Lack of Correspondence Between mRNA Expression for a Putative Cell Death Molecule (SGP-2) and Neuronal Cell Death in the Central Nervous System

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SUMMARY

Neuronal death during nervous system development, a widely observed phenomenon, occurs through unknown mechanisms. Recent evidence suggests an active, destructive process requiring new gene expression. Sulfated glycoprotein-2 (SGP-2), a secretory product of testicular Sertoli cells has been shown to up-regulate in several nonneural tissues undergoing programmed cell death and in several types of neuronal degeneration. In order to determine if this message up-regulates in neurons undergoing developmentally determined cell death, we have studied the expression of SGP-2 mRNA in the developing and adult rat central nervous system (CNS) with *in situ* hybridization. We also report on the expression of this message in nonneural tissues from several regions of the developing embryo. The developing and adult rat central nervous system as well as widely varied tissues in the rat embryo express SGP-2 mRNA in a pattern that does not correlate with regions undergoing developmental cell death. In the nervous system, SGP-2 mRNA is expressed in neuronal populations including motor neurons, cortical neurons, and hypothalamic neurons at ages when the period of developmental cell death has passed. In a nonneural tissue (palatal shelve epithelium) for which a developmental cell death period has been described, SGP-2 mRNA was not present in the region where cell death occurs. We conclude that SGP-2 mRNA expression cannot be correlated with programmed cell death in neural or nonneural tissues. The results of this study as well as recently reported SGP-2 homologies indicate a possible role for this protein in secretion and lipid transport.

INTRODUCTION

The developing nervous system gives rise to many more neurons than will remain in the mature animal (Hamburger, 1982; Oppenheim, 1985). Regulation of neuronal population size occurs largely through the process of developmental neuronal death (Oppenheim, 1985). The cellular mechanisms underlying the death of neuronal subpopulations are not understood. Recent evidence supports the concept of developmental neuronal death as an active process requiring new gene expression within the dying cell. Inhibition of new mRNA or protein synthesis retards developmental neuronal death of chick motoneurons and dorsal root ganglion cells, *in vivo* (Oppenheim, Prevette, Tytel, and Homma, 1990), and prevents cell death that results from deprivation of neurotrophic factors in cultured chick sensory and autonomic neurons (Martin et al., 1988; Scott and Davies, 1990). Two mutations in the nematode, *Caenorhabditis elegans, ced-3,* and *ced-4,* produce a phenotype lacking programmed neuronal death (Ellis and Horvitz, 1986). Genetic mosaic analysis has shown that loss of *ced-3* or *ced-4* function from the neurons destined to die prevents the process of programmed neuronal death specifically in those cells that contain the mutation (Yuan and Horvitz,

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1990). During metamorphosis of the moth, *Manduca sexta,* steroid hormone signals initiate protein synthesis-dependent programmed cell death of neurons and muscle (Truman, 1984; Schwartz, 1990). All of the above findings support the hypothesis that developmental neuronal death results from the active expression of genes whose functions are to eliminate the neuron.

Programmed cell death in nonneural tissue is also dependent on protein synthesis (Cohen and Duke, 1984; Wyllie, Morris, Smith, and Dunlop, 1984; Pratt and Green, 1976). This observation has lead investigators to attempt to identify molecules necessary for initiation of the cell death program through the techniques of subtractive hybridization and cDNA cloning. For example, prostatic epithelial cells deprived of testosterone undergo programmed cell death. One of the most abundant new transcripts in regressing prostatic tissue, testosterone repressed prostate message-2 (TRPM-2) (Leger, Monpetit, and Tenniswood, 1987), encodes a molecule previously characterized as sulfated glycoprotein-2 (SGP-2), a 70 kD protein secreted from testicular Sertoli cells (Buttyan et al., 1989; Bettuzzi, Hiipakka, Gilna, and Liao, 1989). Other tissues undergoing cell death have been reported to express SGP-2/TRPM-2, such as regressing interdigital webs of embryonic feet (Buttyan et al., 1989). These observations have led to the suggestion that SGP-2 may play an active and specific role in programmed cell death or apoptosis, the histologic characterization of programmed cell death (Buttyan et al., 1989).

SGP-2 mRNA expression has also been studied in several systems undergoing neuronal degeneration. SGP-2 mRNA increases in scrapie-infected hamster brain (Duguid, Bohmont, Liu, and Tourtellotte, 1989). A human homologue of SGP-2 mRNA is elevated in Alzheimer's diseased (AD) hippocampus (May, Johnson, Poirer, Lampert-Etchells, and Finch, 1989). Excitotoxic or deafferenting lesions to rat hippocampus also induce an up-regulation of SGP-2 mRNA (May et al., 1990). Because SGP-2 message is up-regulated during programmed cell death in some nonneural tissues and is also elevated in degenerating neural tissues, it was hypothesized that SGP-2 mRNA induction may also be involved in developmental neuronal death.

To address this question, we have studied the expression of SGP-2 mRNA in the developing and adult rat central nervous system (CNS) and in embryonic nonneural tissues. We report that SGP-2 message is expressed widely in both the developing and mature rat CNS in a spatio-temporal pattern unrelated to developmental cell death. Rather, induction of SGP-2 mRNA expression in developing neural tissue coincides with advancing differentiation. Furthermore, nonneural tissues of the developing rat embryo express SGP-2 mRNA in a pattern that does not co-localize with regions of apoptosis. We conclude that SGP-2 mRNA expression does not have a specific role in developmental cell death. However, the widespread expression of this molecule in neural tissue and the timing of its induction with neuronal differentiation indicate an important function for SGP-2 in the CNS.

Recent reports identify the human homologue of SGP-2 as an apolipoprotein (de Silva, Harmony, Stuart, Gil, and Robbins, 1990) and an inhibitor of the complement system (Jenne and Tschopp, 1989; Kirszbaum et al., 1989). The bovine homologue of SGP-2 was also reported as an adrenal chromaffin granule-associated molecule (Palmer and Christie, 1990). The widespread expression we observed in the CNS and embryonic tissues is discussed in the context of possible functional roles of SGP-2 and its human and bovine homologues.

MATERIALS AND METHODS

Northern Blot Analysis

Total RNA isolation from rat testes and embryonic day (E) 15 rat embryo was carried out in guanidinium salts according to the method of Han et al. (1987). Poly A RNA isolation, electrophoresis, and blotting onto Genescreen membranes were carried out employing methods previously described (Heuer et al., 1990). The membrane was exposed to 1200 μCi/cm^2 ultraviolet (UV) radiation for 2 min in order to cross-link the RNA to the blot. The plasmid pGEM-60HE, contains an 1850 base pair cDNA for SGP-2 cloned into the vector pGEM (Promega, Madison, WI). The SGP-2 cDNA was isolated from the plasmid by digestion with *EcoRI* and *Hind III* followed by electrophoretic separation of the cDNA and plasmid vector in 1% Seaplaque agar (FMC, Rockland, ME). Isolated SGP-2 cDNA was used to generate a ^{32}P random-labeled probe using a random labeling kit (Boheringer Mannheim, Indianapolis, IN). The probe was purified over a G_{50} Sephadex (Pharmacia, Piscataway, NJ) column. Blot and probe were hybridized for 16 h at 42°C in 50% formamide, 1.8 *M* NaCl, 10× Denhardt's solution, 1% SDS, 10% dextran sulfate, 20 m*M* Tris, 5 m*M* ethylenediminetetraacetate (EDTA), 250 μg/ml salmon sperm DNA, and 500 μg/ml yeast tRNA. The blot was washed twice at room temperature in 2× SSPE, 1% SDS for 30 min, followed by a

65°C wash in the above solution for 30 min. The blot was exposed to X-ray film for 14 days at −70°C.

In situ Hybridization

In situ hybridization on 8- to 10-μm paraffin-embedded sections of rat embryos (embryonic ages: E12, E15, and E18) and isolated spinal cord (postnatal ages: P2, P20, and adult) was performed as described previously (Heuer, Fatemie-Nainie, Wheeler, and Bothwell, 1990). Sections mounted on poly-L-lysine-coated slides were deparaffinized, rehydrated, post-fixed in 4% paraformaldehyde, treated with protease K, tetraethylammonium (TEA), and TEA/acetic anhydride. Frozen sections through adult and neonatal rat forebrains were treated in a very similar manner with the exception of the omission of protease K treatment from the prehybridization steps. ^{35}S-labeled riboprobes were generated from pGEM-60HE linearized with *Hind III* or *EcoR1*. For antisense probes, Hind III-linearized cDNA was combined with T7 RNA polymerase, and sense probes were generated from *EcoR1*-linearized cDNA with SP6 RNA polymerase. Slides were hybridized with a probe solution containing 2,000,000 c.p.m. for 16 h at 55°C. After hybridization the slides were treated with RNase A for 30 min and washed several times with the highest stringency being 0.1× SSC at 65°C. After autoradiography, slides were counterstained with hematoxalin/eosin or thionin. Adjacent sections were labeled with either antisense probe or the control sense probe. Sections probed with the sense strand showed only a low level of label without any discrete regional localization.

RESULTS

Specificity of the SGP-2 Probe

In order to verify the specificity of the SGP-2 probe, a Northern blot containing poly-A-selected RNA from E15 rat embryos and adult rat testes was probed with the SGP-2 cDNA. A single band at approximately 2.0 kb was obtained for both RNA samples (data not shown). This result agrees with the previously reported molecular weight of SGP-2 mRNA (Collard and Griswold, 1987) and demonstrates that the probe specifically recognized SGP-2 mRNA in the E15 rat embryo.

Expression in Spinal Cord Development

To assess the possible role of SGP-2 in neuronal cell death, we examined mRNA expression in brachial spinal cord motoneurons, a neuronal population for which developmental cell death has been carefully characterized (Oppenheim, 1986). We studied the expression of SGP-2 mRNA in the ventral horn of the brachial spinal cord employing *in situ* hybridization at developmental stages before, during, and after the period of motoneuron death. At E12, before the period of developmental neuronal death has begun, the neural progenitors are not labeled for SGP-2 mRNA [Fig. 1(A)]. Between E15 and E18, when cell death is occurring, SGP-2 mRNA expression has begun [Fig. 1(B,C)]. Nearly every cell in the ventral column is labeled. At this time, the degree of labeling is quite homogeneous. In the postnatal animals, when the period of cell death has ended, silver grains continue to localize in the ventral column cells [Fig. 1(D) and Fig. 2]. In P20 and adult spinal cords the majority of label concentrates over large neuronal cell bodies, and the number of grains per cell has increased dramatically. At these later ages, radially oriented glia in spinal cord white matter also express SGP-2 (Fig. 2).

Expression in Other Embryonic Structures

Neural Expression. The general pattern of SGP-2 mRNA in neural development is one of increasing expression with advancing differentiation. In the E12 embryo, SGP-2 mRNA is generally absent from neuroectodermal cells. By E15, strong SGP-2 mRNA expression is found in some nonneuronal neuroectodermal structures such as the choroid plexus and retinal pigmented epithelium [Fig. 3(A,B)]. This mRNA is also seen in forebrain and hindbrain structures at E15. In both E15 and E18 animals, a similar pattern of neural expression is seen. Little SGP-2 mRNA is detected in the proliferative or mantle zones of developing cortex. However, in cells that have migrated away from the ventricular epithelium, SGP-2 mRNA has appeared. In the E18 embryo [Fig. 3(C)], cortical development has progressed to a thickness of several cell layers. The oldest and most differentiated cells in the E18 cortex are in the marginal zone, the most superficial layer, and in the subplate zone, the deepest cell layer. Cells in the marginal and subplate zones are the most strongly labeled cells in the E18 cortex. A similar pattern of SGP-2 mRNA expression is recapitulated in the spinal cord, where the first cells to express SGP-2 mRNA are also the first cells to migrate away from the ventricular epithelium. Exceptions to this pattern exist where expression is found in confined regions of the neuroepithelium lining the third and fourth ventricles.

In the peripheral nervous system, the level of mRNA expression also increases with advancing

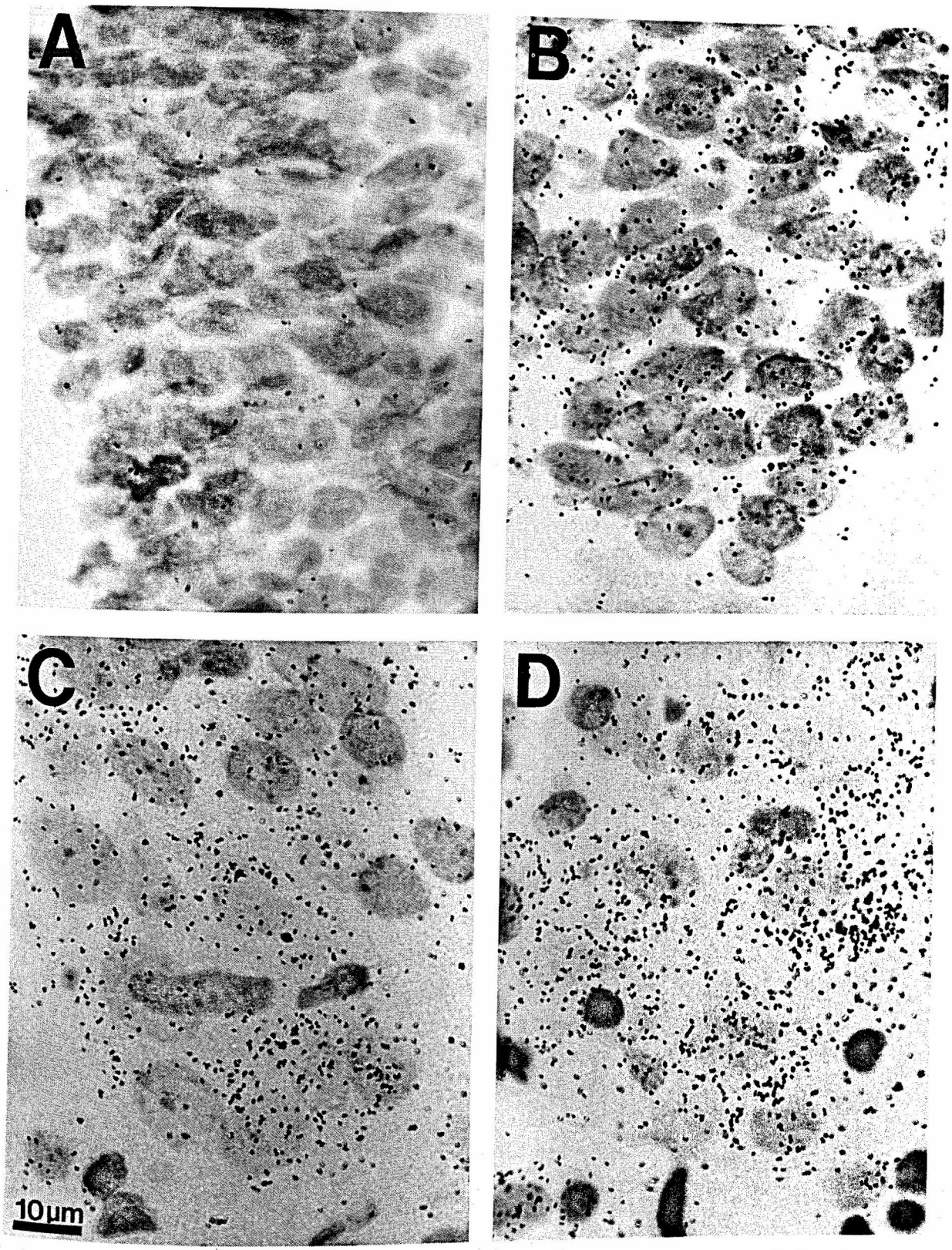

Figure 1 High-power photomicrographs of cells in the ventral portion of the spinal cord labeled for SGP-2 mRNA. (A) A section from an E12 embryo shows only background levels of silver grains in the ventral region of the developing spinal cord. In (B) E15 embryo, (C) E18 embryo, and (D) P2 neonatal animal, SGP-2 label is present in a fairly homogeneous pattern over cells in the ventral spinal cord.

E12
E15
E18
P2
P20
ADULT
A
B

development. In the E12 embryo, neural crest cells are not labeled. At E15, some cranial ganglia are positive, but brachial dorsal root ganglia and sympathetic ganglia show no SGP-2 mRNA expression. By E18, the dorsal root and sympathetic ganglia begin to express SGP-2 mRNA. The concentration of silver grains over DRG cells increases through the other developmental stages examined (P2 and P20).

Nonneural Structures. The results that we will discuss are limited to structures found in the brachial or cranial regions of the embryo. Some of the structures and tissue types that demonstrate SGP-2 expression in at least one of the three ages examined are listed in Table 1. In the E12 animals, positive cells are mainly of mesodermal origin with the heart being the site of most abundant SGP-2 mRNA expression (Fig. 4). Lung mesenchyme, cartilage lining, pericardium, heart, and arterial endothelium are examples of mesodermal cells expressing SGP-2 message at E15 (Fig. 5). At E18, these structures all continue to express SGP-2 mRNA, and additional positive mesenchymal structures include skeletal muscle lining (Fig. 6), meninges, and some chondrocytes. The expression profile seen in the paw at E18 is well after the period of interdigital web cell death (E13–E15) during which the expression of SGP-2 message has been reported (Buttyan et al., 1989). At all three stages, isolated cells in regions of undifferentiated mesoderm also express SGP-2 mRNA. The only endodermal site of expression seen is the E15 liver (Fig. 5). None of the sections from E18 animals contained hepatic tissue. However, previous investigators have reported expression of SGP-2 mRNA in adult rat liver (Collard and Griswold, 1987). Ectodermal mRNA expression is limited to neuroectodermal structures previously mentioned with the exception of epithelial cells lining the forming middle ear in E15 and E18 animals.

Regions of apoptosis from all three embryonic stages do not uniformly express SGP-2 mRNA. In the E15 embryo, epithelia that line the palatal shelves prior to their fusion are entering a period of programmed cell death (Pratt and Green, 1976) (Fig. 7). At high magnification many apoptic cells can be seen in this region [Fig. 7(C)]. However, no SGP-2 expression was seen associated with this region of apoptosis. The dark-field representation of this region [Fig. 7(B)] shows that SGP-2 mRNA is associated with cartilage-forming regions on the lateral sides of the palatal plates, but the medial region, where the dying epithelial cells are located, is not labeled.

Expression in the Adult Central Nervous System

Many regions in adult rat brain express SGP-2 mRNA. Some examples are shown in Figure 8. The strongest concentration of silver grains was found over the choroid plexus [Fig. 8(A)]. Ventricular epithelium (ependymal cells) and the pineal body also label strongly. In forebrain structures, nearly every neuron demonstrated a low level of expression of SGP-2 mRNA. Certain regions, such as the supraoptic nucleus [Fig. 8(B)], show enhanced levels of expression. The majority of cell bodies seen in white matter were not labeled. However, a small proportion of glial cells resident in fiber tracts, such as the corpus collosum and optic tract, have a fairly heavy concentration of SGP-2 mRNA. In hindbrain regions, populations labeling strongly for SGP-2 message include the hypoglossal nucleus [Fig. 8(C)] and the facial motor nucleus. Other hindbrain structures, such as the cerebellum and the more dorsally located sensory nuclei, are weakly labeled. Cerebellar granule cells did not demonstrate any label above the background level obtained with the sense probe control.

DISCUSSION

SGP-2 Expression Is Not a Marker of Cell Death

In this study, we sought to determine if SGP-2 has a specific role in developmental neuronal death.

Figure 2 (A) A high-power view of neurons in the adult ventral spinal cord revealing the high concentration of silver grains over these cells. (B) The ventral spinal cord from an adult animal labeled for SGP-2 mRNA is shown in this dark-field photomicrograph. Large motor neurons in the ventral grey matter and radially oriented glia in white matter are strongly labeled. (C) A schematic representation of the distribution of SGP-2 message label in the spinal cord at all six ages studied.

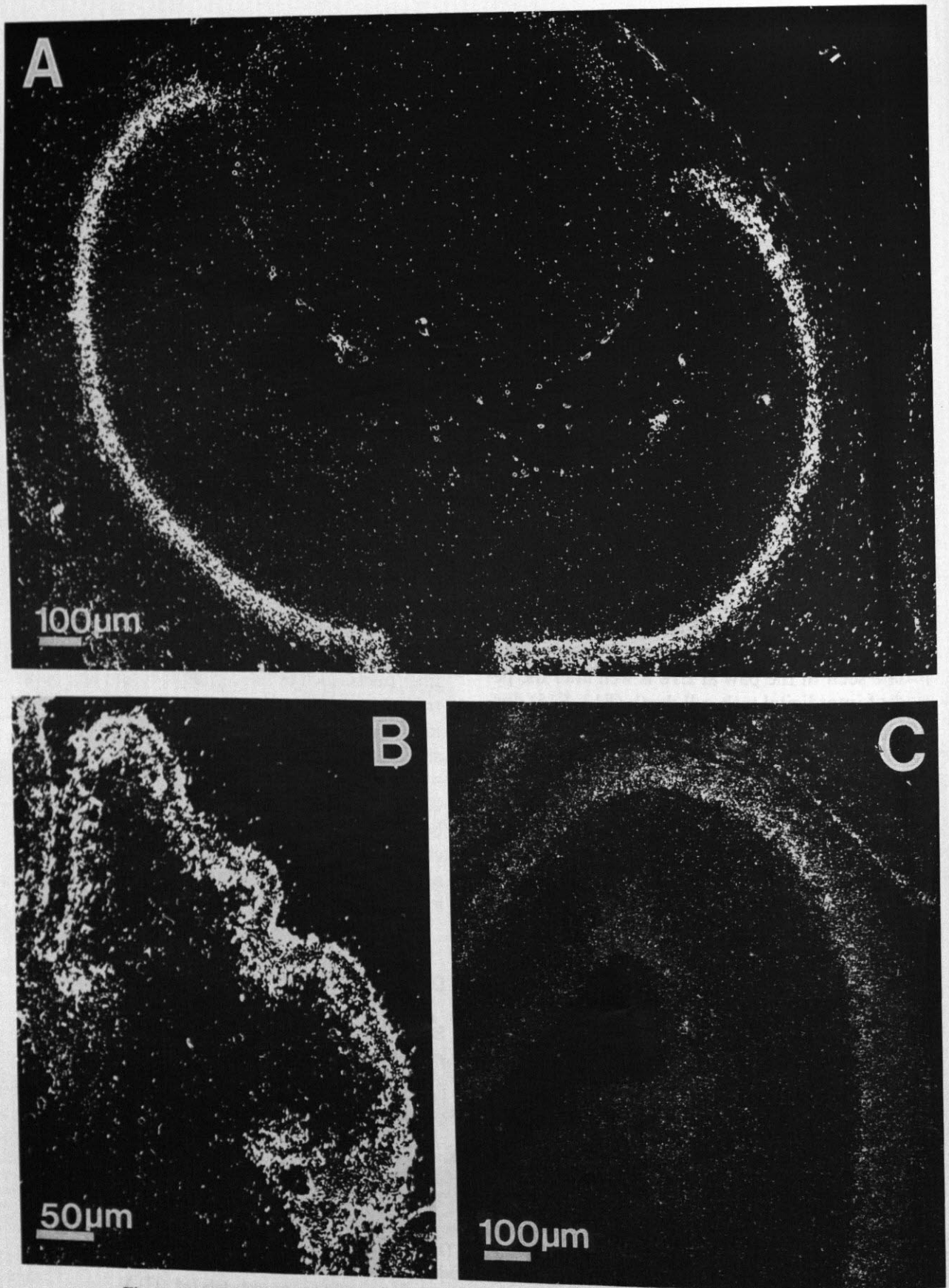

Figure 3 Expression of SGP-2 mRNA in embryonic neural structures. (A) The eye from an E15 embryo shows label in the pigmented epithelium. (B) Choroid plexus from an E15 embryo is strongly labeled. (C) Cortex from an E18 embryo shows expression in the most differentiated layers.

Table 1 Localization of SGP-2 Expression in the Rat Embryo

	Ages		
	E12	E15	E18
Mesodermal structures			
Undifferentiated mesenchyme	+	+	+
Heart/sinus venosous	+	+	+
Vascular endothelium	+	+	+
Lung mesenchyme	−	+	+
Cartilage lining	−	++	+
Differentiated chondrocytes	−	−	+/−
Skeletal muscle	−	−	++
Meninges	−	−	++
Pleura	NS	−	+
Pericardium	−	+	+
Endodermal structures			
Liver	NS	+	NS
Ectodermal structures			
Choroid plexus	−	+++	+++
Pigment epithelia of retina	+/−	+++	+++
Ventricular neuroepithelium	−	−	+/−
Neural crest	−	NS	NS
Differentiated neurons	NS	+	+
DRG	NS	−	+
Sympathetic ganglia	NS	−	+/−
Ventral spinal cord	−	+	+

Note: NS = not studied; DRG = dorsal root ganglia; + = labeled; − = unlabeled; +/− = inconsistantly labeled; ++ = strongly labeled; +++ = very strongly labeled.

We present three lines of evidence that indicate that SGP-2 mRNA expression in neurons is *not* correlated with neuronal death. First, in developing spinal motor column, SGP-2 mRNA expression progressively increases during and after the period when developmental cell death occurs. At postnatal stages, when no significant cell loss takes place (Oppenheim, 1986), SGP-2 message expression continues to increase. Dorsal root ganglia (DRG) neurons demonstrate a similar pattern of increasing postnatal expression. DRG neuron death has not been well characterized in the rat, but the period of DRG death is an early event in chicken development (E5–E8). By extrapolation, DRG cell death is unlikely to occur postnatally in rat (Gorin and Johnson, 1979). Second, nearly every neuron in the adult forebrain and a large portion of hindbrain and spinal cord neurons are positive for SGP-2 mRNA. These cells do not undergo cell death in the mature animal. Finally, during CNS development, SGP-2 mRNA expression generally is more prominent in regions of advanced differentiation. SGP-2 message expression is seen at embryonic day 18 in subplate neurons that differentiate early in cortical development. However, subplate neurons undergo developmental neuronal death between postnatal days 2 and 8 (Al-Goul and Miller, 1989), many days after they begin to express SGP-2 mRNA. Because SGP-2 mRNA expression commences at the time of neuronal differentiation rather than at the time of developmental cell death, and because cellular expression reaches its highest level in mature neurons, the SGP-2 product is unlikely to be a specific indicator of neuronal cell death.

In nonneural embryonic tissue, SGP-2 mRNA expression also does not coincide with regions undergoing programmed cell death. Embryonic tissues that express SGP-2 mRNA are widely varied. The majority of cells expressing SGP-2 mRNA are of mesodermal origin. However, expression in endoderm and ectoderm is also seen. One well-characterized site of embryonic programmed cell death, the palatal shelf epithelium, displayed the histologic appearance of apoptosis but not the expression of SGP-2 mRNA. In the developing embryo, we have described examples of programmed cell death where SGP-2 mRNA is not seen as well as examples of SGP-2 message in cells that will not undergo programmed cell death. These data suggest that the function of SGP-2 is not specific to programmed cell death in nonneural tissues.

SGP-2 as a Multifunctional Protein

The results presented in this study lead to several questions. First, what role does this widely expressed molecule play in nervous system function? Second, as SGP-2 mRNA is expressed in developing tissues originating from all three embryonic layers and in many different organ systems, what function can this secretory molecule possess in the developing embryo? Finally, why is SGP-2 message so strongly up-regulated in some systems undergoing programmed cell death if its function is not specific to that cellular process? Several recent reports on SGP-2 structure, message distribution, and sequence homology provide a basis for speculation regarding answers to these questions.

Analysis of the peptide structure of SGP-2 has revealed that it contains several possible functional domains. Four domains with sequence similarity to the myosin heavy chain have been described (Tsuruta, Wong, Fritz, and Griswold, 1990; Jenne and Tschopp, 1989; de Silva et al., 1990). These myosin-like domains may fold into an α-helical coiled-coil structure that could generate one

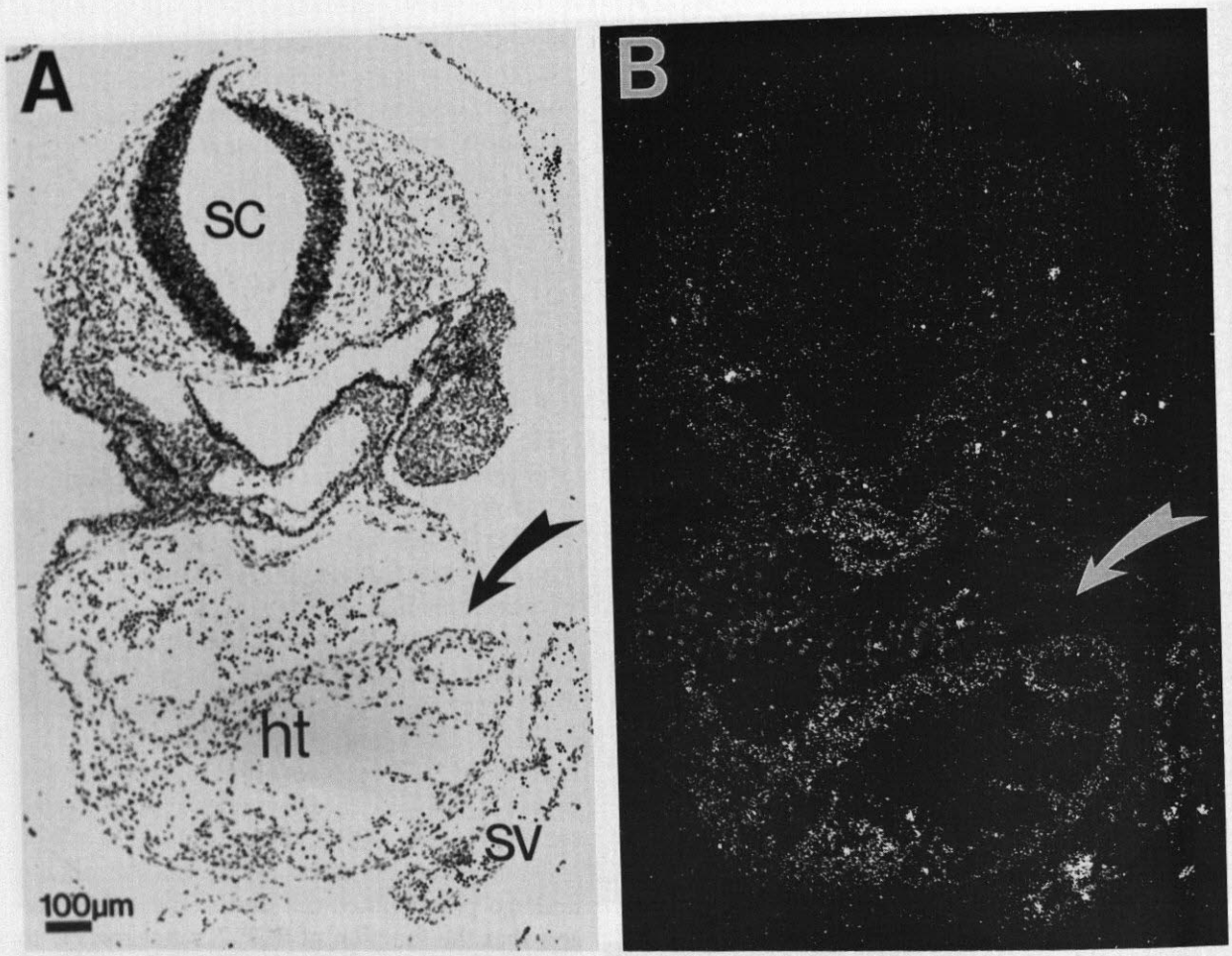

Figure 4 (A) Bright-field and (B) dark-field photomicrographs of a transverse section through the brachial region of an E12 embryo. The arrows point to the only region strongly labeled, which is cardiac tissue. sc = spinal cord; ht = heart; sv = sinus venosus.

(Jenne and Tschopp, 1989), three (de Silva et al., 1990), or four (Tsuruta et al., 1990) amphiphilic helices. The amphiphilic portions predicted in secondary structure probably provide SGP-2 with the ability to interact with the lipid bilayer in the absence of long stretches of hydrophobic amino acids in the primary structure. The amino acid sequence of human and rat SGP-2 also contain four heparin-binding domains found adjacent to or within regions of postulated amphiphilic helices (de Silva, 1990). These regions may confer on SGP-2 the ability to bind to extracellular matrix molecules or to molecules associated with the extracellular surface membrane.

The different functional domains of the SGP-2 protein indicate that it is possible that SGP-2 may possess more than one physiologic function. Further evidence to support this notion comes from the widely varied systems from which SGP-2 homologues have been isolated. The bovine homologue was isolated from adrenal chromaffin granules (Palmer and Christie, 1990). The human homologue has been isolated four times: twice as an inhibitor of the soluble complement cytolysis complex (sC5b-9) (Jenne and Tschopp, 1989; Kirszbaum et al., 1989), most recently as a component of the high-density lipoprotein (HDL) complex (de Silva et al., 1990), and as one of several clones up-regulated in Alzheimer's diseased hippocampus (May et al., 1989). When one considers these homologies to SGP-2 and their structural similarities, it becomes possible to postulate several putative roles for this family of glycoproteins.

SGP-2 in Secretion

One SGP-2 homologue, a bovine adrenal chromaffin granule component labeled glycoprotein III, or GpIII (Palmer and Christie, 1990), is reported to be associated with several endocrine glands. It exists both as a membrane-associated and soluble component of chromaffin granule preparations.

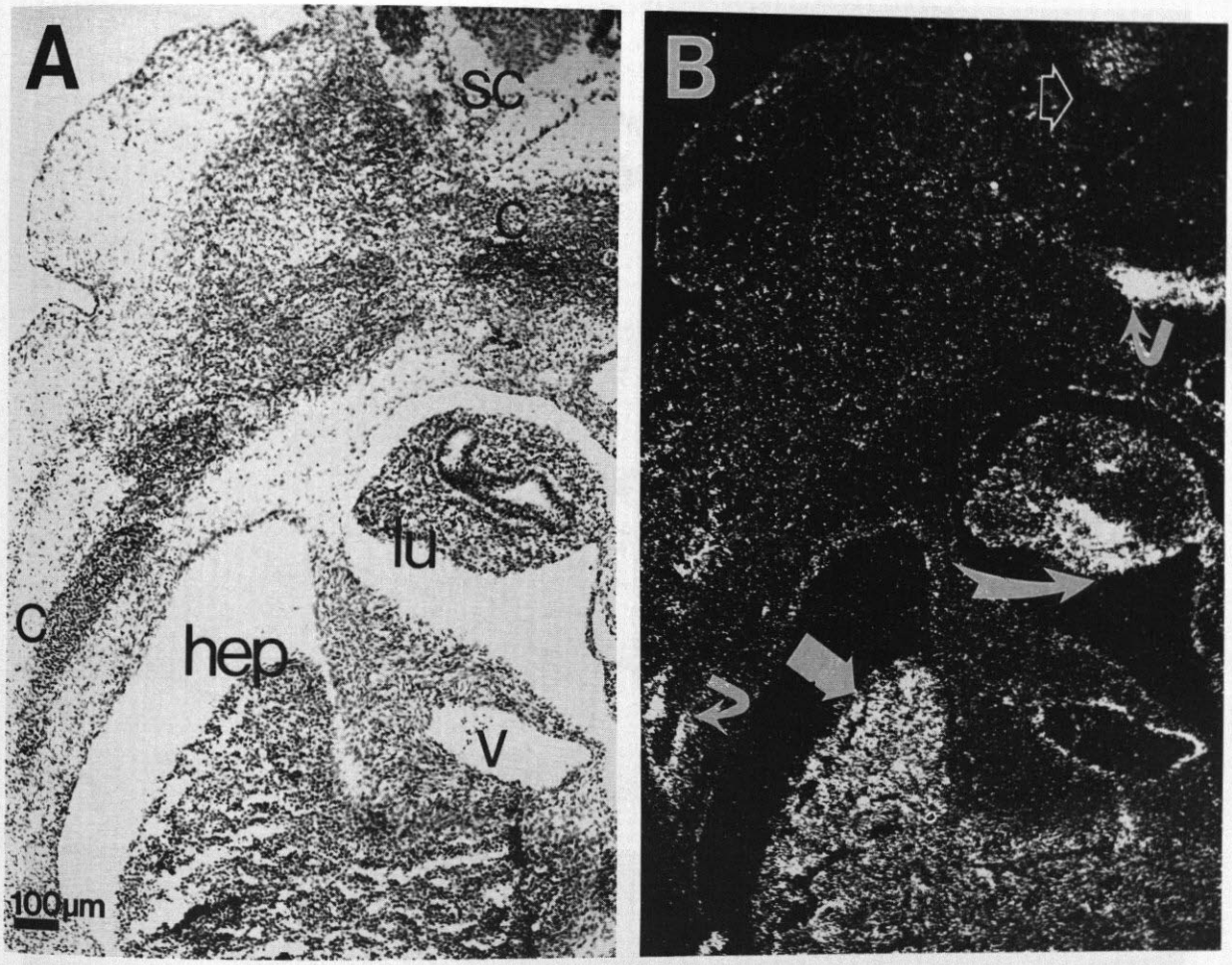

Figure 5 (A) Bright-field and (B) dark-field photomicrographs of a transverse section just caudal to the brachial region in an E15 embryo. The open arrow points to the ventral spinal cord which is labeled. The bent arrows point to the label in cells lining the cartilage of the forming rib and vertebra. The curved arrow points out a region of lung mesenchyme, which is labeled. The large arrow points to labeled hepatic tissue. c = cartilage; lu = lung; hep = liver; v = vessel; other abbreviations as per Figure 4.

GpIII is released from chromaffin granules in an acetylcholine-dependent fashion. The membrane-associated form remains localized to the vesicular membrane during recycling of the vesicle in the Golgi complex (Patzak and Winkler, 1986). GpIII has also been immunochemically localized to the posterior pituitary (Fischer-Colbrie, Zangerle, Frischenschlager, Weber, and Winkler, 1984). This finding suggests that GpIII protein is localized to terminals of neurons projecting from nuclei associated with the posterior pituitary such as the supraoptic nucleus. These results agree with our findings of strong label for SGP-2 mRNA in the supraoptic nucleus. Possible functions for SGP-2 in rat neurons may be in the process of neurotransmitter secretion or secretory vesicle formation. A function related to neurotransmission fits with the pattern of SGP-2 expression in the developing nervous system because neurons differentiate primarily after they migrate away from the ventricular epithelium.

SGP-2 in Immune System Function

One of the postulated functions of SGP-2 may also explain why certain tissues, such as the testis and choroid plexus, transcribe such large amounts of this message. SGP-2 is reported to be a rat homologue of the human protein SP-40,40 or complement cytolysis inhibitor (Jenne and Tschopp, 1989; Kirszbaum et al., 1989). The amino acid sequence homology between SGP-2 and SP-40,40 is 76% which indicates that SP-40,40 is very likely the human cognate of SGP-2. SP-40,40 was coisolated with vitronectin, as a potent (active at physiologic concentration) inhibitor of the cytolytic activity of

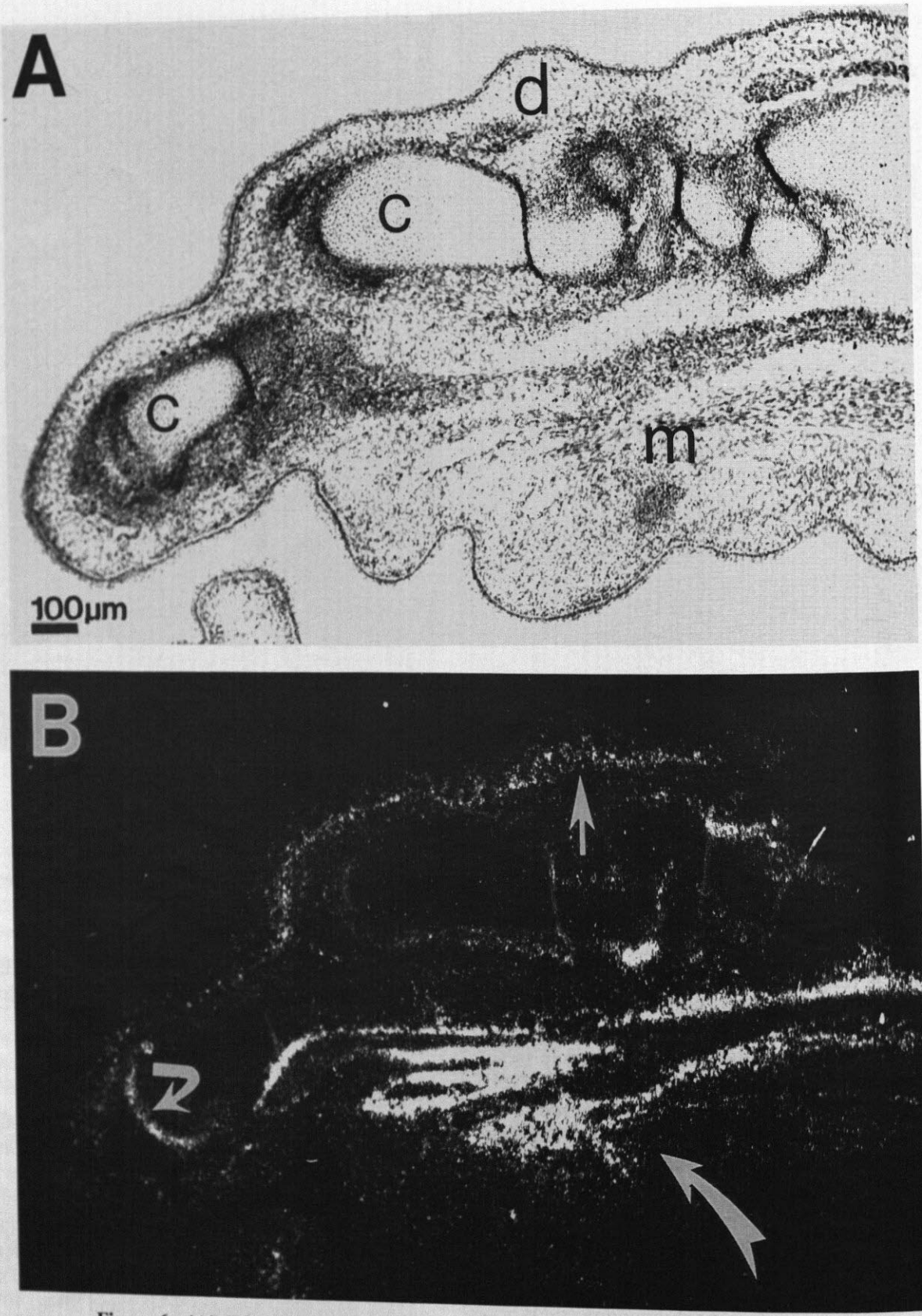

Figure 6 (A) Bright-field and (B) dark-field photomicrographs of a transverse section through the paw of an E18 rat embryo. The bent arrow points out cells labeled in the region between the cartilaginous bone-forming regions of the digits. Cells surrounding carpals and metacarpals are also labeled. The straight arrow points to labeled cells in the dermis. The curved arrow points to a labeled region that appears to outline forming skeletal muscle. d = dermis; m = skeletal muscle; other abbreviations as per Figure 5.

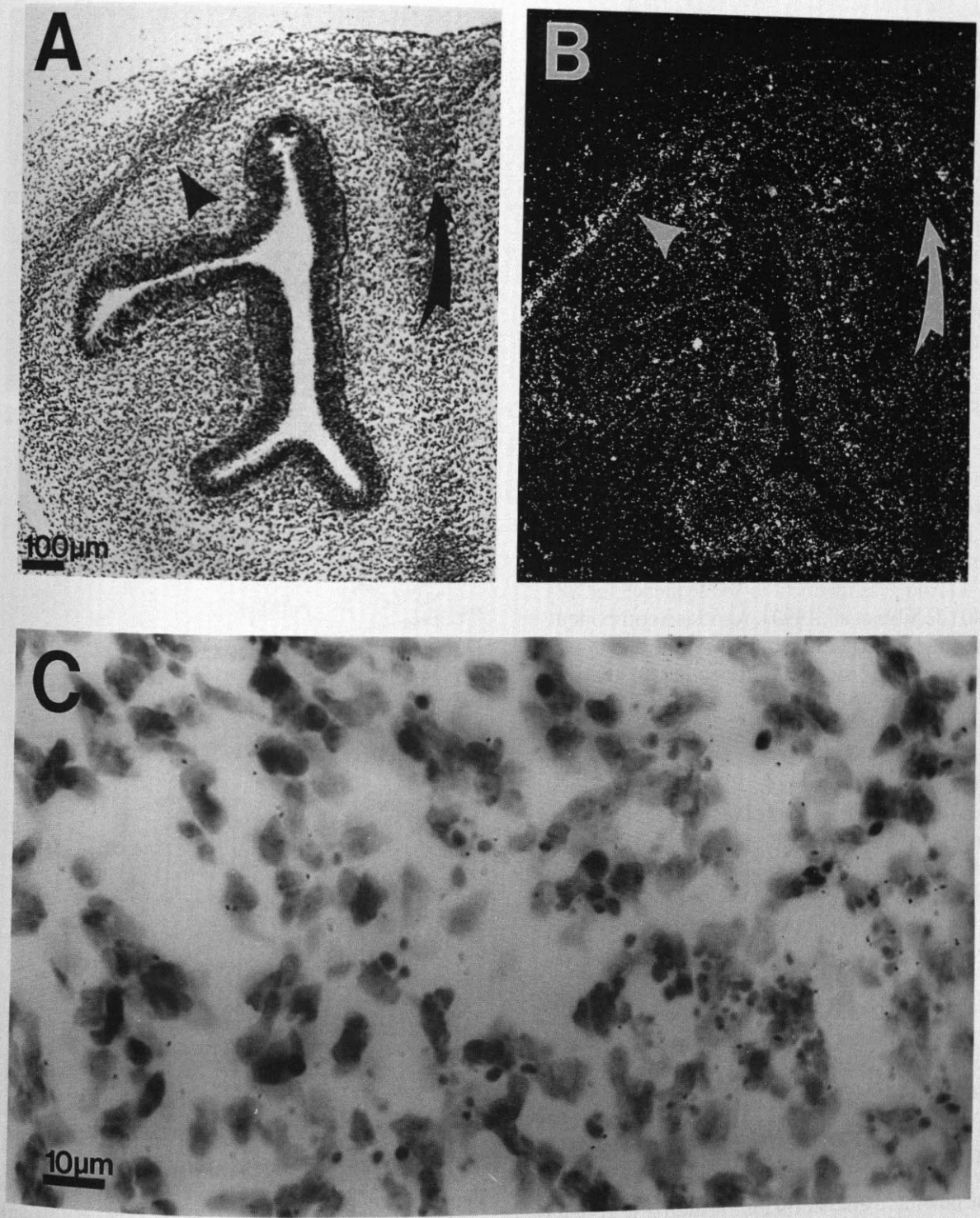

Figure 7 (A) Bright-field photomicrograph of a cranial section through an E15 embryo showing the palatal shelves. The right side of the figure includes the midline region where fusion of the two palatal shelves is taking place. The curved arrow points to the region seen at high power in C. (B) Dark-field illumination of the same image seen in A. Silver grains are not present in the midline region where programmed cell death is occurring. Label is present in cells lining cartilage, pointed out by the arrowhead. (C) The region of the midline between the palatal shelves pointed out by the curved arrow in A and B is shown at high magnification. Many pyknotic nuclei and apoptic bodies are seen, while only background levels of silver grains are apparent.

soluble complement complex SC5b-9. A possible function for SGP-2 in semen is to protect sperm from complement-mediated cytolysis in the vagina. SGP-2 secreted from choroid plexus may also provide the CNS with an additional level of immune privilege by inhibiting complement activation in those tissues confined to the cerebrospinal fluid compartment. It has been suggested (May et al., 1990) that the increased expression of human SGP-2 in Alzheimer's Disease (AD) brains may be a compensatory response to complement components seen in AD plaques.

SGP-2 in Lipid Transport

SP-40,40 shares the property of inhibiting SC5b-9 with other serum proteins such as vitronectin, antithrombin III, HDL and apolipoproteins A1 and A2. It has recently been reported that a cDNA sequence coding for the HDL-associated apolipoprotein J (Apo J) is identical to the sequence for SP-40,40 (de Silva et al., 1990). Apo J is a component of specific subclasses of human HDL and has a very wide tissue distribution, similar to that reported for rat SGP-2. The role of Apo J in HDL function is not known. SGP-2 may possess a lipid transport function for developing sperm in semen or for the nervous system via the cerebrospinal fluid. Another HDL-associated apolipoprotein, Apo E, also has a structure involving amphiphilic and heparin-binding domains. Apo E has been shown to up-regulate in nonneural cells surrounding axonal degeneration (Boyles et al., 1989). A mechanism of SGP-2 action in degenerating tissues may therefore be related to transporting the lipid from dying cells. This function for SGP-2 may also explain its up-regulation in hippocampal neurons after deafferenting or excitotoxic lesions (May et al., 1990).

A Role for SGP-2 in Cell Death

One possible explanation for the dramatic up-regulation of SGP-2 mRNA during prostate programmed cell death or lesion-induced neurodegeneration in hippocampal neurons can be postulated from work done on the removal of apoptic neutrophils from the blood stream. Macrophages recognize and phagocytose apoptic neutrophils in culture (Savill, Dransfield, Hogg, and Haslett, 1989). It has been demonstrated that macrophage recognition and phagocytosis of senescent neutrophils is dependent on neutrophil surface vitronectin binding to the macrophage vitronectin receptor (Savill et al., 1990). Though vitronectin and SGP-2 are not evolutionarily related members of a gene family, these proteins conceivably could have evolved similar functional properties through common elements of secondary structure. The two molecules both contain heparin-binding domains and a cys-

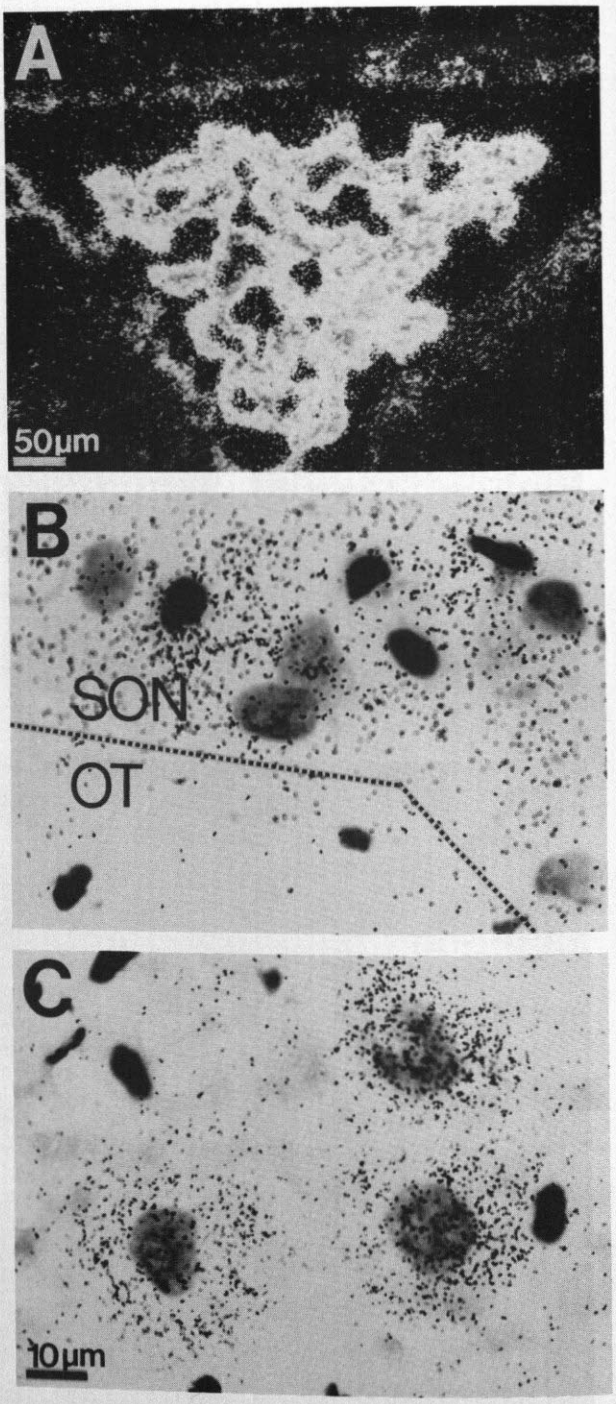

Figure 8 Expression of SGP-2 mRNA message in adult neural structures. (A) Choroid plexus is shown in a dark-field photomicrograph. (B) Cells in the SON are expressing SGP-2 mRNA, while cells in the OT do not label for SGP-2. (C) Neurons in the hypoglossal nucleus strongly express SGP-2 mRNA. SON = supraoptic nucleus; OT = optic tract.

tine-rich region that may have a vestigial relationship to other complement proteins (Kirszbaum et al., 1989). Vitronectin and human SGP-2/SP-40,40 are both potent inhibitors of the cytolytic SC5b-9 complement complex. The same commonalities that allow both SGP-2 and vitronectin to inhibit SC5b-9 could also provide both proteins with the ability to stimulate phagocytic removal of apoptic cells.

If the importance of SGP-2 up-regulation in dying cells is to signal the impending death of the cell to the phagocytes, an analogy can be drawn between this proposed function for SGP-2 and the *ced-1* gene in the nematode *C. Elegans.* When the *ced-1* gene is mutated, neurons in the developing nematode go through a normal series of programmed cell death. The mutation results in the failure of surrounding cells to remove the dead cells from the tissue (Ellis and Horvitz, 1986). SGP-2 may be one of several vertebrate proteins, including vitronectin, that play a role similar to the nematode *ced-1* gene product. The caveat to this hypothesis is that SGP-2 cannot act alone as a specific signal for phagocytosis of dying cells because of its presence in normal healthy cells.

CONCLUSIONS

We conclude that SGP-2 does not have a specific role in developmental neuronal death. Widespread expression of SGP-2 in developing and adult neural tissues indicate an important role for this protein in nervous system function. Whether SGP-2 function in neural tissue is related to secretion, complement inhibition, lipid transport, or an undescribed property of this molecule remains to be determined. This highly interesting and possibly multifunctional molecule warrants further study to discover the purpose behind its up-regulation in neurodegenerative processes.

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