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Serotonin in the human infant carotid body

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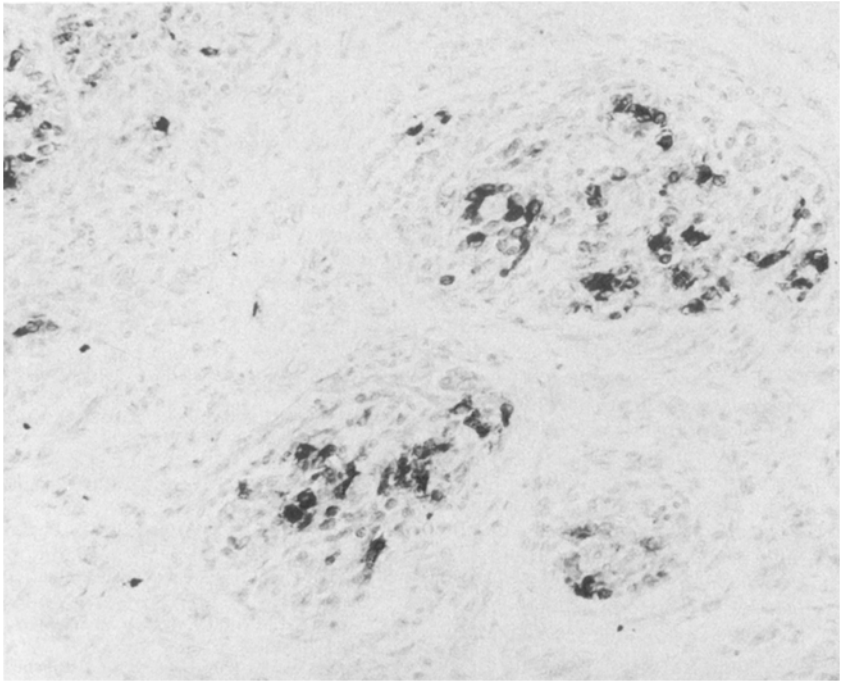
Summary. By immunocytochemistry serotonin was localized in the chief cells of the carotid body in human infants. Radioenzymatic measurement of the serotonin concentration revealed that it represents a significant proportion of the total amine content of the carotid body.
Key words. Carotid body; serotonin; human infant.

The carotid bodies (CB) are principal peripheral chemoreceptors involved in respiratory responses to hypoxia, hypercapnea and acidosis[1]. The morphology and physiology of CB has been reviewed previously[1-7]. The principal cells of CB are glomus or chief cells surrounded by sustentacular cells. The chief cells, considered to be the chemosensory element of CB, contain biogenic amines and peptides stored within the cytoplasmic dense core vesicles[8-16]. Dopamine and norepinephrine are thought to be the predominant amines in the CB of most animal species[8-14]. Although serotonin has been detected in CB of some species, including human, its concentration has been reported to be low compared to the other amines[15,16]. The content and cellular localization of serotonin in the human CB has received only limited study[16]. To our knowledge serotonin levels in the infant CB have not been reported. In this study, we report on the localization and measurement of serotonin in CB of normal infants using immunocytochemistry and a highly specific and sensitive radioenzymatic assay for serotonin.

Materials and methods. The CB were obtained from eight infants at autopsy 11–48 h after death (21.5 ± 4.1 h; mean ± standard error of mean). The infants died as a result of accidents or due to acute illnesses, and either were dead on arrival (DOA) at hospital or were treated for a short time prior to death. The age of infants ranged from 1–11 months (4.9 ± 1.3 months) and included five females and three males. In three patients the cause of death was acute meningitis (one of them DOA), two died from gastroenteritis (one of them DOA), and one each died from cold exposure, cardiac tamponade and a brain tumor.



Immunoperoxidase staining for serotonin in the carotid body of a 3-month-old infant who died from cold exposure. Note that both single and clusters of chief cells within the glomic area stain positively. The surrounding stroma is negative. × 75.

Under a dissecting microscope, the CB were dissected free of surrounding tissue and one was placed in Bouin's fixative for serotonin immunostaining, the other was immediately frozen and stored at −70 °C until biochemical analysis. An immunoperoxidase method according to Hsu et al.[17] using monoclonal antibody against serotonin (Sera Lab., UK) was employed for localization of serotonin in the CB. The immunostaining method and the specificity of the serotonin antibody have been previously reported[18]. Control studies for the immunoperoxidase method included omission of primary antiserum or replacement of primary antiserum with non-immune rabbit serum. The serotonin content of the CB was measured by the radioenzymatic method of Hussain and Sole[19].

Results. Sections of CB immunostained for serotonin showed a positive reaction localized exclusively within the cytoplasma of chief cells (fig.). The surrounding connective tissue, sustentacular cells and stroma were negative. The positively-stained chief cells were randomly distributed throughout the lobules and appeared as either single cells or as small clusters. The staining intensity varied between individual cells and in some cells short cytoplasmic processes were also positively stained.

The mean ± the standard error of the mean concentration of serotonin in the CB was 817.9 ± 130.5 ng/gm tissue; ranging from 416.0–1269.0 ng/gm tissue. Expressed as ng/single CB, the mean serotonin content was 5.51 ± 1.04 ng/single CB; ranging from 1.2–11.1 ng/CB. The mean weight of single CB was 7.1 ± 0.76 mg.

No significant differences in serotonin content of CB were found within the age range studied or with the length of the post-mortem interval.

Discussion. The presence of serotonin in the CB of the human and several animal species has been previously demonstrated[12, 15, 16, 20]. The overall conclusion from these studies is that the serotonin content in the CB represents only a small proportion of the total amine content. Hellstrom reported that serotonin accounts for only 14% of the total amine content in the CB of the adult rat, while dopamine and norepinephrine represent 54% and 32% respectively[12]. Furthermore, Bock and Lassmann reported that serotonin in the rat CB is not localized in the chief cells but instead is present in mast cells scattered within the interstitial tissue[21]. In adult human CB the presence of serotonin in chief cells has been previously demonstrated by the formaldehyde-induced fluorescence method[22] and serotonin was reported to represent 19% of the amine content, while dopamine and norepinephrine accounted for 64% and 15% respectively[16]. In the present study the mean serotonin content was found to be 5.51 ng/CB in infants less than one year of age. In a previous study we reported on the catecholamine content in CB from infants of a similar age; the mean content of dopamine, norepinephrine and epinephrine was 0.88 ng/CB, 1.10 ng/CB, and 0.14 ng/single CB respectively[23]. Using this data it appears that serotonin may represent up to 72% of the total amine content of the CB in infants of this age group. The relatively high proportion of serotonin found in our study (as compared to previous reports) may be due to several factors. Firstly, the serotonin concentration in CB of infants may be higher and subsequently change with development and maturation. Secondly, we have studied CB from infants who died acutely with little or no treatment prior to death; previous studies have shown that certain disease states and medications may affect serotonin levels in the human CB[16].

Immunostaining confirmed that a large proportion of chief cells stained positively for serotonin. In addition, serotonin was localized only in the chief cells and not in mast cells as reported for some animal species[24]. Based on a rough estimate, the percentage of serotonin immunoreactive chief cells appear to correlate with the biochemical determination of the serotonin content, i.e., serotonin represents a substantial proportion of the amine content. Whether each chief cell contains only one or more than one amine is unknown.

The presence of dopamine, norepinephrine and serotonin in CB is well established[8–16], however, the precise role for these monoamines in CB function has not been defined. It is generally accepted that dopamine exerts an inhibitory effect on respiration in most animal species[25–29]. In the human, administration of dopamine appears to inhibit respiration[30] as well as to dampen the ventilatory response to hypercapnic hypoxia[31]. The role of serotonin in the CB is less certain. In some animal species such as the dog, serotonin appears to stimulate respiration[26, 29]. Administration of serotonin in the dog[29], cat[32] and rabbit[33] has been shown to cause excitation of CB chemoreceptor neural activity followed by inhibition. Based on these studies it would appear that dopamine and serotonin exert opposite effects on the CB's control of respiration, dopamine being inhibitory, and serotonin stimulatory. The presence of relatively high concentrations of serotonin in CB of normal human infants is of interest and suggests an important role (possibly stimulatory) for this monoamine in the regulation of CB chemoreceptor function during this stage of development.

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The pineal gland is very large and active in newborn antarctic seals[1]

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Summary. The pineal gland of newborn elephant seals and Weddell seals is larger than in adult females. The gland is considerably larger at birth in Weddell seals than in elephant seals. The former experience greater extremes of temperature. Plasma melatonin concentrations in excess of 2000 pg/ml were recorded in the first days of life, compared with 20–50 pg/ml in adults.
Key words. Pineal gland; melatonin; thermoregulation.

Considerable emphasis has been placed on the role of the pineal gland in mediating the influence of the photoperiod on the neuroendocrine-reproductive axis[2,3]. At times this has been assumed to be the primary role of the gland, but it appears that the role may be more general than this: as currently perceived, the function of the pineal gland is to serve as an intermediary between the external environment, especially the photoperiod, and the organism as a whole[4,5].

For several reasons, the pineal gland may be expected to be small and relatively inactive in newborn seals. In those mammals whose pineal ontogeny has been investigated, the gland is small and lacks organization at birth, but undergoes considerable enlargement postnatally[6,7]. Plasma and pineal melatonin concentrations have been measured in only a few species during early postnatal development, but in all they are lower than in adults[8,9]. Pineal activity in subadult male southern elephant seals, *Mirounga leonina* is greatest during periods of long scotophase, as judged by organ size, size of pinealocytes and plasma melatonin concentration[10,11], but the peak of births of elephant seal and Weddell seal *(Leptonychotes weddelli)* pups occurs in October when scotophase is very short (less than 10 h for elephant seals and virtually nonexistent for Weddell seals). Nonetheless, the pineal gland of foetal (G.C. Liggins, pers. comm.) and newborn seals is very large[12] (fig. 1). We report observations on the pineal gland of neonatal elephant and Weddell seals, revealing that it is also an extremely active gland. We conclude that it may play an important role in thermoregulation about the time of birth and may be vital to survival in the harsh environments in which these seals live. The Weddell seal occurs in greatest abundance near the coast of Antarctica[13], and experiences the greatest degree of cold of any mammal, while the southern elephant seal inhabits mainly subantarctic waters[14].

Pineal glands of seven elephant seal pups from birth to 45 days of age, and two Weddell seal pups seven and 23 days old, were weighed during dissection. Each pineal was hemisected, and fixed in Bouin's fixative or 10% neutral buffered formalin, dehydrated, and embedded in wax. A 5 µm median section from each half gland was stained by the Heidenhain Azan technique. The pinealocyte densities were estimated using the method of Aherne[15]. In adults, densities were determined for both cortex and medulla[12] since, unlike in pups, these regions have different pinealocyte concentrations. The pinealocyte population of the total gland was estimated from a consideration of total gland weight, pinealocyte density, and volumetric proportions for cortex and medulla.

Venous blood was collected from neonatal elephant seals from birth to three weeks, and neonatal Weddell seals from birth to five weeks of age. Collections were made between 11.00 and

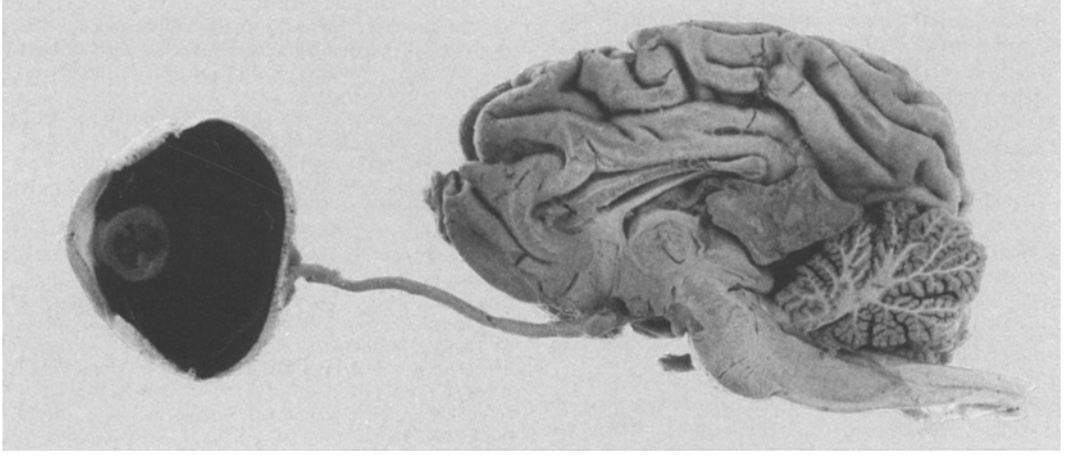

Figure 1. Median section of the brain of a newborn Weddell seal, with the orbit attached by the optic nerve. The pineal gland (p), which is extraordinarily large, lies between the cerebrum and the cerebellum. The gland is very active at birth, and subsequently is influenced by daily and seasonal variation in light intensity.