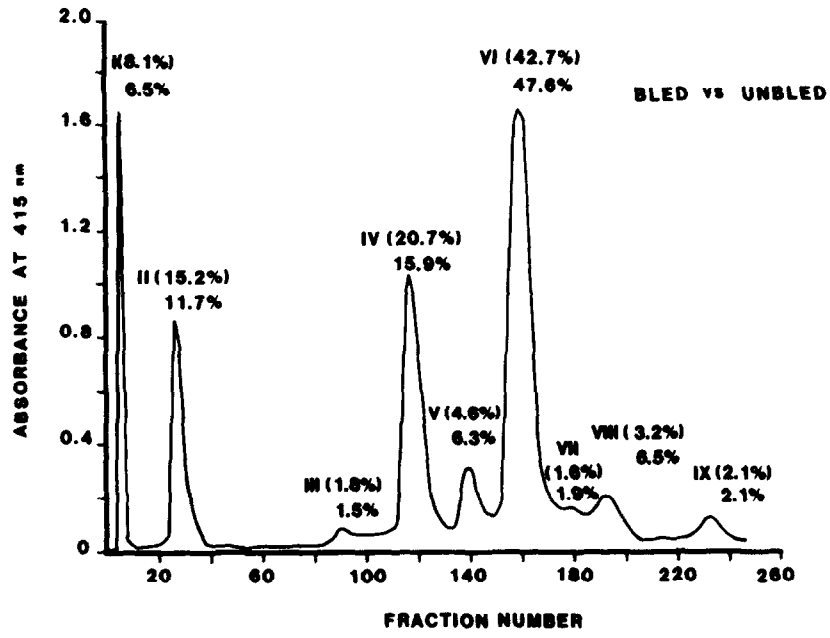

Fig. 1. The proportions of different Hb components in an adult bled normal rat. The values in parentheses represent the anemic proportions, while the other corresponding values indicate nonanemic proportions resolved from the first drawn sample of the same rat.

sample, while components V, VI, and VIII are higher in the first drawn sample from the same rat. A similar pattern was found when blood samples from newborn and normal adults were compared. Components I, II and IV in the newborn showed 52% of total Hb, while in the adult they were 35%. Components V, VI and VIII were less in the newborn (42%) than in the adult (59%), and these differences were statistically significant as reported earlier (2). An early report using starch gel electrophoresis indicated that anemia in rats caused changes in Hb proportions toward newborn values (14). The method, however, could resolve only 4 hemoglobins, as opposed to the separation and quantitation of 10 Hb components by our procedure of DEAE-cellulose chromatography (2). Table 1 further confirms that a good degree of anemia in bled adult rats partially reverses the Hb proportions toward newborn values involving the same six Hb components as is observed between newborn and normal adults. Upon correction of anemia the Hb

TABLE 1

A COMPARISON OF PROPORTIONS OF SOME HEMOGLOBIN COMPONENTS BETWEEN BLED AND UNBLED RATS

Peak Number	Adult Bled	Adult Unbled	$p <$ *
I	7.0 ± 0.5	5.7 ± 0.2	0.001
II	17.1 ± 0.9	14.5 ± 0.7	0.05
IV	21.2 ± 1.3	16.9 ± 2.1	0.05
V	5.1 ± 0.4	7.3 ± 0.5	0.01
VI	40.4 ± 1.7	46.1 ± 2.4	0.01
VIII	4.4 ± 0.4	8.5 ± 0.9	0.01

Values are given as percent of total hemoglobin recovered from the column, computed after applying a small correction for rising background. Mean ± standard deviations are shown for all the six concerned peaks (n = 4 for both). * Determined by the t-test.

fractions returned to normal adult proportions (data not shown). Anemia by phlebotomy is a known stimulus for high erythropoietin level. However, the increased formation of fetal Hb by erythropoietin alone seems unlikely (10, 15). Anyway, our observations suggest that the mechanism responsible for the switching of adult Hb to fetal Hb in man might be operative in bled adult rats in changing their Hb components to newborn like proportions.

In an attempt to shed some light on the mechanism of the bleeding-induced changes in rats, we monitored the effect of ASA, an inhibitor of prostaglandin synthesis, on the Hb proportions during anemia. Rats were divided into groups based on ASA consumption. The effects of various doses of ASA on the six hemoglobin components are shown in Table 2. Previously-published values for BB rats (2) agree reasonably well with the present nonanemic values. However, rats receiving ASA antagonize the reverse switching of Hb components either totally (20 gm ASA/day/500 g) or partially during bleeding-induced anemia, except the 5 mg ASA/day/500 mg group. Table 3 shows the quantitative data for two doses of ASA (20 mg vs 75 mg) intake on the changes in Hb proportions which exhibit statistically significant

TABLE 2

EFFECTS OF ANEMIA AND ASA INTAKE ON HEMOGLOBIN COMPONENTS IN NORMAL ADULT RATS

Conditions	Hemoglobin Components (% of total)	
	I + II + IV	V + VI + VIII
Unbled	37.1	57.4
Unbled	36.1	58.3
Unbled	35.6	59.1
Bled	42.8	51.7
Bled	44.7	48.7
Bled	43.1	51.7
Bled	41.0	52.9
ASA (5 mg/day)	45.1	51.8
ASA (5 mg/day)	43.5	52.0
ASA (20 mg/day)	38.3	57.3
ASA (20 mg/day)	35.0	58.7
ASA (40 mg/day)	38.2	55.8
ASA (40 mg/day)	36.2	56.9
ASA (60 mg/day) *	39.1	57.0
ASA (75 mg/day)	41.2	55.8
ASA (75 mg/day)	40.4	55.1

* one rat died before the completion of the experiments.

differences between 20 mg ASA/day group and the corresponding control bled Hb components ($p < 0.02$). On the other hand, the 75 mg ASA/day group, although shows a steady minor difference compared to its control

TABLE 3

A COMPARISON OF TWO DOSES OF ASA INTAKE ON THE CHANGES IN HEMOGLOBIN PROPORTIONS DURING ANEMIA

Conditions	Mean ± S. D. (n = 4) Hemoglobin components (% of total)	
	I + II + IV	V + VI + VIII
Unbled	36.0 ± 0.8	58.4 ± 0.7
Control Bled	42.9 ± 1.5	51.3 ± 1.8
20 mg ASA/day	36.5 ± 1.4 *	57.5 ± 0.9 **
75 mg ASA/day	41.4 ± 0.8	54.8 ± 0.8

* and ** indicate a significant difference between the corresponding control bled Hb components ($p < 0.02$), as determined by using Student's t test.

bled counterpart, fails to hold a statistical significance. The higher ASA dosages were less effective than the 20 mg ASA intake rats, presumably because of larger accumulation of ASA metabolite (i.e. salicylic acid) which in turn could prevent the inhibitory effect of ASA (16, 17). In the absence of anemia all the rats restored the adult Hb values even when ASA was continued, indicating the need for erythroid cell expansion for the expression of ASA action. Since the simultaneous ASA intake antagonizes the bleeding-induced changes in Hb proportions, it is reasonable to propose that one or more prostaglandins are involved in the process of reverse switching of Hb in adult rat erythroid cells during anemia.

CONCLUSION

Data of this paper show that rat hemoglobins do indeed change in proportions toward newborn like values during anemia. Thus, most of the characteristics of the human in terms of hemoglobin switching during development and anemia are shared by the rat. However, rats receiving ASA between 20 mg to 75 mg per day during phlebotomy fail to demonstrate these changes in certain Hb components, either totally or partially. ASA is known to inhibit cyclooxygenase, a key enzyme involved in the synthesis of prostaglandins. Taken together, these results tend to offer a plausible mechanism for the reverse hemoglobin switching in adult rats undergoing acute anemic stress.

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