equally well. Maintaining hydrophobicity in the leader peptide appears to be the primary evolutionary constraint.

Sequences encoding both the mature ANF peptide and the proANF precursor are highly conserved, suggesting that these structures are critical to their functions. While a biological role of ANF may be regulation of salt and water homeostasis, a role for the proANF molecule is less well defined. Extensive sequence homology between human and mouse proANF implies a specific, necessary role. This region has been suggested to encode a separate hormone (cardiodilatin) (*11*); alternatively, these sequences may play a role in the processing and storage of ANF. Atrial granules are thought to be related to ANF production (*1–6*). Perhaps proANF sequences direct the biosynthesis of these granules.

A striking difference between human and mouse ANF is at the carboxyl terminus (coding block III, Fig. 3). The Arg-Arg sequence in rodent ANF is a putative peptidase cleavage signal similar to that in other prohormones. Some preparations of purified rat ANF lack this sequence (*6*), presumably as a result of proteolytic digestion. These data have two implications. First, the larger coding block in rodents suggests that ANF may have evolved from a longer peptide. Perhaps this ancestral proANF molecule, larger than either the rodent or human proANF, required proteolytic processing to form its carboxyl terminus. Subsequent evolution in humans may have introduced a stop codon at the end of the gene, removing the requirement for any proteolytic action. Second, persistence of the carboxyl tyrosine residue, despite rapid evolution of portions in the second intervening sequence (IVS2) and 3′ untranslated regions, suggests a critical role for this residue in ANF activity.

The IVS2 of the human gene is 800 bp longer than the corresponding IVS2 of the murine gene (Fig. 2). A dot matrix comparison (*19*) of these sequences suggests that there is no homology between the IVS2's of the two genes. Two tandem reiterated sequences present only in the human sequence are 80 percent identical to previously characterized Alu sequences.

A potential glucocorticoid receptor binding site is present in the IVS2 of the human gene (Fig. 2). The structure of the glucocorticoid receptor binding site is poorly understood; however, a consensus sequence has been proposed (*20*). Human ANF contains a sequence (residues 1291 to 1306) that is identical to the consensus glucocorticoid receptor binding sequence at 9 of 11 bp. The mouse gene lacks this glucocorticoid receptor binding site. An analogous situation is found in the human growth hormone gene, which contains a functional glucocorticoid receptor binding site that is absent in the murine gene (*21*). Whether or not the putative glucocorticoid receptor binding site can bind the glucocorticoid receptor remains to be determined. Further studies should identify the role of steroid hormones in regulation of ANF gene expression. Studies of the conserved nucleotide sequences should provide insight into the processes that regulate production of the gene and the mechanisms that control intravascular pressure and volume.

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Synapsin I in Nerve Terminals: Selective Association with Small Synaptic Vesicles

Abstract. *Immunocytochemistry revealed that synapsin I is preferentially (and possibly exclusively) associated with small (40- to 60-nanometer) synaptic vesicles and not with large (greater than 60-nanometer) dense-core vesicles in bovine hypothalamus. These observations may explain why synapsin I is found exclusively in neurons, since small synaptic vesicles are specific to neurons whereas large dense-core vesicles in neurons may be considered the equivalent of secretory organelles in endocrine cells.*

Synapsin I is a major neuron-specific protein that is highly concentrated in nerve endings, where it is associated with the surface of synaptic vesicles (*1–4*). Synapsin I is phosphorylated at multiple sites both in vivo and in vitro by Ca^{2+}-calmodulin–dependent and adenosine 3′,5′-monophosphate–dependent protein kinases (*5*) and probably plays an important regulatory role in the function of synaptic vesicles (*3, 4*). Such a role is presumably related to some general aspect of synaptic vesicle function, since synapsin I is present in virtually all synaptic vesicles in most or all nerve endings (*2, 3*). On the other hand, the absence of synapsin I from nonneuronal cells and even from neuron-related cells, such as the chromaffin cells of the adrenal medulla (*6*), indicates that it is involved in some aspect of secretion specific to "bona fide" neurons.

Neurons can, by exocytosis, secrete at least two classes of substances: small nonpeptide molecules (classical neurotransmitters) and peptides (*7, 8*). Classical and peptide neurotransmitters are often released from the same neuron, even though their secretion seems to involve two distinct types of secretory organelles (*7–10*). It appears that typical synaptic vesicles, the small (40 to 60 nm in diameter) synaptic vesicles (SSV's), are storage sites only for classical neurotransmitters, the content of each vesicle representing a quantum of neurotrans-

mitter (*11, 12*). In contrast, peptides appear to be stored in a morphologically distinct population of vesicles, the large (>60 nm) dense-core vesicles (LDCV's), which may also contain classical neurotransmitters (*7–9, 13, 14*).

In a previous study of the localization of synapsin I in nerve endings (*3*), we examined the SSV's, which constitute the majority of synaptic vesicles in the brain. To determine whether synapsin I is also present on LDCV's, we have now used a Protein A–gold technique on synaptosomes prepared from bovine hypothalami and embedded in agarose (*3, 15*). As in our previous study (*3*), the distribution of synapsin I immunoreactivity was studied specifically in those nerve endings in which the plasmalemma had been disrupted before fixation. Rupture of the plasmalemma before fixation not only eliminated the hydrophobic barrier to macromolecule penetration but also allowed elution of most cytosolic proteins [but not of synapsin I (*3, 4*)], thereby preventing fixative-induced transformation of the cytoplasm into a densely cross-linked matrix. Under our experimental conditions, nerve-ending ghosts still retained at least some of their subcellular organelles, including SSV's (often grouped in clusters) and LDCV's (Fig. 1, A and B) (*16*).

Immunolabeling of agarose-embedded homogenates for synapsin I produced a prominent decoration by colloidal gold particles of all SSV's in all disrupted nerve endings; in contrast, only scattered gold particles were visible on LDCV's (Fig. 1). SSV's relocated outside the nerve ending and isolated in the agarose matrix were, in general, heavily labeled, whereas LDCV's in such ectopic locations were often free of label.

A morphometric analysis (*17*) carried out on vesicle profiles not adjacent to other structures confirmed these observations, revealing a greater than fivefold difference in the density of specific label on SSV's relative to LDCV's. Thus, SSV's showed 49.27 gold particles per micrometer (57.85 and 8.58 in immune and control samples, respectively) and LDCV's 8.85 particles per micrometer (17.9 and 9.05 in immune and control samples, respectively). These data indicate that synapsin I has a strong preference for SSV's, but do not exclude the possibility of low concentrations of synapsin I on LDCV's. However, the low level of specific labeling on LDCV's may be due to limitations of the immunocytochemical technique: SSV's out of the plane of section, or unavoidable spillover of marker molecules from the heavily labeled SSV's during the washing steps of the labeling procedure, might have been responsible for the higher counts of gold particles on LDCV's in immune samples relative to controls.

Experiments on the neurohypophysis suggest that selective association of synapsin I with SSV's may be a general phenomenon. Synapsin I is present in high concentrations in the neurohypophysis (*6*), which is composed primarily of terminals of peptidergic neurons. Immunocytochemical studies of agarose-embedded nerve terminals of the posterior pituitary indicate that synapsin I is not present on the LDCV's containing the peptide neurohormones (*10, 18*), but rather is present on the small (40- to 60-nm) vesicles that coexist in the same endings (*19*). Although the physiological role of these small vesicles is still unknown, they resemble typical SSV's of nerve terminals with respect to size, spatial organization in the ending, and ability to be labeled with extracellular tracers (*19, 20*).

The traffic (*21*) of synaptic vesicles in the nerve endings is undoubtedly regulated by specific and controlled interactions of some of their surface molecules with other structural components of the cell (*22*). We previously hypothesized that phosphorylation of synapsin I might be involved in the control of synaptic vesicle traffic and in some of the events that lead to synaptic vesicle exocytosis (*3, 4*). The present results suggest that, if synapsin I is involved in the control of vesicle traffic, such a role is specific for SSV's.

Differences in the biogenesis of mature LDCV's and SSV's suggest different patterns in the traffic of the two types of vesicles. Classical neurotransmitters can be synthesized and packaged into vesicles in the perikaryon or the nerve ending (*7–9, 11*). In contrast, peptide neurotransmitters or their precursors appear to be synthesized and packaged into vesicles only in the perikaryon (*7–9, 18, 22*). Thus, while recycling of LDCV membrane components to assemble a new LDCV implies their shuttling between the nerve ending and the perikaryon (*7–9, 18, 22*), reassembly of new SSV's may occur through a peripheral recycling of their membrane components (*7–9, 11, 23*). In addition, there is direct experimental evidence that release from LDCV's and SSV's might be differently regulated (*8, 23*).

The mechanism by which synapsin I regulates SSV traffic requires investigation. Synapsin I may, among other functions, provide a link between SSV's and a cytoskeletal matrix that specifically controls the mobility of SSV's. The tendency of SSV's to occur in clusters that often exclude LDCV's (Fig. 1, A and B) indicates the existence of a cytoskeletal matrix that specifically recognizes SSV's.

Large dense-core vesicles but not SSV's can be considered the neuronal equivalent of secretory granules in nonneuronal cells. The morphology, content, and mechanism of assembly of

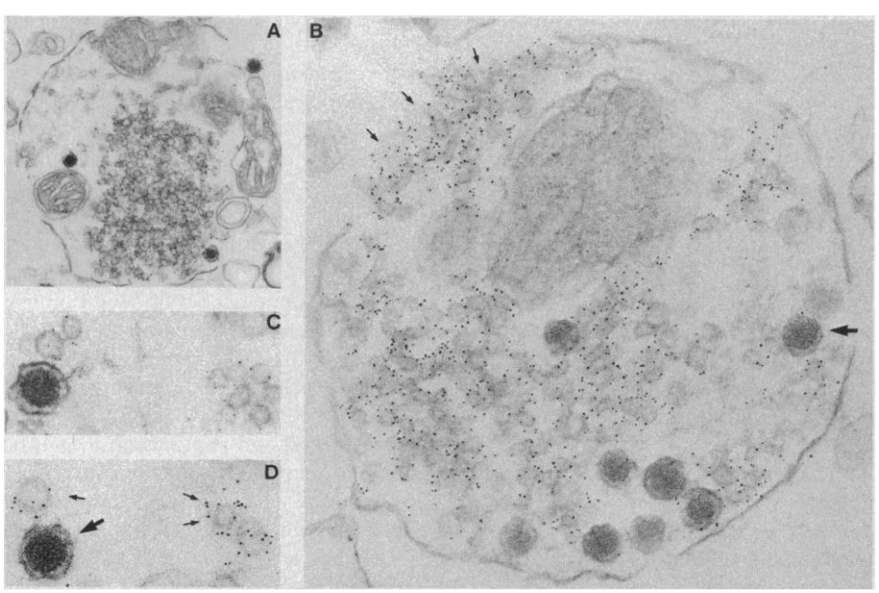

Fig. 1. Electron micrographs showing localization of synapsin I by immunoreactive gold in isolated disrupted nerve endings of bovine hypothalami. (A, B, and D) Preparations reacted with antibody to synapsin I IgG's followed by Protein A–gold complex; (C) preparation in which antibody to synapsin I IgG's was replaced by nonimmune IgG's. In (A), (B), and (D) colloidal gold particles are highly concentrated on SSV's (small arrows). Other organelles, including LDCV's (large arrows), are labeled only by scattered gold particles or are totally unlabeled.

brain LDCV's seem analogous to those of secretory granules of endocrine cells (*18, 22, 24*). In contrast, SSV's—for example, secretory vesicles that are continuously recycled without involvement of the central "headquarters" of the cell—do not have an equivalent in nonneuronal cells. Thus the presence of synapsin I only in neurons may be related to the fact that these secretory vesicles are present only in neurons and are absent from other secretory cells.

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15. Bovine hypothalami were gently homogenized in four volumes of ice-cold 0.25*M* sucrose in a glass and Teflon tissue grinder (clearance, 0.10 to 0.15 mm). Homogenates were then fixed under isotonic conditions (nonlytic type B fixation) or hypotonic conditions (lytic fixation) (*3*) and embedded in agarose (*3*). This technique, in which nerve endings are disrupted in solutions of low ionic strength by mechanical shearing forces (homogenization) or by hypotonic treatments before fixation and agarose embedding, was designed to maximize labeling of cytoplasmic membrane–associated antigens. Virtually identical labeling patterns were obtained in disrupted nerve endings with isotonic and with hypotonic fixation. Total homogenates rather than crude synaptosomal fractions were used in our study to avoid a possible loss of selected nerve terminals during synaptosomal preparation. The hypothalamic region of the brain was chosen because of its high content of peptide neurotransmitters and LDCV's. Immunolabeling was carried out as described (*3*), with the exception that the Protein A–gold complex (5 nm) [prepared according to J. W. Slot and H. J. Geuze, in *Methods in Neurosciences*, C. Cuello, Ed. (Wiley, New York, 1983), vol. 3, pp. 323–346], was used as a probe to detect bound rabbit immunoglobulin G's (IgG's) directed against synapsin I, and that control goat IgG's were omitted from solution A.
16. The ratio of LDCV's to SSV's appeared to be somewhat lower in ruptured than in sealed nerve endings, as if LDCV's were preferentially lost upon lysis of the ending (Fig. 1A).
17. Random electron micrographs (×30,000) of nerve endings containing at least one LDCV were taken. Sections of immune and control preparations had similar thicknesses. Negatives were printed at a final magnification of ×112,500. A line was drawn parallel to portions of SSV's and LDCV's not contiguous to other structures at a distance 250 Å from the vesicle membrane. This distance was selected since synapsin I may partially protrude from the vesicle surface and since 150 Å is approximately the distance to be expected between the colloidal gold particles and the antigenic site [see J. W. Slot and H. J. Geuze, in (*15*)]. Gold particles present in the space between this line and the vesicle surface were counted. Corresponding vesicle profiles were counted with a Zeiss MOP 1 quantitative digital image analyzer. The total length of vesicle profiles was 31 μm for SSV's and 5.5 μm for LDCV's. The data represent ratios of total number of gold particles to total length.
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Cloned Mycoplasma Ribosomal RNA Genes for the Detection of Mycoplasma Contamination in Tissue Cultures

Abstract. *A cloned fragment of the mycoplasma ribosomal RNA operon was used as a molecular probe for the detection of mycoplasmas in cell cultures. According to the conditions of hybridization, the probe can detect prokaryotes in general or mycoplasmas specifically.*

Mycoplasmas are important tissue culture contaminants that exist in close association with the host cell membrane (*1–3*). This infection causes structural damage and changes in host cell metabolism which can interfere with experiments or result in the loss of the infected cell line (*4, 5*). Constant monitoring of cells is therefore imperative (*6*). Several techniques for the detection of mycoplasma contamination have been developed, including cultivation in appropriate media, immunofluorescence, or measurement of uridine-uracil incorporation ratio, and fluorescent staining by DNA-binding dyes such as diaminophenyl indole (DAPI) and Hoechst 33258 (*7*). We describe here a new mycoplasma detec-

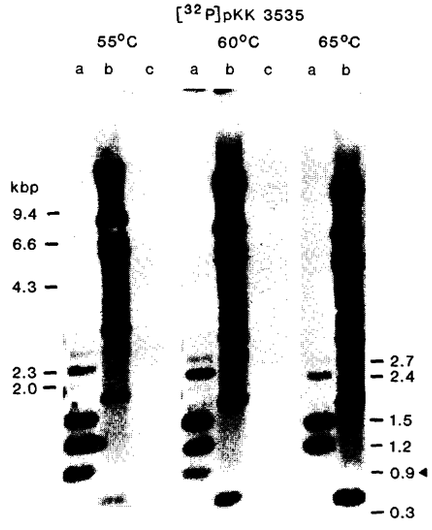

Fig. 1. Identification of rRNA gene fragments in Hind III–digested *M. hyorhinis* DNA by Southern blot analysis. DNA's (2 μg) were digested with Hind III, subjected to agarose gel electrophoresis, and transferred onto nitrocellulose filters (*16, 17*). The filters were baked at 80°C for 2 hours at reduced pressure and hybridized to nick-translated ^{32}P-labeled pKK3535 probe (specific activity 2×10^8 to 4×10^8 cpm/μg). (Lane a) *M. hyorhinis* DNA; (lane b) *E. coli* DNA; and (lane c) HeLa DNA, digested with Hind III. The lengths of the Hind III–digested lambda DNA markers and the six Hind III fragments of the *M. hyorhinis* rRNA operon are indicated in kilobase pairs. The 300-bp fragment is only seen by overexposing the film. The 900-bp *M. hyorhinis* 23*S* rDNA fragment is marked by an arrowhead.