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Transport of Thyroid and Steroid Hormones through the Blood-Brain Barrier of the Newborn Rabbit: Primary Role of Protein-Bound Hormone*

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ABSTRACT. The transport of [^{125}I]T_3 [^{125}I]T_4, [^{3}H]testosterone, and [^{3}H]estradiol through the blood-brain barrier (BBB) of the newborn rabbit was studied with a tissue-sampling, single injection technique. The first pass extractions of T_3 and T_4 were 22 ± 2% and 14 ± 1%, respectively, after carotid injection of Ringer's solution (0.025% albumin). Thyronine transport was saturated and cross-competitive, but was not inhibited by a large excess of leucine, a neutral amino acid. The extraction of T_3 was reduced to 7 ± 1% after injection of hormone mixed in a 10% T_3-specific rabbit antiserum, and this value approximated the extraction of an extracellular space marker, such as sucrose (6 ± 1%). Therefore, antibody-bound T_3 was not available for transport *in vivo*. Conversely, T_3 bound to the plasma proteins in newborn rabbit antiserum, *e.g.* albumin, was transported into the brain. The concentration of plasma protein required to inhibit T_3 transport 50% *in vivo* was 28-fold greater than the serum concentration that resulted in 50% binding of T_3 *in vitro*.

The first pass extractions of testosterone and estradiol after injection of hormone in Ringer's solution were 100 ± 5% and 91 ± 5%, respectively. A 5 g/100 ml concentration of albumin bound about 95% of steroid *in vitro*, but resulted in only a 17–20% inhibition of testosterone or estradiol transport *in vivo*. Similarly, testosterone bound to the progesterone-binding globulin of pregnant guinea pig serum was also transported, although to a lesser extent than albumin-bound hormone. Conversely, testosterone bound to a specific rabbit antiserum, or testosterone or estradiol bound to the sex hormone-binding globulin in human serum was not transported into the brain. These results indicate that the mechanisms of thyroid and steroid hormone transport through the BBB of the newborn rabbit are very similar to those of the adult rat; therefore, the processes mediating BBB transport of protein-bound hormones are firmly established by at least the first 8–24 h of postnatal life. (*Endocrinology* **107**: 1705, 1980)

THE CENTRAL nervous system of the developing organism is influenced by the action of both thyroid and steroid hormones. Thyroid hormone is known to regulate the rate of transport of both glucose and amino acids into the brain of the developing animal (1, 2). In addition, neonatal hypothyroidism is known to cause a depression in both the role of cerebral protein synthesis (3) and brain levels of the enzymes for ketone body metabolism (4). One or all of these metabolic abnormalities may be related in part to the development of mental retardation associated with hypothyroidism in the developing organism (5). Similarly, the steroid hormones, testosterone and estradiol, are known to modulate, at least in the rat, the development of sexually dimorphic behaviors (6). For example, the administration of testosterone to female rats at a critical postnatal period results in the expression of male sexual behavior in adult animals (7).

Since the expression of thyroid or steroid hormone action in the developing brain is probably a function of hormone availability in brain cells, it is important to understand the mechanisms of hormone transport from blood into the brain, *i.e.* hormone transport through the brain capillary wall or blood-brain barrier (BBB). Therefore, the present studies were designed to investigate the permeability properties of the BBB to the thyroid and steroid hormones in the newborn rabbit. Since both thyroid and steroid hormones circulate tightly bound by plasma proteins (8, 9) the investigation of the role of plasma protein binding of the hormones is emphasized in these studies.

Materials and Methods

Radiolabeled L-T_4 ([^{125}I]T_4; 0.8 μCi/pM), L-T_3 ([^{125}I]T_3; 0.1 μCi/pM), [^{14}C]sucrose (3.6 μCi/μmol), [^{3}H]water (1 mCi/g), [1, 2, 6, 7, 16, 17-(*N*)-^{3}H]testosterone (152 Ci/mmol), [2, 4, 6, 7, 16, 17-^{3}H]estradiol (143 Ci/mmol), and *n*-[1-^{14}C]butanol (1.9 mCi/mmol) were all purchased from New England Nuclear Corp. (Boston, MA). The radiochemical purity of the labeled T_4, T_3, testosterone, estradiol, and butanol was at least 98%, as

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judged by either cellulose or Silica gel G thin layer chromatography and radioscanning (Packard Radioscanner, Packard, Downers Grove, IL) using previously reported solvent systems (10–13). Unlabeled L-T_3, L-T_4, L-leucine, bovine albumin (fraction V; nondefatted), and Hepes buffer were purchased from Sigma Chemical Co (St. Louis, MO). Serum was obtained from ether-anesthetized newborn rabbits, barbiturate-anesthetized pregnant guinea pigs, and normal male human volunteers. Testosterone-specific and T_3-specific rabbit antisera were obtained from Endocrine Sciences (Tarzana, CA).

The BBB transport of [^{125}I]T_3, [^{125}I]T_4, and [^{14}C]sucrose, relative to a [^{3}H]water reference, was measured in ether-anesthetized newborn New Zealand rabbits (8–24 h old) using the carotid injection technique of Oldendorf (14), as recently adapted to the newborn rabbit (15). Barrier transport of [^{3}H]-testosterone or [^{3}H]estradiol was measured relative to a [^{14}C]-butanol reference. A common carotid artery of newborn rabbits was cannulated with a 30-gauge needle, and a 0.1-ml bolus of buffered Ringer's solution (pH 7.4; 5 mM Hepes) was rapidly injected (<1 sec). The bolus contained 2.5 μCi/ml ^{125}I isotope or 25 μCi/ml [^{3}H]water in studies of T_3, T_4, or sucrose transport. Measurements of estradiol or testosterone transport involved an injection bolus consisting of 2–5 μCi/ml ^{3}H-labeled steroid and 0.4–1.0 μCi/ml [^{14}C]butanol. After injection of ^{125}I-labeled compounds in Ringer's solution, various concentrations of unlabeled T_3, T_4, or leucine were added to the injection bolus to test for saturation or competition of T_3 or T_4 transport. In addition, dilutions of various sera were added to the bolus to assess the effects of plasma protein binding on the transport of T_3, testosterone, or estradiol.

Fifteen seconds after carotid injection, a time period which permits a single circulatory pass of the bolus through the brain (15), the rabbits were decapitated, and the cerebral hemisphere ipsilateral to the injection was removed from the cranium and solubilized in 1.0 ml Soluene-350 (Packard); a sample of the injection solution was similarly prepared for double isotope liquid scintillation counting. The brain uptake index (BUI) was calculated (14, 15) as follows:

$$\text{BUI} = \frac{(\text{test dpm/reference dpm})\text{brain}}{(\text{test dpm/reference dpm})\text{injectate}} \times 100$$

The BUI = E_T/E_R (11, 13), where E_T is the percent extraction of unidirectional influx of the test compound (T_3, T_4, sucrose, testosterone, or estradiol) and E_R is the percent extraction of the reference compound (water or butanol).

The E_R 15 sec after carotid injection is a function of the maximal percent extraction of the unidirectional influx of the reference, which occurs during the initial 5 sec after injection (15), and the rate of efflux from brain to blood of the labeled reference during the subsequent 10 sec of the total 15-sec circulation period. Estimates of both the maximal percent extraction and the reference efflux rate were determined by simultaneously injecting [^{3}H]water (5 μCi/ml) and [^{14}C]butanol (1.0 μCi/ml) and decapitating the animals 0.25, 1, 2, and 4 min after carotid injection. Both the log normal BUI of [^{3}H]water, relative to [^{14}C]butanol and the log normal percent of injected [^{14}C]butanol per g brain were plotted *vs.* time after carotid injection. The intercepts and slopes of these plots provided data on both the E_R for water or butanol and the rate of cerebral blood flow (see *Results*).

The *in vitro* binding of [^{125}I]T_3, [^{3}H]testosterone, or [^{3}H]-estradiol by neonatal rabbit serum was determined by equilibrium dialysis for 20 h at 37 C, as reported previously (11, 13). The albumin concentration in the rabbit serum was determined by a colorimetric method (bromcresol green) using reagents obtained from Sigma Chemical Co.

Results

The initial extraction and subsequent efflux of the [^{3}H]water and [^{14}C]butanol internal references are depicted in Fig. 1. Since the initial extraction of butanol is 100% (16), the intercept at zero time of the plot of $E_{HOH}/E_{butanol}$ gives the maximal percent extraction of the [^{3}H]water reference (91%; Fig 1). The slope (K_{net} = 0.10 min^{-1}; Fig. 1) of the $E_{HOH}/E_{butanol}$ plot is equal to ($K_{butanol} - K_{HOH}$), where K is the individual rate constant (per minute) of butanol and water efflux from brain to blood (17). The $K_{butanol}$ was determined directly by plotting the rate of change of butanol radioactivity in the brain (Fig. 1). Given $K_{butanol}$ = 0.28 min^{-1} (Fig. 1), then K_{HOH} = 0.18

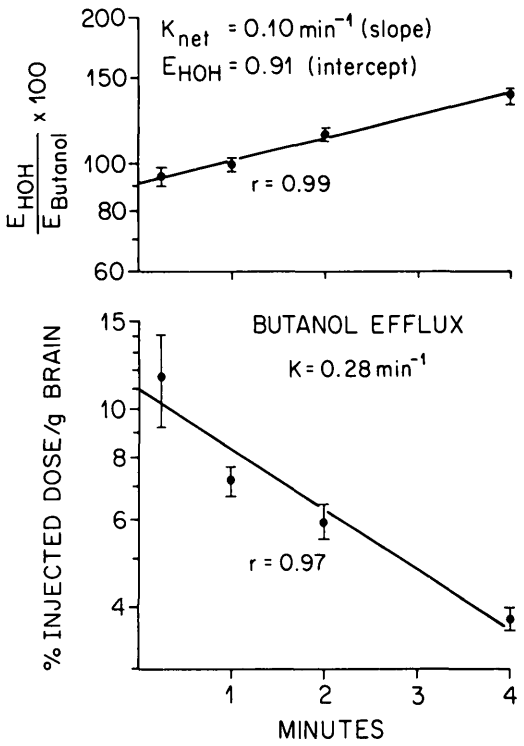

FIG. 1. *Top panel*, The ratio of extraction (E) of water to extraction of butanol is plotted *vs.* time after carotid injection. The intercept at zero time is equal to the maximal fractional extraction of the [^{3}H]water, since the maximal fractional extraction of the [^{14}C]butanol is 1.0 (16). The slope (K_{net}) is equal to $K_{butanol} - K_{HOH}$, where K is the efflux rate constant for butanol and water, respectively (*bottom panel*). The $K_{butanol}$ is determined directly from the slope of the relationship between[^{14}C]butanol radioactivity in brain *vs.* time after carotid injection. Since $K_{butanol}$ = 0.28 min^{-1}, then K_{HOH} = 0.18 min^{-1}. These efflux constants provide information as to the rate of washout of the [^{3}H]-water and [^{14}C]butanol references as well as to cerebral blood flow (see text). Data are the mean ± SE (n = 3).

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min^{-1}. Based on the K values and the initial extractions for butanol or water, the calculated E_R values 15 sec after carotid injection for the [^{14}C]butanol and [^{3}H]water references are 97% and 89%, respectively. Given these estimates for E_R, the respective E_T values 15 sec after injection may be determined from the relationship, $E_T = (BUI)(E_R)$, as described in *Materials and Methods*. The efflux constant for butanol (0.28 min^{-1}) may also be used to calculate cerebral blood flow (F) in the newborn rabbit by the formula, $F = KV'/E_R$ (17), where $E_R = 1.0$ (16), $K = 0.28$ min^{-1}, and $V' = 0.89$ ml/g, which is the ratio of the volume of distribution of butanol in the brain to that in blood (17). Substitution of these values into the equation for flow indicates $F = 0.25$ ml/g·min for the ether-anesthetized newborn rabbit brain. Therefore, cerebral blood flow in the newborn rabbit is about half the flow rate in the adult barbiturate-anesthetized rat (18), but is comparable to brain blood flow in the newborn dog, which is 0.23 ml/g·min (19).

The E_T values for T_3 and T_4 are given in Table 1 and all are at least 2-fold the extraction for sucrose, an extracellular space marker. Similar to the adult rat brain (13), the transport of T_3 and T_4 is saturable and cross-competitive, but T_3 transport is not significantly inhibited by a large excess of leucine, a neutral amino acid (Table 1).

The effect of plasma protein binding on T_3 transport was investigated by adding various dilutions of either neonatal rabbit serum or a T_3-specific rabbit antiserum to the injection solution (Fig. 2). Increasing concentrations of neonatal rabbit serum resulted in increased binding of T_3 and, thus, decreased transport. However, the observed decrease in T_3 transport associated with higher concentrations of serum proteins was considerably less than would be expected if only the fraction that was free (dialyzable) *in vitro* was free and available for transport *in vivo* (Fig. 2). The percent of T_3 bound *in vitro*, as determined by equilibrium dialysis, was compared to the

TABLE 1. Transport of [^{125}I]T_3, [^{125}I]T_4, and [^{14}C]sucrose through the BBB of the newborn rabbit

Isotope	Injection vehicle	E (%)
[^{125}I]T_3[a]	Ringer solution	22.0 ± 1.8
	5 μM T_3	19.8 ± 1.1
	50 μM T_3	13.2 ± 0.9[b]
	50 μM T_4	15.8 ± 1.1[b]
	1000 μM L-leucine	19.8 ± 2.0
[^{125}I]T_4[a]	Ringer solution	13.5 ± 0.4
	50 μM T_3	10.1 ± 0.2[b]
[^{14}C]Sucrose	Ringer solution	5.9 ± 0.6

Values given are the mean ± SEM (n = 4–6).

[a] Bovine albumin (0.025%) was added to all ^{125}I-labeled thyronine injection solutions.

[b] Significantly different ($P < 0.05$, by Student's t test) from Ringer's solution control.

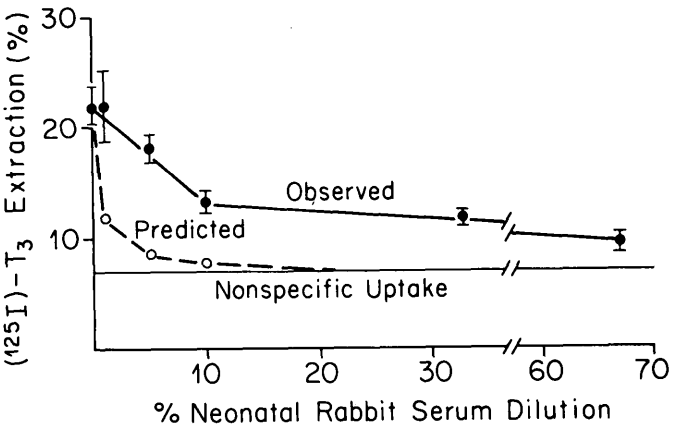

FIG. 2. The extraction of [^{125}I]T_3 is plotted *vs.* the concentration of neonatal rabbit serum in the carotid injection solutions (Observed). The Predicted line represents the expected transport if only the percent of hormone that was free *in vitro* was transported *in vivo*; the Predicted line was obtained by multiplying the maximal percent extraction (22 ± 2%) times the fraction of free (dialyzable) T_3 at each respective serum dilution (Table 2). The nonspecific extraction (7.0 ± 0.5%) represents the extraction of [^{125}I]T_3 after injection of labeled hormone in 10% T_3-specific rabbit antiserum (see text). The concentration of [^{125}I]T_3 in the final injection solution ranged from 4–6 nM. Data are the mean ± SE (n = 6).

TABLE 2. Comparison of free [^{125}I]T_3 *in vitro vs. in vivo*

Newborn rabbit serum conc. (%)	% Bound *in vitro*[a]	% Bound *in vivo*[b]
1	67.1 ± 1.6	<1
5	89.7 ± 0.7	26 ± 2
10	94.4 ± 0.2	58 ± 4
33	>95	69 ± 4
67	>95	84 ± 8

Values are the mean ± SEM (n = 4–6).

[a] Determined by equilibrium dialysis at 37 C.

[b] Determined by the following equation: % bound = $(E_0 - E) \div (E_0 - E_{NS}) \times 100$, where E_0 is the extraction of T_3 after injection of Ringer's solution (Table 1), E is the extraction of T_3 after injection of respective dilutions of newborn rabbit serum, and E_{NS} is the nonspecific extraction of T_3 after injection of a 10% T_3 rabbit antiserum (Fig. 2).

percent of T_3 bound *in vivo*, as estimated from the percent inhibition of T_3 transport, at each respective dilution of serum (Fig. 2 and Table 2). A double reciprocal plot (data not shown) of 1/B *vs.* 1/dilution, where B is the bound fraction *in vitro* (Table 2), was linear (r = 0.99), with an intercept of 1.0 (predicted intercept, 1.0) and a slope of 0.48% rabbit serum; that is, 50% binding of T_3 was observed at a serum dilution of 0.48%. Since essentially all of the T_3 in rabbit serum is bound to albumin (20), the albumin concentration in newborn rabbit serum was measured [2.28 ± 0.07 g/100 ml (mean ± SE); n = 5] which is equivalent to 335 μM albumin (mol wt, 68,000). Therefore, the dissociation constant (K_d) of albumin binding of T_3 *in vitro* is equal to (0.48%) × (335 μM) or

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K_D = 1.6 μM, which agrees well with the value of 3.7 μM reported by Robbins and associates (8). A 1/B *vs.* 1/ dilution double reciprocal plot of the transport data in Fig. 2 may be used to analyze the kinetics of ligand binding *in vivo*; this plot (data not shown) was linear (r = 0.96), with an intercept of 0.9 (predicted intercept, 1.0) and a slope of 13.7% serum or 46 μM albumin (13.7% × the albumin concentration of 335 μM). Therefore, the apparent K_d of albumin binding of T_3 *in vivo* (46 μM) is 29-fold greater than the K_d *in vitro*. These results are comparable to those found in studies of the adult rat brain, in which the apparent K_d of bovine albumin binding of T_3 in vivo is 16-fold greater than the K_d value *in vitro* (13).

The BBB transport of testosterone and estradiol after injection of Ringer's solution is rapid, *e.g.* the E_T values are 100 ± 5% and 91 ± 5%, respectively, (Fig. 3). Similar to studies with the adult rat brain (11), albumin-bound sex steroid was almost freely transported into the newborn rabbit brain (Fig. 3). A 5 g/100 ml concentration of albumin resulted in only 17% and 20% inhibition, respectively, of testosterone and estradiol transport; however, this concentration of albumin binds 93% and 97% of testosterone and estradiol, respectively, *in vitro* (11). Similarly, 67% neonatal rabbit serum resulted in only 24% and 18% inhibition of testosterone and estradiol transport, respectively (Fig. 3), which is considerably less inhibition than would be expected if the amount of steroid hormone bound *in vitro* was inaccessible to transport *in vivo*. For example, the free (dialyzable) fractions of testosterone and estradiol in the presence of 100% neonatal rabbit serum were 2.55 ± 0.24% and 3.13 ± 0.16% (mean ± SE, n = 3), respectively. Human serum resulted in 46% and 40% inhibition of testosterone and estradiol transport, respectively (Fig. 3), which is consistent with the presence of sex hormone-binding globulin (SHBG) in human serum, which in the normal male binds approximately half of circulating testosterone or estradiol (21). Pregnant guinea pig serum resulted in a selective inhibition of testosterone transport (Fig. 3) due to the high concentration of progesterone binding globulin (PBG) in pregnant guinea pigs; PBG is known to bind testosterone but not estradiol (22). Finally, a testosterone-specific rabbit antiserum (Fig. 3) resulted in a virtual total inhibition of testosterone transport into the newborn rabbit brain. These results are similar to those found in studies with the adult rat brain which indicate that antibody-bound hormone is not transported into tissues (23).

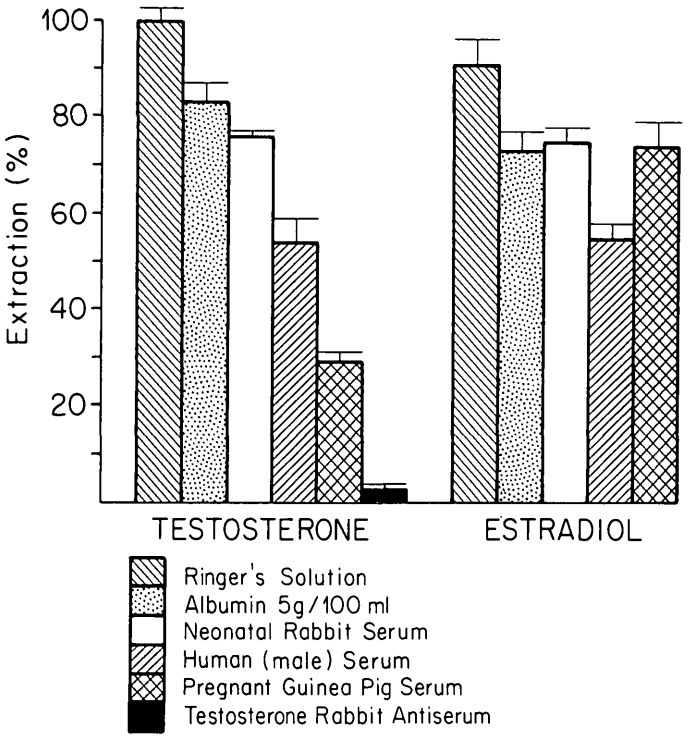

FIG. 3. The extraction of [^{3}H]testosterone and [^{3}H]estradiol after carotid injection of labeled hormone mixed in 1) Ringer's solution, 2) 5 g/ 100 ml bovine albumin, 3) a 67% solution of serum obtained from neonatal rabbits, normal human male volunteers, or pregnant guinea pigs, and 4) a 67% solution of a testosterone-specific rabbit antiserum. The final injection solution concentration of labeled testosterone or estradiol ranged from 6–12 nM. Data are the mean ± SE (n = 4).

Discussion

The present studies indicate that the mechanisms mediating BBB transport of thyroid and steroid hormones in the newborn rabbit are very similar to mechanisms described previously for the adult rat brain (11, 13, 17, 21, 23). With regard to thyroid hormones, T_3 and T_4 traverse the BBB via a saturable, carrier-mediated mechanism that is present in both adult (13, 24, 25) and newborn brains. The intrinsic permeability of the BBB to thyroid hormones is unusually low, and were it not for the presence of the thyroid hormone carrier, very little T_3 or T_4 would enter the brain from the blood. Considering the high lipid solubility of T_3 and T_4, *e.g.* the octanol/Ringers partition coefficients for T_3 and T_4 are 180 and 91, respectively (10), the low intrinsic permeability of the BBB and the need for carrier mediation are somewhat surprising. However, it is probable that the 3–4 iodine moieties of T_3 or T_4 make the molecule more lipid soluble but, perhaps owing to steric considerations (26), render the hormones less diffusible through biological membranes. For example, iodinated contrast agents are highly lipid soluble (27) but do not normally cross the BBB (28). In addition, the permeability of the placenta to thyroid hormones is very low (29), reflecting the presumed absence in this membrane of a thyroid transport system. Conversely, T_3 and T_4 readily cross the hepatocyte cell membrane via a nonsaturable mechanism that is presumed to be free diffusion (10); the rapid

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transport of T_3 and T_4 into liver via free diffusion is probably due to the very large surface area of the hepatocyte plasma membrane, which has a profusely mamillated microvillar surface (30).

The presence of a T_3 transport system within the BBB of the newborn rabbit allows the BBB to compete with circulating albumin for binding of T_3 and, to a lesser extent, T_4. The competition between the T_3 carrier and albumin for T_3 binding may be quantitatively described as follows (13): K_d (app)/K_d = 1 + app C/Km, where K_d (app) is the apparent K_d of albumin binding of T_3 *in vivo* (*i.e.* in the presence of the competing BBB binding system), K_d is the dissociation constant of albumin binding *in vitro* (*i.e.* in the absence of competing binding systems), app C is the apparent binding capacity of the BBB carrier [which is formally defined (31) as app C = C_T (1 + k_5t), where C_T is the local capillary carrier concentration, k_5 is the rate constant of the carrier movement through the BBB membrane, and t is the capillary transit time], and Km is the dissociation constant of the BBB carrier binding of T_3. Given K_D(app) = 46 μM and K_d = 1.6 μM, the ratio of app C/Km = 28, as opposed to the ratio of 16 calculated for the adult rat brain (13). Therefore, the apparent binding index of the T_3 carrier (app C/Km) is nearly 2-fold greater in the newborn brain than in the adult brain. However, the affinity of the newborn T_3 carrier is lower than that of the adult BBB; although the Km of T_3 transport was not formally calculated in the present studies, the results in Table 1 indicate that T_3 transport is half-saturated at about 50 μM compared to the Km of 1.1 μM calculated for the adult brain (13). Therefore, the apparent binding capacity of the newborn BBB must be about 100-fold greater than that of the adult. Owing to the reduced rate of cerebral blood flow in the newborn (see *Results*), the capillary transit time (t) is increased; however, substantial increases in either carrier concentration (C_T) or carrier mobility (k_5) are also to be expected for the newborn to account for the increased carrier capacity. Similar increases in capacity and decreases in affinity have been observed for newborn BBB transport of neutral amino acids (15); however, the lack of competition by 1000 μM leucine indicates that T_3 and neutral amino acids traverse the BBB via separate transport systems.

With regard to the steroid hormones, both testosterone and estradiol are freely transported through the BBB in the absence of plasma protein. The inhibition of BBB steroid transport by steroid-binding plasma proteins is characterized by a spectrum of effects. At one end of the spectrum is albumin, which may bind 95% of testosterone or estradiol *in vitro* (11), yet this plasma protein causes relatively little inhibition of steroid transport *in vivo*. Conversely, antibody-bound testosterone is not transported through the BBB of the newborn rabbit, which is similar to BBB steroid hormone transport in the adult rat (23). Similarly, testosterone or estradiol bound to the SHBG of human serum is not appreciably transported through the BBB of either the neonatal rabbit or the adult rat (21). The fact that 54% and 60% of the plasma testosterone and estradiol in human serum, respectively, were transported through the newborn BBB (see *Results*) may be attributed to the transport of albumin-bound steroid into the brain, *e.g.* approximately half of the circulating testosterone or estradiol in human male serum is albumin bound (21). The SHBG of rabbit serum is primarily a testosterone-binding globulin (TeBG) which binds estradiol only weakly (32). However, the TeBG of rabbit serum has a modest affinity for estriol and estrone (32), two estrogens which, like estradiol, are elevated in plasma during the first 24 h of postnatal life (33). The observation that neonatal rabbit serum inhibited testosterone transport into the newborn brain by only 24% may reflect a relative saturation of TeBG by the high levels of estrogen in the postnatal period. In addition, the lack of substantial inhibition of BBB estradiol transport by newborn rabbit serum indicates that the α-fetoprotein of the newborn rabbit lacks the estradiol-binding properties of the α-fetoprotein in neonatal rat serum (11). In contrast to the case of SHBG-bound or antibody-bound steroid, where globulin-bound hormone is not transported into either the adult or newborn brain, steroid hormone (*e.g.* testosterone) bound to the PBG of pregnant guinea pig serum is transported into either the newborn or adult brain (23). The testosterone binding index (*i.e.* binding site concentration ÷ dissociation constant) of PBG is at least 10-fold greater than the testosterone binding index of albumin (22). Therefore, since virtually all of the testosterone in pregnant guinea pig serum is bound by PBG, the observation that 29% of the testosterone in pregnant guinea pig serum is transported into the newborn brain indicates that PBG-bound steroid is transported through the BBB.

As discussed previously for the adult rat brain (11, 23), the transport into the newborn rabbit brain of albumin-bound or PBG-bound steroid hormone is due to the rapid rate of steroid debinding relative to the brain capillary transit time. Conversely, the debinding reaction of SHBG-bound or antibody-bound steroid hormone is slow relative to the brain capillary transit time, and this accounts for the absence of BBB transport of steroid bound to an antibody or to the SHBG of human serum (11, 21, 23). Therefore, the physical basis to the wide spectrum of protein-bound steroid hormone transport into either the newborn or adult brain is the relative relationships between ligand debinding rates and brain capillary transit time (10–13, 21, 23, 31).

Finally, the observation that testosterone or estradiol bound to the SHBG of human serum is not transported

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into the newborn rabbit brain suggests that globulin-bound steroid may similarly not be transported into the human fetal or neonatal brain. Therefore, changes in fetal or postnatal levels of unbound SHBG, which is a function of both the total SHBG level and the high circulating level of estradiol, would be expected to modulate the albumin-bound fraction of circulating testosterone and estradiol and thereby alter the transport of these two steroid hormones into the developing brain in man.

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References

1. Moore TA, Lione AP, Regen DM 1973 Effect of thyroid hormone on cerebral glucose metabolism in the infant rat. Am J Physiol 225: 925
2. Daniel PM, Love ER, Pratt OE 1975 Hypothyroidism and aminoacid entry into brain and muscle. Lancet 2:872
3. Geel SE, Valcana T, Timiras PS 1967 Effect of neonatal hypothyroidism and of thyroxine on L-[^{14}C]leucine incorporation in protein *in vivo* and the relationship to ionic levels in the developing brain of the rat. Brain Res 4:143
4. Patel MS 1979 Influence of neonatal hypothyroidism on the development of ketone-body-metabolizing enzymes in rat brain. Biochem J 184:169
5. Wechsel ME 1978 Thyroid hormone replacement therapy in the perinatal period: neurologic considerations. J Pediatr 92:1035
6. Ehrhardt AA, Meyer-Bahlburg HFL 1979 Prenatal sex hormones and the developing brain: effects on psychosexual differentiation and cognitive function. Annu Rev Med 30:417
7. Gorski RA 1973 Perinatal effects of sex steroids on brain development and function. Prog Brain Res 39:159
8. Robbins J, Rall JE, Gorden P 1974 The thyroid and iodine metabolism. In: Bondy PK, Rosenberg LE (eds) Duncan's Disease of Metabolism. Saunders, Philadelphia, p 1023
9. Anderson DC 1974 Sex-hormone-binding globulin. Clin Endocrinol (Oxf) 3:69
10. Pardridge WM, Mietus LJ 1980 Influx of thyroid hormones into rat liver *in vivo*: differential availability of T_4 and T_3 bound by plasma proteins. J Clin Invest 66:367
11. Pardridge WM, Mietus LJ 1979 Transport of steroid hormones through the rat blood-brain barrier. Primary role of albumin-bound hormone. J Clin Invest 64:145
12. Pardridge WM, Mietus LJ 1979 Transport of protein-bound steroid hormones into liver *in vivo*. Am J Physiol 4:E367
13. Pardridge WM 1979 Carrier-mediated transport of thyroid hormones through the rat blood-brain barrier: primary role of albumin-bound hormone. Endocrinology 105:605
14. Oldendorf WH 1970 Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. Brain Res 24: 372
15. Braun LD, Cornford EM, Oldendorf WH 1980 Newborn rabbit blood-brain barrier is selectively permeable and differs substantially from the adult. J Neurochem 34:147
16. Oldendorf WH, Braun LD 1976 Tritiated (^{3}H) tryptamine and tritiated ^{3}H-water as diffusible and internal standards for measuring brain extraction of radio-labeled substances following carotid injections. Brain Res 113:219
17. Pardridge WM, Moeller TL, Mietus LJ, Oldendorf WH 1980 Blood-brain barrier transport and brain sequestration of the steroid hormones. Am J Physiol 239:E96
18. Pardridge WM, Oldendorf WH 1975 Kinetics of blood-brain barrier transport of hexoses. Biochim Biophys Acta 382:377
19. Hernandez MJ, Brennan RW, Vannucci RC, Bowman GS 1978 Cerebral blood flow and oxygen consumption in the newborn dog. Am J Physiol 234:R209
20. Sutherland RL, Brandon MR 1976 The thyroxine-binding properties of rat and rabbit serum proteins. Endocrinology 98:91
21. Pardridge WM, Mietus LJ, Frumar AM, Davidson BJ, Judd HL 1980 Effects of human serum on the transport of testosterone and estradiol into rat brain. Am J Physiol 239:E103
22. Westphal U 1978 Steroid-binding serum globulins: recent results. In: O'Malley BW, Birnbaumer L (eds) Receptors and Hormone Action. Academic Press, New York, p 443
23. Pardridge WM, Mietus LJ 1980 Effects of progesterone-binding globulin *versus* a progesterone antiserum on steroid hormone transport through the blood-brain barrier. Endocrinology 106:1137
24. Hagen GA, Solberg Jr LA 1974 Brain and cerebrospinal fluid permeability to intravenous thyroid hormones. Endocrinology 95: 1398
25. Dratman MB, Crutchfield FL 1978 Synaptosomal ^{125}I triiodothyronine after intravenous ^{125}I thyroxine. Am J Physiol 235:E638
26. Diamond JM, Wright EM 1969 Molecular forces governing non-electrolyte permeation through cell membranes. Proc Roy Soc Lond [Biol] 172:273
27. Rapoport SI 1976 Blood-brain barrier. In: Physiology and Medicine. Raven Press, New York, p 166
28. Fischer HW 1974 Contrast media. In: Newton TH, Potts DG (eds) Radiology of the Skull and Brain Angiography. Mosby, St. Louis, p 893
29. Fisher DA 1975 Thyroid function in the fetus. In: Fisher DA, Burrow GN (eds) Perinatal Thyroid Physiology and Disease. Raven press, New York, p 21
30. Goresky CA, Rose CP 1977 Blood-tissue exchange in liver and heart: the influence of heterogeneity of capillary transit times. Fed Proc 36:2629
31. Pardridge WM 1979 Tryptophan transport through the blood-brain barrier: *in vivo* measurement of free and albumin-bound amino acid. Life Sci 25:1519
32. Mickelson KE, Petra PH 1978 Purification and characterization of the sex steroid-binding protein of rabbit serum. J Biol Chem 253: 5293
33. Winter JSD, Faiman C, Reyes FI, Hobson WC 1978 Gonadotrophins and steroid hormones in the blood and urine of prepubertal girls and other primates. Clin Endocrinol Metab 7:513

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