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# Intact and Carboxyterminal PTH Do Not Cross the Blood–Cerebrospinal Fluid Barrier[1] (41894)

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*Abstract.* In order to examine whether parathyroid hormone (PTH) enters the cerebrospinal fluid (CSF), the blood levels of the hormone were acutely elevated either by infusion of parathyroid extract or by stimulation of the parathyroid glands by hypocalcemia. Despite marked elevations in the blood levels of the hormone, PTH could not be detected in the CSF. The data indicate the intact PTH or its carboxyterminal fragment do not cross the blood–CSF interface of the blood–brain barrier. The results, therefore, suggest that the action of PTH on brain must be mediated by an effect on the blood–brain interface of the blood–brain barrier.

The cerebrospinal fluid (CSF) and brain compositions are vigorously protected and changes in their constituents are prevented during marked alterations in the chemistry of blood (1–4). This is achieved by the blood–brain barrier which encompasses the processes involved in the exchange occurring at the CSF–blood (5), CSF–brain (6), and blood–brain interface (7, 8). Parathyroid hormone (PTH) has been shown to augment brain calcium content and affect brain function in acute or chronic clinical and experimental conditions associated with excess PTH (2, 3, 9–12). Thus, it appears that PTH overcomes the blood–brain barrier but the particular interface of this system which is affected by the hormone is not completely delineated.

Available data indicate that the concentration of ionized calcium and phosphorus and the product of ionized calcium–phosphorus in CSF are not affected in conditions associated with excess or deficiency of PTH (2, 3, 13–15). These observations suggest that the hormone does not disrupt the blood–CSF interface of the blood–brain barrier. The present study was undertaken to examine whether PTH enters the CSF compartment and through such a movement may act on the CSF–brain interface.

**Methods.** Eleven adult female mongrel dogs weighing 15–20 (18 ± 0.5 (SE) kg were studied. The animals were anesthetized with 30 mg/kg pentobarbital which was administered intravenously and additional amounts of the drug were given as needed. The dogs were intubated and mechanical respiration was maintained by a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 25 strokes/min and tidal volume to keep $pCO_2$ at about 35 mm Hg. Catheters were inserted in peripheral veins of both hind limbs; one was used for collections of blood samples and the other for infusion of disodium ethylenediaminetetracetate (EDTA) or PTH. A spinal trocar was inserted into the cisterna magnum and it was left *in situ* throughout the experiment for the collections of CSF samples.

In six dogs, parathyroid extract PTE (Eli Lilly & Co., Indianapolis, Ind.) dissolved in 250 ml of normal saline was infused over a period of 4 hr delivering 6 units of PTE/kg body wt/hr. Blood and CSF samples were obtained before and every hour during the infusion of the hormone.

In five dogs, EDTA dissolved in 500 ml of 5% dextrose was infused for 2 hr delivering 75 mg of EDTA/kg body wt/hr. Blood and CSF samples were obtained before, hourly, during the infusion of EDTA and every 2 hr for the 4-hr period following EDTA infusion.

Ionized calcium was measured with Orion Electrode Model SS-20 (Orion Biomedical, Cambridge, Mass.). PTH was measured with

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radioimmunoassay using sheep antisera 478 (kindly supplied by Dr. Claude Arnaud), $^{125}$I-labeled bovine PTH, and pooled sera from patients with chronic renal failure as standard. This antibody reacts predominantly with an immunological determinant in the carboxyl region of the PTH, and it will detect both the intact and the carboxy-terminal fragment. The value of this assay for 63 normal samples ranged from undetectable to 15 μleq/ml (5.7 ± 0.7 (SE) of the detectable values), and the levels were detectable in 33 of the 63 samples (52%). Elevated blood levels of PTH were found by this assay in all samples obtained from uremic patients. Statistical evaluation utilized repeated measures analysis of variance. These were performed to determine if there were significant differences over time. The Scheffe method was used to make multiple comparisons between means (16).

**Results.** The effects of PTH or EDTA infusions on the blood concentrations of ionized calcium and PTH and the PTH concentration in CSF are given in Table I. The *P* values reported in the text are obtained by repeated measures analysis of variance. The significance between means obtained by multiple comparisons according to the Scheffe method is indicated in Table I. There was a progressive and significant ($P < 0.01$) rise in the concentrations of ionized calcium in blood from 4.5 ± 0.1 mg/dl to 6.5 ± 0.2 mg/dl by the end of the PTH infusion. The PTH levels in blood were influenced by the weight of the dogs. After adjusting for dog weight, the blood levels of PTH also displayed significant ($P < 0.01$)

TABLE I. BLOOD CONCENTRATIONS OF IONIZED CALCIUM AND LEVELS OF PARATHYROID HORMONE IN BLOOD AND CEREBROSPINAL FLUID DURING PARATHYROID EXTRACT AND EDTA INFUSIONS

| | Hours | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| I. Parathyroid hormone infusion (6 Units/kg body wt/hr) | | | | | |
| Blood $Ca^{2+}$* (mg/dl) | 4.5 ± 0.1 | 5.2 ± 0.12 | 5.6 ± 0.2 | 6.0 ± 0.2 | 6.5 ± 0.2 |
| PTH** (μleq/ml) | UD | 565 ± 277 | 798 ± 379 | 1351 ± 645 | 1913 ± 980 |
| CSF PTH (μleq/ml) | UD | UD | UD | UD | UD |
| | EDTA infusion period | | | EDTA withdrawal | |
| | 0 | 1 | 2 | 4 | 6 |
| II. EDTA infusion (75 mg/kg body wt/hr) | | | | | |
| Blood $Ca^{2+}$*** (mg/dl) | 4.8 ± 0.06 | 3.4 ± 0.1 | 2.8 ± 0.1 | 4.4 ± 0.1 | 4.6 ± 0.1 |
| PTH† (μleq/ml) | UD | 6.0 ± 0.4 | 9.0 ± 2.0 | 6.0 ± 0.3 | 4.0 ± 0.2 |
| CSF PTH (μleq/ml) | UD | UD | UD | UD | UD |

*Note.* Data are printed as means ± SEM; UD = undetected; $Ca^{2+}$ = ionized calcium; PTH = parathyroid hormone. Scheffe's method was used to make multiple comparisons between means.

* Comparisons of 4 hr to control significant by different overall ($P < 0.05$).

** All pairwise significant overall ($P < 0.05$) after adjustment for dog weight.

*** Pairwise comparisons significant overall ($P < 0.05$), are control vs 1, 2, and 4 hr, 1 hr vs 2 hr, 4 hr vs 1 and 2 hr, and 6 hr vs 1 and 2 hr.

† All comparisons to control significant and 2 hr significantly different from 6 hr, overall ($P < 0.05$).

elevations and were high throughout the 4 hr of the study. Despite the profound increment in the blood levels of PTH, the concentration of the hormone in the CSF remained undetectable.

There was a significant ($P < 0.01$) fall in the levels of ionized calcium in blood from $4.8 \pm 0.06$ to $3.4 \pm 0.1$ by the end of the first hour of EDTA infusion and a further significant (<0.01) decrease to $2.8 \pm 0.1$ mg/dl by the end of the second hour. The level of ionized calcium returned to control values two hours after the termination of EDTA infusion. The blood levels of PTH increased with the fall in the concentration of ionized calcium during EDTA infusion and began to decline after the termination of the infusion. The increments in endogenous PTH levels were modest. However, the PTH concentrations in CSF remained undetectable.

**Discussion.** The results of the present study demonstrate that an increase in the blood levels of PTH produced by PTH infusion or by stimulation of endogenous release of the hormone is not associated with a rise in the concentration of PTH in CSF. These data show that the intact PTH or its carboxyterminal fragment do not cross the blood–CSF interface of the blood–brain barrier.

Previous studies have shown that the concentrations of calcium and phosphorus in CSF remain stable despite marked changes in their concentrations in the blood (2–4) and in the absence or the presence of normal or excess PTH (2, 3, 13–15). These data and the results of the present study, therefore, indicate that the changes in brain calcium content in the presence of excess PTH could not be due to an action of the hormone on the CSF–brain interface of the blood–brain barrier. One must conclude that the effect of PTH on brain must occur at the blood–brain interface of the blood–brain barrier.

The mechanisms by which PTH mediates calcium accumulation in brain through an action at the blood–brain interface are not addressed in the present study. One may, however, consider several possibilities. The hormone may stimulate a brain adenylate cyclase system, affect phospholipid turnover of cell membrane or stimulate synthesis of a carrier protein. Studies are required to explore these possibilities.

Other studies in our laboratory have shown that the electroencephogram (EEG) may be normal in the presence of increased calcium content of brain if excess PTH is absent (17). These observations suggest that PTH is required before an abnormality in EEG develops and they are consistent with an effect of PTH on brain independent of the hormone-mediated calcium accumulation. Indeed, *in vitro* studies have shown that PTH stimulates brain microsomal ATPase (18), and inhibits oxidative phosphorylation by mitochondria from various cells (19–22). For such action of PTH to occur *in vivo,* the hormone or one of its fragments must enter the brain cells. Nordquist and Palmeiri (23) have demonstrated that PTH enters the cells of the renal proximal tubule and localizes in the mitochondria. It is possible that a similar event exists in the brain, and if it does, it must occur at the blood–brain interface.

Although our studies demonstrate that PTH does not enter CSF despite marked increment in the blood levels of the hormone, such a phenomenon may not be applicable to other hormones. Several investigators have shown that the CSF contains thyroxine and triiodothyronine (24, 25), steroid hormones (26), prolactin (27), calcitonin (28, 29), insulin (30, 31), growth hormone (32), and gastrin (32). The origin of these hormones found in the CSF is not clear. A positive correlation between the blood and CSF levels of prolactin (27), insulin (31), and growth hormone (32) were found but not for those of calcitonin (28) and gastrin (33). These observations suggest a selectivity of the blood brain barrier in the modulation of the passage of hormones from blood to CSF.

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