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β-Adrenoceptor Function in White Blood Cells from Newborn Infants: No Relation to Plasma Catecholamine Levels

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ABSTRACT. The maturity of β-adrenoceptors in newborn infants was studied in relation to the catecholamine surge during labor. Umbilical blood was collected at birth from 12 infants delivered vaginally and 13 infants delivered by elective cesarean section. Granulocytes and lymphocytes were isolated. Receptor numbers and binding affinity were determined in the granulocytes by incubation with ^{125}I-iodohydroxybenzylpindolol. Receptor responsiveness was tested by assessing isoproterenol-induced cyclic AMP accumulation in lymphocytes. Significantly higher plasma noradrenaline, adrenaline, and dopamine concentrations were found in infants born vaginally (108; 8.9; 0.9 nmol/liter, respectively, median values) as compared with those delivered by cesarean section (11.0; 2.4; 0.2 nmol/liter). No significant differences in β-adrenoceptor binding sites (receptor number: 39.2 ± 2.6 *versus* 44.7 ± 5.9 fmol/mg protein and binding affinity: 66.6 ± 7.8 *versus* 65.0 ± 6.2 pM) or responsiveness (maximal isoprenaline induced cAMP formation 52.4 ± 10.3 versus 40.6 ± 8.9 pmol/10^6 cells) were found between the two groups of infants. Lymphocyte β-adrenoceptor sensitivity was similar to that found in adults.The β-adrenoceptors on whole blood cells seem to be mature at birth and have the same responsiveness as in adults. The higher catecholamine surge during vaginal delivery as compared to elective cesarean section does not seem to affect β-adrenoceptor function. Our results do not support the idea that reduced β-adrenoceptor function is the cause of the previously observed inappropriately small cardiovascular and metabolic responses to the exceptionally high plasma catecholamine concentrations at birth. (*Pediatr Res* 20: 1152–1155, 1986)

Abbreviations

CA, catecholamines
B_{max}, receptor number
K_D, binding affinity
cAMP, cyclic adenosine monophosphate
^{125}IHYP, ^{125}I-iodohydroxybenzylpindolol

The fetal sympathoadrenal system is markedly activated during vaginal delivery and particularly during perinatal asphyxia (1–5). The plasma CA concentrations found in the umbilical artery after uneventful vaginal deliveries are about 20-fold higher and, during perinatal asphyxia, up to 100-fold higher than in resting adults (1, 6). This CA surge might be of importance for neonatal adaptation after birth. CA have been demonstrated to stimulate lung liquid absorption (7, 8), enhance surfactant release (7), increase dynamic lung compliance (9), mobilize glucose and free fatty acids (10, 11), increase cardiac performance (12), and mediate shunting of blood to the most vital organs (13, 14). The cardiovascular and metabolic responses to these enormous concentrations of CA are, however, relatively small. Thus, blood pressures and heart rates are only slightly higher in vaginally delivered infants than in infants delivered by elective cesarean section (14) despite a 5- to 6-fold difference in CA levels (4). The plasma glucose and glycerol concentrations are lower in relation to the CA concentrations than would be expected from results obtained in adults (11).

One possible explanation for these findings may be fetal shunting of blood. Other possibilities to be considered are immaturity of the adrenergic receptors (15) or down-regulation of the β-adrenoceptors due to the CA surge. A reduced number of β-adrenoceptor binding sites and reduced responsiveness (isoproterenol induced cAMP accumulation) have been reported in neonatal granulocytes, as compared to those of adults (16).

The aim of the present study was to investigate further the relationship between β-adrenoceptor function and sympathoadrenal activity in newborn infants. We studied the relationship between plasma CA levels and the responsiveness of the β adrenoceptors by comparing vaginally delivered infants, who were expected to have high CA levels, with those delivered by elective cesarean section, who were expected to have lower CA levels. Maturation of β-adrenoceptor-mediated function may involve both formation of the receptors, coupling to second messenger systems and intracellular mechanisms. Therefore, we studied isoprenaline-induced cAMP formation as a measure of receptor mediated functional responsiveness and more specific receptor characteristics, as obtained by receptor ligand binding techniques. To ensure assessments of both receptor binding and responsiveness when the amount of placental blood sampled was limited, we isolated both granulocytes, which were used to determinate B_{max} and K_D values for ^{125}IHYP, and lymphocytes, which were used for studies of β-adrenoceptor responsiveness. This approach, using two cell types, does not limit the comparisons between vaginally delivered infants and those delivered by cesarean section. For example, both granulocytes and lymphocytes have been reported to be desensitized by exposure to β-agonists (17, 18).

MATERIALS AND METHODS

Patients. Fetal placental and mixed umbilical blood was collected with a special funnel from 25 full-term infants, delivered

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after uncomplicated pregnancies. None of the mothers had received any medication except iron and vitamins. Twelve of the infants were delivered vaginally and 13 by elective cesarean section, usually under epidural analgesia as previously described (4). The parents were informed about the study which was approved by the local Ethics Committee.

Cell preparation. Lymphocytes were isolated by density gradient centrifugation using Ficoll-Paque (Pharmacia, Uppsala, Sweden) according to instructions from the company. Blood was collected from the umbilical cord and placenta. The blood was mixed with an equal volume of a balanced salt solution containing heparin. This mixture was carefully layered on the Ficoll-Paque solution and centrifuged at 400 × *g* at 18°C for 30 min. After removing the majority of the plasma layer, the lymphocyte coat, was carefully collected using a siliconized pasteur pipette. This cell suspension was washed twice and finally resuspended in Dulbecco's phosphate-buffered saline containing 5.5 mM glucose. After removal of the Ficoll-Paque layer, the pellet containing erythrocytes and granulocytes was resuspended in 10 ml 0.9% NaCl and thereafter mixed with 25 ml 6% Dextran T 70 with 10% bovine serum albumin in saline. The erythrocytes were left to sediment for 30 min after which the supernatant was collected and centrifuged for 10 min at 200 × *g* at 4°C. The remaining erythrocytes were lysed by a brief exposure to ice cold 0.2% NaCl. After removal of the lysed erythrocytes, granulocyte membranes were prepared by lysis and homogenization of the cells (using a Polytron homogenizer). The membrane preparation was stored frozen at −80°C until the β_2-adrenoceptor binding assay was performed.

Cell incubation. Lymphocytes (2–2.5 × 10^6 cells/ml) were incubated without (basal) or with isoproterenol (10^{-9}–10^{-4} M) in a final volume of 200 μl at 37° C for 20 min. The incubations were performed in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (0.5 mM) and were run in triplicates at each isoproterenol concentration. The reaction was stopped by heating to 95° C for 3 min and the samples subsequently frozen and stored at −20° C until analyzed for cAMP contents.

Binding assay. Granulocyte membranes were incubated in duplicate in the presence and absence of 1 μM propranolol at six concentrations (10–200 pM) of ^{125}IHYP. The incubation buffer consisted of 0.9% NaCl with 10 mM Tris, pH 7.5 containing 1 mM ascorbic acid, 50 μM phentolamine, and 1 μg albumin in a final volume of 300 μl. Samples were incubated for 1 h at 37° C, after which the incubates were diluted by adding 10 ml 0.9% NaCl containing 10 mM Tris, pH 7.5 and rapidly filtered through Whatman GFC filters presoaked in this buffer solution. The filters were immediately (within 10 s) washed with 10 ml buffer. The binding fulfilled conventional criteria for saturability and stereo-selectivity. Values for K_D and B_{max} were calculated from Scatchard plots. The *r* values for the 6 point regression lines were all above 0.88 and the majority were above 0.95. Figure 1 shows a representative saturation curve and Scatchard plot from one experiment.

Other assays. Plasma catecholamines were determined by high-performance liquid chromatography with electrochemical detection (19). Cyclic AMP was determined by a protein binding method (20) and the protein contents of the granulocyte membrane preparations were determined according to Lowry *et al.* (21).

Statistics. All results are expressed as median and range values or as mean values ± SEM. Mann-Whitney U test, or Student's *t* test, when appropriate, were used for statistical comparisons. A level of $p < 0.05$ was considered as significant.

RESULTS

Patient data and plasma CA concentrations are summarized in Table 1. None of the vaginally delivered infants had an Apgar score below 6 at 1 min although some of the infants were acidotic as usually found in a "normal" material (22). The numbers of

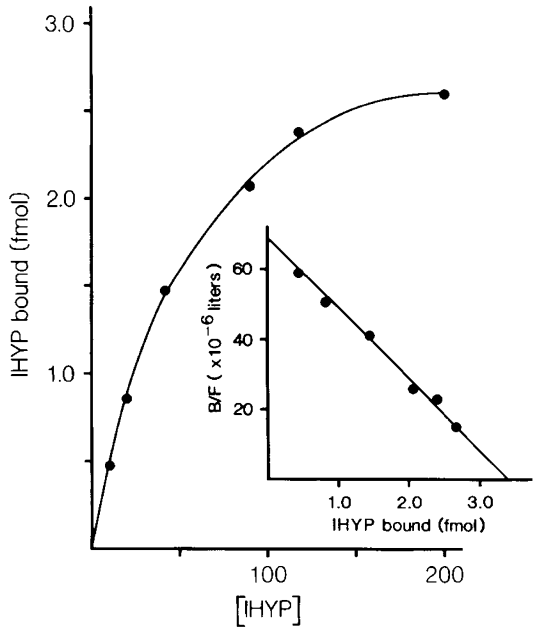

Fig. 1. Binding of ^{125}IHYP to granulocyte membranes from one of the assays in the study. The amount of specifically bound ^{125}IHYP (fmol) was saturable with increasing concentration of ^{125}IHYP (pmol). The *inset* shows a Scatchard plot of data, from which the K_D and B_{max} (49.2 pM and 63.0 fmol/mg protein, respectively, in this experiment) can be calculated.

Table 1. *Patient data (median and range)**

	Vaginal delivery	Cesarean section
Birth wt (g)	3367 (2970–4180)	3583 (3110–4640)
	n = 12	*n* = 13
Placental wt (g)	560 (420–730)	651 (450–820)
	n = 12	*n* = 13
Sex (M/F)	2/10	8/5
pH	7.18 (7.02–7.34)	7.30 (7.29–7.31)
	n = 8	*n* = 5
Hematocrit (%)	51.9 (45.0–57.0)	44.2 (30.0–56.0)
	n = 10	*n* = 9
Catecholamines (nmol/liter)		
Noradrenaline	108.0 (6.8–539.3)	11.0 (1.8–37.4)
	n = 11	*n* = 12
Adrenaline	8.9 (0–35.9)	2.4 (0–11.8)
	n = 11	*n* = 12
Dopamine	0.9 (0–2.6)	0.2 (0–0.8)
	n = 11	*n* = 12

* The differences in catecholamine levels between the two patient groups were statistically significant ($p < 0.05$, Mann-Whitney U test) for all catecholamines. pH and hematocrit values were statistically different ($p < 0.05$, Student's *t* test for unpaired variates) between the two groups.

the receptor binding sites in the granulocytes and their affinities, as well as the basal and maximally stimulated cAMP concentrations in the lymphocytes are presented in Table 2. The plasma CA concentrations were higher, in individual cases considerably higher, in the vaginal group than in the infants delivered by cesarean section. However, no significant differences in B_{max} or K_D for ^{125}IHYP or isoproterenol-stimulated cAMP formation were observed. The individual results concerning isoproterenol-stimulated cAMP formation are shown in Figure 2. The lymphocytes from one infant responded with exceptionally high cAMP formation (Fig. 2, *top curve*). This infant was delivered vaginally and was asphyxiated (pH 7.11, Apgar score 6 at 1 min) and had the highest CA level of all babies (539 nmol/liter noradrenaline and 22.0 nmol/liter adrenaline).

Table 2. *B_{max} and K_D of β-adrenoceptors of granulocytes (A) and isoproterenol-induced cAMP formation in lymphocytes (B) [mean ±SEM (n)]*

	Vaginal	Cesarean section
A.		
B_{max} (fmol/mg protein)	39.2 ± 2.6 (11)	44.7 ± 5.9 (12)
K_D (pM)	66.6 ± 7.8 (11)	65.0 ± 6.2 (12)
B.		
Concentration (pmol/10^6 cells)		
Basal	3.8 ± 1.1 (11)	4.6 ± 1.6 (7)
Maximal	52.4 ± 10.3 (11)	40.6 ± 8.9 (7)
EC_{50} (nM)	32.4 ± 8.9 (11)	19.6 ± 4.3 (7)

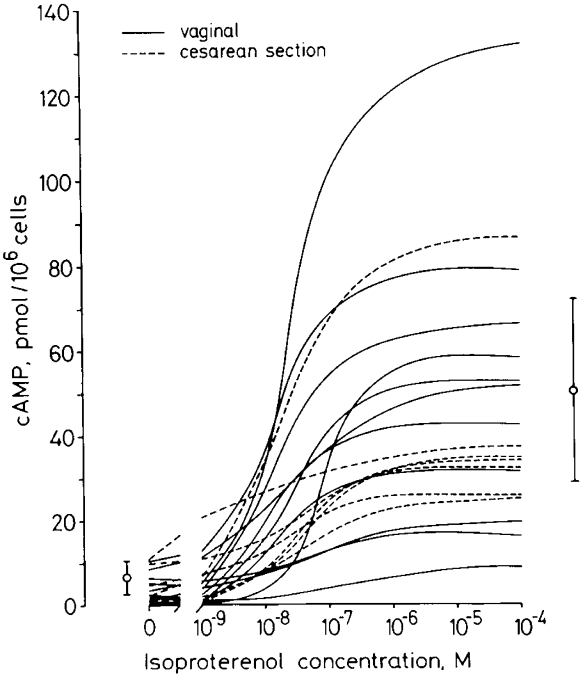

Fig. 2. Stimulation of cAMP formation by isoproterenol in the presence of the phosphodiesterase inhibitor IBMX. Values (mean ± SD) for adult lymphocytes obtained in the same laboratory are also shown for comparison (*left vertical bar*: resting values without isoproterenol = 7.1 ± 4.1, $n = 22$; *right vertical bar*: values at an isoproterenol concentration of 10^{-4} M = 51.1 ± 21.5, $n = 22$).

No significant correlations were found between either noradrenaline or adrenaline concentrations in plasma at birth and lymphocyte β-adrenoceptor responsiveness whether absolute values or logarithmically transformed CA values were used in the calculations. No sex differences were observed with regard to B_{max} or K_D values. Plasma CA levels and lymphocyte responses to isoproterenol were slightly higher in the boys than in the girls, but the differences were not significant.

The infants were also grouped according to gestational age: less than 39 or 39 wk and more. No significant differences with regard to beta-adrenoceptor numbers or responsiveness were found. On the other hand, there was a significant inverse relationship between cAMP formation and birth weight (Fig. 3).

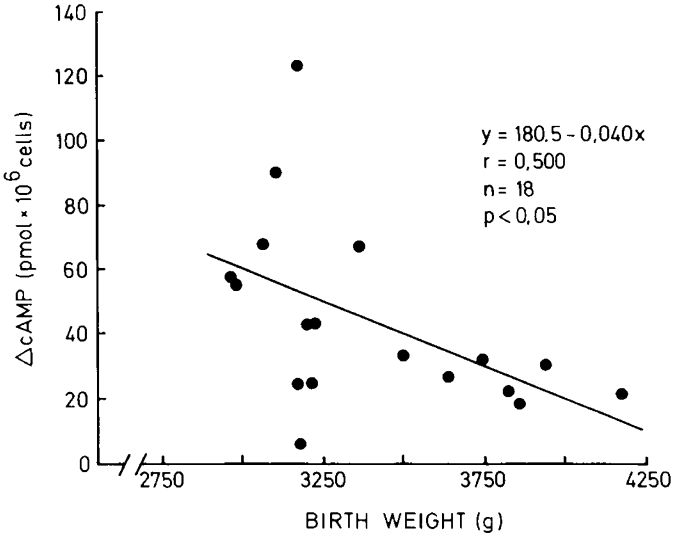

Fig. 3. Correlation between maximal increase in isoproterenol-induced cAMP formation and birth weight of the infants.

DISCUSSION

We studied β-adrenoceptor function in white blood cells from newborn infants by determining the numbers and affinities of β-adrenergic binding sites in granulocytes as well as β-adrenoceptor responsiveness of lymphocytes isolated from fetal placental blood. The properties of the white blood cell receptors were found to be similar to those in adults, in contrast to an earlier study (16) which reported that granulocytes from six newborn babies contained fewer β-adrenoceptors and also a reduced cAMP response to isoproterenol stimulation than those from six healthy adults. However, the β-adrenoceptor numbers reported for the adults in that study are considerably higher than those reported in previous studies from the same group (23, 24), suggesting that the choice of controls may have influenced the results and interpretations.

In a recent report O'Hara *et al.* (25) found fewer binding sites for the β-adrenoceptor ligand ^{125}iodocyanopindolol in intact lymphocytes from vaginally delivered infants than in adult lymphocytes. They also found lower cAMP levels in lymphocyte incubates with and without isoproterenol. Our results, obtained in granulocyte membranes and intact lymphocytes, respectively, are clearly at variance with these previous reports and do not support the idea of a reduced β-adrenoceptor function in the neonatal period.

To study whether the CA surge during labor (6, 26) could modify β-adrenoceptor function, we compared infants delivered vaginally with those born by cesarean section, since the latter group of infants has been shown to have considerably lower CA levels (4). However, we found no significant differences in either B_{max}, K_D, or stimulated cAMP-formation between the two groups of infants, despite the expected differences in plasma CA levels. Furthermore, there was no significant relationship between CA levels at birth and β-adrenoceptor numbers and properties.

We cannot exclude the possibility that the enhanced CA surge in the vaginal group induced a minor change in agonist affinity for the β-adrenoceptors, as the EC_{50} values for isoprenaline-induced cAMP formation were somewhat higher (although not significantly) in the vaginal group. Thus, Feldman *et al.* (27) reported that white blood cells from subjects changing from the supine position to being ambulatory showed a decrease in high affinity binding sites for agonists with a parallel decrease in adenylate-cyclase function, without changes in the total number of receptors. This was suggested to be caused by the increase in CA levels when rising from the supine position.

One asphyxiated infant with extremely high CA levels also had the highest β-adrenoceptor responsiveness (isoproterenol-induced cAMP formation). This might be due to the transient up-regulation of receptors which has been found to precede down-regulation in connection with epinephrine or isoproterenol exposure (28) and also after exercise in adults (29). Except in one of our patients, the higher CA levels during vaginal delivery did not seem to have modified β-adrenoceptor function.

No sex differences in β-adrenoceptor properties were found in this study. However, it is difficult to draw any definitive conclusion since the sex distribution in the two groups was uneven. This question is of some interest since enhanced maturation of β-adrenoceptors in the lung due to the influence of sex steroids might be one factor explaining why newborn girls less frequently develop respiratory problems (30).

There was no relationship between gestational age (from the 36th wk) and β-adrenoceptor properties in the present study. On the other hand, there was a significant inverse relationship between β-adrenoceptor function and birth weight. Growth-retarded fetuses have elevated plasma corticosteroid and thyroid hormone levels both of which may influence β-adrenoceptor function (15, 31). None of the infants in the present study was growth retarded according to conventional clinical criteria, but it is possible that a slight but clinically insignificant retardation of growth in some of these infants was sufficient to stimulate steroid hormone release and subsequently enhance β-adrenoceptor formation.

One of the main reasons for undertaking the present study was the previous observation that the high CA surge during vaginal birth is associated with relatively modest effects on circulation and metabolism (11). The present results indicate that these small circulatory and metabolic responses were not caused by immaturity of the β-adrenoceptors. However, we only studied β-adrenoceptors in white blood cells and it is possible that the function of these receptors may differ from the function of those in other tissues. However, one animal study (32) has clearly shown that there are parallel changes of β-adrenoceptors in granulocytes and other tissues, such as the heart and lung, during treatment with propranolol. In man, the receptor number on leucocytes have been reported to correlate with changes in cardiac responsiveness to isoproterenol (33). Thus, it seems reasonable to speculate that our results indicate a normal maturity of the neonatal β-adrenoceptors also in other tissues.

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