Permeability of the Pineal Organ of the Golden Hamster (Mesocricetus auratus) to HRP with Special Reference to Different Types of Blood Capillaries

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Summary. The pineal organ of the golden hamster consists of deep and superficial portions which are connected to each other by a stalk. The permeability of capillaries for horseradish peroxidase (HRP) injected intravenously was examined in sections of entire portions of the gland that cut either along coronal or sagittal planes. Two distinct portions of the parenchyma, i.e., dorsal major and ventral minor ones, were found in the superficial gland. Most of the capillaries in the dorsal portion were of the continuous type of endothelium, whereas those in the ventral portion were fenestrated. In the dorsal portion, HRP readily crossed the endothelium, permeated the basal lamina, flowed into the perivascular connective tissue space and intruded into the intercellular clefts of the parenchyma. In contrast, HRP was not found to penetrate through the endothelium of the capillaries in the ventral portion to reach the perivascular area, thereby leaving the intercellular clefts of the parenchyma free of HRP. In the deep gland the capillaries were exclusively of the non-fenestrated type. Intravenously injected HRP was prevented from crossing the endothelium by the tight junction. In some areas, HRP penetrated through the capillaries in the pia mater, and crossed the outer limiting membrane to reach the intercellular clefts of the parenchyma and the basal lamina of the capillaries in the peripheral region of the deep pineal gland. The junctions between endothelial cells were not penetrated by HRP. The observations indicate that the type of capillary, absence of perivascular spaces, and permeability in the deep pineal are all similar to these factors in the general brain tissue; they differ from these in the superficial pineal gland, in which the dorsal portion shows characteristics found in other endocrine glands, but the ventral zone exhibits a unique situation: the presence of a blood-pineal barrier with a pericapillary connective tissue area.

The types of capillaries in the mammalian pineal gland show considerable interspecies variability. The fenestrated type of endothelial cell is present in the superficial pineal gland of the rat and ground squirrel (Matsushima and Reiter, 1975), ground squirrel (Povlishock et al., 1975), and mouse (Matsushima et al., 1989), whereas a continuous type is found in the gland of the cow and sheep (Anderson, 1965), monkey and cat (Wartenberg, 1968), pocket gopher (Sheridan and Reiter, 1973), human fetus (Møller, 1974), Mongolian gerbil (Welsh, and Reiter, 1978), and pig (Wyrzykowski, 1989). Conflicting results have been reported for capillary types in the superficial pineal gland of the golden hamster (Sheridan and Reiter, 1968; Clabough, 1971; Sheridan and Rollag, 1983; Hewing and Bergmann, 1985). They form the rationale for further studies on capillaries by systematic scanning over whole sections of the pineal organ in the golden hamster instead of the examination of randomly selected portions of the gland, the method hitherto conducted by most investigations.

One of the important potential routes of secretory products from pineal organ to target organs is the blood stream (Rollag et al., 1978; Clark et al., 1985). In relation to secretion into the blood stream, the permeability of the vascular system in the pineal gland is of interest. Using intravenous injections of horseradish peroxidase (HRP) and microperoxidase, the capillaries in the pineal gland of the mouse (Møller et al., 1978) and Mongolian gerbil (Welsh and Beitz, 1981) have been shown permeable to proteins and polypeptides, thus confirming the absence of a blood-brain barrier (BBB) in these species. The BBB has also been reported to be absent from the pineal gland.
of the rat (Wislocki and Ludec, 1952; Azzì et al., 1990; Rühle et al., 1992), rabbit (Møller, 1974), and Djungarian hamster (Møller and Van Veen, 1981), but present in the pineal gland of the cat (Møller, 1974).

As there have been no ultrastructural investigations on the permeability of proteins in pineal capillaries of golden hamsters, the purpose of the present investigation was to determine the types and permeability of blood capillaries in the superficial and deep pineal glands of adult golden hamsters having been intravenously injected with HRP. Preliminary findings of the study were reported at the symposium on "Melatonin and the Pineal Gland" in Hong Kong, 1988.

MATERIALS AND METHODS

Twenty adult male hamsters (Mesocricetus auratus) during the months from March to September were used. They were maintained in an environment of controlled photoperiods (14L: 10D, lights on 0500h) and temperature (23±2°C). Animals were killed between 0900-1100h. Anesthesia was induced with pentobarbital (40mg/100 g body weight) intraperitoneally. HRP (type VI, Sigma MO) administered at a dosage level of 10-12 mg/100 g body weight was dissolved in 0.5 ml normal saline. The tracer solution was injected into the femoral vein over a 30 sec period, and allowed to circulate for 1, 5, 10, 15 or 30 min. Most of the glands were fixed by immersion in the fixative, which consisted of 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4), whereas a few, by perfusion through the cardiac ventricle. The brain was removed, and tissue blocks containing the pineal complex were excised and fixed for an additional 90 min. Coronal or sagittal vibratome sections (50 or 100 μm thick) were incubated with 3,3′-diaminobenzidine tetrachloride (DAB) in 0.05 M Tris-HCl buffer and 0.01% H2O2. The 50 μm sections were picked up on slides and examined under a light microscope. For electron microscopy, the 100 μm sections were postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h. The tissue was then treated en bloc for 1 h with 2.5% uranyl acetate in maleate buffer, dehydrated in a graded series of ethanol, and embedded in Epon. Thin sections, which contained the whole area of the gland, were picked up on 150-mesh copper grids and stained lightly with lead citrate and scanned systematically in a Hitachi HU-12A electron microscope.

Three hamsters injected with 0.5 ml normal saline were used as controls for endogenous peroxidase.

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Fig. 1 a and b. Light photomicrographs of DAB-incubated thick sections of the superficial pineal gland from HRP-injected hamsters. a. A sagittal section, immersion fixation; circulation time, 15 min. Vascular networks, as shown by reaction products in the lumina, are much denser and reaction product in the extravascular matrix is darker in the dorsal major portion (above the dotted line) than that in the ventral narrow region (below the dotted line). b. A coronal section, perfusion fixation; circulation time, 30 min. Parenchyma of the dorsal region appears grey (brownish hue under the microscope, above the dotted line), whereas that in ventral zone is pale (colorless, below the dotted line). a, b: × 60

Abbreviations used in figures

A unmyelinated axon, CE choroid epithelial cell, E endothelial cell, G glial cell, L lumen, N nerve fiber, Pn perineurium, PP process of pinealocyte, PR pineal recess, RBC red blood cell, S Schwann cell, SPR suprapineal recess, * perivascular space.
Vibratome pineal sections from these animals were treated with the same procedures as those from the hamsters injected with HRP.

RESULTS

Pineal glands of control animals demonstrated no endogenous peroxidase in the parenchyma; peroxidase activity was observed only in the erythrocytes within the vascular lumen. There was no significant detectable difference in the distribution of the tracer among specimens from animals subjected to different circulation times of the injected HRP.

Superficial pineal gland

The sagittal (Fig. 1a) or coronal (Fig. 1b) sections of the superficial pineal gland from HRP-injected hamsters displayed two different tinctorial regions of the parenchyma after incubation in DAB as chromogen. A narrow ventral zone appeared colorless, whereas the remaining larger dorsal area exhibited a lightly brownish staining under the light microscope. The tinctorial difference between both regions was more readily observed in the material fixed by perfusion (Fig. 1b) than by immersion (Fig. 1a). Scanning by electron microscope of the entire area of thin sections of the superficial gland from three hamsters revealed a majority of capillaries in the dorsal portion to be of the continuous type, whereas most of those in the narrow ventral zone showed fenestrated endothelia (Table 1). Except for the luminal contents of the vessels, no significant differences in the fine structure resulted from the different methods of fixation. As will be described in detail later, the majority of continuous capillaries in the dorsal portion were seen permeable to HRP; a pronounced distribution of HRP reaction product occurred intercellular clefts of the dorsal parenchyma. In contrast, almost no HRP penetrated through the endothelium of the capillaries in the ventral portion. It is readily conceivable that the reaction product of HRP extravasated and permeated through the intercellular spaces of the parenchyma was responsible for tinging the dorsal area a brownish color, as shown by the light microscopy mentioned above. Also under the light microscope, the border between the dorsal and ventral portions was rather sharp; in the electron microscope the disappearance of reaction product present in the intercellular clefts of the dorsal parenchyma was abrupt, and no special device or structure to stop the passage of HRP was noticed at the border between the two portions.

Dorsal portion

As shown in Table 1, most of capillaries in this portion were of the continuous type and a few were fenestrated. In the endothelium of the continuous type, many HRP-containing vesicles were found near, or open to, the surface of either the luminal or the abluminal plasmalemma (Figs. 2, 3). Additionally, there were transendothelial channels apparently formed by the fusion of such vesicles (Fig. 3). Reaction product was also seen in the intercellular clefts of the endothelium. HRP permeated the basal lamina, flowed into the wide perivascular connective tissue area, and then into the intercellular spaces of the parenchyma (Fig. 3). Pinocytic uptake of HRP was observed in the pinealocytes and the glial cells (Figs. 2, 3).

In the fenestrated capillaries, HRP was present on both sides of the fenestrae, over the entire length of the interendothelial clefts, and in the perivascular space (Fig. 4). But in occasional fenestrated capillaries, in spite of HRP appearing on both sides of the fenestrae, its flow was seen stopped at the tight junction between endothelial cells. (Fig. 5).

It is of interest to note that reaction product could

<table>
<thead>
<tr>
<th>Capillary type</th>
<th>Dorsal portion</th>
<th>Ventral portion</th>
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<tr>
<td></td>
<td>Continuous</td>
<td>Fenestrated</td>
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<tr>
<td>HRP leakage</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hamster 1</td>
<td>32</td>
<td>0</td>
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<tr>
<td>2</td>
<td>34</td>
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<td>3</td>
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A plus sign (+) indicates HRP-leakage and minus sign (−) no leakage in the pericapillary area.

Table 1. Frequencies of the two types of capillaries and their permeability to HRP in dorsal and ventral portions of the superficial pineal gland as observed on single sections from three hamsters.
Figs. 2 and 3. Continuous capillaries in the dorsal portion of the superficial pineal gland; circulation time, 10 min. HRP is seen throughout the entire length of the interendothelial cleft (arrows), and in luminal and abluminal caveolae and vesicles, wide perivascular spaces, and intercellular spaces of the parenchyma. Pinocytotic vesicles of HRP are seen in the pinealocytes and glial cells. HRP is also present between nerve fibers (Fig. 2) and in the transendothelial channel (arrowheads) (Fig. 3). Inset: A higher magnification of the transendothelial channel (arrowheads), which is filled with HRP. Fig. 2: ×11,000, Fig. 3: ×10,700, Inset: ×40,000
Figs. 4 and 5. Fenestrated capillaries in the dorsal portion of the superficial pineal; circulation time, 5 min, HRP is present on both luminal and abluminal sides of fenestrae (arrowheads), and in perivascular spaces, and intercellular spaces of pineocytes. HRP is also present in the interendothelial clefts (arrow) (Fig. 4). Portions of the interendothelial cleft (arrow) are free of HRP (Fig. 5). Inset: A higher magnification of the interendothelial cleft (arrow) shows tight junctions, which prevent the flow of HRP. Fig. 4: ×28,000, Fig. 5: ×20,000, Inset: ×30,000

be seen in the intrapineal nerves containing both myelinated and non-myelinated nerve fibers; HRP permeated through the perineurial layer, pervaded the endoneurial spaces, and infiltrated between Schwann cells and unmyelinated axons (Fig. 6). HRP was not, however, observed penetrating the inner mesaxon of the myelinated nerve fibers.

Following the intravenous injection of HRP, the tracer crossed the fenestrated endothelium of capillaries in the dorsal pia mater (Fig. 9), entered the connective tissue area, and then the intercellular spaces of the dorsal pineal parenchyma. It was also present in the spaces of the basal infoldings and the basolateral intercellular spaces of the choroid epithe-
lium lining the suprapineal recess. Reaction product was also present in many vesicles and caveolae which were open to the basal plasmalemma of the epithelial cells. HRP was prevented from entering the suprapineal recess by the tight junction between the choroid epithelial cells.

**Ventral portion**
The vascular network in the narrow ventral portion of the superficial gland was less dense than in the dorsal one, and most of the capillaries observed were of the fenestrated type (Table 1, Figs. 7, 8). HRP was localized only within the lumen. The outer surface of the fenestrae along the abluminal plasma membrane of the endothelium, the basal lamina, and the pericapillary area were free of HRP. The endothelium was provided with tight junctions at which the flow of HRP stopped (Fig. 8). The intercellular clefts of the parenchyma were devoid of the reaction product. In the ventral pia mater too, HRP was restricted to the lumen of the fenestrated capillaries (Fig. 10).

**Deep pineal gland**
Light microscopy of sections of the deep pineal gland showed the HRP reaction product confined to be to the vascular lumen (Fig. 11). Capillaries were exclusively of the nonfenestrated type, and the endothelium was provided with tight junctions which prevented HRP from entering the intercellular clefts of the parenchyma (Figs. 12, 13). Endothelial cells displayed few HRP-containing vesicles. No pericapillary connective tissue area was present, and the processes of glial cells besieged the basal lamina of the capillaries.

HRP leaked from the fenestrated capillaries in some areas of the pia mater (arrows in Fig. 11), permeated through the outer limiting membrane to reach the intercellular spaces of the pineal parenchyma near the surface, and occasionally extended to the basal lamina of the capillary and the perivascular area of larger vessels (Fig. 14). It seemed that the extravascular HRP in such parenchymal areas was not derived from capillaries in the deep pineal, because the latter were provided with tight junctions.
DISCUSSION

One of the most interesting findings in the present study was that two distinct portions of the parenchyma were discernible in the DAB-treated thick sections of the superficial pineal from HRP-injected hamsters: the dorsal major and the ventral minor portions of the gland. Electron microscopy allowed us to observe that HRP passed the capillaries in the dorsal portion via interendothelial clefts and transendothelial channels which were formed by the fusion of vesicles and caveolae associated with the luminal or abluminal endothelial cell membranes. In contrast, most of the capillaries in the ventral portion did not allow HRP to cross the endothelium, the presence of fenestrae notwithstanding. Our findings established the existence of a difference in permeability to the exogenous tracer between the dorsal and ventral portions of the superficial pineal gland.

Morphological investigations on capillaries of the mammalian pineal gland have revealed considerable interspecies differences, including the presence or absence of endothelial fenestrations, the extension and contents of the perivascular area, and the permeability of the capillaries (Waterberg, 1968; Matsumiya and Reitter, 1975; Vollrath, 1981; Hewing and Bergmann, 1985; Bhatnager, 1988). The present observations substantiate that both continuous and fenestrated capillaries occur in the superficial pineal gland, while only the continuous type occurs in the deep pineal of the golden hamster (Hewing and Bergmann, 1985).

Owing to topographical scanning of the hamster superficial gland, the present study is first to reveal that the majority of capillaries in the dorsal portion are of the continuous type, whereas those in the ventral portion are fenestrated. This can explain the conflicting results in hamster pineal glands of previous investigations that apparently have disregarded the topographic difference in capillary type (Sheridan and Reitter, 1968; Clabough, 1971; Sheridan and Rollag, 1983; Hewing and Bergmann, 1985). It is therefore advisable in the study of the pineal gland...
organ is usually believed to be homogeneous (BEARER and ORCI, 1985). However, it may vary considerably in different organs; exogenous tracers cannot pass through the fenestrated capillary at all in the choroid of the eye (PINO and ESSNER, 1981), while they can freely permeate peritubular fenestrated capillaries of the kidney (VENKATACHALAM and KARNOVSKY, 1972). Variance in endothelial permeability has been shown within intestinal villi from the ileo-cecum of the rat (HART and PINO, 1985). The fenestrated capillaries at the tips of villi are more restrictive to the exogenous tracer molecules than those at the bases of villi. The authors have suggested that this apical restrictiveness functions to prevent excessive loss of plasma proteins to the intestinal lumen while degenerative epithelial cells are being sloughed off.

In the present study HRP was seen on both sides of the fenestrae and in the perivascular space of fenestrated capillaries located in the dorsal portion. The HRP outside of such capillaries might have come
from the interendothelial clefts of the same fenestrated capillary or from those of the continuous type present in the adjacent regions. Although our investigation could not determine whether or not the fenestrae of the endothelium in the dorsal portion could provide a route for HRP transport, a significant difference in HRP distribution around the fenestrated capillaries indicates that the permeability to the exogenous protein in the dorsal major portion was different from that in the ventral minor one of the superficial pineal. In the meanwhile, we also observed a similar regional difference in the permeability of fenestrated capillaries between the dorsal and ventral pia mater. These results indicate that the presence of endothelial fenestrae can not be used as a criterion for the determination of the permeability of capillaries to proteins.

It might be admitted that the parenchyma in the

**Fig. 10** a-c. A fenestrated capillary in the ventral pia; circulation time, 10 min. a. HRP is confined to the lumen, the luminal side of the interendothelial junction (arrow), and the fenestrae (arrowheads). The basal lamina (double arrow) and extravascular spaces (*) are free of HRP. b. A higher magnification of the tight junction (arrow) in the interendothelial cleft shown in the upper rectangle in Figure 10a. The tight junction prevents HRP from permeating through the interendothelial cleft. c. A higher magnification of the diaphragms of fenestrae (arrowheads) shown in the lower rectangle in Figure 10a. HRP reaction product is present only on the luminal side of the diaphragms of fenestrae. a: ×8,000, b: ×13,000, c: ×16,000

**Fig. 11.** Light photomicrograph of the DAB-incubated thick section of a frontal section through the deep pineal gland from an HRP-injected hamster; circulation time, 5 min. HRP reaction products is present in some areas (arrows) at the periphery of pineal parenchyma (see text). ×50
Figs. 12 and 13. Continuous capillaries in the deep pineal gland; circulation time, 5 min. The boxed area in Figure 12 is shown at a higher magnification in Figure 13. HRP is present in cytoplasmic vesicles and the luminal side of the intercellular cleft (arrow) of the endothelium. The basal lamina (double arrows) and the pineal parenchyma are free of HRP. The interendothelial cleft is closed by a tight junction (arrow), which stops HRP penetration. Fig. 12: ×7,500, Fig. 13: ×25,000

Fig. 14. A small vein near the pial surface of the deep pineal. HRP is seen in portions of the interendothelial cleft (arrows), between which there is a lack of HRP due to tight junctions. HRP is also present in the perivascular area and intercellular clefts (white arrows) of parenchyma. ×48,000
deep pineal and the narrow ventral zone of the superficial pineal gland would be less readily accessible to blood compounds as compared with that in the larger dorsal portion of the superficial pineal gland. In view of the reports on different morphological features of pinealocytes between the superficial and deep glands (Boeckmann, 1980, Matsushima et al., 1990) or between the cortical (peripheral) and medullary (central) portions of the superficial pineal gland (Vollrath, 1979), it would be of interest to examine whether or not the differences in vascular permeability are correlated with the functional organizations of parenchymal cells in the different areas of the gland.

As shown in the present study, the arrangement of the wide perivascular space in the superficial pineal with what appeared as extensions of this space into the pineal parenchyma allows for a large area of communication between the perivascular regions and the pineal gland proper. Wide perivascular spaces and their extensions into enlarged intercellular spaces have been described in the pineal gland of some mammals including the mouse (Ito and Matsushima, 1968, Matsushima et al., 1989), rat (Matsushima and Reiter, 1975), rabbit (Romin, 1973), and guinea pig (Lues, 1971). The perivascular space may be wide enough to harbor terminals of pinealocyte and glial processes, and collagenous fibers in addition to nerve fibers. The presence of IHRP reaction products throughout perivascular areas and intercellular spaces in the dorsal portion of the superficial pineal gland demonstrates a nonobstructed communication between the vascular system and the pineal parenchyma composed of pinealocytes and glial cells which can take up proteins from the blood. Although the present study has shown only the uptake of the peroxidase from the vasculature, one cannot exclude the possibility that the reverse could also be true, i.e., the pineal is capable of secreting its products into the vascular system via the same route.

In summary, as far as the type of capillary, the presence or absence of perivascular spaces, and permeability properties are concerned, the deep pineal gland displays the same features as the central nervous system, whereas in the superficial pineal gland the dorsal portion shows characteristics of the peripheral endocrine glands, and the ventral zone exhibits a unique situation: the presence of a blood-pineal barrier with a pericapillary connective tissue area.

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