Brain Research, 554 (1991) 229–236

ADONIS 000689939116800V

BRES 16800

Transport of iodothyronines from bloodstream to brain: contributions by blood:brain and choroid plexus:cerebrospinal fluid barriers

Mary B. Dratman[1–3], Floy L. Crutchfield[1] and Monika B. Schoenhoff[2]

[1]*Department of Medicine, Medical College of Pennsylvania,* [2]*Department of Psychiatry, University of Pennsylvania and* [3]*Medical Research Service, Philadelphia VA Medical Center, Philadelphia, PA 19104 (U.S.A.)*

(Accepted 19 February 1991)

Key words: Thyroid hormone; Thyroxine; Triiodothyronine; Circumventricular organ; Transthyretin; Retinol binding protein; Cerebral blood flow

Thyroid hormone entering the brain from the cerebral circulation must first cross barriers at the the blood:brain and choroid plexus:cerebrospinal fluid interfaces. The route taken after entry through those barriers might bring about selective delivery of hormone to different regions of the brain and those differences might be crucial for the ultimate functional effects of the hormone. To determine whether and how distribution of hormone in the brain might vary according to the route of entry, film autoradiograms of serially sectioned brains were prepared after delivery of a pulse of ^{125}I-labeled thyroid hormone into either the right lateral cerebral ventricle or the femoral vein. The results after intrathecal injection, reflecting the penetration of hormone into brain after crossing the choroid plexus:cerebrospinal fluid barrier, revealed a markedly limited, essentially periventricular distribution of radioactivity at both 3 and 48 h after hormone administration. Results after i.v. administration, which allows hormone access across both barriers, revealed an initial distribution pattern (at 3 h) generally similar to that seen after administration of markers of cerebral blood flow; at 48 h there was strong resolution in selected brain regions never noted to be labeled after intrathecal hormone injection. The functional implications of the differences in results produced by the two different routes of hormone entry are not known. However, ready access to circumventricular organs would appear to be favored by hormone crossing the choroid plexus:cerebrospinal fluid barrier whereas access to the panoply of nuclear triiodothyronine receptors would be favored by hormone crossing the blood:brain barrier. Therefore both routes of barrier transport should be taken into account in assessing the kinetics and actions of thyroid hormones in the central nervous system.

INTRODUCTION

Although the nature of thyroid hormone action in the mature brain is not understood, well confirmed observations demonstrate that thyroxine (T_4) entering the brain is rapidly converted to triiodothyronine (T_3)[8,15] and that T_3 is differentially bound[21] and processed in discrete neural elements of rat central nervous system[11]. Because there is considerable overlap in the regional binding of the hormone and the regional sites of synthesis of nuclear receptors for iodothyronines[3], it is reasonable to anticipate that thyroid hormones would mediate effects on gene expression at those sites. However, efforts to identify such effects have led to inconclusive results[20], and the role of T_3 nuclear receptors in mature brain remains a subject of continuing controversy.

To evaluate the kinetics of thyroid hormone binding to its known and possibly still unknown brain receptors, knowledge of the mechanisms controlling hormone entry into the brain is essential. Some years ago Pardridge demonstrated that T_4 and T_3 could be detected in brain tissue within 15 s after injection as a bolus into the carotid artery[16]. Using the Oldendorf technique, which compares the uptake into brain of the substance under investigation with that of tritiated water, Pardridge identified a saturable, facilitated transport mechanism for thyroid hormones operating at the blood:brain barrier (BBB). While not ruling out a role for the choroid plexus:cerebrospinal fluid (CSF) barrier (CSF:B) in the delivery of these hormones to the brain, it was noted that some regions not proximate to brain ventricles and choroid plexi were nevertheless rapidly labeled after injection of ^{125}I-iodothyronines into the carotid artery.

New information bearing on this issue has recently emerged (comprehensively reviewed in ref. 2) demonstrating that a well-studied serum thyroid hormone-binding protein, thyroxine binding prealbumin, now generally called transthyretin (TTR), is highly localized in the choroid plexus[1] where it is synthesized at rates far exceeding those in liver[7,23]. While TTR made in liver is secreted into the blood stream, TTR in the choroid plexus is secreted directly into the CSF[19]. Moreover, and

Correspondence: M.B. Dratman, Philadelphia VA Medical Center, University and Woodland Avenues, Philadelphia, PA 19104, U.S.A.

most interestingly, TTR synthesis is thought to be separately regulated in liver and choroid plexus. Thus, during acute inflammation, short-term food deprivation and marginal protein malnutrition, levels of TTR in serum fall markedly as a result of reduced hepatic synthesis of the protein whereas under these conditions, TTR levels in CSF do not change[5,24].

When the choroid plexus:TTR system was first described, the possibility arose that it might be an important or even the most relevant system mediating thyroid hormone transport into brain[6,19]. The autoradiographic observations here presented throw light on this issue in that the brain distribution of ^{125}I-iodocompounds after i.v. injection of the isotopically labeled thyroid hormones is compared with that found after direct intrathecal (i.th.) injection. In the former instance the hormones entering the brain can presumably gain access across both BBB and CSF:B. After i.th. injection, however, the entry of hormone into the brain reflects only the route taken by thyroid hormone molecules after they have crossed the choroid plexus CSF:B.

MATERIALS AND METHODS

Preparation of animals and injection of isotope

Adult male rats weighing ~200 g were received from Zivic Miller Laboratories 4 days after thyroparathyroidectomy; they were maintained in the animal facility under standard conditions of temperature and diurnal lighting and were given ad libitum access to regular rat chow and drinking water containing 0.5% calcium chloride. As in our previous autoradiographic experiments[9-11] the present studies were carried out within 7 to 10 days following removal of the thyroid gland.

High sp.act. ^{125}I-iodothyronines (~3000 μCi/μg) in 50% propylene glycol were received from Abbott Laboratories and analyzed for purity by means of descending paper chromatography (tertiary amyl alcohol:hexane:2 N ammonia 5:1:6); the isotopic preparation was diluted 1:10 with saline and delivered into the right lateral cerebral ventricle as a single dose (~1 μCi in a volume $\leq$ 4.0 μl). This dose was chosen because it approximates the amount of isotopically labeled hormone which accumulates in the brain of a 200 g rat after i.v. injection of 200 μCi labeled hormone (i.e. 1 μCi per g b. wt.). Using an injection procedure similar to that described previously[13], rats were anaesthetized with i.p. Nembutal (40 mg/kg) and atropine (0.1 mg/kg); when unresponsive to stimulation, the rats were placed in a stereotactic apparatus and, using a Hamilton syringe, 4.0 μl of the diluted hormone solution was introduced through a burr hole into the right lateral cerebral ventricle (coordinates 0.6 mm posterior to bregma, 1.5 mm lateral to sagittal sinus and 3.0 mm below dura); rats were decapitated at either 3 or 48 h after i.th. injection of the labeled hormone. A total of 14 animals successfully injected i.th. with labeled hormone and surviving in an apparently healthy state throughout the period of observation were used for autoradiographic studies. The time intervals chosen were based on previous autoradiographic observations[9,10] demonstrating that at 3 h, i.v.-delivered hormone has labeled all sites in which cerebral blood flow has largely determined the limits of hormone distribution (although not of hormone resolution). At 48 h, on the other hand, strong resolution in some regions and complete loss of previously localized hormone in others has led to a distribution pattern which appears by all criteria to be correlated with factors other than blood flow. Moreover, the autoradiographic maps prepared at the 48 h time interval after i.v. hormone delivery show considerable similarity to the maps obtained from in situ hybridization studies of message for c-*erb* A receptor subtypes[3].

Autoradiograms prepared from 16 rats given i.v. labeled hormones and decapitated at the same time intervals were used as the basis for comparison with autoradiograms from i.th.-injected animals. To control for the effects of anaesthesia during the early period of hormone uptake and processing in the brain in the i.th.-injected animals, some rats were given i.v. hormone while under the influence of Nembutal and atropine, exactly as described in the protocol used for i.th. hormone injection. To determine whether distribution of the radioisotope would differ in ^{125}I-T_4 (T_4*) as compared with ^{125}I-T_3 (T_3*)-injected rats, some rats were injected i.th. with T_4*. The autoradiographic results obtained were then compared with those found after i.th. T_3* administration. Because no qualitative differences were observed, the results refer collectively to the iodothyronines, T_3 and T_4. Only the more complete data from the T_3*-labeled rats are shown in the figures.

Preparation of autoradiograms

Methods for preparing film autoradiograms of rat brain after injection of labeled thyroid hormones have been described in detail[9,10,22]. Briefly: following decapitation by guillotine, brains were rapidly removed, frozen in liquid freon, placed on a chuck and serial 20 μm thick coronal sections cut in a cryostat were placed on coverslips and dried at 50 °C; in the darkroom, under safelight, the sections were apposed to tritium-sensitive no-screen film for periods of time which were inversely correlated with the dose of labeled hormone administered (approximately 15 days/1.0 μCi/1.0 g b. wt. for i.v.-treated rats and 15 days/1.0 μCi/200 g rat receiving the labeled hormones via the i.th. route). Following development with D-19 developer and Kodak rapid fix, the films were air dried and examined in a Jena microfilm enlarger to facilitate qualitative comparisons between results obtained from the i.th. vs the i.v. route of hormone administration.

Presentation of autoradiographic data

To demonstrate details of the labeling patterns, photographs of individual brain sections were taken at the level of (1) the original injection site (right lateral ventricle), which level provided views of the contralateral ventricles as well as coronal views of cerebral cortex, anterior thalamus, hypothalamus and 3rd ventricle; (2) the 4th ventricle, providing views of the cerebellum and brainstem.

Estimation of extent of hormone recycling from brain to blood to brain after i.th. administration of labeled thyroid hormones

In previous experiments, we compared immunoassayable T_3 levels in serially collected venous blood samples drawn at intervals after i.v. vs i.th.-T_3 injection[13]. On the basis of the dose of i.th. T_3* used in the present experiments, it was possible to calculate how much hormone would be expected to enter the general circulation from the brain over the 48 h period of observation, and whether any of that circulating T_3* would be expected to recycle through the brain. A similar approach is valid for estimating the potential for T_4 recycling after i.th. injection.

RESULTS

Note regarding the nature of labeled iodocompounds in brain after i.v. injection of ^{125}I-iodothyronines

As discussed in previous reports from our laboratory, autoradiographic data obtained after administration of labeled ligands provide limited biochemical information in that they reflect only the distribution of *total* radioactivity in the tissue. To address this problem, we carried out biochemical analyses of brain radioactivity at fre-

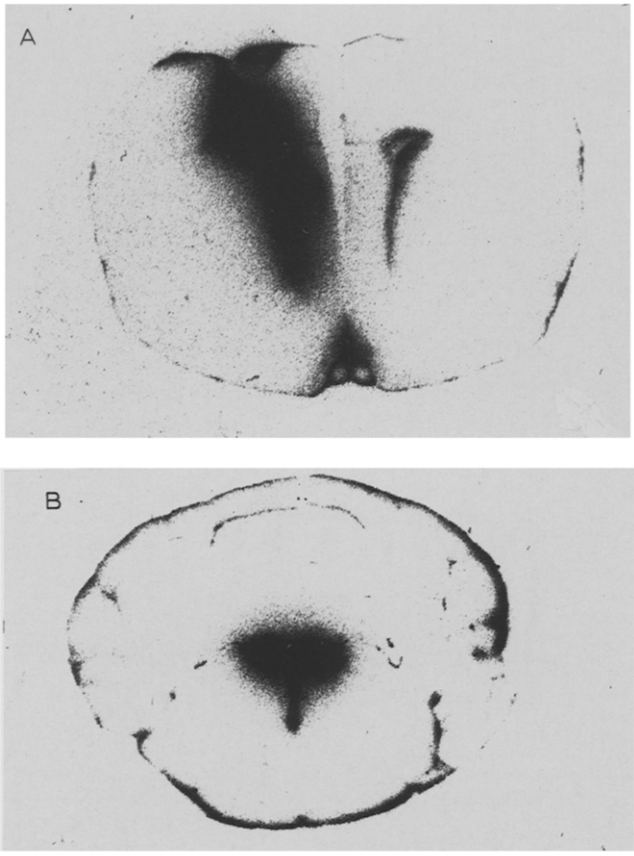

Fig. 1. Distribution of radioactivity in brain 3 h after i.th. injection of ^{125}I-T_3. Animals were handled and autoradiographic procedures were performed as described in Materials and Methods. Injection was made through the overlying cortex and corpus callosum into the right lateral ventricle. A: activity is noted at the site of injection, in the contralateral ventricle, in the third ventricle, in the tissues immediately surrounding the labeled ventricles and in the arachnoid membrane. Note lack of labeling of optic nerves despite high degree of activity in the immediate surround. B: shows dense accumulation of label in the region of the 4th ventricle as well as in the arachnoid membranes surrounding brainstem and lobules of cerebellar cortex. Label in caudal regions of arachnoid is more intense than that in more rostral regions (cf A).

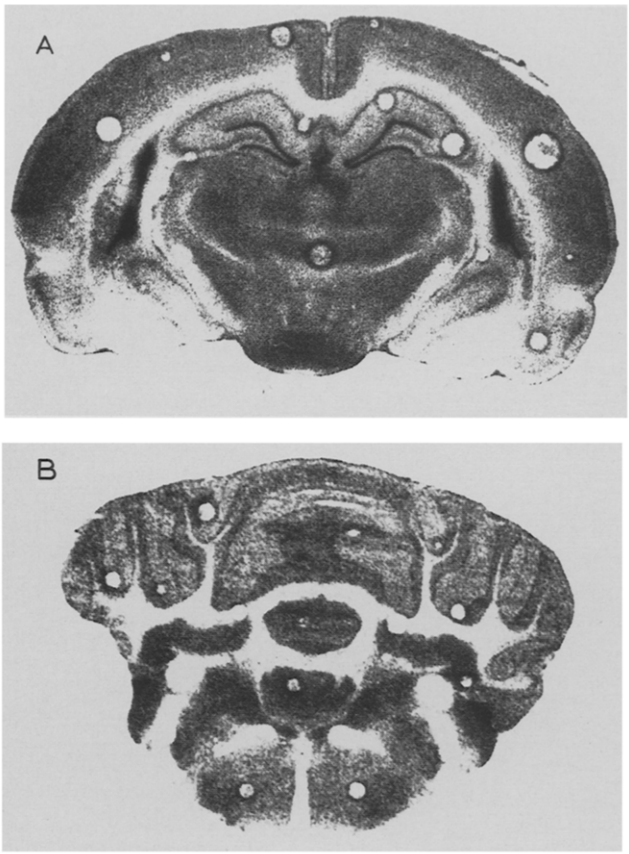

Fig. 2. Distribution of radioactivity in brain 3 h after i.v. ^{125}I-T_3 injection. A: section shows strong labeling in the lateral ventricles as well as localization, with some degree of resolution, in cortex, dentate gyrus, habenulae, thalamic nn, and hypothalamus (for more details of autoradiographic features found at this time interval see ref. 10). B: strong labeling is seen in 4th ventricle and in brainstem and cerebellar cortical parenchyma. Note that resolution in the granule cell layer is already detectable at this time.

quent intervals after i.v. injection of labeled T_3[10] and T_4[9]. In those experiments, chloroform:methanol extraction and HPLC analyses revealed that more than 85% of radioactivity in brain was, at both 3 and 48 h, due to iodothyronine (unmetabolized labeled T_3 in the case of injected T_3*; labeled T_4 + labeled T_3 in the case of injected T_4*). The analyses also demonstrated that labeled iodide concentrations were usually less than 5% and did not exceed 7% of total tissue radioactivity. Although both T_3 and T_4 are rapidly metabolized in the rat brain, their metabolites must be rapidly cleared from the brain parenchyma. Thus, the autoradiographic patterns noted after i.v. injection, which allows delivery across both BBB and CSF:B, are mainly due to labeled iodothyronines.

Comparison of results after i.v. and i.th. injection of labeled iodothyronines

Early time interval observations. Autoradiograms prepared after i.th. iodothyronine injection demonstrate the limited extent to which hormone circulating in CSF penetrates into the brain. As determined from study of film autoradiograms derived from serially sectioned brains obtained 3 h after i.th. T_3* injection, localization is primarily at the site of hormone deposition in the right lateral ventricle and its surround, and in the vicinity of the 3rd and 4th ventricles, whereas labeling in the tissue surrounding the contralateral ventricle is relatively less. Selected views of the film autoradiograms at the level of the injection site and the 4th ventricle are seen in Fig. 1. These show the intense and rather exclusive labeling of periventricular regions which develops within 3 h after i.th. administered hormone.

In contrast to the foregoing results, autoradiographic patterns emerging 3 h after i.v. hormone delivery show that labeling is prominent in the ventricles, but is also

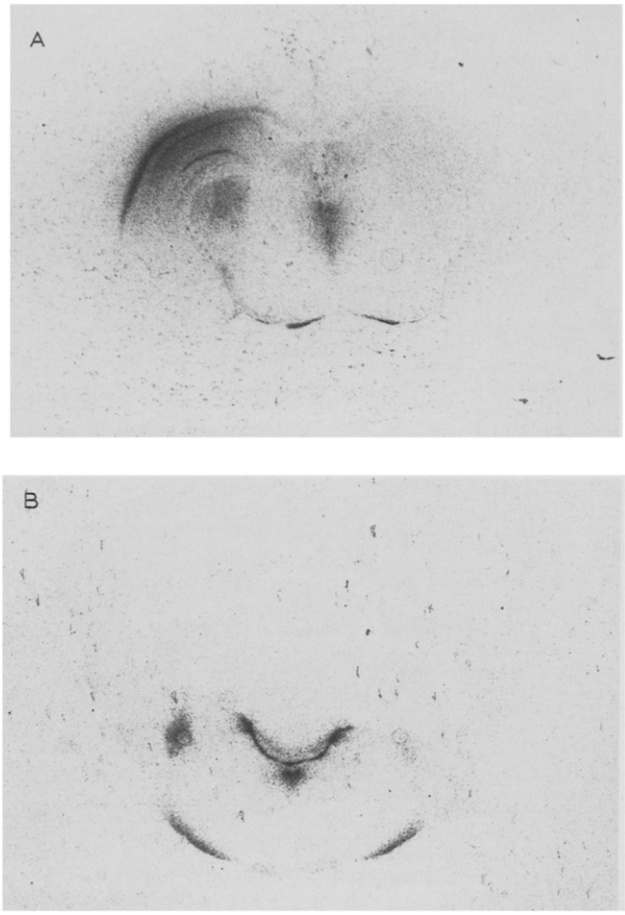

Fig. 3. Distribution of radioactivity in brain sections processed 48 h after i.th. injection of ^{125}I-T_3. A: to highlight to the maximum the labeling pattern seen in this autoradiogram, photographic exposures were prolonged, resulting in a considerable increase in background noise. Label is noted in the right lateral and 3rd ventricles and in the hippocampus, now considerably resolved in the granular and pyramidal cell layers. Radioactivity has also extended beyond the site of injection to produce labeling of mediodorsal and (homolateral) ventromedial thalamic nn; label is also noted in the supraoptic nn proximate to the (no longer detectably labeled) arachnoid membranes. B: this similarly overexposed photograph of the autoradiogram at the level of the cerebellum reveals labeling of both cochlear nn but with greater prominence on the side homolateral to the injection site. Note label in the trapezoid bodies with a suggestion of labeling in the pyramids, both structures proximate to the arachnoid membranes.

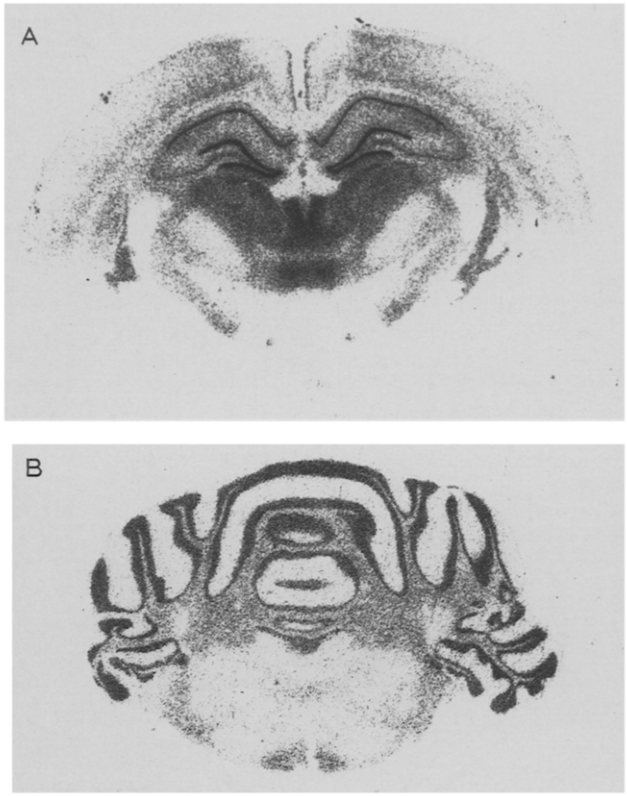

Fig. 4. Distribution of radioactivity in brain sections obtained 48 h after i.v. ^{125}I-T_3 injection. A: at the level of the hippocampus, labeling is most prominent in the dentate gyrus, showing strong resolution in the granule cell layer and crisply resolved labeling of the pyramidal cell layer. Note differential activity from one thalamic nucleus to another. Fornix and other fiber tracts are also selectively labeled (see ref. 10 for further details). B: the cerebellar cortex shows a high degree of resolution of radioactivity in the granular layer and almost complete disappearance of label from the molecular layer. Some regions of cerebellar white matter are now labeled as are the cerebellar peduncles, spinal tract of the Vth cranial nerve and the pyramids.

generally (albeit selectively) distributed throughout the grey matter, as seen after administration of markers of cerebral blood flow. Label is particularly prominent in cerebral cortex, basal ganglia, thalamus, colliculi, brainstem and cerebellum. All of these regions are entirely unlabeled at the 3 h time interval after i.th. injection, even when film and photographs are greatly overexposed (data not shown). In Fig. 2, showing autoradiograms from i.v.-injected rats, evidence of beginning resolution in hippocampal and cerebellar cell layers is in marked contrast with the unresolved periventricular activity seen in autoradiograms prepared after i.th. injection (as seen in Fig. 1).

Late time interval observations. Results obtained 48 h after i.th. injection of labeled hormone demonstrate that local distribution at the injection site persists as the most prominent of the labeled regions, with a high degree of activity noted in hippocampus (especially in dentate gyrus and region of the fornix) and to a lesser degree, in the region of 3rd and 4th ventricles. As seen in Fig. 3, poorly resolved activity in periaqueductal grey, subiculum, medial geniculate nucleus and fornix indicates that the hormone has penetrated into or has persisted in some regions beyond the periventricular regions. These results contrast with the considerable degree of resolution found within selected hippocampal laminae (pyramidal and granule cell layers of dentate gyrus). Moreover, prominent labeling in the trapezoid bodies and supraoptic nn suggests that these structures may take up and concentrate CSF-localized hormone circulating in the subarach-

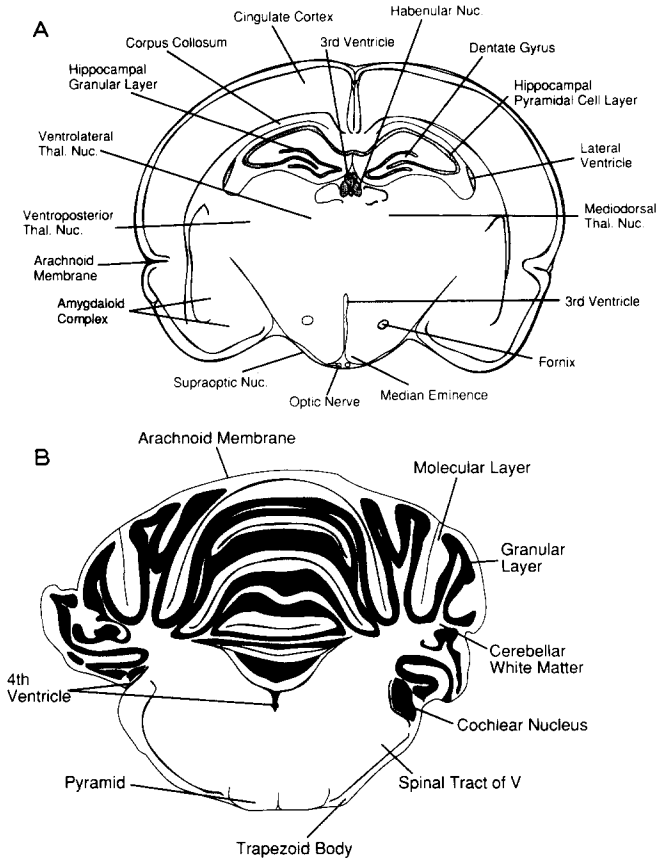

Fig. 5. Schematic representation of brain sections and brain structures described in Figs. 1–4. A: brain section taken at the level of the lateral and 3rd ventricles showing hippocampus, thalamus and hypothalamus. B: brain section taken at the level of the 4th ventricle, showing cerebellum and brainstem.

noid space. Because labeling of the arachnoid appears to be more evident in caudal vs rostral brain sections, subcompartments of the CSF pool may exist or different regions of the brain membranes may differ in their capacity for binding the hormone.

Views of corresponding brain sections labeled by means of i.v.-injected hormone (Fig. 4) show that after 48 h, differential labeling is strongly delineated in cortical laminae, hippocampus, thalamic nuclei, cerebellum and selected fiber tracts, as characterized more fully in ref. 10. However, despite the necessarily limited views provided by these autoradiograms, they serve to highlight the marked differences which characterize the late-appearing labeling patterns engendered by i.th. as compared with i.v. delivery of hormone.

Comparison of results after injection of T_3 vs T_4**

If TTR in choroid plexus epithelial cells has the same binding specificities as TTR in serum, T_4 rather than T_3 transport into the CSF will be favored. Although transport across the choroid plexus CSF barrier is not an issue in experiments involving direct i.th. injection of hormone, TTR is also abundant in CSF where T_4 rather than T_3 binding would presumably also be favored. Therefore, autoradiographic results after i.th. injection might differ according to the iodothyronine used for injection.

Previous autoradiographic studies carried out after bolus i.v. injections of ^{125}I-iodothyronines demonstrated that except for a time lag, and longer duration of ventricular (presumably choroid plexus) labeling in the case of T_4*, patterns of labeling after T_3* and T_4* were similar[9]. Parallel biochemical studies revealed that T_4 reaching the brain was rapidly and efficiently converted to T_3[8]. If, on the other hand, the process of intracerebral conversion of T_4 to T_3 was inhibited (as is the case in ipodate-treated animals), marked disruption of brain T_4* labeling patterns resulted. These observations indicate that well-resolved labeling of discrete brain regions noted after T_4* administration reflects the localization in these regions of T_3* generated in situ from T_4*. As expected, the present studies showed that labeling patterns after i.th. T_4* were qualitatively similar to those found after i.th. T_3* even when the observations were prolonged for as long as 72 h. To simplify the presentation, all of the autoradiographic data shown in the figures are from T_3*-injected animals.

Effect of anaesthesia on labeling patterns after i.v. hormone delivery

To prevent the additional trauma to the brain which the use of an implanted catheter would entail, we injected the i.th. dose of ^{125}I-iodothyronine through a Hamilton syringe equipped with a 27 gauge needle while the rats were in the stereotaxic apparatus, under the influence of Nembutal anaesthesia. As a result, the early phase of hormone processing took place in the i.th.-injected rats while they were in, or slowly emerging from anaesthesia. To control for the effect of anaesthesia, some rats were given i.v. labeled hormone while under the same conditions of anaesthesia with Nembutal and atropine. Autoradiograms prepared from the brains of the latter animals at the 3 h post-injection time showed no discernible changes in hormone distribution patterns in brain as compared with those from conscious animals. However, the kinetics of hormone uptake and disposal were found to be altered by the anaesthesia, as discerned from an approximately 1.6-fold higher level of serum radioactivity and a 2.5-fold greater level of brain radioactivity in the anaesthetized as compared with unanaesthetized rats. Not unexpectedly, effects of anaesthesia were no longer discernible in the rat brains examined at the 48 h post-injection period, at which time both sets of animals showed similar amounts of serum and brain hormone levels as well as similar patterns of hormone distribution in brain.

Does hormone recycling play a role in results after i.th. hormone injection?

Evidence previously presented[13] demonstrates that a considerable fraction of an i.th.-administered dose of T_3 appears in the blood stream within 3 h after i.th.-injection. Therefore the possibility of hormone recycling through the BBB after i.th. hormone injection was considered. However, the actual quantity of hormone delivered from brain to blood is trivial when compared to the size of the serum hormone pool and there is no theoretical basis for concern about recycling. In these experiments 1 μCi of labeled hormone was delivered into the lateral ventricle. Even if all of the injected hormone had found its way back into the serum within the period of observation, less than 0.01% of this amount would have reentered the brain over a 48 h period.

DISCUSSION

Hormone released from the thyroid gland soon equilibrates throughout the vascular system. Within the cerebral circulation, iodothyronines encounter the choroid plexus CSF:B and BBB. The free T_4 fraction in the blood flowing rapidly through the choroid plexus capillaries — 5-fold faster than through the entire brain[4] — may pass through the fenestrated choroid plexus capillary endothelium into the choroid plexus stroma and from there, by lipid partitioning, into the epithelial cell layer lining the ventricular surface[6]. However, outflow into the CSF from this layer (which constitutes the morphologic component of the CSF:B) would require a mechanism other than lipid partitioning because the concentration of free hormone in CSF is greater than that in blood (approximately 75 pM and 30 pM respectively). The mechanism which operates in the service of T_4 transport across this barrier has been studied extensively by Schreiber and his colleagues[19] who have shown that TTR is synthesized within and secreted from the ventricular surface of the epithelial cells into the CSF. Because TTR synthesis in the choroid plexus appears to be controlled separately from that of liver TTR[5,24] it is possible that its putative role in T_4 transport across the CSF:B may contribute importantly to thyroid hormone homeostasis and action in brain. Evidence consistent with a functional role for T_3 entering the brain via the CSF is provided by observations showing that a single dose of T_3 (1 μg/100 g b. wt.) delivered into the lateral cerebral ventricle, produced earlier and greater heart rate effects in hypothyroid rats than the same dose given i.v.[13].

In the case of the BBB, the tight junctions of brain capillaries exert differential limitations on entry, according to the nature of the substances seeking admission from the intravascular to the brain extracellular fluid compartment. As proposed by Pardridge[16,17], a saturable capillary endothelium-localized, carrier-mediated transport mechanism for iodothyronines competes with serum binding proteins for free iodothyronines oscillating in and out of the free state as the blood moves relatively slowly through the extensive brain capillary network. Additionally, and possibly most importantly, some tissue factor (either released from the capillary endothelium or ensuing from direct interaction of the hormone binding protein with elements of the endothelium) promotes enhanced dissociation of binding protein:hormone complexes in the cerebral capillaries.

It is noteworthy that after i.v. administration, autoradiographic patterns seen during the early phases of T_3* and T_4* uptake into brain are qualitatively similar to (though certainly not identical with) those derived from i.v.-injected markers of cerebral blood flow[9,18]. While the mechanism of hormone transport across the BBB may be uniform across the cerebral capillary bed, the distribution of iodothyronines carried across the tightly joined capillary endothelial cells will, at least initially, be determined by the characteristics of the cerebral circulation[25]. Therefore the autoradiographic evidence indicates that during early times after hormone administration, iodothyronine entry into the brain extracellular fluid space is largely due to BBB transport.

Nevertheless, even at the earliest time intervals studied, (< 1 min; data not shown) choroid plexus labeling was prominent. We originally assumed that this feature of brain labeling was largely due to radioactive iodide which, having been generated by enzymatic deiodination of labeled iodothyronine, was on its way from brain to blood through the agency of the choroid plexus iodide transport mechanism[26,27]. New information showing that choroid plexus synthesizes and secretes TTR provides another mechanism whereby labeling of this tissue (and iodothyronine transport into the brain via the CSF) might be promoted, namely through iodothyronine* binding to TTR. On this basis, significant contributions of the choroid plexus:CSF system to the process of hormone delivery to the brain are expected, and are likely to occur within a time frame which overlaps with that required for the hormone to cross the BBB. Differentiation between the two sources of hormone entry into brain cannot be made on the basis of observations derived only from data obtained after i.v. injection, which allows delivery of hormone across both barriers.

The data shown in Figs. 1–4 casts considerable light on this issue in that marked differences in brain labeling patterns were noted in rats given i.th. as compared with i.v. doses of hormone. In the former instance, labeling is most intense at the site of hormone deposition, which may to some extent, be due to leakage of hormone into

the brain through the track left by the needle as it was withdrawn from the lateral ventricle. However, all of the brains from i.th.-injected rats exhibited similar degrees of enhanced labeling in brain tissue surrounding the injected ventricle. This result suggests that each choroid plexus might play a distinctive role in contributing to one vs another periventricular region of brain tissue[14]. At the same time, evidence for considerable, though not necessarily uniform circulation of CSF-localized thyroid hormones through the ventricular system, is provided by results showing strong labeling in brain regions surrounding all of the brain ventricles as well as some brain regions proximate to the subarachnoid space. Clearly, in the normal course of events, all choroid plexi would participate at essentially the same time (although not necessarily at the same rate) in the transfer of hormone to the CSF. To appreciate the consequences, one would need to envisage the confluence of brain tissue labeling which would occur if delivery of hormone from each ventricle were occurring essentially simultaneously.

Among the many unresolved questions regarding thyroid hormone action in brain, the issue of iodothyronine access to sites involved in transduction of the hormone message is obviously important. Until more information is available, all aspects of this issue will be of interest. One example of the possible implications of hormone transport by TTR comes to mind: Forman et al.[12] have reported that retinoic acid and T_3, each bound to its (highly homologous) receptor, can form a heterodimer capable of binding to thyroid hormone response elements (TREs) upstream of the growth hormone gene. In view of the capacity of TTR to bind retinol binding protein, as well as T_4, coordinated retinol and thyroid hormone access to the brain might be a consequence of TTR localization in, and CSF-directed secretion from the choroid plexus. Thus, while it appears that iodothyronine transport across the BBB probably accounts for the major dynamics of iodothyronine transport into brain parenchyma, the results overall militate against a unitary hypothesis of thyroid hormone access to the brain.

Acknowledgements. This work is supported by the Pat Kind Fund for Brain Research and Public Health Service Research Grants MH45252 and MH44210. We thank Effie Erlichman for excellent technical assistance.

REFERENCES

1 Aleshire, S.L., Bradley, C.A., Richardson, L.D. and Parl, F.F., Localization of human prealbumin in choroid plexus epithelium, *J. Histochem. Cytochem.*, 81 (1983) 608–612.
2 Bartalena, L., Recent advances in studies of thyroid hormone binding proteins, *Endocr. Rev.*, 11 (1990) 47–64.
3 Bradley, D.J., Young III, W.S. and Weinberger, C., Differential expression of α and β thyroid hormone receptor genes in rat brain and pituitary, *Proc. Natl. Acad. Sci. U.S.A.*, 86 (1989) 7250–7254.
4 Cserr, H.F., Physiology of the choroid plexus, *Physiol. Rev.*, 51 (1971) 273–311.
5 Dickson, P.W., Aldred, A.R., Marley, P.D., Bannister, D. and Schreiber, C., Rat choroid plexus specializes in the synthesis and the secretion of transthyretin (prealbumin). Regulation of transthyretin synthesis in choroid plexus is independent from that in liver, *J. Biol. Chem.*, 261 (1986) 3475–3478.
6 Dickson, P.W., Aldred, A.R., Menting, J.E., Marley, P.D., Sawyer, W.H. and Schreiber, G., Thyroxine transport in choroid plexus, *J. Biol. Chem.*, 262 (1987) 13907–13915.
7 Dickson, P.W., Howlett, G.J. and Schreiber, G., Rat transthyretic (prealbumin): molecular cloning, nucleotide sequence and gene expression in liver and brain, *J. Biol. Chem.*, 260 (1985) 8214–8219.
8 Dratman, M.B. and Crutchfield, F.L., Synaptosomal [^{125}I]-triiodothyronine after intravenous [^{125}I]thyroxine, *Am. J. Physiol.*, 235 (1987) E638-E647.
9 Dratman, M.B. and Crutchfield, F.L., Thyroxine, triiodothyronine, and reverse triiodothyronine processing in the cerebellum: autoradiographic studies in adult rats, *Endocrinology*, 125 (1989) 1723–1733.
10 Dratman, M.B., Crutchfield, F.L., Futaesaku, Y., Goldberger, M.E. and Murray, M., [^{125}I]triiodothyronine in the rat brain: evidence for neural localization and axonal transport derived from thaw-mount film autoradiograpghy, *J. Comp. Neurol.*, 260 (1987) 392–408.
11 Dratman, M.B., Futaesaku, Y., Crutchfield, F.L., Berman, N., Payne, B., Sar, M. and Stumpf, W.E., Iodine ^{125}I-labeled triiodothyronine in rat brain: evidence for localization in discrete neural systems, *Science*, 215 (1982) 309–312.
12 Forman, B.M., Yang, C.R., Au, M., Casanova, J., Ghysdael, J. and Samuels, H.H., A domain containing leucine-zipper-like motifs mediate novel in vivo interactions between the thyroid hormone and retinoic acid receptors, *Molec. Endocrinol.*, 3 (1989) 1610–1626.
13 Goldman, M., Dratman, M.B., Crutchfield, F.L., Jennings, A.S., Maruniak, J.A. and Gibbons, R., Intrathecal triiodothyronine administration causes greater heart rate stimulation in hypothyroid rats than intravenously delivered hormone, *J. Clin. Invest.*, 76 (1985) 1622–1625.
14 Gross, P.M., *Circumventricular Organs and Body Fluids. Vols. I–III*, CRC Press, Boca Raton, 1987.
15 Kaplan, M.M. and Yaskoski, K.A., Phenolic and tyroxyl ring deiodination of iodothyronines in rat brain homogenates, *J. Clin. Invest.*, 66 (1980) 551–562.
16 Pardridge, W.M., Carrier-mediated transport of thyroid hormones through the blood-brain barrier: primary role of albumin-bound hormone, *Endocrinology*, 105 (1979) 605–612.
17 Pardridge, W.M. and Landaw, E., Tracer kinetic model of blood-brain barrier transport of plasma protein bound ligand. Empiric testing of the free hormone hypothesis, *J. Clin. Invest.*, 74 (1984) 745–752.
18 Sakurada, O., Kennedy, C., Jehle, J., Brown, J.D., Carben, G.L. and Sokoloff, L., Measurement of local cerebral blood flow with iodo[^{14}C]antipyrine, *Am. J. Physiol.*, 234 (1978) H59–H66.
19 Schreiber, G., Aldred, A.R., Jaworowski, A., Nilsson, C., Achen, M.G. and Segal, M.B., Thyroxine transport from blood to brain via transthyretin synthesis in choroid plexus, *Am. J. Physiol.*, 258 (1990) R338–345.
20 Schwartz, H.L., Effect of thyroid hormone on growth and development. In J.H. Oppenheimer and H.H. Samuels (Eds.), *Molecular Basis of Thyroid Hormone Action*, Academic Press, New York, 1983, pp. 413–444.
21 Schwartz, H.L. and Oppenheimer, J.H., Nuclear triiodothyronine receptor sites in brain: probable identity with hepatic receptors and regional distribution, *Endocrinology*, 103 (1978) 267–273.

22 Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M., Potlack, C.S., Pettigrew, K.D., Sakurada, O. and Shenohara, M., The ^{14}C deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anaesthetized albino rat, *J. Neurochem.*, 28 (1977) 897–916.
23 Soprano, S.R., Herbert, J., Soprano, K.J., Schon, E.A. and Goodman, D.S., Demonstration of transthyretin mRNA in the brain and other extrahepatic tissues in the rat, *J. Biol. Chem.*, 260 (1985) 11793–11798.
24 Wade, S., Bleiberg-Daniel, F. and Le Moullac, B., Rat transthyretin: effects of acute short-term food deprivation and refeeding on serum and cerebrospinal fluid concentration and on hepatic mRNA level, *J. Nutr.*, 118 (1988) 199–205.
25 Weiss, H.R., Buckweitz, E., Murtha, T.J. and Awletta, M., Quantitative regional determination of morphometric indices of the total and perfused capillary network in the rat brain, *Circ. Res.*, 51 (1971) 494–503.
26 Welch, K., Active transport of iodide by choroid plexus of the rabbit in vitro, *Am. J. Physiol.*, 202 (1962) 757–760.
27 Wright, E.M., Active transport of iodide and other anions across the choroid plexus, *J. Physiol.*, 240 (1974) 535–566.