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BRIEF COMMUNICATION

CSF-Plasma Relationships for DSIP and Some Other Neuropeptides

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BANKS, W. A. AND A. J. KASTIN. *CSF-plasma relationships for DSIP and some other neuropeptides.* PHARMACOL BIOCHEM BEHAV **19**(6) 1037–1040, 1983.—The relationships between CSF and plasma hormonal levels of several peptides were studied in the same samples of simultaneously obtained plasma and CSF. A significant correlation existed between CSF and plasma levels of DSIP as well as gastrin. Preliminary results also showed a correlation between CSF and plasma levels of NT, but not VIP or calcitonin. CSF/plasma ratios and the effect of BBB disruption also varied from peptide to peptide. These diverse CSF/plasma relationships are not easily explained by models of nonspecific passage but may indicate individual systems or axes that could be involved in the central effects of peripherally administered peptides.

Cerebrospinal fluid Blood-brain barrier Brain Delta sleep-inducing peptide (DSIP) Vasoactive intestinal peptide (VIP) Gastrin Calcitonin Neurotensin (NT) Gut-brain axis Neuropeptide(s) Peptide(s) Neurohormone(s)

CURRENT evidence suggests that many peptides may serve as hormones in the periphery and as neurotransmitters in the CNS. The existence of such peptides raises the possibility of an interaction between brain and periphery based on this common language. Such an interaction would probably be reflected in and perhaps mediated by cerebrospinal fluid (CSF) and by peripheral blood levels and could help to explain such phenomena as the behavioral effects of peripherally administered peptides.

No clear picture, however, currently exists for the plasma-CSF dynamics of peptides. In this study, aliquots of simultaneously obtained peripheral blood and CSF from the posterior fossa of dogs was assessed for correlations. The relationship among peptides in CSF and plasma and the effect of disruption of the blood-brain barrier (BBB) was also examined.

METHOD

Animals and Samplings

Mongrel dogs were anesthetized with sodium pentobarbital 30 mg/kg IV. CSF was obtained from the posterior fossa with a 3.8 cm long 22 gauge needle and blood from a foreleg vein with an intermittent infusion set. CSF (3 ml) was collected over a 10 min period and blood was obtained halfway through this period (4–6 min). All samples were collected into chilled polypropylene tubes with the blood tubes containing EDTA and Trasylol. Morning (AM) samples were collected from six dogs between 0900 and 1100 and a second set of samples was taken one hour later. Evening (PM) samples were collected from four dogs between 1830 and 2030 and a second set of samples was taken one hour later. PM samples were also taken from two dogs in which the BBB had been disrupted by a forceful tap of the posterior fossa. The CSF and plasma samples were spun at 2300 g for 10 min and the supernatants divided into 0.5 ml aliquots and stored at −70°C until time of assay. All CSF samples were perfectly clear and no RBC's were noted after centrifugation except in the disrupted BBB samples that had packed cell volumes between 0.1 and 1.0%. We have previously shown that the unaided eye can readily detect blood in the CSF at a concentration of 1 part in 2000 [3].

Radioimmunoassays

Our RIA for delta sleep inducing peptide (DSIP) has been previously described and shown to detect DSIP levels in dog CSF and plasma with a sensitivity of about 135 pg/ml [3]. The DSIP antibody is highly specific, requiring eight of the nine amino acids in DSIP for binding. Dilution curves of immunoactivity in CSF and plasma are parallel to the standard curve and recovery of synthetic DSIP added to CSF is about 100%. For these studies, 0.1 ml of CSF or 0.01 ml of plasma was assayed and tubes were counted for 1 min.

RIA kits for gastrin 1–17, vasoactive intestinal peptide (VIP), neurotensin (NT), and calcitonin were obtained from Immunonuclear Corporation (Stillwater, MN). Sample sizes of 0.1–0.2 ml were measured with the kit assays and the assay tubes counted in a gamma counter for 10 min to improve accuracy. According to the supplier, sensitivity and specificity are as follows. The antibody for gastrin reacts with gastrin$^{1-17}$, but not with gastrin$^{1-12}$, gastrin$^{1-34}$, or cholecystokinin (CCK), and has a sensitivity of 40 pg/ml. The antibody to NT is specific for NT$^{1-13}$ with only 1.2%

TABLE 1
SUMMARY OF CSF-PLASMA RELATIONSHIPS

	DSIP	Gastrin	Neurotensin	Calcitonin	Vasoactive Intestinal Peptide
CSF	428 ± 6.4	59.0 ± 3.7	2.1 ± 0.34	5.65 ± 0.90	5.9 ± 2.5
Plasma	3118 ± 90.8	146 ± 4.7	26.1 ± 2.0	95.0 ± 5.9	21.8 ± 3.7
Ratio	0.143 ± 0.003	0.404 ± 0.098	0.089 ± 0.013	0.069 ± 0.012	0.25 ± 0.093
a	0.049	0.456	0.0601	No	No
b	276	−7.4	0.567	Correlation	Correlation

CSF, plasma, and ratio values are means ± SE in pg/ml. a and b are values in the equation $y=ax+b$ where x is plasma value and y is CSF value in pg/ml and are given for peptides that had statistically significant correlations between CSF and plasma values.

cross reactivity with NT^{8-13} and sensitivity is reported as 15 pg/ml. The antibody to calcitonin reacts with human and rat calcitonin, but not with salmon calcitonin and has a sensitivity of 15 pg/ml. The antibody to VIP is specific for VIP with less than 0.1% cross reactivity with most peptides tested including $gastrin^{1-17}$ and neurotensin; its sensitivity is 5 pg/ml.

Statistics

The BMDP2V program was used to determine F values (ANOVA) for comparisons between first and second samples, AM vs PM samples, intact vs. disrupted BBB samples, and covariance of CSF and plasma peptide levels. The BMDP1R multiple linear regression program was used to test for correlations among peptide levels in the CSF or plasma or among their ratios. Means are reported with their standard errors.

RESULTS

No statistically significant differences occurred between the first and second samples, nor between AM and PM samples. The AM, PM, 1st and 2nd samples, therefore, were combined for further statistical evaluation.

Delta Sleep-Inducing Peptide

The mean CSF level was 428±6.4 pg/ml, the mean plasma level was 3118±90.8 pg/ml, and the mean CSF/plasma ratio was 0.143±0.003 (Table 1). These values are well within the sensitivity of the assay and are in the range expected with this antibody [3]. A significant correlation existed between CSF and plasma ratios ($y=0.049x + 276$, $F(1,6)=5.9$, $p<0.05$). Disruption of the BBB did not effect CSF, plasma, or ratio values.

Kit Assays

The dilution curves for gastrin, NT, VIP, and calcitonin in plasma were parallel to their standard curves. The mean value for CSF gastrin was 59.0±3.7 pg/ml and for plasma gastrin was 146±4.7 pg/ml (Table 1) and are within the sensitivity limits of the assay. For gastrin, the plasma values are in the expected range for this kit, but CSF values are higher than those previously described in lumbar CSF from human beings [14]. The mean CSF/plasma ratio was 0.404±0.098. A significant correlation existed between plasma (x) and CSF (y) gastrin values ($y = 0.456x - 7.4$, $F(1,6)= 10.6$, $p<0.05$). Disruption of the BBB significantly reduced the CSF gastrin level to 18.1±8.5 pg/ml, $F(1,10)= 20.7$, $p<0.01$, and the CSF/plasma ratio to 0.122±0.058, $F(1,10)= 19.6$, $p<0.01$, but had no effect on plasma levels.

The mean CSF value for NT of 2.1±0.34 pg/ml was well below the sensitivity of 15 pg/ml specified by the supplier, but the mean plasma value was 26.1±2.0 pg/ml and the ratio was 0.089±0.013 (Table 1). Plasma values are in the expected range, but CSF levels are much lower than the previously reported mean of 235 pg/ml [16]. A significant correlation existed between CSF and plasma levels ($y = 0.0601x + 0.567$, $F(1,6)= 19.5$, $p<0.01$). Disruption of the BBB did not cause a significant change in CSF or ratio values but did result in a significant increase in plasma values to 37.4±2.4 pg/ml, $F(1,10)= 7.3$, $p<0.05$.

For calcitonin, the mean CSF value of 5.65±0.90 pg/ml was below the supplier's specified sensitivity of 15 pg/ml but the mean plasma level was 95.0±5.9 pg/ml (Table 1). These values are in the upper range of those previously reported for plasma [4] and in the lower range for human lumbar CSF [20]. The mean ratio was 0.069±0.012. No correlation existed between CSF and plasma levels. Disruption of the BBB significantly increased CSF (17.7±4.9 pg/ml, $F(1,10)=20.0$, $p<0.01$) and ratio (0.157±0.0394, $F(1,10)=9.2$, $p<0.05$) values but did not affect blood levels.

The mean levels of VIP in CSF of 5.9±2.5 pg/ml and in plasma of 21.8±3.7 pg/ml were above the sensitivity of 5 pg/ml specified by the supplier, with a mean CSF/plasma ratio of 0.25±0.093 (Table 1). The CSF levels are much lower than those previously reported in lumbar CSF from normal human beings [7]. No significant linear relationship was found to exist between CSF and plasma values. Disruption of the BBB did not produce statistically significant changes in the plasma or CSF values or their ratios.

The levels of peptides appeared to vary independently of one another. This was also observed for the ratios, but for plasma values a significant inverse relationship was found between gastrin and calcitonin ($p<0.01$) and a positive relationship between calcitonin and VIP ($p<0.05$).

DISCUSSION

Significant correlations between CSF and plasma levels were found for DSIP and gastrin. A positive correlation also was observed for NT, but not for calcitonin or VIP; the sensitivity of the kit assays for these peptides was, however,

at or above the level found for their mean CSF levels, so that any results involving those peptides must be considered preliminary at best. Other studies examining CSF and plasma levels for correlations have usually found the two to be unrelated, but most of these studies have used CSF obtained by lumbar puncture. CSF from the lumbar area is known to mix very slowly with CSF from the brain and is felt to reflect spinal, rather than brain, neurochemistry [5,10]. This may limit the applicability of studies using lumbar fluid for understanding brain-blood interactions and may partially account for different peptide values found here in cisternal CSF.

Previous work examining peptide levels in lumbar CSF and blood have found correlations for vasopressin [11,15], while other work has denied such a relationship for ACTH [1], β-endorphin [18], gastrin [14], calcitonin [4, 17, 20], NT [16], VIP [7], and even vasopressin [12]. By contrast, when the CSF was taken from the cisterna magna or ventricles, CSF levels correlated with plasma levels for insulin [22], CCK [2], and DSIP [3], although not for β-endorphin [21] or ACTH [6].

The CSF/plasma relationships seemed to vary greatly among the different peptides. Not only did these values correlate for some peptides while not for others, but the slopes describing the CSF-plasma correlations varied almost tenfold from 0.456 for gastrin to 0.049 for DSIP. The other two aspects of CSF-plasma relationships (CSF/plasma ratio, effect of BBB disruption) also differed among the peptides. Since the various peptides were measured in aliquots of the same sample, it is possible that each CSF/plasma relationship is independent.

This diversity is further illustrated by the wide range of CSF/plasma ratios. The CSF/plasma ratios for DSIP and gastrin were 0.140 ± 0.004 and 0.404 ± 0.098, respectively; for VIP, NT, and calcitonin, the three peptides whose CSF values were determined preliminarily with less accuracy, the ratios were 0.251 ± 0.0930, 0.0837 ± 0.0134, and 0.0692 ± 0.0115 respectively. Based on the observation that for hydrophobic substances such as peptides, hydrodynamic volume is a good predictor of CSF/plasma ratios [8], it has been stated that a CSF/plasma ratio of greater than 0.08 reflects either active transport across the blood-CSF barrier or direct neurosecretion into the CSF [19]. Although physiochemical factors like molecular charge [9], half-life in CSF and plasma, protein binding, and lipophilicity [3,13] probably influence this ratio, the 0.08 level might be used to represent the crossing of an inert substance by nonspecific mechanisms. In our study, four of the five peptides tested exceeded this level.

A diversity in response to BBB disruption was also noted in preliminary experiments. CSF and ratio values increased significantly after disruption only for calcitonin while these values decreased for gastrin but remained unchanged for DSIP. Generally, it seemed that the lower CSF and ratio values tended to increase and the higher values tended to decrease after disruption of the barrier. For plasma levels, only NT seemed to change significantly, the others remaining unaltered. Such observations are not easily explained by a purely "restrictive" role for the BBB but suggest an active role for the BBB in CSF/plasma dynamics.

This diversity in CSF/plasma relationships is not readily understood by current models of peptide BBB penetrance. Neither the molecular sieve model nor the pinocytotic model would easily account for the apparently independent variations among the three aspects of the CSF/plasma relationship investigated. Thus, the correlation between CSF and plasma values, CSF/plasma ratios, and effect of BBB disruption all suggest other systems of regulation.

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