the carboxypeptidase-B proteolysis product of human, canine, and rabbit tissue MM creatine kinase showed lysine to be the C-terminal amino acid. Identical results were obtained with human and canine tissue MM creatine kinase. These results confirm that lysine is the C-terminal amino acid of rabbit MM creatine kinase[13] as well as the C-terminal amino acid for both human and canine myocardial MM creatine kinases. We conclude that multiple subforms of MM creatine kinase are produced in plasma by hydrolysis of the C-terminal lysine from 1 polypeptide chain of tissue MM creatine kinase (MM_3) to produce MM_2. Hydrolysis of the C-terminal lysine from the second polypeptide chain of MM_2 produces MM_1. It is most probable that carboxypeptidase-N is the responsible agent since it is found in plasma[14] while carboxypeptidase-B is found primarily in the pancreas[15]. Since the detection of MM_3 indicates recent release into plasma it can be used as a marker for recent myocardial injury or vessel patency following thrombolytic therapy.

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0014-4754/84/111275-03$1.50 + 0.20/0

Exocytosis of secretory granules – a probable mechanism for the release of neuromodulators in invertebrate neuropiles

D.W. Golding and E. Bayraktaroglu*

Department of Zoology and Dove Marine Laboratory, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU (Great Britain), 28 November 1983

Summary. Presynaptic terminals typically contain secretory granules, usually 100–200 nm in diameter, in addition to the smaller synaptic vesicles. Evidence is presented that granule exocytosis is a widespread phenomenon in invertebrate neuropiles. Such secretory release is apparently associated with morphologically unspecialized regions of the plasmalemma, rather than synaptic thickenings.
Key words. Invertebrate neuropile; exocytosis; synapses; neuromodulators.

It has long been known[1,2] that presynaptic terminals whether in vertebrates or invertebrates, in central or peripheral nervous systems, typically contain dense-cored granules, usually 100–200 nm in diameter, in addition to the smaller synaptic vesicles. Neurosecretory endings within neurohemal organs are identical in ultrastructure[3–5]. Secretory release from both types of terminals is thought to occur by exocytosis – i.e., following fusion of the bounding membranes of the secretory inclusions with the plasmalemma. However, whereas discharge from presynaptic terminals is generally assumed to involve synaptic vesicles[6,7]; release within neurohemal organs such as the corpus cardiacum of insects and the neurohypophysis of vertebrates involves the larger granules (ref. 8, 9 for review). Work in this laboratory has previously shown that a process of granule exocytosis apparently identical in most respects to that occurring within neurosecretory fibers is a feature of synaptic terminals in annelids[10,11] and we now provide evidence that this form of secretory activity, which was virtually unknown with respect to central nervous systems until recently, is a widespread phenomenon in invertebrate neuropiles (see also Roubos et al.[12]).

Cerebral ganglia of *Dendrocoelum lacteum* (Platyhelminthes), *Lumbricus terrestris* and other earthworms (Annelida) and *Helix aspersa* (Mollusca) were prepared for electron microscopy following fixation in 1% OsO_4 in 0.1 M phosphate buffer, pH 7.2, sometimes following 2.5% glutaraldehyde in the same buffer. In addition, some specimens were stimulated by incubation for various periods of time in K^+-rich (50 mM) Ringer solution or 1 mM 4-aminopyridine (4AP) in Ringer solution[13]. Some were processed using the tannic acid-glutaraldehyde-OsO_4 (TAGO) method[14,15] using 1% tannic acid and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 1 h, followed by 1% OsO_4 in the same buffer.

The neuropiles we have examined are richly endowed with secretory granules (fig. 1A). These inclusions show great variety in size, form, electron density of the core, etc., and can be used as a basis for classifying the axon varicosities and synaptic boutons in which they are contained. Synaptic vesicles show less variation and, unlike the granules, they are usually concentrated adjacent to synaptic thickenings.

Sites of granule exocytosis within the neuropiles have been recognized by the incidence of 'omega profiles' together with the presence of electron dense material within the indentations (fig. 1B, C). They have been encountered in each of the ganglia examined (and also within the cerebral ganglion of the arthropod *Balanus hameri* – A.S. Clare, personal communication).

Various stages in the process can be recognized as dissolution of the dense material occurs and the fiber recovers its normal profile. In rare cases, such massive secretory discharge has apparently occurred that the rate of dissolution has not been able to match that of release, resulting in the formation of expansive pools of extracellular material (fig. 1D). This is probably not an artifact of fixation since the individual fibers concerned were surrounded by apparently inactive terminals. TAGO fixation facilitates the detection of release sites since it intensifies the staining of extracellular materials, including the cores of secretory granules exposed to the extracellular medium by exocytosis.

'Compound exocytosis' involving the fusion of two or more granules and the confluence of their contents with the extracellular space has been encountered, as within certain neurohaemal organs[16].

The finding with respect to annelids[11] that granules undergo exocytosis at morphologically unspecialized sites rather than at synaptic thickenings has been confirmed in this wider study. Not one of the hundreds of exocytotic figures we have encountered has involved discharge of the contents of a secretory granule into a synaptic cleft.

Gershon et al.[17] have classified synapses, with reference only to synaptic vesicles, as 'directed' when the vesicles are concentrated adjacent to synaptic thickenings, and 'undirected' when the vesicles within varicosities discharge their contents at morphologically unspecialized sites. Adapting these categories we conclude that the majority of nerve fibers we have observed form both directed synapses with reference to their vesicular inclusions, and undirected junctions with respect to their granules (see Golding and May[11] for references to other configurations).

Preliminary observations suggest that the incidence of granule exocytosis is markedly affected by stimulation. Thus in *Dendrocoelum*, omega profiles could be found only after diligent scrutiny in control ganglia, and not at all in anesthetized specimens (several sections of each of three specimens were examined). In contrast, they were abundant in stimulated ganglia, particularly following exposure to 4 AP (fig. 2A) when in excess of 20 sites were observed in several sections of each of three specimens.

Granule exocytosis is not restricted to fibers of the 'neurosecretory type' in which large granules are abundant, but is encountered in connection with a wide range of the types present, including those whose granules are smaller and/or overshadowed by an abundance of synaptic vesicles. However, the phenomenon is apparently more common with respect to 'peptidergic-type' elements: it may have a less prominent role in cells which, like at least some neurones specialized for cholinergic[18] and aminergic function[19] in vertebrates, nevertheless store peptides within their secretory granules.

Differences between fibers apparently equally well endowed

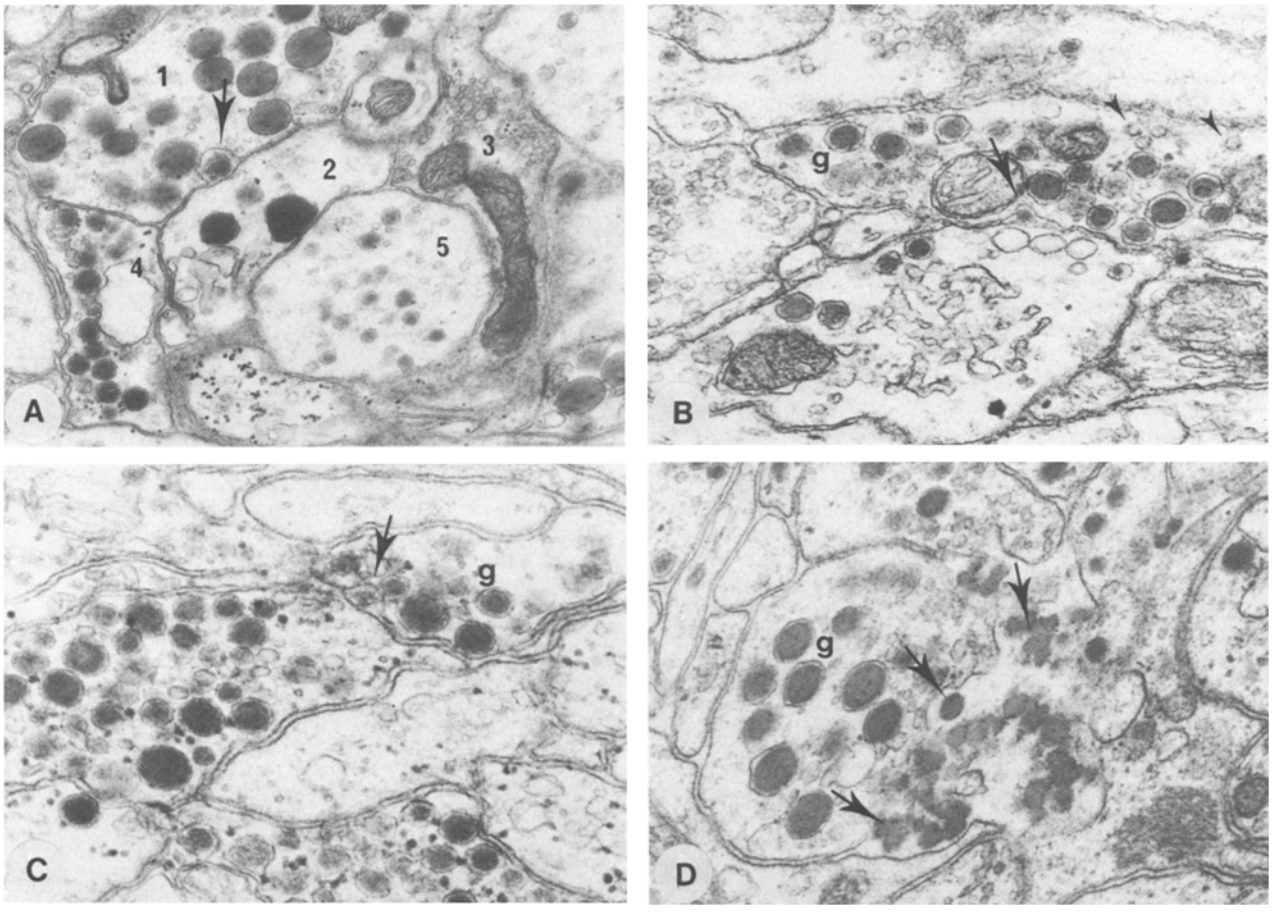

Figure 1. *A*) *L. terrestris*, OsO_4. Note the abundance and variety of secretory granules within axon varicosities (Nos 1–5) in the neuropile. Arrow, site of granule exocytosis. × 24,000. *B* and *C*) Sites of exocytosis (arrows) in *D. lacteum*, glutaraldehyde/OsO_4, (B) and *H. aspersa*, OsO_4, (C). Arrowheads, coated pits associated with sites of granule exocytosis identified in adjacent sections. *g*, secretory granules within axon varicosities. × 50,000. *D*) *Allolobophora longa*, OsO_4. Accumulated pool of secretory material (arrows) in intercellular space. *g*, undischarged granules. × 40,000.

with large granules have also been noted. Some individual fibers examined in serial sections could be seen to have several exocytotic sites, whereas adjacent fibers had none. Similarly, terminals of one type may show abundant activity, whereas in others omega profiles are rare. This applies not only to unstimulated specimens, in which the differences may reflect contrasting levels of activation of the respective neurones, but also in ganglia subjected to the supposedly unspecific stimulus of 4AP.

Probable signs of membrane retrieval are frequently encountered in association with sites of granule exocytosis, in the form of coated pits surmounting the omega profiles. Even in TAGO-fixed specimens, these have lucent contents. The diminutive size (approximately 40 nm) of many of the coated vesicles associated with sites of exocytosis (fig. 1B, 2B), together with the observation that two or more coated pits may form in association with individual exocytotic profiles, indicates that membrane retrieval in invertebrate neuropiles is commonly mediated by the formation of 'microvesicles'. However, coated pits which are similar in size to secretory granules are sometimes observed (fig. 2C). In such cases, the process probably resembles that in the mammalian neurohypophysis involving larger 'vacuoles'[20].

One notable feature is the formation, in rare cases, of reciprocal endocytotic profiles (fig. 2D) – i.e., involving internalization of membrane from sites of granule discharge not only by the secretory ('presynaptic') partner, but also by the adjacent ('postsynaptic') fiber. The latter may be a reaction to the distortion of the plasmalemma resulting from the extrusion of secretory material into the intercellular space, but more probably, it is a manifestation of receptor-mediated endocytosis[21] – the mechanism by which target cells internalize hormone-receptor complexes prior to recycling of the latter.

Granule exocytosis by non-endocrine nerve terminals, together with the well established role of synaptic vesicles, provides the basis for a 'dual vesicle hypothesis'[11] according to which neurones typically possess two highly distinctive mechanisms for neurochemical mediator storage and release. Both mechanisms involve discharge direct from within the secretory inclusions into the extracellular space. Synaptic vesicle membrane is probably retrieved in the form of coated vesicles of similar size, possibly at a site distant from the site of exocytosis, which rapidly rejoin the pool of synaptic vesicles, whereas secretory granule membrane is apparently often retrieved in the form of 'microvesicles', from the exact site of fusion, and is degraded rather than recycled[11]. Given the recognition of influences on nervous function other than those corresponding to the actions of classical neurotransmitters[22,23] and the storage of peptides within granular inclusions (e.g. Kiss and Williams[24]), even by cholinergic and aminergic neurones[18,19], the phenomenon of granule exocytosis at central sites is most readily interpreted as a mechanism by which neuromodulators are released within the CNS.

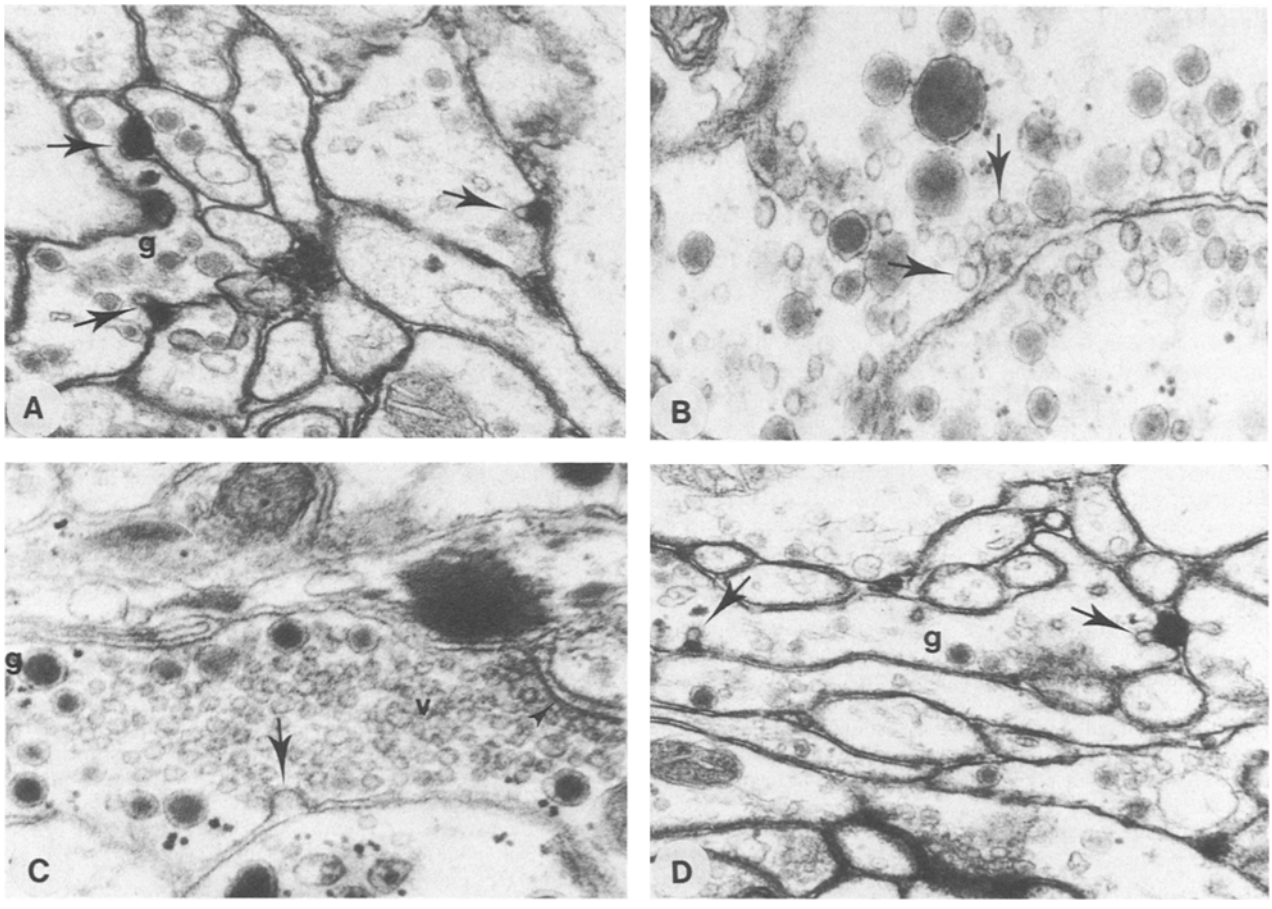

Figure 2. *A*) *D. lacteum*. TAGO-fixed specimen showing 'stained' granule cores (arrows) discharged into the intercellular space following 4AP stimulation. The cores may be compared with those of undischarged granules (*g*) and those of discharged granules after conventional fixation (e.g., in fig. 2B, 2C). The outer leaflet of plasma membranes is also darkened. × 55,000. *B*) *L. terrestris*, OsO_4. 'Microvesicles' (arrows) associated with site of granule exocytosis (confirmed in adjacent sections). × 60,000. *C*) *L. terrestris*, OsO_4. Coated pit (arrow) similar in size to secretory granules (*g*). *v*, synaptic vesicles; arrowhead, synaptic cleft. × 55,000. *D*) *D. lacteum*. TAGO-fixed specimen showing membrane retrieval at two sites (arrows), one of which forms reciprocal figure. *g*, undischarged granule. × 47,000.

Acknowledgments. The authors gratefully acknowledge the assistance of Mr R.M. Hewit and Mrs. S. Al-Yousuf. Supported by research grant GR/A88743 from the S.E.R.C., U.K., to D.W. Golding.

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0014-4754/84/111277-04$1.50 + 0.20/0

Independent responses of two fruit characters to developmental regulation in *Microseris douglasii* (Asteraceae, Lactuceae)[1]

S. Mauthe, K. Bachmann, K.L. Chambers and H.J. Price

Premedical Biology Program, University of Heidelberg, Im Neuenheimer Feld 504, D-6900 Heidelberg 1 (Federal Republic of Germany), Department of Botany, Oregon State University, Corvallis (Oregon 97331, USA), and Department of Plant Sciences, Texas A&M University, College Station (Texas 77843, USA), 22 August 1983

Summary. The 'hairy achenes' and 'yellow achenes' characters are expressed only in peripheral fruits on *Microseris* capitula. Segregation in interstrain hybrid D37 shows that the genes responsible for these characters respond independently to developmental regulation.
Key words. Microseris douglasii, fruit, peripheral; achenes phenotype; genetic analysis; morphogen gradient.

The detailed course of the development of leaves, flowers and other organs in herbaceous plants is usually dependent on the position of the developing organ with respect to the apical meristem or, more exactly, on the position of the organ primordium in the sequence of primordia elaborated by the meristem. If there are marked morphological changes in sequentially formed organs, the effect is termed 'heterophylly' for leaves or 'heterocarpy' for fruits. Bachmann[2] has discussed various manifestations of this effect and suggested that it can be understood as the response of the developing primordium to concentration differences of a morphogen (phytohormone?) that depend on the diffusion gradient of that morphogen and/or the time course of its production. Even where this system is not amenable to direct physiological experimentation, it can be analyzed in considerable detail by genetic dissection of its components. Specifically, 2 types of genes should be found; genes that participate in the establishment of the morphogen gradient and genes that are expressed under the influence of the gradient.

The flowering head (capitulum) of the Compositae (Asteraceae) is a particularly suitable system for such a genetic analysis. The development of each of the many individual florets and of the fruits (achenes) that mature from them can be considered a bioassay for the morphogen gradient that can be read in the mature fruiting head. With tens or even hundreds of achenes present on a single capitulum, the gradient can be probed very precisely. We have used a specific fruit character, the 'hairy' fruit wall of the outer, older achenes in contrast to the smoother wall of the inner ones, to probe the genetic basis of heterocarpy in the annual species of *Microseris* (Asteraceae: Lactuceae)[3,4].

At least 2 genes interact in the analyzed strains to determine the relative number of hairy achenes. These appear to be genes determining the gradient[2]. Their interaction suggests that the most effective concentration (the origin of the gradient?) is around the rim of the capitulum. From there centripetally a more or less broad ring of hairy achenes is found that encircles an inner field of smooth ones. The existence of 'half-hairy' achenes at the border between outer and inner ones suggests that the response to the gradient is cell-specific and can vary across a single organ primordium[2].

The 'hairy achenes' phenotype is one of many characters under the influence of this gradient. These include the shape, color and color pattern of the flower petals, the shape, color, and color pattern of the fruits and the structure of the pappus. The analysis of these other characters is very difficult, because the flower characters must be scored during the short period of flowering, most fruit characters cannot be scored in sterile aborted fruits that usually occur in most capitula, and the pappus characters show a remarkable environmental plasticity obscuring a complex genetic basis. The analysis of a coordination (or a lack of it) among the responses of different genetically determined characters to the morphogenetic gradient across the capitulum is therefore limited to exceptional favorable cases. Such a case is presented here. We shall demonstrate segregation for the dependence of fruit color on the position of