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R Paradisi, … , E Galassi, C Flamigni

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Research Article

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High Concentrations of Catecholamines in Human Hypothalamic-Hypophysial Blood

R. Paradisi,* G. Frank,‡ G. Grossi,§ S. Venturoli,* E. Porcu,* M. Capelli,§ E. Galassi,‡ and C. Flamigni*

**Institute of Reproductive Physiology and Pathology, Department of Obstetrics and Gynecology, S. Orsola Hospital, University Alma Mater Studiorum, Bologna, Italy I-40138; ‡2nd Division of Neurosurgery, Bellaria Hospital, Bologna, Italy I-40139; and §Central Laboratory, S. Orsola Hospital, Bologna, Italy I-40138.*

Abstract

While the hypothalamic-hypophysial portal system has been extensively studied in laboratory animals, equivalent studies have not been performed in humans. Here, we present an experimental procedure for collecting suprapituitary blood in man. To solve the question on the origin of such blood we investigated specific markers of hypothalamic secretory activity: the catecholamines (CAs). We found (*a*) norepinephrine (NE), dopamine (DA), and epinephrine (E) concentrations from ~ 1.5 to 2.5, 3.5 to 4.5, and 6- to 10-fold higher, respectively, in suprapituitary than peripheral blood, (*b*) different NE/DA and NE/E ratios in favor of DA and E in suprapituitary blood, and (*c*), a complete (100%) group separation (suprapituitary vs. peripheral) when discriminant analysis included only DA and E. These data indicate that suprapituitary blood composition is different from that of the peripheral blood, and is particularly rich in CAs and claimed differences between DA and E release on one hand and NE release on the other in suprapituitary blood also are observed. We advance the hypothesis of a hypothalamic source of such amines draining via median eminence into portal vasculature, and name this blood "hypothalamic-hypophysial blood." Besides serving as "classical" neurotransmitters, CAs may also have a direct neurohormonal role in the regulation of the human hypothalamic-hypophysial function.

Introduction

The hypothalamic-hypophysial portal system has been extensively studied in several mammal species. Portal blood has been collected by different techniques (1–3), by different surgical approaches (2, 4) and in different topographic sites (4). In man, no reports on suprapituitary blood collections have been reported in literature; only studies on intrapituitary blood (4–6) or blood from inferior petrosal sinuses (7, 8) exist.

We have developed an experimental method for collecting blood from the suprapituitary region in men with nonfunctioning pituitary disease that makes use of transsphenoidal microsurgery. This procedure should make it possible to gain new insights into the endocrine mechanisms regulating the human hypothalamic-hypophysial axis. However, the question regarding the origin of the collected blood and the need of locating, with precision, its source immediately arises. To address this question we investigated specific markers of the hypothalamic secretory activity. Catecholamines (CAs)[1] are abundant in discrete regions of the rat brain including the hypothalamus (9) and median eminence (10). In particular, reticulo-hypothalamic norepinephrine (NE), and epinephrine (E), neuron systems project terminals to various hypothalamic areas including the arcuate nucleus (11), and the tuberoinfundibular dopamine (DA) neuron system is present in the arcuate nucleus with projections to the median eminence (12). These CA neuron systems also exist in human brain (13). Anterior pituitary tissue contains very low or undetectable CA concentrations, and posterior pituitary tissue contains assayable amounts of DA in rats (9, 10).

The vascularization of the human pituitary gland has long been debated, however, it is commonly accepted that most of the blood supply to the adenohypophysis is derived from the hypothalamic-hypophysial portal system (14). Considering that CA concentrations in hypothalamic-hypophysial portal blood may reflect hypothalamic secretory patterns, we deliberately assumed that the CA concentration gradient in suprapituitary vs. peripheral blood is an index of hypothalamic catecholaminergic function. To test this assumption, we have made use of a highly sensitive and fully automated analytical procedure that utilized high performance liquid chromatography with electrochemical detector (HPLC-ED) (15, 16). This method makes it possible to quantify DA, NE and E simultaneously in minute volumes of plasma, a requirement for investigations on suprapituitary blood in man.

Methods

12 normotensive subjects (Table I), with clinical appearance and radiological findings (high resolution computed tomography and magnetic resonance imaging) of pituitary adenoma, but with no claimed endocrine disturbances of hypothalamic-hypophysial function, entered the study after informed consent was obtained. A detailed endocrine workup was obtained in all subjects. Plasma growth hormone (GH), thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), 17β-estradiol, testosterone, progesterone, cortisol, dehydroepiandrosterone sulfate, thyroxine, and triiodothyronine

Address reprint requests to Dr. R. Paradisi, Institute of Reproductive Physiology and Pathology, Department of Obstetrics and Gynecology, University of Bologna, Massarenti 13, I-40138 Bologna, Italy.

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1. *Abbreviations used in this paper:* CA(s), catecholamine(s); DA, dopamine; E, epinephrine; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; HHB, hypothalamic-hypophysial blood; HPLC-ED, high performance liquid chromatography with electrochemical detector; LH, luteinizing hormone; PRL, prolactin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

Table I. Clinical Features, Immunohistochemical Findings, and CA Concentrations in Peripheral (P) and Suprapituitary (SP) Blood

							DA		NE		E	
							P	SP	P	SP	P	SP
Case No.	Sex, age	Height	Weight	Clinical features	Immunohistochemical diagnosis	Days	Blood		Blood		Blood	
	yr	*m*	*kg*				*pmol/liter*					
1	L.M.G.	F, 26	1.59	57	VD,	GH− PRL− ACTH− FSH− LH− TSH−	−3	63		603		106	
							0	63	535	520	709	256	852
							+3	54		627		128	
2	M.O.B.	F, 39	1.63	75	VD, O, Li,	GH− PRL− ACTH− FSH− LH− TSH−	−3	215		620		65	
							0	129	517	863	969	66	2,098
							+3	86		686		492	
3	T.P.	F, 51	1.68	53	VD, PM,	GH− PRL− ACTH− FSH− LH− TSH−	−3	241		627		213	
							0	163	714	863	1,359	94	710
							+3	161		346		256	
4	S.L.	M, 41	1.77	92	VD, H, O,	(Transitional meningioma)	−3	106		680		213	
							0	107	347	1,176	1,359	86	595
							+3	106		660		85	
5	A.L.	F, 35	1.60	85	H, O,	GH− PRL− ACTH− FSH− LH− TSH−	−3	76		549		77	
							0	61	171	959	840	187	1,020
							+3	86		597		203	
6	D.F.	M, 36	1.71	82	VD,	GH+ PRL+ ACTH− FSH− LH− TSH−	−3	125		443		131	
							0	86	315	512	744	361	437
							+3	52		289		118	
7	G.G.	M, 61	1.70	68	H, V,	GH− PRL− ACTH− FSH− LH− TSH−	−3	118		520		93	
							0	64	306	603	756	213	352
							+3	103		401		256	
8	G.S.	M, 39	1.68	83	VD, H, O,	GH− PRL+ ACTH+ FSH+ LH+ TSH+	−3	187		703		180	
							0	105	428	691	614	128	511
							+3	104		520		150	
9	A.A.G.	F, 58	1.58	70	VD, PM,	GH− PRL− ACTH− FSH+ LH− TSH+	−3	129		1,371		263	
							0	191	172	1,399	1,158	146	1,967
							+3	107		1,152		105	
10	I.D.	F, 40	1.69	64	VD,	GH− PRL− ACTH− FSH− LH− TSH−	−3	86		378		98	
							0	52	172	443	591	524	3,511
							+3	104		242		213	
11	P.C.	F, 45	1.52	82	H, O,	GH− PRL− ACTH− FSH− LH− TSH−	−3	131		1,004		207	
							0	372	653	1,194	4,137	109	546
							+3	118		656		109	
12	A.Z.	F, 62	1.68	70	VD, H, PM,	GH− PRL− ACTH− FSH− LH− TSH−	−3	137		774		191	
							0	242	1,436	999	2,778	109	547
							+3	248		928		110	

VD, visual disturbances; H, headache; O, obesity; PM, postmenopause; Li, lipothymia; V, vertigo. − All negative cells, + Occasionally scattered positive cells, ++ Diffuse positive cells, +++ All positive cells.

were measured serially by radioimmunoassay. Pituitary responses to gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) stimulation were also tested. The majority of the subjects presented with neurological symptoms due to mass effect of the tumor (Table I). Only one subject (No. 2) had symptoms of hypopituitarism: slight signs of hypothyroidism and normal-low adrenal steroids. Four patients had mildly elevated PRL levels from stalk compression due to the frequent suprasellar extension of the tumor. On the whole, they can be considered as having quasinormal endocrine hypothalamic-hypophysial function. On the basis of histological examination of pituitary tissue, 11 subjects were rediagnosed as having pituitary adenomas, and 1 as having transitional meningioma of the sella. The adenomas were classified as being hormonally "nonfunctioning pituitary adenomas" and immunohistochemically as "nonreactive null cell adenomas" (17). Immunohistochemical examinations were carried out on multiple sections of the tumor tissue using the peroxi-

dase-antiperoxidase method (18) with commercially available antisera against GH, PRL, ACTH, LH, FSH, TSH (Dakopatts, Glostrup, Denmark). Immunohistochemistry yielded either negative results for all adenohypophysial hormones in eight adenomas or a few scattered cells that immunostained for various hormones in three adenomas (Table I).

None of the patients had received medication for at least six months before surgery. Patients were premedicated with diazepam "7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one" 20 mg i.m. and atropine "endo-(±)-α-(hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester" (0.5 mg i.m.). Operations were performed under general anesthesia induced by thiopental sodium "5-ethyldihydro-5-(1-methyl-butyl)-2-thioxo-4,6(1H,5H)-pyrimidinedione monosodium salt" (3–5 mg/kg) and maintained with nitrous oxide-oxygen (70/30) and isoflurane "1-Chloro-2,2,2-trifluoroethyl difluoromethyl ether" (1%). Replacement therapy consisting in cortisone acetate (25–37.5 mg/d) per os was given in the early postoperative period.

The human pituitary stalk and gland region was approached via a transsphenoidal route (19). Tumor removal was carried out selectively and as radically as possible. Collection of suprapituitary blood was accomplished under both direct microscopic and fluoroscopic control. It consisted of periodically aspirating blood by a microsuction apparatus (Fig. 1), just after tumor removal. The tip of the microcannula of the aspirator was kept in the postero-superior corner of the sella turcica at the junction of the diaphragm sellae with the dursum sellae where the pituitary stalk, against which the tip was leaning, passes. Under microscopic control, the infundibular stem and process of the neurohypophysis could be distinguished from adenohypophysis, even though a thick collar of the residua pars tuberalis covered the infundibular stem. From the microsuction apparatus, blood was directed into small polypropylene tubes containing appropriate preservatives for different hormonal studies, without substantially prolonging the surgical procedure. In particular, six blood samples of 0.5–0.6 ml each were collected over a 30- to 60-s period at 2-min intervals. The problems of blood contamination were minimized by the following: (*a*) filtration of any bits of nervous or tumor tissue by means of an in-line support system (Millipore Corp., Bedford, MA) containing 100-mesh nylon gauze (Schweiz Seidengazes Fabrik AG, St. Gallen, Switzerland), which was interposed between the microaspirator and the collecting tubes, (*b*) simultaneous use of a second, larger suction device, situated in the sella, to remove extraneous blood (Fig. 1), and (*c*) evaluation of microhematocrits of each aspirate to exclude dilution due to mixing with cerebrospinal fluid.

In all subjects, six control samples (each of 5 ml, 30 ml total) of peripheral venous blood were collected simultaneously by the anesthesiologist (day 0, operative condition). Moreover, additional samples of peripheral blood were obtained (0800 to 1000 hours) 3 d before (day −3, basal condition) and 3 d after the operative (day +3, replacement therapy condition). After inserting a venous line the patients were kept for at least 60 min in semirecumbent position before sampling. A blood sample (30 ml) was collected and, then, divided in six aliquots of 5 ml each.

In all blood samples, plasma was immediately separated and stored at −70°C until analyzed. DA, NE and E concentrations were determined by HPLC-ED (Fig. 2) (15, 16). Briefly, plasma CAs were complexed with diphenylboronic acid "2-aminoethyl diphenylborinate" and the complex was extracted on reversed-phase C 18 cartridge. The cartridge was eluted by column-switching with the HPLC-ED mobil phase, and the CAs were separated by the HPLC analytical column. The analyzer diluted 300-μl aliquots of peripheral plasma with 600 μl of buffer containing the complexing agent and 100- or 50-μl aliquots of suprapituitary plasma with 800 or 850 μl, respectively, of buffer, depending on sample volume (Fig. 2). The intraassay (interassay) variability were 1.9% (3.5%), 2.1% (3.8%), and 2.0 (3.7%) for NE, E, and DA, respectively. The minimum plasma concentration detectable was: 6, 11, and 20 pmol/liter for NE, E, and DA, respectively.

Statistical analysis was performed by using nonparametric paired Wilcoxon rank test. Discriminant analysis (Wilks' method in SPSS discriminant procedure on log-transformed variables) was also performed to distinguish between suprapituitary and peripheral (day 0) blood (20).

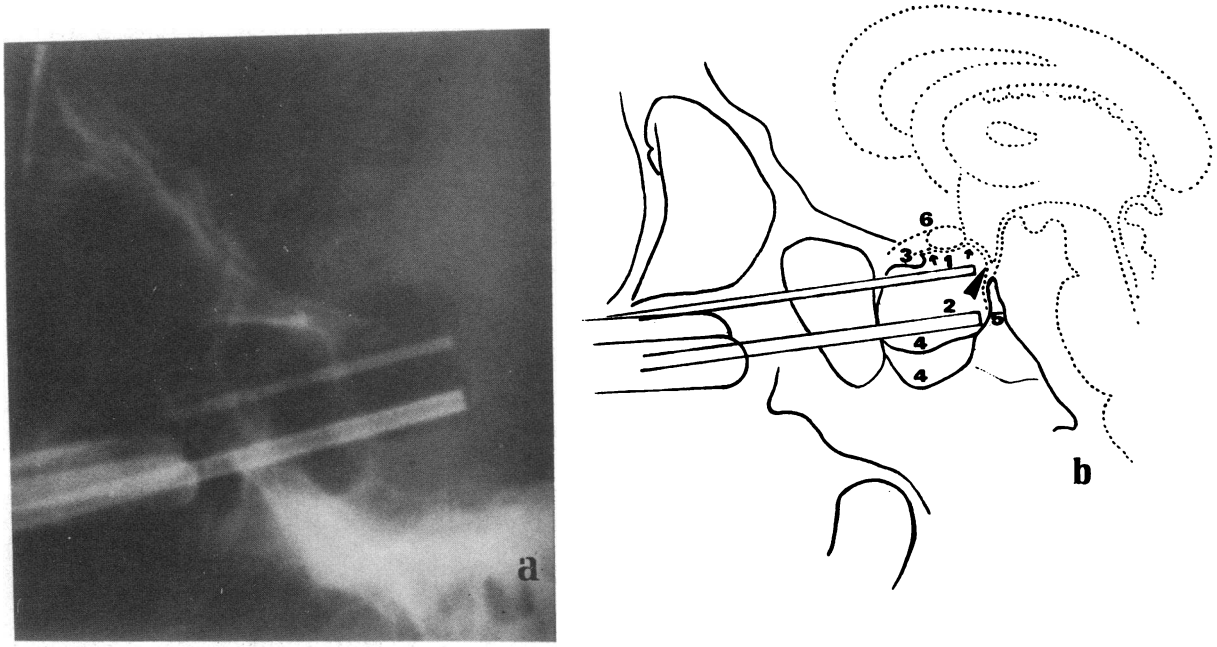

Figure 1. Experimental operative condition of suprapituitary blood collection via transsphenoidal route from human pituitary stalk region. *a*, Intraoperative x-ray control. *b*, Artist's drawing: *1*, microaspirator; *2*, second aspirator; *3*, anterior clinoid process; *4*, double sellar floor; *5*, dursum sellae; *6*, optochiasmatic complex; arrows, diaphragm sellae; arrowhead, pituitary stalk.

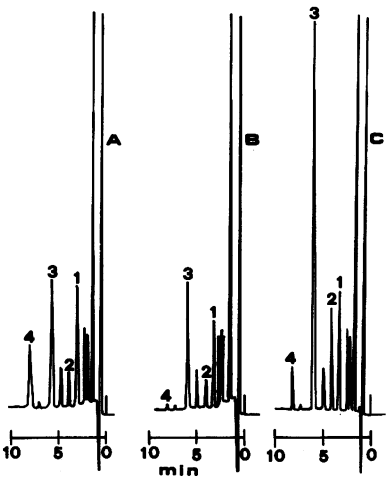

Figure 2. Plasma analytical profile of CAs by HPLC-ED. (*A*) Plasma standard. (*B* and *C*) Peripheral (day 0) and suprapituitary plasma of one subject (case No. 1). Peaks: *1*, NE; *2*, E; *3*, internal standard (3,4-dihydroxybenzilamine); *4*, DA. *A*, plasma standard containing: NE: 738, *E:* 341, internal standard: 909, DA: 816 pmol/liter, respectively (300 μl extracted). *B*, peripheral plasma containing: NE: 520, *E:* 256, internal standard: 909, DA: 63 pmol/liter, respectively (300 μl extracted). *C*, suprapituitary plasma containing: NE: 709, E: 852, internal standard: 909, DA: 535 pmol/liter, respectively (100 μl extracted). Analytical conditions: HPLC-ED mobil phase: methanol-acetonitrile-50 mM phosphate buffer, pH 2.8, 15:8:77, containing 200 mg/liter of sodium dodecylsulfate; flow rate: 1.5 ml/min; analytical column: two 30 × 4 mm, HS-3 C-18, 3 μm (Perkin-Elmer Corp., Norwalk, CT); detector (Coulochem, ESA, Bedford, MA): guard cell, +0.45 V; detector 1, +0.10 V; detector 2, −0.30 V, response 2, gain 100 × 10 (*A* and *B*), 100 × 30 (*C*).

Results

Individual and mean CA concentrations are reported in Table I and Fig. 3, respectively. The mean (±SEM) CA concentration gradient in suprapituitary vs. peripheral blood in day 0 was 4.0±0.6, 1.5±0.2, and 7.7±2.4 for DA, NE, and E, respectively. In particular, suprapituitary CA concentrations were markedly (DA, $P < 0.003$ and E, $P < 0.002$) and less markedly (NE, $P < 0.04$) higher than peripheral concentrations (day 0) (Fig. 3). No significant differences among the three peripheral conditions were observed for DA and E, whereas differences ($P < 0.01$) were observed for NE between day 0 and both day −3 and +3.

The mean (±SEM) NE/DA and NE/E ratios were significantly different in peripheral (day 0) vs. suprapituitary blood (7.7±1.0 vs. 3.2±0.5, $P < 0.005$, for NE/DA; 7.0±1.3 vs. 2.1±0.6, $P < 0.03$, for NE/E), whereas E/DA ratio showed no significant difference.

Finally, a discriminant function was calculated in order to evaluate the ability of the variables, DA, NE, E, to distinguish between suprapituitary and peripheral (day 0) blood. Only DA and E were entered into the function (F = 1.587 × log DA + 1.619 × log E − 18.039). Based on the discriminant scores, all the subjects (100%) were correctly classified (Fig. 4).

Discussion

In the study CA concentrations in peripheral conditions are somewhat more uniform and consistent with those reported in man by radioenzymatic (21, 22) or by the same HPLC-ED techniques (23). The manifest gap in CA concentrations and the different inter-CA ratios in suprapituitary versus peripheral blood are a novel finding in man. Since both suprapituitary and peripheral blood were collected simultaneously during surgery, anesthesia and surgical stress do not interfere with the concentration gap, even though such stressors activate peripherally (day −3 to day 0) catecholaminergic stimulation, in which NE release predominates (Fig. 3), confirming previous observations (21). The arterial-venous differences observed in the periphery (24) also do not explain the CA concentration gradient. Furthermore, no gross contamination with pieces of infundibular tissue could contribute to these differences because the blood was filtered during collection. Since surgery was performed at different hours, the simultaneous sampling of blood on day 0 obviates interference due to circadian rhythms of plasma CAs (22). Hemodynamic parameters also may influence CA regulation (25), but the subjects studied

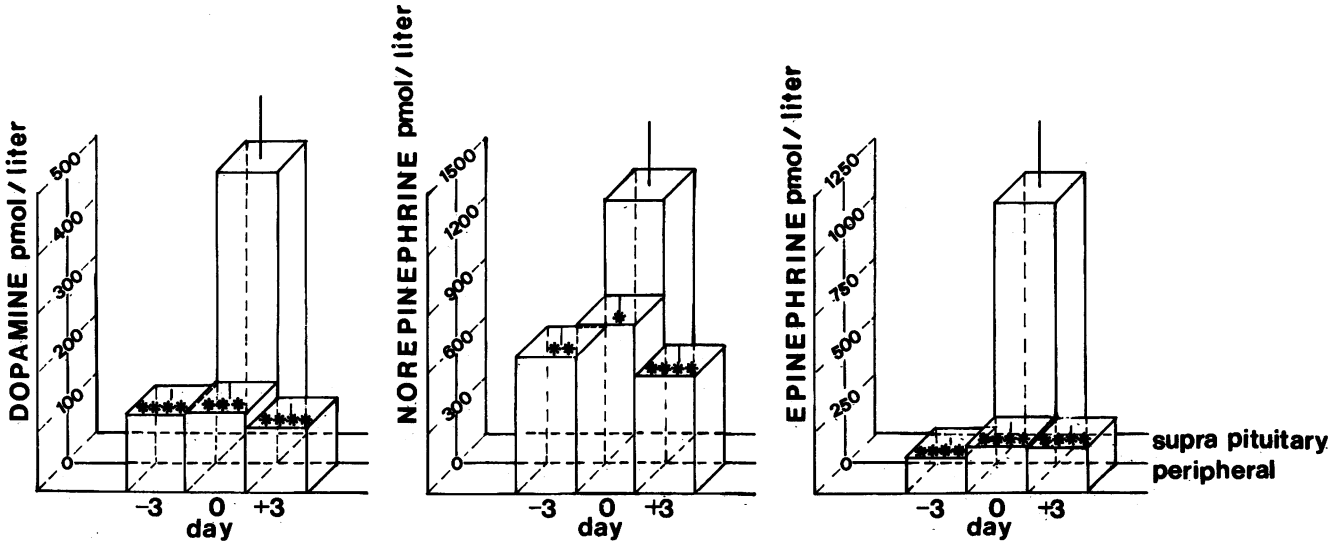

Figure 3. DA, NE and E concentrations in the study period in peripheral (preoperatively: day −3; operatively: day 0; postoperatively: day +3) and suprapituitary blood. Each bar represent the mean±SEM for 12 subjects. Note the higher CA concentrations in the suprapituitary blood than in the peripheral. *$P < 0.04$, **$P < 0.007$, ***$P < 0.003$, ****$P < 0.002$ vs. suprapituitary blood.

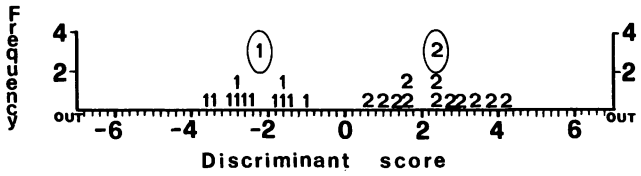

Figure 4. Stacked histogram of discriminant scores for groups 1 (peripheral blood) and 2 (suprapituitary blood). Circled figures indicate the position of group centroids. A complete (100%) group separation is apparent. Wilks' method in SPSS discriminant procedure on log-transformed variables was used.

were normotensive and mean arterial pressure did not change substantially during surgery.

Once these possible interfering causes are excluded, the "quantitatively" high CA concentrations and the "qualitatively" different inter-CA ratios in suprapituitary blood, and the highly significant results detained by discriminant analysis provide strong evidence that different CA concentrations exist in suprapituitary versus peripheral blood. Therefore, on the basis of hypothalamic CA secretory patterns, the suprapituitary blood most likely is "hypothalamic-hypophysial blood" (HHB) and the CAs contained in this blood probably are preferentially derived from the hypothalamus. Our findings are strengthened in part by studies on laboratory animals, in which more refined portal blood collecting procedures have been performed (3). DA concentrations in rats are several (4–40)-fold higher (26–28), E concentrations are also higher (27) in hypophysial portal than in peripheral blood, whereas NE concentrations are similar (28).

The data lead us to emphasize that in man, besides from a peripheral source, such as adrenal medulla for E and sympathetic neurons for NE (29), as well as other peripheral tissues for DA (30), large amounts of CAs may enter plasma from a central source: the brain. The tubero-infundibular DA (12) and reticulo-hypothalamic E and NE (11) most likely released from median eminence in HHB. Moreover, the differences of NE/DA and NE/E ratios in favor of DA and E in HHB provide further evidence that most of the DA and E in this blood are central in origin, whereas the peripheral fraction seems predominate in NE. The 100% cases correctly classified by a discriminant function that evaluated only DA and E further support this possibility. Therefore, CA release from hypothalamic neurons in blood appears more selective for DA and E, while the reason for a minor release of the central-NE fraction remains uncertain. More likely, higher numbers of DA terminals and only few NE terminals draining directly on the fenestrated capillaries of the portal vessels may be hypothesized. Hence with degrading enzymes such as monoamine oxidase and catechol-*O*-methyl-transferase present in tissue, it is likely that little NE ever reaches the portal vessels intact. E is an enigma. The possibility exists that NE, which is released from NE terminals in median eminence in the presence of high levels of phenylethanolamine-*N*-methyl-transferase, may be converted to E and spill over into the portal vessels.

The report represents the first direct evidence in man that a greater and differential CA release occurs in HHB than in peripheral blood. The hypothesis of a central (hypothalamic) source of these biogenic amines, particularly DA and E, draining via median eminence into portal vasculature could be properly advanced. Besides serving as "classical" neurotransmitters, CAs may also have a direct neurohormonal role in the regulation of the human hypothalamic-hypophysial function.

Now that we have stated that blood collected with this procedure has a majority contribution from central-hypothalamic source, it may be useful in human neuroendocrine research to study other substances, such as neuropeptides or neurohormones.

Acknowledgments

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