Present Status of Paraneuron Concept

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Summary. Paraneurons are receptosecretory cells producing substances of neurons. Like neurons, they are characterized by membrane-bound granules and vesicles which contain bioactive (and inactive) peptides, amines or other classic messengers, adenine nucleotides and acidic carrier proteins including chromogranins.

The cell-biological significance of the carrier proteins in the secretory granules and the utility of immunohistochemical detection of chromogranins as reliable markers of paraneurons are emphasized.

Cells "ectopically" producing bioactive peptides and/or amines, including ANP producing muscle cells of the heart, do not contain chromogranin immunoreactivity.

The boundary between the neuron and paraneuron and that of the paraneuron are unclear because the cells comprise gradational spectra from typical ones to atypical ones. It is arbitrary whether to include the mast cell in the paraneurons, although it seems reasonable to exclude it by defining a paraneuron as being epithelial in nature.

PARANEURON: CRITERIA AND MEMBERS

Paraneurons are endocrine and sensory cells which share structural, functional and metabolic features with neurons. They produce substances identical with or related to neurotransmitters (including neuromodulators) or neurohormones. They also possess neurosecretion-like and/or synaptic vesicle-like granules. The paraneurons are receptosecretory in function: they recognize adequate stimuli with receptors on the receptive portion of the cell surface and release secretions through the secretory portion of the cell.

The main members of the paraneuron, most of which are the cells dealt with in this symposium, are as follows:

1. Gastro-enteric endocrine cells; bronchopulmonary paraneurons; urogenital paraneurons.

2. Peptide/amine producing endocrine cells, including parafollicular, parathyroid, adenohypophyseal and pancreatic endocrine cells.

3. Chromaffin cells of the adrenal medulla; carotid body chief cells which are also chromaffin in nature; SIF (small intensely fluorescent) cells in the sympathetic ganglia.

4. Gustatory cells of the taste bud.

5. Olfactory cells; receptor cells of the vomeronasal organ.

6. Merkel cells in the skin.

7. Hair cells of the inner ear and lateral line organ (fish); electroreceptor cells of the ampullary and tubular organs (fish).

8. Visual cells of the retina; pinealocytes.

Different types of paraneurons comprise a spectrum if one arranges them according to structural and functional similarities. This paraneuron spectrum continues to that of the neurons, showing some cell types belonging to both spectra. These include olfactory cells, SIF cells, and chromaffin cells of the adrenal medulla and carotid body. Still other cells show structures and functions which are at the same time neuronal and paraneuronal. Neurons and paraneurons are thus continuous and overlapping (FUJITA et al., 1988).

Although our initially proposed criteria for paraneurons included the pronouncement that paraneurons are neuroectodermal in origin (FUJITA, 1976), this was later deemed inappropriate because advances in experimental embryology, especially by LE DOUARIN (1982) revealed non-neuroectodermal origins for gastro-entero-pancreatic and some other paraneurons. The paraneurons are therefore of multiple origin (FUJITA and KOBAYASHI, 1979; FUJITA, 1980). Some of them are neuroectodermal but many are simply ectodermal or entodermal. Even mesenchymal cells may be considered to be located on the outskirts of the paraneuron community (see below).

CELLS ON THE MARGIN OF THE PARANEURON GROUP

The boundary of the paraneuron is vague. The *melanocyte*, for instance, is closely related to neurons in the sense that it is originated from the neural crest and produces the monoamine derivative, melanin. Its dendritic processes as well as the granules, melanosomes, are additional neuron-like features. We, however, prefer to exclude the melanocyte from paraneurons, because the cell does not seem to actively release its granules in response to adequate stimulus. Neither does it seem that melanin acts as a signal substance to a target cell.

The *mast cell* has occasionally been considered an "associate member" of the paraneuron. This is due to its receptosecretory nature and secretion of histamine and other amines, as well as ATP and other adenine nucleotides (for review see FUJITA et al., 1988), though its production of VIP proposed by CUTZ et al. (1978) has not been confirmed by later authors. The fact that the early work injecting Ca^{2+} into the cell to cause secretion release was performed by the use of this cell has been taken into account (KANNO et al., 1973). NGF has been demonstrated to increase rat mast cells *in vivo* (ALOE and LEVI-MONTALCINI, 1977). In spite of the above-mentioned paraneuronal features of the mast cell, I was reluctant to include it as a "regular" or genuine member of the paraneuron, mainly due to its nature as a connective tissue element and its close relation to blood cells. The delusion in this context was complicated by disputes concerning the origin of the mast cell, until elegant transplantation study by KITAMURA et al. (1977, 1979) determined its derivation from the bone marrow. As far as its mesenchymal origin is concerned, the mast cell is clearly outside of the ordinary paraneurons which are usually epithelial in origin.

It seems now appropriate and timely to define paraneurons as being epithelial in nature. From their phylogenetic origin onward, paraneurons have been located in the epithelia in order to receive information from the external milieu, to transduce it into chemical messengers and to release them into the internal milieu. There are a number of paraneurons located sub- or extraepithelially, but they have originally been born in the epithelium and secondarily been evaginated or migrated to those sites, retaining their epithelial nature.

Even from previous stages of research, we excluded the mast cells from being fully qualified members of the paraneuron group, and stressed that it depends

upon the consideration of the researcher whether he includes this cell into the paraneuron. To repeat: the boundary of the paraneuron family is unclear and cells there comprise a gradation in cell-biological features towards non-epithelial cells. This optionality and indecisiveness seem rather unwelcomed by some readers. Some reviewers of our monograph (FUJITA et al., 1988) accused us of classifying the mast cell as a paraneuron (PEARSE, 1988; LIEBERMAN, 1988). These criticisms may be answered by certain sentences in the book itself (p. 12): "In the outskirts of paraneurons such untypical members like the *mast cell* and *melanocyte* are located. More peripherally follows the megakaryocyte-platelet system secreting monoamines and adenine nucleotides.... Where to draw the line limiting a group of cells is always arbitrary."

What is important in this context is not whether to call the mast cell a paraneuron or not, but to acknowledge that the cell, in spite of its mesenchymal origin, expresses paraneuron-like features and, due to its easy isolation from murine ascites, has served and is serving toward cell-biological investigations, including the cell's amine handling mechanism, stimulus-secretion coupling and receptor research, all potentially applicable to neurons and paraneurons.

ECTOPIC EXPRESSION OF PARANEURONAL FEATURES

The production of peptide/amine messengers has been recognized in some cells which can be regarded as significantly different in nature from the paraneurons.

The atrial muscle cells producing cardiodilatin (FORSSMANN et al., 1983) or ANP, atrial natriuretic peptides (KANGAWA and MATSUO, 1984), are most important both in the amount of the cells and their product and in functional significance (for details see FORSMANN et al., in this volume). There then follow the murine salivary gland, especially its granular ducts, producing NGF (nerve growth factor), EGF (epidermal growth factor), renin, angiotensin, neurotensin and many other bioactive peptides (for reviews see BARKA, 1980; MURPHY et al., 1980), and frog cutaneous exocrine cells producing different bioactive peptides such as caerulein, bombesin, tachykinin and sauvagine as reviewed by ERSPAMER (1983), an explorer in this field (see RENDA et al., in this volume).

It is noteworthy that ANP- and cardiodilatin-like

Fig. 1. Spectrum of neurons (*left*) and paraneurons (*right*) continuous and overlapping with each other. 1 Enteric intramural neuron, which represents the prototype of neurons—"archineuron"; 2 multipolar autonomic neuron; 3 sensory neuron; 4 pyramidal neuron; 5 Purkinje cell; 6 "neurosecretory" or "peptidergic" neuron; 7 CSF-contacting neuron; 8 olfactory cell; 9 gut endocrine cell, which represents the prototype of paraneurons—"archiparaneuron"; 10 carotid body chief cell and SIF cell; 11 adrenal chromaffin cell; 12 adenohypophysial, parafollicular, or other endocrine cell; 13 pinealocyte; 14 visual cell; 15 Merkel cell; 16 inner ear hair cell; 17 bronchopulmonary paraneuron; 18 gustatory cell. Reproduced, under permission, from T. FUJITA, T. KANNO and S. KOBAYASHI: *The Paraneuron*, Springer-Verlag, 1988

immunoreactivities have been identified in neurons of a ganglion in the snail (NEHLS et al., 1985) in some neurons in the central and peripheral nervous system of various mammals (FORSSMANN and MUTT, 1985; KAWATA et al., 1985), as well as in adrenomedullary cells and in SIF cells of sympathetic ganglia in the rat (INAGAKI et al., 1986). The research group of MATSUO revealed the occurrence of BNP (brain natriuretic polypeptide), which is closely related but different in amino acid sequence from ANP, in the brain (SUDOH et al., 1988) and, though in much smaller amounts, in the atrial muscle cells (see FORSSMANN et al., this volume). As to the peptides in the frog skin, the same or quite similar molecules have been known to be shared by neurons in the nervous tissue and/or endo-

crine cells (paraneurons) in the gut epithelium (for review see RENDA et al., this volume).

In addition to mast cells and basophil leukocytes, bioactive monoamines are also produced by blood platelets which contain large amounts of serotonin. The exocrine cells in the frog skin also contain serotonin in the identical granules as those possessing bombesin or other peptides.

In Summary, muscular, exocrine-glandular and blood cells may produce bioactive peptides and/or amines identical with those produced by neurons and paraneurons. Theoretically, every cell of the body may have the potentiality to produce those substances with the genes they share, but in reality the genes are expressed predominantly in a specific cell

group of neurons and paraneurons, and, ectopically, only in several cell types outside the group.

It seems now appropriate to consider the secretions of neurons and paraneurons and to compare them with those of the ectopic amine/peptide producing cells.

SECRETIONS OF NEURONS AND PARANEURONS

Neurons and paraneurons produce and release several categories of substances. These include: 1) fragments of prepropeptides, some of which are bioactive messengers; 2) amines or other "classic" messengers; 3) ATP and other adenine nucleotides; 4) chromogranins and other acidic carrier proteins; 5) protein-processing and amine-converting enzymes; 6) glycosaminoglycans; 7) lipids; and 8) calcium and other ions. As detailed accounts of these substances have been given elsewhere (FUJITA et al., 1988), we will here concentrate on the items 3, adenine nucleotides and 4, chromogranins.

ATP and other adenine nucleotides are contained in the secretory granules of every paraneuron as well as the larger and smaller granules/vesicles of every neuron, as a light microscopic quinacrine reaction, electron-microscopic uranaffin reaction and biochemical analysis of isolated granules indicate (BURNSTOCK, 1972; RIBEIRO, 1978; BÖCK, 1980; IJIMA and HASEGAWA, 1985; for review see FUJITA et al., 1988; PAYNE, this volume). These adenine nucleotides are believed to exert significant, though not precisely known, biological effects after being released by exocytosis (KURODA, 1978; see PAYNE in this volume).

Acidic carrier proteins reacting metachromatically in toluidine blue and other basic dyes have been recognized first in pancreatic islet cells (SOLCIA and SAMPIETRO, 1965; FUJITA and TAKAYA, 1968) and later proposed by us to occur universally in endocrine cells now categorized as paraneurons (FUJITA and KOBAYASHI, 1973). These acidic carrier proteins correspond to chromogranins, which were first obtained from the secretory granules of adrenal chromaffin cells (BLASCHKO et al., 1967; SCHNEIDER et al., 1967; WINKLER, 1976) and recently recognized to be contained in the granules of a large variety of paraneurons (O'CONNOR et al., 1983; LLOYD and WILSON, 1983).

Chromogranins are at present known to comprise slightly different molecules: chromogranins A, B and C. Chemical studies, on the other hand, have revealed that the acidic protein obtained from parathyroid

granules, called secretory protein I (SP-1) or parathyroid secretory protein (PSP), closely resembles chromogranin A in molecular structure, while secretogranins I and II extracted from rat pheochromocytoma correspond to chromogranins B and C, respectively (for details see WINKLER et al., 1986; ELDEN et al., 1987).

CHROMOGRANINS AS PARANEURON MARKERS

It would make for clearcut identification of paraneurons if chromogranins and/or related proteins would be demonstrable in every paraneuron. The present available data do not satisfy this expectation. The immunoreactivities for chromogranins occur heterogeneously even in one and the same paraneuronal tissue. In the adenohypophysis (RINDI et al., 1986; RUNDLE et al., 1986) and pancreatic islets (GRUBE et al., 1986, 1989, this volume; EHRHART et al., 1986), for instance, only part of the paraneuronal cells reveal a positive immunoreactivity, some only weakly reacting and others being negative. The population and types of the immunopositive cells vary according to species, and if one examines a given cell type in a variety of animal species with an antiserum against chromogranin A (which is the type distributed most extensively among the three chromogranins), one will find a positive reaction in some of the species.

The heterogeneous chromogranin immunopositivity in paraneurons may be ascribed either to differences in chromogranin molecules among species and cell types or to the antigenic site on an chromogranin molecule being masked by other granule components (GRUBE et al., 1986; FUJITA et al., 1988). At any rate, a positive immunoreactivity of a given paraneuron in whatever animal must be taken as the paraneuronal marker of the cell type concerned.

In fact, the cell types immunoreactive for chromogranins are increasing with examinations in various materials. The Merkel cell as a representative sensory paraneuron has been successfully immunostained in the pig snout (Fig. 2, see also HARTSCHUH et al., this volume).

We examined the cells ectopically secreting amine/peptide messengers, including the atrial muscle cells, frog cutaneous glands and granulated ducts of murine submandibular gland, and found that their granules were all immunonegative for chromogranins.

Neurons generally do not immunoreact for chromogranins. We, however, recognized a clear immunoreactivity in the intramural neurons of the pig,

Fig. 2. Immunoreactivity for chromogranin A as demonstrated by the PAP method in some paraneuronal cells. **a.** Rat adrenal gland with its cortex (right hand) and medulla. The medullary cells are immunostained heterogenously for chromogranin A. The nuclei are slightly counterstained with hematoxylin in this section. $\times 300$. **b.** A portion of the antral mucous membrane in the snake (*Elaphe quadrigata*). Essentially every endocrine cell immunoreacts for chromogranin A. $\times 740$. **c.** Snout mucous membrane of the pig. Merkel cells located at the bottom of the epithelium (the left half of the picture) are immunoreactive with their secretory granules gathered to the cell base. $\times 1,300$. **d.** A spread preparation of a layer of porcine jejunum containing the myenteric nerve plexus. Globular elements forming the beaded nerve fibers are immunostained for chromogranin A. $\times 270$ (The antiserum used was raised against porcine SP-1 and was purchased from Immuno Nuclear Corporation, MN, USA. The micrographs were prepared by Dr. T. IWANAGA)

especially intense in their beaded fibers (Fig. 2). This may possibly reflect the fact that the neurons in the gut wall represent a primitive type, sharing some features with paraneurons (FUJITA et al., 1988). As for neurons of other types, they probably possess acidic carrier proteins of more or less different molecular structure, so that available chromogranin antibodies can not demonstrate them. It is important in this context to remember that neurophysins have long been known as acidic carrier proteins of granules as well, in magnocellular neurosecretory cells of the hypothalamus.

NEURON-SPECIFIC PROTEINS AS PARANEURON MARKERS

The cytosol of paraneurons may contain proteins which have been isolated from the brain and believed, until recently, to be specific for neurons. These neuron-specific proteins include neuron-specific enolase (NSE), neurofilament protein (NFP), neuronal tubulin, and spot-35 or Purkinje cell specific protein. The distribution of these proteins in different types of paraneurons will be dealt with by IWANAGA and FUJITA in this volume. Here, it shall only be pointed out that our examinations using various mammals have shown that none of the above-mentioned substances seem to be contained in the atrial muscle cell or other "ectopically" amine/peptide producing cells.

PHYLOGENY OF NEURONS AND PARANEURONS

Recent electron-microscopic and immunocytochemical studies have indicated that intraepithelial bipolar paraneurons as well as multipolar neurons occur in hydra, the most primitive coelenterate (WESTFALL and KINNAMON, 1978; GRIMMELIKHUIJZEN, this volume) and that both types of cells are widely distributed in metazoa of the protostomian as well as deuterostomian series (for review see FUJITA et al., 1988). For this reason the open type basal-granulated cells in the gut epithelium retained in vertebrates deserve the name of archiparaneurons, while the multipolar intramural neurons which actually are characterized by different primitive features may be called archineurons. The gut is regulated by these neurons and paraneurons which have changed their structure, function and messenger substances only slightly through the 500,000,000 years of metazoan evolution (for review see FUJITA et al., 1988).

Various types of neurons and paraneurons have meanwhile differentiated to be added to the archineurons and archiparaneurons, along with the development of various kinds of nervous, sensory and endocrine organs.

REFERENCES

- ALOE, L. and R. LEVI-MONTALCINI: Mast cells increase in tissues of neonatal rats injected with the nerve growth factor. *Brain Res.* 133: 358-366 (1977).
- BARKA, T.: Biologically active polypeptides in submandibular glands. *J. Histochem. Cytochem.* 28: 836-359 (1980).
- BLASCHKO, H., R. S. COMLINE, F. H. SCHNEIDER, M. SILVER and A. D. SMITH: Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. *Nature* 215: 58-59 (1967).
- BÖCK, P.: Identification of paraneurons by labelling with quinacrine (Atebrin). *Arch. Histol. Jap.* 43: 35-44 (1980).
- BURNSTOCK, G.: Purinergic nerves. *Pharmacol. Rev.* 24: 509-581 (1972).
- CUTZ, E., W. CHAN, N. S. TRACK, A. GOTH and S. I. SAID: Release of vasoactive intestinal polypeptide in mast cells by histamine liberators. *Nature* 275: 661-662 (1978).
- EHRHART, M., D. GRUBE, M. F. BADER, D. AUNIS and M. GRATZL: Chromogranin A in the pancreatic islet: Cellular and subcellular distribution. *J. Histochem. Cytochem.* 34: 1673-1682 (1986).
- EIDEN, L. E., W. B. HUTTNER, J. MALLET, D. T. O'CONNER, H. WINKLER and A. ZANINI: A nomenclature proposal for the chromogranin/secretogranin proteins. *Neuroscience* 21: 1019-1021 (1987).
- ERSPAMER, V.: Amphibian skin peptides in mammals —looking ahead. *Trends Neurosci.* 6: 200-201 (1983).
- FORSSMANN, W. G. and V. MUTT: Cardiodilatin-immunoreactive neurons in the hypothalamus of *Tupia*. *Anat. Embryol.* 172: 1-5 (1985).
- FORSSMANN, W. G., D. HOCK, F. LOTTSPEICH, A. HENSCHEN, V. KREYE, M. CHRISTMANN, M. REINECKE, J. METZ, M. CARLQUIST and V. MUTT: The right auricle of the heart is an endocrine organ: cardiodilatin as a peptide hormone candidate. *Anat. Embryol.* 168: 307-313 (1983).
- FORSSMANN, W.-G., K. NOKIHARA, M. GAGELMANN, D. HOCK, S. FELLER, P. SCHULZ-KNAPPE and F. HERBST: The heart is the center of a new endocrine, paracrine, and neuroendocrine system. *Arch. Histol. Cytol.* 52, Suppl.: 293-315 (1989).
- FUJITA, T.: The gastro-enteric endocrine cell and its paraneuronic nature. In: (ed. by) R. E. COUPLAND and T. FUJITA: *Chromaffin, enterochromaffin and related cells*. Elsevier Scientific Publ. Co., Amsterdam-Oxford-New York, 1976 (p. 204-208).
- : Concept of paraneurons. *Arch. Histol. Jap.* 40

- Suppl.: 1-12 (1977).
- FUJITA, T.:** Paraneuron, its current implications. *Biomed. Res.* 1, Suppl.: 3-9 (1980).
- FUJITA, T. and S. KOBAYASHI:** The cells and hormones of the GEP endocrine system—The current of studies. In: (ed. by) T. FUJITA: *Gastro-entero-pancreatic endocrine system: A cell-biological approach*. Igaku-Shoin, Tokyo, 1973 (p. 1-16).
- : Current views on the paraneurone concept. *Trends Neurosci.* 2: 27-30 (1979).
- FUJITA, T. and K. TAKAYA:** Metachromatic reaction of pancreatic B cells to toluidine blue; influence of pH on staining. *Stain Technol.* 43: 329-332 (1968).
- FUJITA, T., T. KANNO and S. KOBAYASHI:** The paraneuron. Springer-Verlag, Tokyo-Berlin-Heidelberg, 1988.
- GRIMMELIKHUIJZEN, C. J. P., D. GRAFF and I. C. MC FARLANE:** Neurons and neuropeptides in coelenterates. *Arch. Histol. Cytol.* 52, Suppl.: 265-276 (1989).
- GRUBE, D., D. AUNIS, F. BADER, Y. CETIN, A. JORNS and S. YOSHIE:** Chromogranin A (CGA) in the gastro-entero-pancreatic (GEP) endocrine system. I. CGA in the mammalian endocrine pancreas. *Histochemistry* 85: 441-452 (1986).
- GRUBE, D., G. BARGSTEN, Y. CETIN and S. YOSHIE:** Chromogranins in mammalian GEP endocrine cells: Their distribution and interrelations with co-stored amines and peptides. *Arch. Histol. Cytol.* 52, Suppl.: 91-98 (1989).
- HARTSCHUH, W., E. WEIHE and N. YANAIHARA:** Immunohistochemical analysis of chromogranin A and multiple peptides in the mammalian Merkel cell: Further evidence for its paraneuronal function? *Arch. Histol. Cytol.* 52, Suppl.: 423-431 (1989).
- IJIMA, T. and K. HASEGAWA:** Quinacrine-induced dilation of the rat cecum and degeneration of large granular vesicle-containing neurons in the myenteric plexus. *Cell Tiss. Res.* 241: 383-389 (1985).
- INAGAKI, S., Y. KUBOTA, S. KITO, K. KANGAWA and H. MATSUO:** Immunoreactive atrial natriuretic polypeptides in the adrenal medulla and sympathetic ganglia. *Regul. Peptides* 15: 249-260 (1986).
- KANGAWA, K. and H. MATSUO:** Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (α -hANP). *Biochem. Biophys. Res. Comm.* 118: 131-139 (1984).
- KANNO, T., D. E. COCHRANE and W. W. DOUGLAS:** Exocytosis (secretory granule extrusion) induced by injection of calcium into mast cells. *Can. J. Physiol. Pharmacol.* 51: 1001-1004 (1973).
- KAWATA, M., K. NAKAO, N. MORI, Y. KISO, H. YAMASHITA, H. IMURA, and Y. SANO:** Atrial natriuretic polypeptide: topographical distribution in the rat brain by radioimmunoassay and immunohistochemistry. *Neuroscience* 16: 521-546 (1985).
- KITAMURA, Y., M. SHIMADA, K. HATANAKA and Y. MIYANO:** Development of mast cells from grafted bone marrow cells in irradiated mice. *Nature* 268: 442-443 (1977).
- KITAMURA, Y., M. SHIMADA, S. GO, H. MATSUDA, K. HATANAKA and M. SEKI:** Distribution of mast-cell precursors in hematopoietic and lymphopoietic tissues of mice. *J. Exp. Med.* 150: 482-490 (1979).
- KURODA, Y.:** Physiological roles of adenosine derivatives which are released during neurotransmission in mammalian brain. *J. Physiol. (Paris)* 74: 463-470 (1978).
- LE DOUARIN, N. M.:** The neural crest. Cambridge University Press, Cambridge-London-New York, 1982.
- LIEBERMAN, A. R.:** Book review. The paraneuron. *J. Neurocytol.* 17: 871-875 (1988).
- LOYD, R. V. and B. S. WILSON:** Specific endocrine tissue marker defined by a monoclonal antibody. *Science* 222: 628-630 (1983).
- MURPHY, R. A., A. Y. WATSON, J. METZ and W. G. FORSSMANN:** The mouse submandibular gland: An exocrine organ for growth factors. *J. Histochem. Cytochem.* 28: 890-902 (1980).
- NEHLS, M., M. REINECKE, R. E. LANG and W. G. FORSSMANN:** Biochemical and immunological evidence for a cardiodilatin-like substance in the snail neurocardiac axis. *Proc. Nat. Acad. Sci. USA.* 82: 7762-7766 (1985).
- O'CONNOR, D. T., D. BURTON and L. J. DEFTOS:** Chromogranin A: Immunohistology reveals its universal occurrence in normal polypeptide hormone producing endocrine glands. *Life Sci.* 33: 1657-1663 (1983).
- PAYNE, C. M.:** Phylogenetic considerations of neurosecretory granule contents: Role of nucleotides and basic hormone/transmitter packaging mechanisms. *Arch. Histol. Cytol.* 52, Suppl.: 277-292 (1989).
- PEARSE, A. G. E.:** Names and claims. *Nature* 333: 218-219 (1988).
- RENDA, T., L. D'ESTE, A. FASOLO, L. H. LAZARUS, F. MINNITI and V. ERSAMER:** Brain-gut-skin peptides: An update overview. *Arch. Histol. Cytol.* 52, Suppl.: 317-323 (1989).
- RIBEIRO, J. A.:** ATP; related nucleotides and adenosine on neurotransmission. *Life Sci.* 22: 1373-1380 (1978).
- RINDI, G., R. BUFFA, F. SESSA, O. TORTORA and E. SOLCIA:** Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. Distribution, distinction from costored hormones/prohormones and relationship with the argyrophil component of secretory granules. *Histochemistry* 85: 19-28 (1986).
- RUNDLE, S., P. SOMOGYI, R. FISCHER-COLBRIE, C. HAGAN, H. WINKLER and I. W. CHUBB:** Chromogranin A, B, and C: immunohistochemical localization in ovine pituitary and the relationship with hormone-containing cells. *Regul. Peptides* 16: 217-233 (1986).
- SCHNEIDER, F. H., A. D. SMITH and H. WINKLER:** Secretion from the adrenal medulla: biochemical evidence for exocytosis. *Brit. J. Pharmacol.* 31: 94-104 (1967).
- SOLCIA, E. and R. SAMPIETRO:** On the nature of the metachromatic cells of pancreatic islets. *Z. Zellforsch.* 65: 131-138 (1965).
- SUDOH, T., K. KANGAWA, N. MINAMINO and H. MATSUO:** A new natriuretic peptide in porcine brain. *Nature* 332: 78-81 (1988).

- WESTFALL, J. A. and J. C. KINNAMON:** A second sensory-motor-interneuron with neurosecretory granules in Hydra. *J. Neurocytol.* 7: 365-379 (1978).
- WINKLER, H.:** The composition of adrenal chromaffin granules; an assessment of controversial results. *Neuroscience* 1: 65-80 (1976).
- WINKLER, H., D. K. APPS and R. FISCHER-COLBRIE:** The molecular function of adrenal chromaffin granules: Established facts and unresolved topics. *Neuroscience* 18: 261-290 (1986).

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