Trends in NeuroSciences

Does the kallikrein-like enzyme gene family code for a group of peptide hormone-processing enzymes?

The cloning of genes which code for a family of kallikrein-like enzymes may provide a basis for understanding the substrate specificity of peptide-processing enzymes.

The biosynthesis of a peptide hormone is a complex process (for review see Ref. 1) involving many co- and post-translational events, the foremost among them being the proteolytic cleavage(s) of the larger propeptide hormone to its biologically active peptide(s). In many cases, there must be multiple proteolytic cleavages to liberate the bioactive peptide hormones; for example, there are 12 cleavage sites required to release all of the Met- and Leu-enkephalins from the proenkephalin A precursor protein[2].

In the majority of cases the proteolytic cleavages occur at sites directly flanking the peptide hormone consisting of a pair of amino acids, either lysine or arginine, as shown in Fig. 1. Usually the carboxy-terminal amino acid in this pair is arginine. In a few precursors, such as pro-vasopressin/neurophysin and pro-somatostatin, one of the proteolytic cleavages occurs at a single arginine. These endolytic (internal) cleavage(s) are then followed by an exolytic (external) cleavage(s) at the newly created carboxy terminus of the peptide hormone, digesting the single or double basic amino acid segment with a carboxypeptidase B-like specificity.

For many years scientists have been studying proteases that may be responsible for these proteolytic events. In general, the classical approach has been to make a synthetic substrate analogue of the cleavage site in the prohormones and use it in an enzymatic assay for purification of the enzyme from those tissues known to express the prohormone-processing enzymes. Alternatively, natural substrates such as the prohormones themselves have been synthesized or isolated and used for purifying enzymes capable of processing the prohormone. Using both approaches, many different proteolytic enzymes have been identified and/or isolated. There appears to be no general consensus on the characteristics of the enzymes that are responsible for these cleavages, but one characteristic of particular interest is the pH at which these different enzymes work most effectively. This pH is usually indicative of the pH at which they work intracellularly and thus an indication of where they may be active intracellularly. In some cases the enzymes that have been identified have slightly acidic pH activity optima, suggesting that they may be performing their cleavages in peptide hormone-containing secretory granules which have an internal pH of 5.5–6.0. In other cases the enzymes appear to have slightly alkaline pH activity optima which may suggest that they elicit their proteolytic activity within

Continued on page 106

Fig. 1. *Model propeptide hormone. Abbreviations: PH = peptide hormone; L = lysine; A = arginine.*

Birth of olfactory neurons

Lifelong neurogenesis

Over the past 13 years a steady stream of papers on the vertebrate olfactory mucosa has issued from the laboratory of P. P. C. Graziadei at Florida State University in Tallahassee. These reports have shown that the olfactory neurons – the receptor cells in the nose that sense odors and transmit action potentials into the olfactory bulb – are continually dying and being replaced from a resident proliferative population, the basal cells. This turnover has been demonstrated in all classes of vertebrates, including adult mammals. The olfactory system seems to be the only place in the mammalian nervous system where such spectacular neurogenesis and axonogenesis continue throughout life. For this reason an understanding of the process may ultimately prove useful to the clinical neuroscientists who would like to be able to stimulate regenerative neurogenesis following central lesions. Such an extension of the basic scientific findings is still only a hope, and if it is to be realized at all it wife and collaborator, G. A. Monti standing of the olfactory neuron and its differences from others. The unique features of this neuron have been the subject of Graziadei's previous work; most recently his attention has turned to its early development.

A few years ago Graziadei and his wife and collaborator, G. A. Monti Graziadei, reviewed the development of the olfactory mucosa[1]. It is a relatively simple epithelium with three classes of cells: supporting cells, basal cells, and neurons. Single pulsed injections of tritiated thymidine initially labeled the basal cells and a small fraction of the supporting cells. When the animals were allowed to survive longer the labeled nuclei were found progressively more superficially in autoradiograms and had the cytological characteristics of neurons. Eventually, after about 30

Continued on page 106

© 1984, Elsevier Science Publishers B.V., Amsterdam 0378 – 5912/84/$02.00

Kallikrein family, continued from page 105

the Golgi apparatus or condensing vacuoles, the immediate precursors to secretory granules. It is with this latter group of enzymes that several exciting new developments have recently arisen.

For a long time it has been known that there is a group of structural proteases with slightly alkaline pH optima which are capable of cleaving endolytically at the carboxy-terminal side of an arginine residue. These enzymes are members of the kallikrein-like enzyme family[3] which is a subgroup of the larger serine protease family, so called because the amino acid serine is always involved in the catalytic reaction[7]. Kallikrein itself is a kidney enzyme that is responsible for cleaving kininogen to kinin, a peptide that causes vasodilation. But several of the kallikrein-like enzymes have also been implicated in the processing of prohormones to their end-products. The best example of this is the proteolytic processing of proepidermal growth factor (pro-EGF) and pro-β nerve growth factor (pro-βNGF) by alkaline proteases. These proteases by amino acid sequence analysis are very homologous and enzymatically very similar to kidney (often referred to as glandular) kallikrein thus making them members of the kallikrein-like enzyme family (reviewed in Ref. 4).

A research team at the Australian National University in Canberra previously reported the finding of an mRNA in the submaxillary gland of the mouse which would code for a kallikrein-like protein[5]. Since the submaxillary gland is a major site of NGF and EGF synthesis in the mouse, it is very possible that the product of this gene could be involved in processing pro-βNGF and/or pro-EGF to their component peptides. Of greater interest was their use of this kallikrein-like cDNA to probe by Southern blot analysis the mouse genome for the presence of gene sequences which would be homologous to this cDNA[6]. They found that not only was there a gene which would encode their cDNA but, much to their surprise, there were in addition 20–30 more genes that were very homologous to the kallikrein-like cDNA that they had isolated from the submaxillary gland. They subsequently isolated several of these genes and determined their DNA sequence, showing them to be at least 75% homologous. It should be pointed out that although the presence of a gene in the genome does not ensure expression of that gene and the resultant protein product, these genes have all the characteristics which would allow for normal expression under appropriate conditions.

When comparing the different amino acid sequences of the proteins which would be derived from these genes, several areas were consistently non-homologous and several consistently homologous. The homologous areas were those that had previously been shown to be necessary for the protein to function as a serine protease[7,8]. The non-homologous areas, on the other hand, included those that had been previously identified as lining the substrate-binding pocket of the enzyme[9–11], suggesting differential substrate specificity. This observation raises the exciting possibility that this family of enzymes, a group of related proteases which have the capability of cleaving at arginine residues depending on the flanking amino acid sequence, may indeed be involved in the processing of the many propeptide hormones to their end-products.

In addition to the efforts at purification of the enzymes previously mentioned, several molecular biologists have now become interested in using their techniques to study this problem. The genes for many of the prohormones have been isolated, and it is now possible to transfer these genes back into cells which do not ordinarily express them and see if the prohormones are processed properly. Indeed, by transferring these genes back into tumor cells which express another kind of prohormone, such as pro-opiomelanocortin in AtT20 cells or proenkephalin A in PC12 cells, it will be possible to see if the enzymes in that cell which properly process POMC or proenkephalin A are now capable of processing in the intact cell the prohormone coding for another peptide hormone. Using this approach, a research group at the University of California, San Francisco, recently found that proinsulin appears to be processed to insulin-sized molecules when expressed in the AtT20 mouse tumor cells, suggesting that the protease(s) which cleave POMC can also cleave proinsulin[12].

Although conclusive evidence identifying the kallikrein-like proteases as a major group of enzymes responsible for the proteolytic processing of prohormones to their end-product peptides is not yet available, they have many of the characteristics which would be required of such enzymes. They cleave carboxy terminal to arginine residues, are active between pH 6.5 and 9.0, exhibit highly specific and different substrate speci-

Continued on page 107

Olfactory neurons, continued from page 105

days, there were no more heavily labeled neurons. These results were interpreted to mean that the basal cells were a resident population of presumptive neurons and the neurons that they produced had a limited lifetime, of about 30 days. It is unknown why these particular nerve cells should have such a short lifetime; perhaps the exposed position that they occupy renders them susceptible to damage, and the constant neurogenesis is a response to the disappearance of the longest lived of the mature neurons. There is some support for this idea from the observation that when the mature neurons are killed *en masse* (either by chemicals applied to the epithelium or by cutting the olfactory nerve) the mitotic activity of the basal cells increases.

Once having made its terminal division the new neuron sends an axon through the olfactory nerve into the olfactory bulb, where it forms synapses on the dendrites of the mitral cell in an arrangement called a 'glomerulus', visible using light microscopy. The olfactory axon is surprisingly autonomous and non-selective in its synaptogenesis. If the natural target – the olfactory bulb – is removed in a neonatal mouse, for example, the olfactory nerve innervates the spared portions of the forebrain and forms glomerular synapses in this embryologically incorrect location. This apparent indifference to the identity of its postsynaptic partner has been amplified in a very recent paper by Monti Graziadei[2]. The bulb was removed from a mouse, thus severing the olfactory nerve fibers and killing the mature olfactory neurons. Then the region of the lesion was packed with gel-foam to provide a physical block to the entry of the new axons into the remaining telencephalon. The basal cells produced new neurons as expected, but it was anticipated that differentiation would be altered by the absence of postsynaptic partners. The results indicated otherwise; even under these conditions the olfactory marker protein, a small immunologically identifiable protein found only in olfactory neurons and expressed late in their development, was detected. Thus, the neuron appears to be programmed to grow, differentiate, and perhaps to die, independently of the formation of postsynaptic contacts.

These diverse studies demonstrate that this is a most unusual neuron but its embryonic origins have only recently been described. In an anatomical study of the early differentiation of the

Continued on page 107

Kallikrein family, continued from page 106

ficities, and – as recently confirmed by a molecular biological approach – there are many of them.

Reading list

1 Mains, R. E., Eipper, B. A., Glembotski, C. C. and Dores, R. M. (1983) *Trends Neuro-Sci.* 6, 229–235
2 Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Hirose, T., Inayama, S., Nakanishi, S. and Numa, S. (1982) *Nature (London)* 295, 202–206
3 Fiedler, F. (1979) in *Handbook of Experimental Pharmacology* (Erdos, E., ed.), Vol. XXV (Suppl.), p. 103, Springer-Verlag, Berlin
4 Bothwell, M. A., Wilson, W. H. and Shooter, E. M. (1979) *J. Biol. Chem.* 254, 7287
5 Richards, R. I. *et al.* (1982) *J. Biol. Chem.* 257, 2758–2761
6 Mason, A. J., Evans, B. A., Cox, D. R., Shine, J. and Richards, R. I. (1983) *Nature (London)* 303, 300–307
7 Young, C. L., Barker, W. C., Tomaselli, C. M. and Dayhoff, M. O. (1978) in *Atlas of Protein Sequence and Structure, Vol. 5, Suppl. 3* (Dayhoff, M. O., ed.), pp. 73–93, National Biomedical Research Foundation, Silver Springs, MD
8 Krieger, M., Kay, I. M. and Stroud, R. M. (1974) *J. Mol. Biol.* 83, 209–230
9 Tschesche, H., *et al.* (1979) in *Kinins II: Biochemistry, Pathophysiology and Clinical Aspects* (Fujii, S., Moriya, H. and Suzuki, T., eds), pp. 245–260, Plenum Press, New York
10 Stroud, R. M., Krieger, M., Koeppell, R. E., Kossiakoff, A. A. and Chambers, J. L. (1975) in *Proteases and Biological Control* (Reich, E., Rifkin, D. B. and Shaw, E., eds), pp. 13–32, Cold Spring Harbor Laboratory, New York
11 Hartley, B. S. and Shotton, D. M. (1971) in *The Enzymes, Vol. 3* (Boyer, P. D., ed.), pp. 323–353, Academic Press, New York
12 Moore, H. H., Walker, M. D., Lee, F. and Kelly, R. B. (1983) *Cell* 35, 531–538

JAMES L. ROBERTS
DOLAN PRITCHETT

Department of Biochemistry and Center for Reproductive Sciences, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA.

Olfactory neurons, continued from page 106

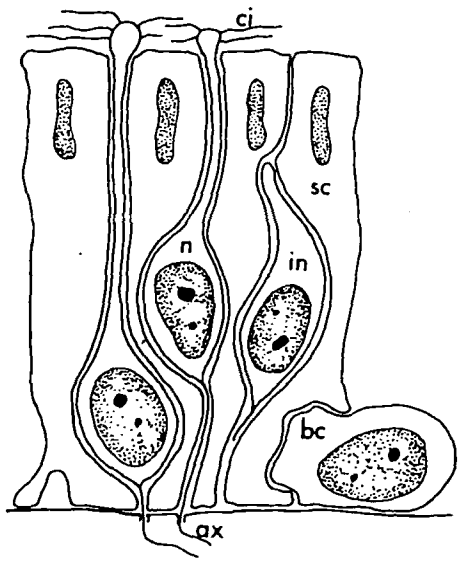

Fig. 1. *Diagram of the olfactory neuroepithelium with its basic cellular components. Neurons (n) are provided with cilia (ci) at the distal, bare end of the dendrite. Supporting cells (sc) extend from the free surface to the basal lamina and separate the neurons. The basal cells (bc), which have been recognized as the staminal cells of the neurons lie close to the basal lamina and immature neurons (in) are present in the neuroepithelium at all times. Axons (ax) of the receptor neurons leave the neuroepithelium to reach the olfactory bulb where they establish synaptic contacts with the mitral cell dendrites.* (Reproduced with kind permission from P. P. C. Graziadei.)

olfactory mucosa in the amphibian *Xenopus laevis*[3], S. L. Klein and P. P. C. Graziadei have shown that the neural and non-neural cells in the mature olfactory mucosa originate from two cell types which are distinguishable by light-microscope criteria very early in embryogenesis.

In amphibians, the presumptive neural ectoderm is divided into two laminae, the inner 'nervous layer' and the outer 'non-nervous ectoderm'. In the other dimension, on the embryo's surface, the neural plate is divided into three regions: the central presumptive neural tube; the two flanking strips of neural crest; and lateral and anterior to the crest, the U-shaped primitive placodal thickening (PPT). The latter, certainly the least well known of the three, borders the somatic ectoderm. As neurulation proceeds, parts of the anterior PPT regress to leave three placodal thickenings, visible as bumps on the embryo's head. The hypophyseal placode is on the midline; lateral to it are the paired olfactory placodes which invaginate to become the olfactory pits and mucosae.

The authors used light and electron microscopy (both transmission and scanning) to examine closely spaced stages, from (Nieuwkoop and Faber)

Continued on page 108

Sinistral findings in schizophrenia

High dopamine in the left amygdala

While of little immediate practical importance, the quest for the neuroanatomical location of schizophrenic illness has substantial heuristic value. The recent report[1] by Gavin Reynolds, from the Cambridge MRC group, that there is gross asymmetry of dopamine concentrations in the amygdalae of post-mortem brain from schizophrenic patients promises to be a milestone in this process. In itself, the report is nice in its simplicity and, taking a broader view it provides the map reader with a triangulation point, in company with neurological and neuropsychological evidence, to focus attention on the left temporal lobe.

Dopamine (DA) has never been far from centre stage in aetiopathological theories of schizophrenic illness but recently seemed to have run out of steam. Clinical investigation of central DA transmission in unmedicated schizophrenic patients has largely failed to show anything of interest, and results from post-mortem neurochemical investigations – while demonstrating increased DA concentrations and DA receptor densities in limbic and striatal regions – have been devalued somewhat by differences of opinion as to whether the DA receptor abnormalities are due to illness or chronic neuroleptic treatment[2,3]. The special relationship of DA with schizophrenia has also recently been challenged in favour of noradrenaline (NA)[4]. Reynolds, simply asked whether there is an imbalance in DA or NA concentrations between left and right amygdalae and caudate nuclei within post-mortem schizophrenic brains? This approach nicely controls out the complicating variables of ante-mortem drug therapy and post-mortem handling, on the reasonable assumption that any such influences would be expected to act symmetrically. On the basis of results from two separate series of brains from the Brain Bank the answer is that left amygdala from schizophrenic brain stands out as having abnormally high concentrations of DA. Otherwise the results from schizophrenic brain are impressively similar to control brain; DA concentrations in right amygdala are normal, DA concentrations in caudate are normal and symmetrical, and NA concentrations are normal and symmetrical in both caudate and amygdala.

This development has not only revived flagging interest in DA, it has made imperative the re-examination of many neurochemical variables in terms of laterality, and it has brought post-mortem neurochemistry into company with a wealth of data from quite unconnected disciplines in pointing to disturbance of the left temporal lobe in schizophrenia. The concept of laterality imbalance in schizophrenia dates back at least to the 1930s in the opinions of Kleist who felt that high-order language dysfunction

Continued on page 108

© 1984, Elsevier Science Publishers B.V., Amsterdam 0378 – 5912/84/$02.00

Sinistral findings, continued from page 107

was central to the illness and that the source lay in the left temporal lobe. Hemispheric imbalance has been implicit in the controversial areas of inter-hemispheric conductance[5] and handedness[6] in schizophrenia, but in relation to disorder of the left temporal lobe the work of Flor-Henry is perhaps best known. He and his colleagues have consistently postulated a dominant hemisphere temporal dysfunction in schizophrenic illness, based on extensive studies involving tests of language and detailed EEG records[7]. Similar conclusions have been reached by Taylor and colleagues who demonstrated left-hemisphere disorder on the basis of linguistic and motor-perceptual tests[8]. Trimble and his group have re-emphasized and refined the association between schizophrenia and temporal lobe epilepsy by demonstrating that complex partial seizures in the dominant temporal lobe are associated with schizophrenic phenomena, including auditory hallucinations[9]. Studies of asymmetry in the galvanic skin response (GSR) in schizophrenic patients have likewise been interpreted to reflect dysfunction in deep structures of the left temporal lobe[10], and it may be relevant that amygdala lesions have been specifically linked to GSR abnormalities in experiments on primates[11]. The advent of sophisticated radiographic scanning techniques has allowed structural analysis of the brain *in situ*, and recent computerized tomography (CT) scan studies show a tendency towards left-hemisphere abnormality[12], left anterior areas in particular[13]. None of these clinical investigations can point specifically to the amygdala but it is in the correct neighbourhood and is certainly a nodal point in limbic-cortical communication.

If dopaminergic transmission is abnormal in the left temporal lobe in schizophrenic illness (and the post-mortem DA abnormalities cannot really be interpreted to distinguish between over- or under-activity), it is reasonable to ask whether this might reflect a primary predisposition to abnormal temporal function or an end result of such dysfunction. That is at present impossible to answer but it would seem more likely to be the latter, perhaps a consequence of chronic coarse electrical disturbance whose origins lie in early brain damage. One source of such damage might be perinatal birth injury which has been shown to correlate with GSR abnormality and with the likelihood of breakdown in the offspring of schizophrenic mothers – representing perhaps one of the environmental insults which can provoke frank illness in the genetically predisposed[14]. Another topical candidate is viral infection[15].

The trouble, of course, with an asymmetric abnormality is finding an aetiological model which can allow for the production of a lateralized effect. Altogether an intriguing jigsaw, and the attraction of the report by Reynolds is not just its consonance with data from diverse other sources but also its stimulus to further neurochemical studies of laterality in schizophrenia.

Reading list

1 Reynolds, G. P. (1983) *Nature (London)* 305, 527–529
2 Mackay, A. V. P., Iversen, L. L., Rossor, M., Spokes, E., Bird, E. D., Arregui, A., Creese, I. and Snyder, S. H. (1982) *Arch. Gen. Psychiatry* 39, 991–997
3 Crow, T. J. (1982) in *Disorders of Neurohumoral Transmission* (Crow, T. J., ed.), pp. 287–340, Academic Press, London
4 Hornykiewicz, O. (1982) *Nature (London)* 229, 484–486
5 Jones, G. H. and Miller, J. J. (1981) *Br. J. Psychiatry* 139, 553–557
6 Gruzelier, J. H. (1981) *Psychol. Medicine* 11, 219–227
7 Flor-Henry, P. (1976) *Ann. NY Acad. Sci.* 280, 777–780
8 Taylor, M. A., Greenspan, B. and Abrams, R. (1979) *Am. J. Psychiatry* 136, 1031–1034
9 Perez, M. and Trimble, M. R. (1980) *Br. J. Psychiatry* 137, 245–249
10 Gruzelier, J. H. and Venables, P. H. (1974) *Biol. Psychiatry* 8, 55–73
11 Bagshaw, M. H., Kimble, D. P. and Pribram, K. H. (1965) *Neuropsychologia* 3, 111–119
12 Toone, B. K., Dawson, J. and Driver, M. V. (1982) *Br. J. Psychiatry* 140, 244–248
13 Golden, C. J., Graber, B., Coffman, J., Berg, R. A., Newlin, P. B. and Bloch, S. (1981) *Arch. Gen. Psychiatry* 38, 1014–1017
14 Parnas, J., Mednick, S. A. and Moffitt, T. E. (1981) *Trends NeuroSci.* 4, 262–264
15 Crow, T. J. (1983) *Lancet* i, 173–175

A. V. P. MACKAY
Physician Superintendent, Argyll and Bute Hospital, Lochgilphead, Argyll PA 31 8LD, UK.

THE NEUROSCIENCE PROGRAM OF WAYNE STATE UNIVERSITY
presents a *Colloquium*
OLD BRAINS: NEW IDEAS
22 May 1984
WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE
Scott Hall, 9.15 a.m. – 5.00 p.m., Detroit, MI.

The speakers are: *Drs T. MAKINODAN, P. McGEER, D. SELKOE, L. SOKOLOFF and R. WURTMAN.* Topics include selective neuronal vulnerability, molecular pathology, neuroimmunology, and metabolic probes of functional activity of the CNS in age related diseases, including Alzheimer's disease. *Contact Dr H. Goldman, Department of Pharmacology, or Dr J. Hazlett, Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201. 5 CME Credits.*

Olfactory neurons, continued from page 107

23, when the placode is still bilaminar, to 39, when the olfactory pit has acquired its mature characteristics. They exploited the initial lamination of the placode, the different staining and ultrastructural properties of the two cell types, and a detailed knowledge of the mature tissue in order to infer the developmental sequence.

The first step is the delamination of the placode, as the inner cells send processes up through the outer layer to reach the superficial surface. This apical membrane subsequently develops cilia, a characteristic of the mature olfactory neuron. From the basal end of these same cells, processes extend to become the growth cones of the olfactory axons which exit the placode to form the olfactory nerve and penetrate the subjacent telencephalon. Throughout the time that some of the inner cells are developing along the lines just described, others remain cuboidal and mitotically active at the base of the placode. These are the basal cells that continue throughout life as the source of new neurons. They differ from the better-known neuronal precursors, the neuroepithelial cells in the neural tube, in that the basal cells do not appear to span the epithelium.

Simultaneously with the differentiation and proliferation of the neural cells, the cells of the non-nervous ectoderm send processes inwards towards the basal membrane, but apparently do not reach it. None the less the previously bilaminar placode has now become unilaminar with both dark (neural) and light (non-neural) processes found at all levels.

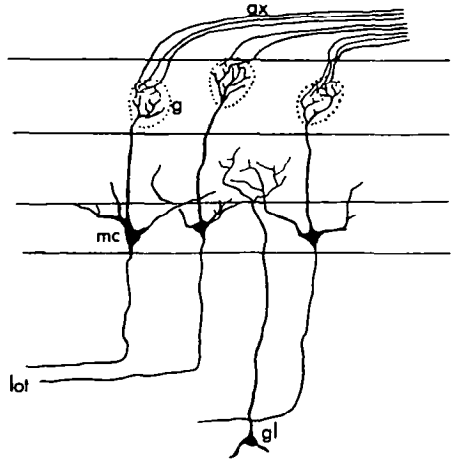

Fig. 2. *Detail of the neural pathway in the olfactory bulb. The sensory axons (ax) reach the glomeruli (g) where they come into contact with the dendritic branches of the mitral cells (mc). The axons of the mitral cells form the lateral olfactory tract (lot). gl = granule cell.* (Reproduced with kind permission from P. P. C. Graziadei.)

Continued on page 109

Olfactory neurons, continued from page 108

The light cells continue to occupy most of the free surface of the epithelium. They develop domes and microvilli, characteristic of supporting cells in the adult olfactory mucosa. Some of the light cells continue to proliferate, thus providing for turnover of the supporting cells.

In summary, two cell types which appear quite different from one another and are segregated into different laminae early in embryogenesis intermingle and differentiate to produce the neural and non-neural cells of the mature olfactory mucosa. This work has traced the development of a unique set of neurons and provides a clear case of the early establishment of two cellular lineages in a sensory receptor.

Reading list

1 Graziadei, P. P. C. and Monti Graziadei, G. A. (1978) in *Handbook of Sensory Physiology, Vol. 9* (Jacobson, M., ed.), pp. 55–83, Springer-Verlag, Berlin
2 Monti Graziadei, G. A. (1983) *Brain Res.* 262, 303–308
3 Klein, S. L. and Graziadei, P. P. C. (1983) *J. Comp. Neurol.* 217, 17–30

STEPHEN S. EASTER JR

The Salk Institute, PO Box 85800, San Diego, CA 92138, and Division of Biological Sciences University of Michigan, Ann Arbor, MI 48109, USA.

Letter to the Editor

We welcome comments from our readers. Short communications stand the best chance of publication. The Editor reserves the right to take extracts from the longer ones.

The importance of vasopressin in memory

SIR:

Gash and Thomas[1] do not really address the points raised in my recent letter to the Editor (*TINS*, March 1984)[2] when they question the use of Brattleboro rats as proper controls. They even admit that the results they obtained depended on the batch of animals used.

The inconsistent effects on avoidance behavior may indeed be related to the conditions of the experiment. All experiments should be carried out under strictly controlled conditions: this holds for biochemical, pharmacological and also for behavioral experiments. According to Gash and Thomas, this seems to compromise and probably invalidates the conclusions.

Gash and Thomas may be right in questioning the term 'memory', if indeed the effect of vasopressin and related peptides can be shown in aversively motivated responses only. We originally used the term 'resistance to extinction of active and passive avoidance behavior'. However, after many years of research on vasopressin we decided to use the term 'memory processes'. We did this because, as stated in my letter[2], vasopressin and related peptides had effects which corroborated the thesis that these peptides affected memory processes.

Gash and Thomas reject these arguments because some studies have not been independently confirmed. However, the thesis on which our concept is based is derived from a number of other studies performed by us and by others. I consider that vasopressin modulates memory processes because it has a long-term effect, the influence is time dependent, and it has an anti-amnesic effect.

In a recent letter to *Nature* my colleagues and I discuss peripheral versus central effects of vasopressin[3].

References

1 Gash, D. M. and Thomas, G. J. (1984) *Trends NeuroSci.* 7, 64–65
2 de Wied, D. (1984) *Trends NeuroSci.* 7, 62–63
3 de Wied, D., Gaffon, O., van Ree, J. M. and de Jong, W. (1984) *Nature* 308, 276–278

D. DE WIED

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

TINS DISCOUNT SUBSCRIPTION RATE

Trends in NeuroSciences is now available at the substantial discount of 30% to all students (undergraduate, graduate, post-graduate, etc.) enrolled at recognized institutions.

A student subscription comprises 12 monthly issues starting from any month in 1984. Prices include air delivery world wide. US $34.00, USA and Canada. £18.00, UK. Dfl. 84.00, Europe. Dfl. 92.00, Rest of World.

Please start my subscription to *Trends in NeuroSciences* from the next available issue (1984)

I enclose my personal cheque for £...../Dfl. ...../$..... made out to Elsevier.

Name ..... Signature .....

Address for dispatch of issues .....

..... Postal code .....

Name of Institution .....

Title of course for which you are enrolled ..... Date of termination of course .....

A photocopy of this form must be sent to the appropriate address accompanied by your personal cheque and valid proof of current educational status (photocopy of Student card, letter from Head of Department, etc.)

Note: Current subscribers who are eligible for student discount may claim the discount only on expiry of their current subscription. No refunds, credit or extensions of the subscription period can be given.

Subscribers in the USA and Canada only (cheque for $US 34.00) to
Elsevier Science Publishing Company Inc.
Attn. *TINS* Subscriptions,
52 Vanderbilt Avenue,
New York, NY 10017, USA.

Subscribers in the UK only (cheque for £18.00) to
Elsevier Publications (Cambridge),
Attn. *TINS* Subscriptions,
68 Hills Road,
Cambridge CB2 1LA, UK.

Subscribers in the Rest of World, (cheque for 84.00 Dutch guilders (Europe) or 92.00 Dutch guilders (outside Europe)) to
Elsevier Science Publishers, B.V.,
P.O. Box 548,
1000 AM Amsterdam, The Netherlands.

For additional order forms, circle no. 190 on the advertising enquiry form.