Virchows Archiv A Pathol Anat (1990) 416:417–422

Virchows Archiv A
Pathological Anatomy and Histopathology
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Augmented enkephalin-immunoreactivity in adrenaline-producing phaeochromocytomas *

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Received September 29, 1989 / Received in revised form November 8, 1989 / Accepted November 9, 1989

Summary. Immunohistochemical studies for methionine- and leucine-enkephalin were performed on 26 phaeochromocytomas to elucidate the patho-physiological roles of enkephalins. Positive reactions were seen in all phaeochromocytomas with varying intensities. The location of methionine- and leucine-enkephalin agreed fairly well with each other. Stronger immunostaining was obtained in phaeochromocytomas secreting both adrenalin and noradrenalin than in those secreting predominantly noradrenalin. Paroxysmal hypertension was frequently observed in patients with adrenalin-secreting phaeochromocytomas, especially those with marked enkephalin positivity. Urinary excretion of metanephrine was significantly correlated with enkephalin positivity. These findings show that all phaeochromocytomas retain the ability to produce enkephalins of the adreno-medullary or extra-medullary chromaffin tissues from which they derive. Augmented enkephalin-immunoreactivity in adrenalin-producing phaeochromocytomas may be interpreted as reflecting a close association of enkephalins with adrenalin under physiological conditions.

Key words: Enkephalin – Phaeochromocytoma

Introduction

Three years after methionine-enkephalin (met-enkephalin) and leucine-enkephalin (leu-enkephalin) were isolated from pig brains (Hughes et al. 1975), met-enkephalin was extracted from human phaeochromocytomas (Sullivan et al. 1978). It is now known that met- and leu-enkephalin are present in the highest concentration in human adrenal medulla and in lesser concentrations in the brain (Imura et al. 1983; Nakao et al. 1984). However, it has been reported that phaeochromocytomas are not necessarily positive immunohistochemically for met- and leu-enkephalin (Lundberg et al. 1979; Hassoun et al. 1984; Hacker et al. 1988). The positive rate for leu-enkephalin is only 50% in the study of DeLellis et al. (1983), and that for met-enkephalin is 62% in the study of Lloyd et al. (1984).

In extraction studies, some authors have reported an association of enkephalins with adrenergic vesicles (Roisin et al. 1983) or adrenalin (A)-producing phaeochromocytomas (Yoshimasa et al. 1983a; Parmer and O'Connor 1988) but others have found no such association (Baldi et al. 1988). From immunohistochemical studies, Livett et al. (1981) found met- and leu-enkephalin in A-producing cells but not in noradrenalin (NA)-producing cells, while Varndell et al. (1982) observed met-enkephalin in both A- and NA-producing cells.

We have found that all phaeochromocytomas are positive for met- and leu-enkephalin, and that enkephalin-immunoreactivity is higher in A-producing phaeochromocytomas than in NA-producing ones.

Materials and methods

Phaeochromocytomas removed surgically from 26 patients during the past 6 years were studied. The resected tumours were fixed in 4% formaldehyde solution, embedded in paraffin and cut at 4 μm thickness. The sectioned specimens were stained with haematoxylin and eosin. Usually, two sets of serial or semiserial sections were prepared from separate parts of the tumour for immunohistochemical study. Immunohistochemical tests for met- and leu-enkephalin were performed by the indirect immunoperoxidase method, with peroxidase antiperoxidase (PAP) kits commercially available from Chemicon International Inc., El Segundo, California, USA.[1]

* This work is supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture (No. 61770949).

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[1] Specificities of anti-met-enkephalin antiserum are indicated as follows: Met-enkephalin, preabsorbed at 10 nmol/ml, abolishes staining. Leu-enkephalin, preabsorbed at 100 nmol/ml, reduces staining. Met-enkephalin-Arg, met-enkephalin-Arg-Phe or β-endorphin, preabsorbed at 100 nmol/ml, respectively, gives no effect. Anti-leu-enkephalin antiserum has no cross-reactivity with enkephalins other than leu-enkephalin.

The positivity of the immunohistochemical staining was scored as +, ++ and +++, depending on the intensity and the extent of the staining; + for weak diffuse staining, or moderate staining in a small part of cells, ++ for moderate diffuse staining, or strong staining in a small part of cells, and +++ for strong staining in a considerable or large part of cells. As a negative control for staining, nonimmunized rabbit serum was used in place of the first rabbit antibody.

Urinary excretions of A, NA, metanephrin (MN) and normetanephrin (NMN) was measured at Mitsubishi Yuka BCL, Tokyo, Japan, by a modified fluorimetric method of high performance liquid chromatography (HPLC). Normal ranges are as follows: A 1–23 μg/day, NA 29–120 μg/day, MN 0.05–0.15 mg/day, NMN 0.1–0.24 mg/day. The amount of A and NA in the phaeochromocytomas was kindly measured, using HPLC, by Dr. Ritsuji Tamada, Department of Clinical Pathology, Dokkyo University School of Medicine, Tochigi, Japan.

Results

A positive reaction for met- and leu-enkephalin was seen in all of the phaeochromocytomas. The distribution pattern of enkephalin-positive cells was usually diffuse with varying staining intensities (Fig. 1), but sometimes sporadic or clustered. Met-enkephalin-positive cells showed good agreement with leu-enkephalin-positive ones with regard to location. When phaeochromocytoma cells were divided into two types, a large pleomorphic type with a variegated, sometimes bizarre, appearance (Fig. 2a) and a relatively small organoid one with a uniform appearance (Fig. 2b), the former gave stronger positive reactions for both met- and leu-enkephalin than the latter. The pleomorphic type cells were observed, at least in part, in most of 16 cases of pheochromocytoma secreting A, as well as NA, (A-secreting pheochromocytomas; Table 1), but in only one (Case 18) of 10 cases of phaeochromocytoma secreting NA predominantly (NA-secreting pheochromocytoma; Table 2). In Case 13, about half of the tumour was occupied by a ganglioneuromatous element, where most of the mature "nerve cells" were devoid of met- and leu-enkephalin activity (Fig. 3).

Positivity of +++ was more frequently observed in A-secreting phaeochromocytomas than in NA-secreting ones (Tables 1, 2), and phaeochromocytomas of +++ positivity tended to have a higher tissue A content than those of ++ or + positivity. Paroxysmal, but not sustained, hypertension was frequently observed in patients with A-secreting phaeochromocytomas, especially of +++ positivity. Enkephalin immunoreactivity did not show any correlation with the diabetic condition of the patient. From a quantitative standpoint, urinary MN excretion exhibited higher correlation with met- or leu-enkephalin positivity than urinary A excretion (Fig. 4). Urinary NA or NMN excretion did not seem to be correlated with the enkephalin immunoreactivity of phaeochromocytomas.

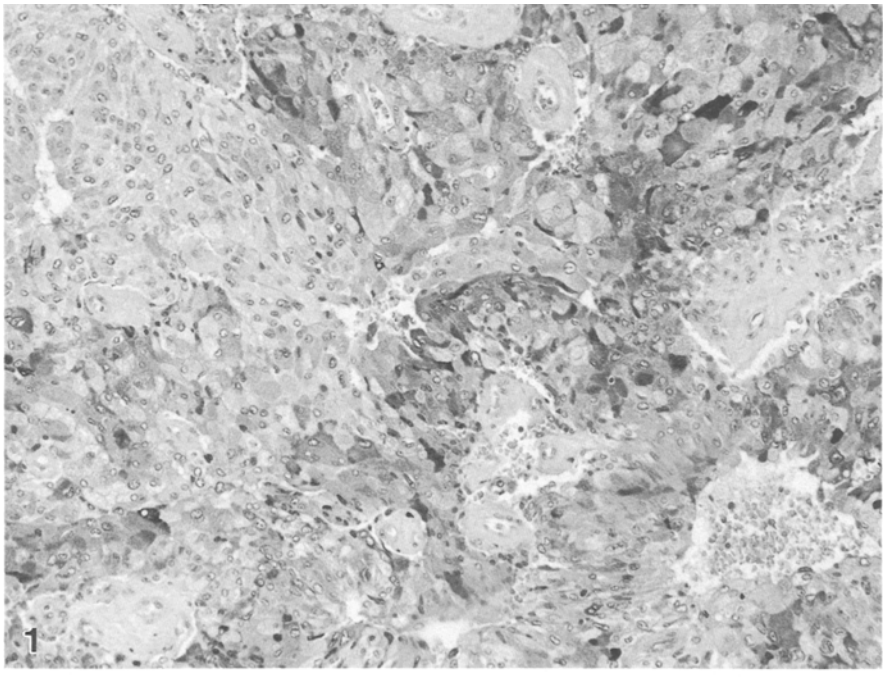
Discussion

Osamura et al. (1984) reported that the same adrenomedullary or phaeochromocytoma cells contained met-enkephalin, leu-enkephalin, met-enkephalin-Arg-Gly-Leu and met-enkephalin-Arg-Phe. According to Yoshimasa et al. (1983b) there were significant positive correlations among the amounts of these substances in

Table 1. Main clinical features, urinary excretion and catecholamine content of tissue, and immunohistochemical reactions in 16 adrenalin-secreting phaeochromocytomas

Case	Age	Sex	Tumour weight (g)	Urinary excretion (μg/day)		A and NA content (μg/g wet tissue)		Immunohistochemical reactions		Hypertension	Diabetes mellitus	Comments
				A	NA	A	NA	Met-enk	Leu-enk			
1	55	M	110	3270	499	7.1	1.2	+++	+++	P[a]	+	
2	83	F	120	712	487	6.6	6.7	+++	+++	P	−	
3	58	F	46	481	970			+++	+++	P	−	
4	50	F	25	408	234			+++	+++	P	+	
5	53	M	87	205	425	3.7	3.6	+++	+++	P	−	
6	16	F	4	27	93			+++	+++	P	−	MEN type 2
7	48	M	190	350	4700	5.9	10.2	++	+++	P	+	
8	77	F	146	325	320			++	+++	P	−	
9	42	F	280	200	2000			+++	++	P	−	
10	53	M	120	508	475			+++	++	−	+	
11	49	M	35	1620	1530			+	+++	S[b]	+	
12	37	M	320	4480	21600	0.1	0.1	++	++	S	−	
13	53	M	102	132	457			++	++	−	−	ganglio-neuroma
14	51	F	190	1240	1290			++	+	S	+	
15	43	M	15	50	175			+	++	P	−	
16	43	M	267	8880	7480			+	+	P	+	bilateral

[a] paroxysmal, [b] sustained



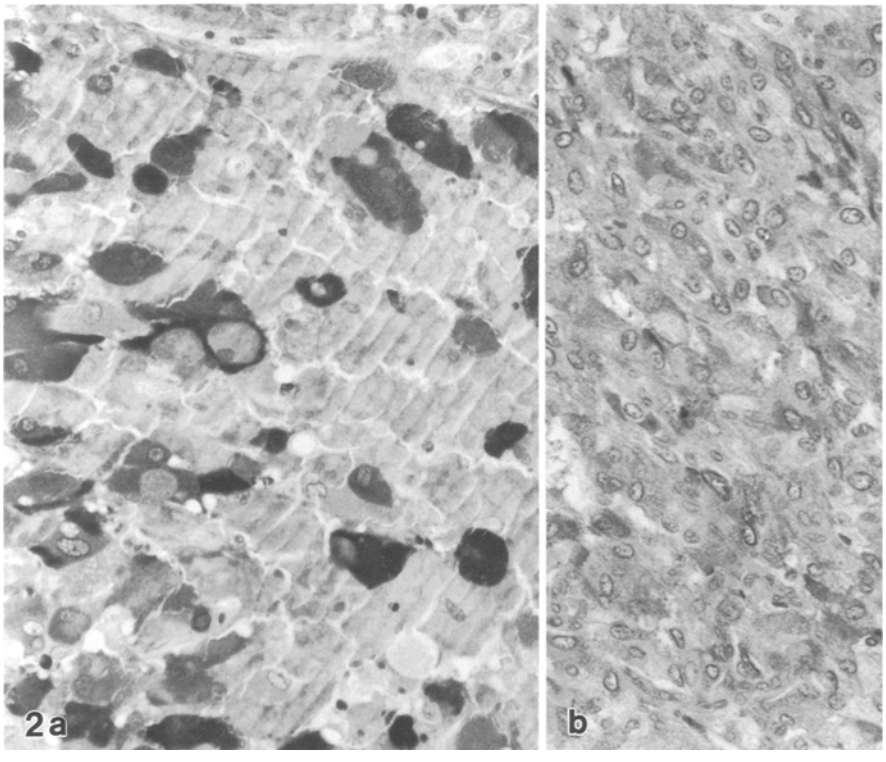


Fig. 1. This case of NA-secreting phaeochromocytoma shows strong positivity for leu-enkephalin exceptionally, being designated as +++. There are pleomorphic cells (right upper corner of the figure). Immunoperoxidase case 18 ×125

Fig. 2. (a) shows typical pleomorphic cells in A-secreting phaeochromocytoma with strong immunostaining, designated as +++. (b) shows organoid cells in NA-secreting phaeochromocytoma with moderate diffuse staining, designated as ++. Leu-enkephalin immunoperoxidase Case 5 (a, ×250) and Case 21 (b, ×250)

phaeochromocytoma tissues. Thus, it is evident that preproenkephalin A, itself, is produced in phaeochromocytomas. Our study shows that all phaeochromocytomas are positive immunohistochemically for met- and leu-enkephalin, retaining the preproenkephalin A-producing ability of the adreno-medullary or extra-medullary chromaffin tissues from which they derive.

In the normal adrenal medulla, enkephalins are stored in and secreted from the chromaffin vesicles, concomitantly with catecholamines (Viveros et al. 1979; Kilpatrick et al. 1980), and the enkephalins circulate as intact pentapeptides in human plasma (Clement-Jones et al. 1980; Yoshimasa et al. 1983a). However, considering the extremely short half-life of enkephalins in plasma and tissues – a few seconds to minutes (Hambrook et al. 1976), it has been suggested that the site of action is close to the site of release (Dupont et al. 1977). It is unlikely that met- and leu-enkephalin could be immun-

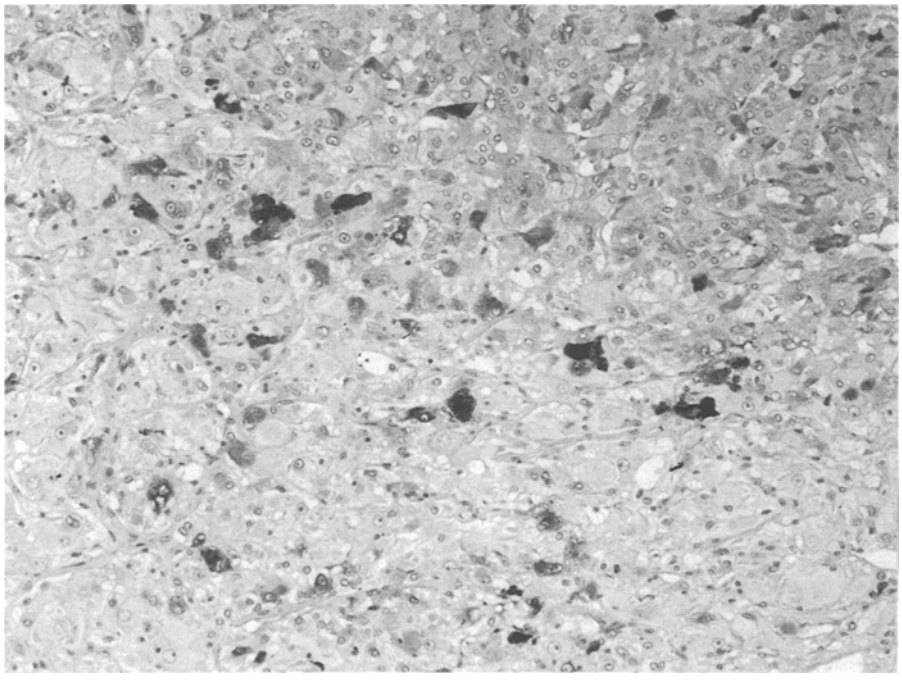


Fig. 3. Note the phaeochromocytoma with ++ immunostaining in the upper half of the figure and the element of ganglioneuroma in the lower half. Most of the ganglionic cells are negative. The scattered strongly positive cells lack neural differentiation. Met-enkephalin immunoperoxidase Case 13 (×125)

Table 2. Main clinical features, urinary extretion and catecholamine content of tissue, and immunohistochemical reactions in 10 noradrenalin-secreting phaeochromocytomas

Case	Age	Sex	Tumour weight (g)	Urinary excretion (μg/day)		A and NA content (μg/g wet tissue)		Immunohistochemical reactions		Hypertension	Diabetes mellitus	Comments
				A	NA	A	NA	Met-enk	Leu-enk			
17	54	F	7	50	4000			+++	+++	S[a]	+	
18	50	M	170	34	340			+++	+++	–	–	extra-adrenal
19	46	M	63	17	1550			++	++	S	+	extra-adrenal
20	65	M	1100	16	577	0.2	0.7	++	++	S	–	malignant
21	39	F	29	9	1440			++	++	S	–	malignant extra-adrenal
22	54	M	72	57	33000			+	++	S	+	malignant extra-adrenal
23	48	F	44	52	1760	1.2	14.7	++	+	P[b]	–	
24	31	M	230	45	1460			+	+	–	+	
25	48	M	95	18	269	0.2	0.3	+	+	–	–	
26	38	F	354	4	1260			+	+	S	–	

[a] sustained, [b] paroxysmal

ohistochemically detected in a free state, because of their relatively low molecular weights and the short half-life in the tissues. Therefore, the demonstration of enkephalins in phaeochromocytomas can reasonably be explained by the presence of these compounds in the chromaffin vesicles, whether or not they are incorporated into the preproenkephalin A molecule.

The adreno-medullary cells are thought to be supplied with blood that has previously perfused the adrenal cortex and contains high concentrations of corticosteroids. The phenylethanolamine N-methyltransferase which triggers the formation of A is induced in the presence of glucocorticoids (Wurtman and Axelrod 1966). Therefore, extra-adrenal or malignant phaeochromocytomas, which are frequently extra-adrenal in origin, secrete NA predominantly. This seems to be one of the reasons why enkephalin-positivity is lower in extra-adrenal or malignant phaeochromocytomas than in benign adrenal ones; the reported positive incidences are 40–80% in the former and 80–100% in the latter (Bostwick

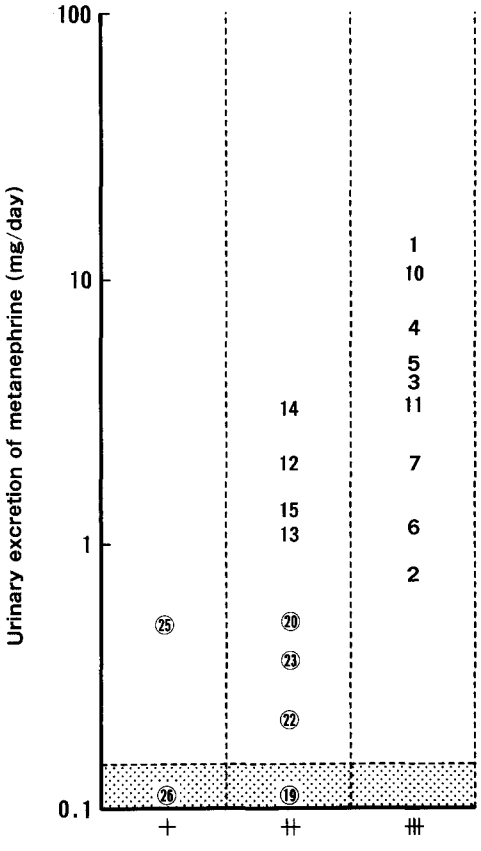


Fig. 4. Association of immunocytochemical reaction for met- or leu-enkephalin with urinary excretion of metanephrine. The higher value for immunostaining was chosen, when there were differences between positivities for met- and leu-enkephalins. The numbers and the encircled numbers denote the number of cases of A-secreting or NA-secreting phaeochromocytomas, respectively. The shaded area shows the normal range of urinary metanephrine excretion, which is expressed on a log scale

et al. 1987; Capella et al. 1988; Linnoila et al. 1988). However phaeochromocytoma does not seem to follow the all or none principle concerning the production of A and NA, as seen in Tables 1 and 2. We consider that the conclusion that a variable but positive reaction for enkephalins is shown in all of our phaeochromocytomas is reasonable, although we do not deny the possibility that highly malignant or exclusively NA-secreting phaeochromocytomas may lack the ability to produce these peptides.

Although the physiological role of enkephalins in adrenomedullary tissue is largely unknown, an inhibitory effect of met-enkephalin on catecholamine secretion from cultured adreno-medullary or phaeochromocytoma cells has been reported (Kumakura et al. 1980; Sainai and Guidotti 1982; Yanase et al. 1986). Our study shows that enkephalin immunoreactivity is associated with urinary excretion of A and MN, which is a major metabolite of A. Patients with A-, but not NA-, secreting phaeochromocytomas frequently have accompanying paroxysmal hypertension, and A-secreting phaeochromocytomas show necrosis or haemorrhagic infarction more often than do NA-secreting ones (Ito 1989). These observations are interpreted as suggesting that uncontrolled A secretion might result in extreme paroxysmal hypertension and auto-infarction of the adrenal tissue even in normal persons without phaeochromocytoma. It may be that enkephalins function as neuromodulators to prevent overflow of A secretion from adreno-medullary cells in a paracrine or autocrine fashion, in response to stimulation.

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