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THE DISTRIBUTION OF CHOLECYSTOKININ AND VASOACTIVE INTESTINAL PEPTIDE IN RHESUS MONKEY BRAIN AS DETERMINED BY RADIOIMMUNOASSAY

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ABSTRACT

The concentration of cholecystokinin (CCK) and vasoactive intestinal peptide (VIP) in dissected cortical and subcortical areas of four rhesus monkeys' brains was determined by radioimmunoassay (RIA). Cerebral cortical samples from one human brain are included for comparison. Preliminary data from two baboon brains are described. The results are similar to previous studies on rat (1-7), human (7-12), porcine (12,13), bovine (3) and guinea pig brains (14) and indicate that: 1) both CCK and VIP are widely distributed in cortical and subcortical areas in these species, 2) CCK is generally more abundant than VIP in primate brain, and 3) the distribution of CCK and VIP in the rat brain parallel those in infrahuman primate and human brain.

INTRODUCTION

CCK (15) and VIP (16), originally discovered as gastrointestinal hormones, both have a wide distribution in the mammalian central nervous system and occur in particularly high concentrations in cerebral cortex (1-14,17). Both have been localized in separate groups of specific cells in cerebral cortex which have an intimate association with cerebral blood vessels (17,18). It has been suggested that these peptides may in some way regulate cerebral blood flow (17,18).

The distributions of CCK and VIP have been studied in detail in many mammalian species including man using both RIA and immunocytochemistry. There are no data, however, on the distribution by RIA of either CCK or VIP in the brains of infrahuman primates. The data presented in this paper on CCK and VIP levels in rhesus monkey brain are intended to fill this gap, and to provide data to compare with previous studies on post-mortem human brain (7-9).

METHODS

A. Tissue dissection and extraction:

Rhesus monkey brains were obtained and dissected as in Lewis et al. (19). The samples were frozen after dissection, stored at -70°C, homogenized in 10 volumes of ice cold 0.1N HCl and clarified by centrifugation. Ten µl aliquots of the supernatant were lyophilized prior to the VIP RIA. Prior to the CCK RIA, the 10 µl aliquots were neutralized with 10 µl of 0.1N NaOH.

The human cerebral cortical samples were obtained from Massachusetts General Hospital, courtesy of Tricia Marshall. The frozen tissue was homogenized at room temperature in methanol:water 9:1 (v/v), centrifuged, and the methanol supernatant evaporated under nitrogen.

B. CCK and VIP radioimmunoassay:

The CCK RIA was performed with antiserum R5 as previously described (2). This CCK antiserum cross-reacts strongly with CCK8 sulfate, CCK33, gastrin and caerulein and weakly with CCK8 desulfate and CCK4. The exact chemical nature of the CCK-like peptides in rhesus monkey brain has not been established. On the basis of gel filtration chromatography and CCK RIA, human cerebral cortex contains mainly CCK8-like peptides (Reference 12 and M. Beinfeld, unpublished observations). The VIP RIA was performed as previously described (20) using a VIP antiserum provided by Gajanan Nilaver. This antisera did not cross-react with secretin, GIP (gastric inhibitory peptide), glucagon, and only about 0.02% with PHI (acronym for a Peptide with a C-terminal Histidine and an N-terminal Isoleucine amide).

RESULTS

The CCK and VIP concentrations in cerebral cortical regions of the rhesus monkey brain are shown in Table 1. These samples were dissected as described before (19), based on the cytoarchitectonic map of von Bonin and Bailey (21). The CCK levels in all regions of the rhesus monkey cerebral cortex are high. In the rhesus monkey, the frontal and orbital regions are higher than the motor and sensory, parietal and occipital regions. A similar rostro-caudal gradient has been reported in the human brain (8). Table 2 presents data on the CCK levels in three cerebral cortical areas from one human brain for comparison with the rhesus monkey data. The data in Table 2 on CCK content of human

cortex agree very well with the rhesus monkey data, and, though it is unsafe to generalize from a single sample, there appears to be a marked rostro-caudal gradient in CCK concentration in the cortex of this individual.

Table 1: CCK and VIP concentrations in cortical areas of the rhesus monkey brain

Area	Cytoarchitectonic Designation	CCK (pg/mg tissue)	VIP (pg/mg tissue)
Inferior prefrontal	FD/FDγ	258 ± 16	100 ± 4
Midlateral prefrontal	FD	311 ± 46	111 ± 14
Dorsal prefrontal	FD	356 ± 86	93 ± 12
Medial prefrontal	FD	458 ± 109	86 ± 9
Midorbital prefrontal	FD	205 ± 42	92 ± 11
Medial orbital	FL	394 ± 85	112 ± 22
Posterior orbital	FF	369 (338, 399)	104 (80, 128)
Olfactory tubercle	FF	327 (271, 383)	64 (63, 64)
Prearcuate	FDΔ	217 ± 40	95 ± 9
Dorsal premotor	FB/FC	316 ± 11 (N=3)	75 ± 12
Ventral premotor	FCBm	254 ± 43 (N=3)	102 ± 19
Frontal operculum	FCop	234 ± 60	104 ± 17
Anterodorsal motor strip	ant. FA	268 (260, 276)	72 (72)
Precentral	post. FA/FBA	144 ± 49	82 ± 18
Postcentral	PB/PC	169 ± 37	96 ± 8
Superior parietal	PE/PEm	357 ± 69 (N=3)	113 ± 10
Inf. parietal ant.	PF	222 ± 23 (N=3)	120 ± 11
Inf. parietal, post.	PG	213 ± 48	112 ± 1
Parietal operculum	PF/PCop	237 ± 69	96 ± 16
Cingulate, anterior	LA	372 ± 32 (N=3)	96 ± 11
Cingulate, posterior	LC	225 ± 72	105 ± 16
Supratemporal plane	TC/TB	252 ± 25 (N=3)	89 ± 19
Superior temporal, post.	TA	226 ± 51 (N=3)	78 (74, 81)
Superior temporal, ant.	TA	209 ± 18 (N=7)	102 ± 12
Temporal pole, dorsal	dor. TG	247 ± 21 (N=3)	90 ± 16
Infer. temporal, post.	TEO	174 ± 40 (N=3)	94 ± 13
Infer. temporal, ant.	TE	177 ± 29 (N=10)	88 ± 5
Temporal pole vent.	vent. TG	244 ± 34 (N=3)	74 ± 4
Periamygdaloid	A	354 (261, 446)	50 (49, 50)
Entorhinal	ant. TH	232 (189, 274)	74 (47, 101)
Parahippocampal	post. TH	341 (337, 344)	84 (84)
Fusiform	TF	274 ± 41	87 ± 11
Insular, ant.	IA	268 (224, 331)	88 (85, 90)
Insular, post.	IB	160 (118, 199)	83 (64,102)
Preoccipital, lat.	lat. OA	201 ± 21 (N=8)	92 ± 7
Preoccipital, med.	med. OA	254 ± 32 (N=8)	98 ± 19
Peristriate, lat	lat. OB	190 ± 20 (N=8)	78 ± 4
Peristriate, med.	med. OB	108 (99, 117)	68 (67, 68)
Striate, lat.	lat. OC	197 ± 16 (N=3)	58 ± 3
Striate, med.	med. OC	123 ± 26	68 ± 13

The number of samples of the same region included in the average and standard error of the mean is four (4) except when indicated.

Table 2: CCK Concentration in Human Frontal, Temporal, and Occipital Cortex

Brain Region	CCK pg/mg tissue	N
Frontal	340.2 ± 39.8	4
Temporal	291.3 ± 23.3	4
Occipital	152.4 ± 23.5	4

Four samples of each cortical area from one 57 year old male taken 8 1/2 hours post-mortem. The samples were extracted in 90% methanol as previously described (2).

In the rhesus monkey cortex, there is about 5-20 times as much CCK as VIP on a molar basis. The areas that are highest in CCK are not highest in VIP though they are both present in all cortical areas examined, their distributions are not correlated.

Some cerebral cortical areas obtained from two baboon brains were assayed for CCK and VIP content (data not shown). Though the dissection differed from that used for the rhesus brains and the sample sizes were smaller, some comparisons can be made. In the baboon cerebral cortex, the CCK : VIP molar ratio is about 2-6 and, like the rhesus, there is no correlation between the distributions of CCK and VIP. In most of the cortical areas examined, the rhesus and baboon levels of CCK were the same within experimental error.

Two interesting trends can be observed in the distribution of CCK in the rhesus monkey cortex. The CCK levels tend to be less than 200 ng/g in the motor and sensory processing areas: pre- and post-central, inferior temporal, posterior insular, striate and peristriate cortices. The CCK levels tend to be greater than 300 ng/g in the cortical areas which receive projections from the magnocellular portion of the nucleus medialis dorsalis of the thalamus: medial prefrontal, medial and posterior orbital and anterior cingulate cortices. The possible functional significance of this observation remains to be determined.

The VIP concentration is high in all rhesus monkey cortical regions. Unlike CCK, there appears to be no rostro-caudal gradient of VIP. Unlike CCK, most of the baboon cortical samples had higher levels of VIP (at most a factor of two) than the corresponding areas in the rhesus monkey.

Table 3: CCK and VIP concentrations in subcortical areas of the rhesus monkey brain

Area	CCK pg/mg tissue	VIP Conc. pg/mg tissue
Caudate	126 ± 52 (N=3)	17 ± 3
Septum	131 (131)	41 (41)
Olfactory bulb	108 (35.5, 180)	
Inferior colliculus	107 (80, 133)	49 (48, 50)
Superior colliculus	32 (11.3, 52)	46 (46, 46)
Putamen	80 ± 12	13 ± 4
Hypothalamus anterior	74 (74)	59 (58, 59)
Thalamus (nuc. med. dors.)	21 (7.3, 34.4)	19 (15, 22)
Cerebellum	19 ± 2	7 ± 2
Globus pallidus	16 (15, 16)	18 ± 2

The number of samples of the same region included in the average and standard error of the mean is four (4) except when indicated.

The CCK and VIP concentrations in subcortical regions of the rhesus monkey brain are shown in Table 3. As in the cerebral cortex, CCK is higher than VIP on a molar basis in the subcortical regions. The CCK content in the baboon subcortical areas was higher than the rhesus in the caudate, hypothalamus, putamen, globus pallidus, while the colliculi and the thalamus were the same.

In the baboon, the VIP level in caudate and putamen was higher than in rhesus monkey while in hypothalamus, thalamus and colliculi, the VIP levels are similar. As in the rat (5,6), the baboon amygdala is high in VIP, similar to cerebral cortical levels.

DISCUSSION

This study was conducted to provide information on the distributions of CCK and VIP in the primate brain obtained under controlled conditions with a minimum post-morten time prior to dissection and storage. The most striking aspect of the distribution of CCK and VIP in infrahuman primate brain is the very high concentration of both peptides throughout the cerebral cortex. This finding is in agreement with the results of previous investigations in a variety of other species, and points to a particularly important role of VIP and CCK in cortical function, such that high cortical levels of these peptides is conserved across species.

The distribution of CCK in human brain has been examined in detail by RIA (8-11). In one study, where CCK was only measured in the temporal lobe (11), levels of CCK were the same as the rhesus. In another report by Geola et al. (9), human CCK levels were much lower than we found in the rhesus (52 ng/g for frontal cortex vs 205-458 ng/g for rhesus). The most detailed study of the distribution of CCK in the human brain by Emson et al. (8) showed consistently higher values for cortical and subcortical CCK in the human than the rhesus (419-1178 ng/g vs 205-458 ng/g). The study of Vanderhaeghen (7), however, gives the best agreement with rhesus data except in the caudate and thalamus where Vanderhaeghen's values are lower than the rhesus. The agreement between the human and rhesus values for CCK, even though the mean autopsy time in the Emson study (8) was 50 hours and the Vanderhaeghen study (7) was 24 hours, demonstrates that CCK immunoreactivity is very stable post-mortem.

The distribution and levels of CCK appear to be well conserved in mammalian species. The rat and guinea pig (1-3,14) CCK levels closely resemble rhesus , except that both rat and guinea pig have more CCK in the caudate and thalamus, while the CCK in the rat cortex and hypothalamus is higher than in the rhesus monkey. Likewise, pig brain is higher in most areas in CCK than the rhesus monkey while cow brain is the same or lower.

The distribution of VIP in human brain has been less extensively studied than the CCK distribution. The data of Samson et al. (10) best resembles the rhesus monkey data, although several of the cortical areas and the caudate were higher in VIP in the rhesus monkey than in the human. The human VIP data of Fahrenkrug (22) also agrees well with the rhesus VIP values in some cortical areas, the caudate, and hypothalamus, although most of the cortical areas have lower VIP than the rhesus.

The VIP data of Bryant (4) is consistently lower for human cortex, while VIP levels in human hypothalamus is similar to rhesus.

Like CCK, the distribution of VIP also is well conserved in the brains of mammalian species. In comparison to rhesus monkey or rat, pig brain cortical and subcortical areas are substantially lower in VIP (13). Rat brain VIP, both in terms of

distribution and levels, is quite similar to rhesus monkey subcortical VIP levels, while the rat cortex is consistently higher in VIP than the cortex of rhesus monkey. These differences in VIP and CCK levels reported from different laboratories may be due to differences in extraction, storage or the use of peptide antisera with different antigenic determinants. The fact that our human and rhesus monkey data for CCK are so similar, despite the use of different extraction methods (though the CCK antiserum was the same), indicates that antiserum differences between laboratories may account for the discrepant values. An additional potential problem with comparison of VIP data between laboratories is the possibility that the VIP antisera used in these previous studies cross-reacts with other newly discovered members of the VIP family of peptides such as secretin (23) or PHI (24), recently detected in mammalian brain.

In comparing the CCK and VIP levels in the brain regions of different mammalian species, some differences do appear and may reflect real species differences in brain VIP and CCK concentration. To examine this properly, however, a comparative study should be done in the same laboratory using the same methods of storage, extraction, RIA, etc.

What is very clear from the comparison that of all the species examined, rat comes the closest to infrahuman primate and human brain in terms of the similarity of both VIP and CCK levels, and their relative distribution.

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