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Age-Related Changes in the Adrenergic Control of Glycogenolysis in Rat Liver: The Significance of Changes in Receptor Density

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ABSTRACT. The present study examined the developmental changes in the adrenergic control of glycogenolysis in the rat model. A relatively new β -adrenergic radioligand, ^{125}I -iodocyanopindolol (ICP), was examined in binding assays with rat liver plasma membrane (LPM). ICP demonstrated both a higher specificity and a greater affinity for β -adrenergic receptors than any previously available β -adrenergic radioligand used to study rat LPM. Utilizing this new ligand it was found that β -adrenergic receptor density decreased from $114 \pm 4 \text{ fmol mg}^{-1}$ in newborn LPM to $19 \pm 3 \text{ fmol mg}^{-1}$ in adult male LPM. In contrast α -adrenergic receptor density examined using ^3H -prazosin increased from $161 \pm 14 \text{ fmol mg}^{-1}$ in the newborn to $554 \pm 59 \text{ fmol mg}^{-1}$ in the adult male. The results of ICP displacement assays employing various β -adrenergic agonists and antagonists indicated that ICP binding sites were β_2 -adrenergic receptors. Both guanosine triphosphate and its nonhydrolyzable synthetic analog guanylyl-imidodiphosphate lowered the affinity of epinephrine for ICP binding sites similarly in newborn and adult LPM. Thus the coupling of receptor to guanine nucleotide regulatory protein appeared to be the same in both age groups examined. In isolated hepatocytes glycogen phosphorylase activation was mediated by β_2 -adrenergic stimuli in the newborn and predominantly α -adrenergic stimuli in the adult male. These results suggest that the change in glycogen phosphorylase activation from β - to predominantly α -adrenergic mechanisms seen with maturation is related to changes in receptor density. (*Pediatr Res* 19:862-868, 1985)

Abbreviations

cAMP, 3':5'-cyclic adenosine monophosphate
 GTP, guanosine triphosphate
 Gpp(NH)p, guanylyl-imidodiphosphate
 ICP, ^{125}I -iodocyanopindolol
 LPM, liver plasma membrane
 Kd, dissociation constant

The mechanism of action of epinephrine on liver glycogenolysis varies depending on a number of factors. In certain species such as the guinea pig and rabbit epinephrine-induced glycogenolysis is mediated by the β -adrenergic receptor system (1, 2) while in the adult male rat the α -adrenergic system predominates (3, 4). In the adult female rat glycogenolysis has recently been shown to be under the influence of both α - and β -adrenergic stimuli (4). Several recent studies have also emphasized the importance of the age of the experimental animals on the adrenergic control of glycogenolysis. In these investigations it was demonstrated that β -adrenergic stimuli resulted in a marked rise in cAMP levels in hepatocytes from juvenile rats but little rise was noted in the hepatocytes from adult male rats. They also showed that glucose release from the cells occurred following both α - and β -adrenergic stimuli in the juvenile hepatocytes but only following α -adrenergic stimulation in the adult hepatocytes (5, 6). In another study using cultured fetal hepatocytes cAMP accumulation and glycogenolysis were mediated solely by β -adrenergic stimuli (7). However, the results of this study must be questioned since it has recently been reported that the culturing of adult male hepatocytes results in an increase in β -adrenergic receptor density and a changeover in adrenergic mediated glycogenolysis from α to β control (8). Nonetheless, several of the latter studies seem

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to suggest that in rat liver the adrenergic control of glycogenolysis undergoes developmental changes; and it has been speculated that this maturational change from β - to predominantly α -adrenergic control may be related to changes in the number or more specifically density of adrenergic plasma membrane receptors. However, initial attempts by us and others (8) to characterize adequately the β -adrenergic receptor in rat LPM have been unsuccessful utilizing ^{125}I -iodohydroxybenzylpindolol because of its high nonspecific binding. In the present study we utilized a new β -adrenergic radioligand, ICP, in binding assays to determine β -adrenergic receptor density in purified plasma membranes from newborn and adult male rats because at least in other species and tissues it has been shown to have a greater affinity and specificity for the β -adrenergic receptor than any previously available radioligands (9). α -Adrenergic receptor density was also investigated in the same membrane preparations using ^3H -prazosin. The results of binding assays on plasma membranes was then correlated with adrenergic stimulation of glycogen phosphorylase in isolated hepatocytes. Our results demonstrate that with maturation β -adrenergic receptor density decreases while α -adrenergic receptor density increases. In addition the adrenergic stimulation of glycogen phosphorylase in isolated hepatocytes was mediated solely by the β -adrenergic system in the newborn and predominantly by the α -adrenergic system in the adult male. These findings support the hypothesis that changes in plasma membrane receptor density are responsible for the maturational changes in the adrenergic control of glycogenolysis. We also were able to show that ICP binding sites are β_2 subtype adrenergic receptors.

MATERIALS AND METHODS

Plasma Membrane preparation. Purified plasma membranes were prepared from the livers of Sprague-Dawley rats obtained from Sasco Inc. (St. Louis, MO) by the method of Neville (10) with slight modifications of several of the centrifugation steps. Animals were sacrificed by intraperitoneal pentobarbital in adults and decapitation in newborns. Pentobarbital did not effect receptor density or Kd in preliminary investigations in adult rats. The removed livers were immediately placed in ice cold 1.0 mM NaHCO_3 buffer. In the adult males, which were older than 60 days and weighed 195–265 g, 10 g of liver tissue from each animal was used in the preparation of each membrane. In the newborns aged 5–7 days weighing 12–16 g each the livers of eight to nine different animals from a given litter were pooled (4–6 g total) in preparing each membrane. After weighing the livers were minced in 19 volumes of 1.0 mM NaHCO_3 buffer and homogenized in a Dounce homogenizer by eight strokes with Pestle A. Each homogenate was filtered through one layer each of gauze and cheesecloth; and after removing samples for protein and 5'-nucleotidase assays, 1.0 mM NaHCO_3 was added to a final volume of 250 ml which was well mixed and then centrifuged for 20 min at $3500 \times g$. The pellets were added to approximately 11 ml of 69% (w/w) sucrose and then adjusted to a final sucrose concentration of 44% by the addition of either more 69% sucrose or buffer solution. Fifteen to twenty milliliters of 42.3% (w/w) sucrose was carefully layered over the resuspended pellets and the samples centrifuged at 4°C in a Beckman SW 27 rotor for 2 h at $90,000 \times g$. The membrane which floated on top of the 42.3% sucrose layer were removed, mixed gently in 25 ml of the buffer solution, and centrifuged at $15,000 \times g$ for 20 min. The pellets were then resuspended in 3–5 ml of the buffer which yielded a final protein concentration of 2–6 mg/ml as determined by the method of Lowry *et al.* (11) using bovine serum albumin as standard. Purification of the samples was assessed by determining protein recovery and 5'-nucleotidase activity as described by Wendell and Unkeless (12). Once prepared, membranes were immediately snap frozen in an acetone-dry ice bath and then stored at -60°C until used in binding

assays. Under these conditions, the protein was stable for binding studies for at least 2 months.

Assay of ^{125}I -ICP binding. Binding assays were carried out in duplicate or triplicate at 37°C by incubating membrane (20–80 μg) in a final volume of 250 μl of 10–50 mM Mg-Tris buffer (pH 7.4) and ICP and/or competing drugs for 1 h. For ICP saturation studies the membrane protein was incubated with increasing final concentrations of ICP ranging from 20–300 pM. For ICP displacement studies membrane protein and ICP (30 pM) were incubated with increasing concentrations of the competing ligand. Specific concentrations are noted in the legends of the appropriate figures. Reactions were terminated by the addition of 4 ml of ice cold Mg-Tris buffer and bound and free ligand were separated by rapid filtration through Whatman GF/B glass fiber filters (Whatman, Inc., Clifton, NJ) which were then washed with an additional 12 ml of the buffer. The filter discs were allowed to completely dry before the radioactivity was measured in a γ counter at 80% efficiency (United Technologies Packard Auto-Gamma 500). Specific binding of the ICP was defined as the amount of the label bound in the absence of competing ligand minus the amount bound in the presence of 10^{-6} M (–)-alprenolol. Under the conditions employed specific binding was a linear function of the protein concentration and was consistently greater than 80% of total binding.

Assay of ^3H -prazosin binding. Binding assays were carried out in duplicate at 25°C by incubating membrane (20–80 μg) in a final volume of 1 ml of 10–50 mM Mg-Tris buffer (pH 7.4) and ^3H -prazosin at a final concentration of 50–600 pM for 30 min. Termination of the assay and separation of bound from free ligand was done as described above for ICP. The dried discs were placed in 7 ml of scintillation fluid in glass vials and the radioactivity measured in a beta counter (United Technologies Packard, Tri-Carb 460). Specific binding was defined as previously mentioned using 10^{-5} M ($\pm$)-phenolamine as the competing ligand and was consistently greater than 70% of total binding.

Hepatocyte preparation. Isolated hepatocytes were obtained by *in vivo* perfusion of the livers with a collagenase-containing Krebs-Ringer bicarbonate buffer pH 7.4. In the adult rats the livers were perfused according to the method of Berry (13). In the newborn rats in which the hepatocytes from two animals were pooled the method of Ferré *et al.* (14) was used. In this case the perfusate enters the liver via a 23-gauge butterfly needle introduced into the inferior vena cava above the renal veins and exits via the severed portal vein. Cephalad flow of perfusate above the liver was avoided by ligation of the inferior vena cava below the heart. Collagenase (35 mg/100 ml) and 0.102 M CaCl_2 (1.14 ml/100 ml) were added to the buffer only after the exiting perfusate was grossly free of blood and then the perfusion was allowed to continue for another 20–30 min during which time the perfusate was continually gassed with a 95%:5% O_2 : CO_2 mixture. The livers were then gently removed into a beaker containing the perfusion media and gently teased to release isolated hepatocytes. After filtering through cheesecloth and centrifugation at $60 \times g$ for 30 s to separate the parenchymal cells the pellet was resuspended in perfusion media in a flask into which a 95%:5% O_2 : CO_2 mixture was continuously introduced. The cells were then placed in a 37°C AquaTherm Water Bath Shaker (New Brunswick Scientific Company, Inc., Edison, NJ) at 100 rev/min for 60–90 min before being used. Viability of the hepatocytes was checked by Trypan blue exclusion immediately before and after stimulation studies and was routinely greater than 90%. Cell concentration was $2\text{--}6 \times 10^6$ cells/ml and contamination by nonparenchymal cells was consistently less than 10% as determined by light microscopy.

Phosphorylase a dose response assay. For the adult assays 500- μl aliquots of the hepatocytes were incubated with either adrenergic agonists or agonists plus antagonists at 37°C in the water bath shaker. Stimulation was stopped at exactly 2 min by removing 200 μl aliquots of the mixture into test tubes on ice as

described by Hutson *et al.* (15). This period of time was chosen because in preliminary studies hormonal stimulation was maximal at 2 min in both newborn and adult hepatocytes. The cells were then homogenized for 10 seconds at half speed on an Ultra-Turrax homogenizer (Tekmar Company, Cincinnati, OH). Newborn assays were performed identically except half volume aliquots were used. Phosphorylase *a* activity was then determined in the stimulated hepatocytes by measuring the incorporation of ^{14}C -G-1-P into glycogen by the method of Gilboe *et al.* (16).

Materials. (–)-Epinephrine, (–)-norepinephrine, (–)-isoproterenol, (–)-alprenolol, and guanosine triphosphate were obtained from Sigma Chemical, St. Louis, MO; (–)-metoprolol and (±)-phenolamine were from Ciba Pharmaceutical, Summit, NJ; ^{125}I -iodocyanopindolol and ^3H -prazosin were obtained from New England Nuclear, Boston, MA; ^{14}C -glucose-1-phosphate from Amersham International Ltd., Amersham, UK; guanylylimidodiphosphate was obtained from Boehringer Mannheim, New York, NY; (±)-epinephrine from Sterling Winthrop Research Institute, Rensselaer, NY; (–)-zinterol from Bristol Myers Company, Evansville, IN; and collagenase, type I from Worthington Pharmaceutical, Freehold, NJ.

RESULTS

Purification of the membranes was assessed by measuring protein recovery and 5'-nucleotidase activity and the results are presented in Table 1. The findings suggest that while both adult and newborn LPM were highly purified the degree of purification was slightly higher in the adult membrane preparations.

In order to determine appropriate conditions for binding assays

Table 1. Membrane purification*

	Newborn	Adult
Protein in crude homogenate (mg protein per g wet liver)	135 ± 8 mg/ml	162 ± 16 mg/ml
% protein recovery (crude homogenate to LPM)	2.6 ± 0.7%	1.3 ± 0.4%
5'-Nucleotidase activity increase (LPM vs crude homogenate)	7 ± 1 times	11 ± 2 times

* Results represent the mean ± SE of five membrane preparations.

and to evaluate ICP binding to rat liver plasma membrane preliminary studies were undertaken. Figure 1 describes the association-dissociation reaction of ICP with liver plasma membrane protein. It was found that equilibrium was reached within 40 min and although not shown in Figure 1 the amount of ICP bound remained the same beyond this point out until 4 h time. In this particular experiment the β -adrenergic antagonist (–)-alprenolol was added to a final concentration of 10^{-6} M at 40 min to examine the dissociation reaction. Insets A and B show logarithmic transformations of the specific binding data for the association and dissociation reactions respectively.

In order to determine if altering the protein concentration would affect ICP binding, 80 pM final concentrations of the radioligand were incubated with various concentrations of membrane protein ranging from 0.04–0.32 mg/ml for 1 h at 37° C. The results which are indicated in Figure 2 demonstrate that under the conditions employed specific binding of ICP to the membrane is directly proportional to protein concentration and represents greater than 85% of total binding.

Fig. 2. ICP binding as a function of protein concentration. Eighty pM concentrations of ICP were incubated with protein concentrations ranging from 0.04 to 0.36 mg/ml for 1 h at 37° C in the absence (● total binding) and presence of 10^{-6} M (–)-alprenolol (Δ nonspecific binding) for the determination of specific binding (○). Data points are the means of triplicate determinations.

Fig. 1. Forward and reverse kinetics of ICP binding to newborn LPM. Membrane (0.2 mg/ml) and ligand (30 pM) were incubated at 37° C; and at 40 min (–)-alprenolol was added to a final concentration of 10^{-6} M to initiate the dissociation reaction. At the times indicated 250- μl aliquots were removed in duplicate and assayed for bound ICP. B_t represents amount of ICP bound at the given times while B_∞ represents the amount bound at equilibrium. The inset (A) is a logarithmic transformation of the binding data of the initial phase of the association reaction where R_o and L_o are unoccupied receptor and free ligand concentrations at the beginning of the reaction and x is the concentration of bound ligand at time t . The association rate constant is determined from the slope of this line by the following equation $K_{on} = \text{slope} (2.303)/[L_o - (R_o)]$. The inset (B) shows $\ln(\text{ICYP})$ bound versus time. By nonlinear regression analysis K_{-1} and K_{-2} were determined (see text).

In another study it was found that the addition of ($\pm$)-phen-tolamine at a final concentration of 10^{-5} M had no effect on either specific or nonspecific binding of ICP to membrane (data not presented). This indicates that, unlike some other iodinated

β -adrenergic radioligands (17, 18), under the conditions employed in our binding assays ICP does not bind to α -adrenergic receptors to any appreciable degree.

Further characterization of the ICP binding sites was determined by a number of displacement studies. As shown in Figure 3 the order of potency for displacing ICP from the membrane preparations was isoproterenol > epinephrine > norepinephrine which is typical for β_2 -adrenergic receptors. The data in Figure 3 also demonstrates that ICP is stereoselectively displaced by the (-) and (+) forms of epinephrine.

That ICP binding sites are β_2 -adrenergic receptors was further deduced by other displacement studies using subtype selective and nonselective β -adrenergic ligands (Fig. 4). It was found that alprenolol, a nonspecific β -adrenergic antagonist, was more effective in displacing ICP than zinterol, a β_2 -adrenergic agonist, and approximately three orders of magnitude more effective than metoprolol, a β_1 -adrenergic antagonist.

Inhibition of ICP binding by epinephrine was also investigated in the absence and presence of GTP and its nonhydrolyzable synthetic analog, Gpp(NH)p. The addition of GTP resulted in a shift of the right in the ICP displacement curve (data not shown) and an increase in the Hill coefficient from 0.46 to 0.71. Similar studies were also carried out using Gpp(NH)p in both adult and newborn membrane preparations (Fig. 5). In both the addition of the GTP analog resulted in a similar shift to the right in the displacement curve and increase in the Hill coefficient as was seen with GTP.

Table 2 summarizes the data obtained from ICP and ^3H -prazosin saturation binding studies. K_d and receptor density ($B_{\max}$) were determined from Scatchard plots of specific binding data, representative examples of which are shown in Figure 6. ICP (β -adrenergic) binding sites declined significantly with age (114 ± 4 fmol mg^{-1} in the newborns *versus* 19 ± 3 fmol mg^{-1} in the adult) while ^3H -prazosin (α -adrenergic) binding sites in-

Fig. 3. Inhibition of ICP binding to newborn LPM by classical adrenergic agonists. Twenty to thirty μg of membrane protein was incubated with ICP (30 pM) and concentrations of competing ligands ranging from 10^{-9} to 10^{-3} M for 1 h at 37°C . Data points are the mean of three experiments each done in triplicate.

Fig. 4. Inhibition of ICP binding to newborn LPM by β -adrenergic ligands. Twenty to thirty μg of membrane protein was incubated with ICP (30 pM) and concentrations of the competing ligand ranging from 10^{-9} to 10^{-3} M for 1 h at 37°C . Data points are the mean of three experiments each done in duplicate.

Fig. 5. Inhibition of ICP binding to newborn and adult LPM by epinephrine in the presence and absence of Gpp(NH)p. Twenty to thirty μg of membrane protein was incubated in the presence (○) and absence (●) of Gpp(NH)p (10^{-5} M) for 1 h at 37°C . Data points are the mean of two to three experiments each done in duplicate or triplicate.

Table 2. Summary of receptor binding in newborn and adult LPM

			^{125}I -iodocyanopindolol		^3H -prazosin	
			K_d	$B_{\max}$	K_d	$B_{\max}$
	<i>n</i>	Wt (g)	(pmol)	(fmol/mg)*	(pmol)	(fmol/mg)*
Newborn	5	12-16	30 ± 2	114 ± 4	103 ± 7	161 ± 14
Adult	5	195-265	31 ± 5	$19 \pm 3^\dagger$	58 ± 11	$554 \pm 59^\ddagger$

* fmol bound per mg purified LPM.

† Adult < newborn $p < 0.001$.

‡ Adult > newborn $p < 0.001$ by Student's *t* test.

Fig. 6. Newborn and adult ICP saturation binding curves and Scatchard plots of specific binding data. Membrane protein (0.2–0.3 mg/ml) was incubated with increasing final concentrations of ICP ranging from 20–300 pM for 1 h at 37° C in the absence (● total binding) and presence of 10^{-6} M (–)-alprenolol (△ nonspecific binding) for the determination of specific binding (○). Data points are the mean of triplicate determination at each ligand concentration. B/F, bound to free ligand concentration.

Fig. 7. Newborn glycogen phosphorylase dose response. Aliquots, 250 μ l, of isolated hepatocytes from 5- to 7-day-old rats were stimulated with either (–)-isoproterenol (●), (–)-epinephrine (○), or (–)-norepinephrine (△) in final concentrations ranging from 10^{-10} to 10^{-3} M for 2 min at 37° C. Results are expressed as percentage of maximum phosphorylase *a* activity versus agonist concentration. Data points are means of four to five separate experiments.

Fig. 8. α and β -Adrenergic stimulation of glycogen phosphorylase in newborn and adult hepatocytes. Isolated hepatocytes from 5-day and adult rats were stimulated with (–)-epinephrine (10^{-5} M) and either (±)-phenolamine (3×10^{-5} M) to represent β -adrenergic stimulation or (–)-alprenolol (3×10^{-5} M) to represent α -adrenergic stimulation for 2 min at 37° C. Results are expressed as percentage of basal phosphorylase *a* activity in the absence of stimulation. The data points are the mean $\pm$ SE of six to eight experiments. * 5 day > adult $p < 0.01$ ○ adult > 5 day $p < 0.01$ by Student's *t* test.

creased significantly (161 ± 14 fmol mg^{-1} in the newborn versus 554 ± 59 fmol mg^{-1} in the adult). The K_d value of approximately 30 pM for ICP represents an affinity three to four times greater than any previously available iodinated β -adrenergic radioligand (9).

The glycogenolytic response of newborn hepatocytes to adrenergic stimulation was assessed by measuring phosphorylase *a* activity and the results are presented in Figure 7. The order of potency for stimulating glycogen phosphorylase was isoproterenol > epinephrine > norepinephrine which is characteristic of β_2 -adrenergic responsiveness. In another series of experiments the relative contributions of α - and β -adrenergic stimulation on glycogen phosphorylase in both newborn and adult hepatocytes was examined (Fig. 8). Hepatocytes were stimulated with a combination of (–)-epinephrine (10^{-5} M) and either (±)-phenolamine (3×10^{-5} M) or (–)-alprenolol (3×10^{-5} M) to represent either β - or α -adrenergic stimulation, respectively. In the 5-day

hepatocytes β -adrenergic stimulation resulted in an almost 100% increase above basal in phosphorylase *a* activity but α -adrenergic stimulation was without effect. In the adult male hepatocytes a significant rise in phosphorylase *a* activity was only seen with α -adrenergic stimulation.

DISCUSSION

Attempts to characterize adequately the β -adrenergic receptor in newborn rat purified LPM have been unsuccessful using previously available radioligands such as ^{125}I -iodohydroxybenzylpindolol because of its high nonspecific binding (8). This radioligand has been reported to bind to serotonin sites in rat cerebral cortex (19) and much of its nonspecific binding can be eliminated in rat glioma cells by the addition of phenolamine, an α -adrenergic antagonist (17, 18). In the present study we utilized a relatively new radioligand, ICP. Under the conditions

employed in our assays this ligand demonstrated rapid, reversible, and stereoselective binding as well as a very high affinity (K_d of 30 pM) and specificity for β -adrenergic receptors. The biphasic nature and shallowness of the ICP dissociation reaction with the addition of antagonist have been observed elsewhere (9). The calculations of the dissociation rate constants are based on the assumption that there are interconvertible receptor conformations resulting in a rapidly formed ICP-receptor complex which can dissociate quickly and a thermodynamically favored ICP-receptor complex that dissociates more slowly as postulated by Engel *et al.* (9). The high affinity and specificity of ICP binding made it possible to demonstrate that there was a significant decline in β -adrenergic receptor density in LPM seen with maturation of the male rat. In addition we were able to determine that ICP binding sites in newborn LPM were β_2 -adrenergic receptors. The α -adrenergic receptor was also examined in these same membranes and it was found that concomitant to the decline in β -adrenergic receptor density there was an increase in α -adrenergic receptor density as measured by 3H -prazosin binding sites. To our knowledge only one other study has been published which specifically examined developmental changes in adrenergic receptor density in rat liver (20). β -Adrenergic receptors were studied using ^{125}I -pindolol and α -adrenergic receptors using 3H -prazosin and 3H -rauwolscine. Although the receptor densities in the study were much lower than ours large increases in α -adrenergic receptor densities and decreases in β -adrenergic receptor densities with maturation were also noted. The lower numbers in the report are most likely due to the fact that a less purified membrane preparation was employed. In addition the reported K_d value for 3H -prazosin binding was at least an order of magnitude larger than what we found in the present study and in previous studies (21). A recent study (11) employing ICP and adult male LPM of similar purity as ours but prepared by an entirely different procedure reported a β -adrenergic density virtually identical to that reported here; and although no actual data were presented they stated that the receptor density was much higher in newborn LPM. Our studies on isolated hepatocytes revealed that the activation of glycogen phosphorylase by epinephrine was mediated exclusively by β_2 -adrenergic mechanisms in the newborn and predominantly by α -adrenergic mechanisms in the adult. Thus it appears that the β -adrenergic control of glycogenolysis which develops during fetal life (7) is carried over into the neonatal period; and only after this time does attenuation of the β -adrenergic response and emergence of the α -adrenergic response occur (3–6). Much data have been accumulating which suggest that the low β -adrenergic receptor density in the adult male is responsible for the lack of a β -adrenergic glycogen phosphorylase activation. In the present study the coupling mechanism between receptor and guanyl nucleotide regulatory protein seemed to be the same in both newborn and adult membrane preparations since the addition of GTP and Gpp(NH)p in competition assays resulted in a shift to the right and an increase in the Hill coefficient of the displacement curves. Recently Nakamura *et al.* (8) have demonstrated the presence of both subunits of guanyl nucleotide regulatory protein in adult male hepatocytes and found that the addition of GTP did not increase adenyl cyclase activity in response to isoproterenol. We (22) and others (6) have also found that, although β -adrenergic stimulation produces only a small rise in cAMP accumulation in adult male hepatocytes, glucagon produces a rise in cAMP and glycogen phosphorylase activity which is similar to that seen in the newborn. These findings when taken together suggest that the most likely explanation for the lack of β -adrenergic responsiveness in adult male hepatocytes is the decreased receptor density since all of the other components of the β -adrenergic-mediated glycogen phosphorylase activation pathway are present and apparently functional. The significance of the developmental changes in rat liver adrenergic responsiveness with development is not clear at present. The cell surface adrenergic receptors may be activated by circulating epinephrine,

norepinephrine, or locally released norepinephrine upon sympathetic nerve stimulation. Innervation of the rat liver is restricted to primarily vessels in the portal space and hilus (23, 24) as opposed to guinea pig, cat, primates, and humans where adrenergic nerves penetrate deep into the parenchyma (23–25). However, there is evidence that glucose output from the liver is directly regulated by the sympathetic nervous system in the rat (26). The development of hepatic innervation in the rat has not been reported to our knowledge and it remains to be studied whether the adrenergic receptor responsiveness changes are related to the developmental changes in innervation. While functionally unimportant in the adult rat, β -adrenergic control of glycogenolysis in the newborn may be essential in maintaining glucose homeostasis. As we have demonstrated, although α -adrenergic receptors are present in the newborn hepatocyte in concentrations even greater than β -adrenergic receptors, their stimulation does not lead to a rise in glycogen phosphorylase activity. In addition sensitivity of the liver to glucagon, the other major hormone involved in glucose homeostasis, is apparently less in the newborn than in the adult (27).

In summary we conclude that 1) ICP appears to be a very useful radioligand for examining the β -adrenergic receptor in rat hepatocytes and probably other tissues and species as well, 2) rat liver ICP binding sites are β_2 -adrenergic receptors, and 3) the maturational change in adrenergic-mediated glycogen phosphorylase activation from β - to predominantly α -adrenergic control is probably related to concomitant changes in the respective receptor densities.

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Differential Toxicity of RCA_{II} (Ricin) on Rabbit Intestinal Epithelium in Relation to Postnatal Maturation¹

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ABSTRACT. The purpose of this work was to assess the toxic lectin ricin (RCA_{II}) as a probe for the study of intestinal permeability in the developing small bowel. Jejunal explants from suckling and adult rabbits were exposed to varying dosages of RCA_{II} for 30 min at 25° C and then cultured in toxin-free medium. The RCA_{II} dose required to inhibit protein synthesis during 6 h of culture increased from 0.1 μ g/ml in 4-day-old rabbits to 25 μ g/ml in weanling rabbits. RCA_{II} cytotoxicity was almost completely blocked by 0.1 M lactulose in all age groups. The kinetics of 125 I-RCA_{II} binding to purified microvillus membranes were determined by incubating a fixed concentration of membrane protein (30 μ g) with increasing concentrations of labeled lectin (2-18 μ g/ml). Binding attained saturation with adult but not with suckling animal membranes. The latter yielded a curvilinear relationship in Scatchard plots, suggesting either several classes of binding sites or negative cooperativity. RCA_{II} binding was confined to the delipidated fraction of the membranes and decreased by 42% from 6 days old to adult age. The extreme sensitivity of colostral epithelium to RCA_{II} is probably related to the high level of endocytosis exhibited by the immature membrane of suckling rabbits. The development of increasing resistance to the toxin, and associated decrease in binding, might be related to disappearance of saccharide sites in productive surface receptors occurring in the developmental course of intestinal glycosylation. (*Pediatr Res* 19: 868-872, 1985)

Abbreviations

RCA_{II}, ricin
PBS, phosphate-buffered saline

The early part of the suckling period in rodents is characterized by a high level of receptor-mediated responses in small intestinal epithelium. This has been clearly shown for the jejunal transport of breast milk immunoglobulin (1, 2) and is also inferred from the high content of trophic factors in breast milk (3, 4). The surface characteristics underlying this high receptor responsiveness in the intestine of neonatal animals are poorly understood. A marked developmental change in surface reactivities to lectins has been described in the small intestine of suckling rats (5-7). In this animal species, the intestinal microvillus membrane undergoes a progressive shift from sialylation to fucosylation of glycoproteins and glycolipids during postnatal development (7). In the present work, we have examined the response of rabbit intestinal epithelium to the toxic action of RCA_{II}. This toxin inhibits protein synthesis in intact cells (8, 9) through a mechanism that requires terminal nonreducing galactosyl (or N-acetyl-galactosaminyl) residues (10) in a glycoprotein receptor capable of transmembrane signaling for toxin internalization (11). This toxin, therefore, "mimics" the mechanism of interaction of trophic hormones (12). The RCA_{II} membrane receptors may be part or be closely associated with cell surface components involved in cell-macromolecule and cell-cell recognition (13). The probing of intestinal epithelium with RCA_{II} may provide insight into the role of glycosylation on receptor-mediated responses in the developing small bowel.

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