National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

The Distribution Of Sulfated Glycoprotein-2 (SGP-2) In The Hypothalamus Of Normal And Estradiol-Lesioned Rats

Kenneth Michael Wener

Department of Anatomy and Cell Biology
McGill University
Montreal, Quebec Canada
July, 1995

A Thesis Submitted to the Faculty of Graduate Studies and Research in Partial Fulfilment of the Requirements for the Degree of Master of Science

ISBN 0-612-12291-3

Short title: SGP-2 distribution in the normal and estradiol-lesioned hypothalamus.

ABSTRACT

Sulfated glycoprotein-2 (SGP-2) is an acidic glycoprotein that has recently been discovered in numerous tissues including brain. This glycoprotein has been shown to be involved in the response to diverse types of neuronal injury and synaptic remodelling. It is well established that estradiol treatment results in selective destruction of ß-endorphin neurons in the hypothalamic arcuate nucleus. The question addressed by this thesis is what effect SGP-2 plays in this estradiol-induced pathology. We have used immunocytochemistry to elucidate the distribution of SGP-2 in the normal and estradiol-lesioned hypothalamus. Unexpectedly, SGP-2-immunopositive perikarya occurred in a highly specific distribution in the normal rat hypothalamus. SGP-2-reactive neurons were localized to the medial preoptic area (MPOA), supraoptic nucleus (SON), paraventricular nucleus (PVN), dorsomedial nucleus (DMN) and perifornical area (PA) of the hypothalamus. Both the male and female hypothalamus exhibited similar distributions of immunoreactivity. The neuropil as well as small cell profiles consistent with glia appeared free of SGP-2 labelling. Estradiol-induced loss of ß-endorphin resulted in a greater than two-fold increase in the number of SGP-2-reactive neurons only within the MPOA. Other populations of SGP-2-positive neurons remained unchanged. Small cell profiles appeared labelled, after estradiol-valerate (EV) treatment, mainly within the MPOA and immunopositive deposits were associated with the neuropil of the PVN, PA and MPOA. Since, vitamin E, which blocks the neurotoxic effect of estradiol also prevents the EV-induced changes in SGP-2 distribution, the increase of SGP-2-immunopositive cells and presence of neuropil deposits result from the deafferentation engendered by the estradiol-induced lesion rather than a direct effect of estradiol. The

exact role SGP-2 plays in the hypothalamus is still unknown, however we suggest SGP-2 acts as a mediator of synaptogenesis in both the normal and pathological states. We conclude that the SGP-2 response in the MPOA is possibly related to the selective up-regulation of mu-opioid receptors following the EV-induced deafferentation.

RÉSUMÉ

La glycoprotéine sulfatée-2 (SGP-2) est une glycoprotéine acide présente dans plusieurs tissus y compris le cerveau. La SGP-2 semble impliquée dans divers types de lésions neuronales et le remodelage des synapses. Il est déjà bien établi que l'administration du valérate d'estradiol (VA) produit une déstruction sélective de la ß-endorphine des neurones du noyau hypothalamique arqué. La présente recherche consiste à étudier le rôle de la SGP-2 dans les lésions neuronales produites par le VA. C'est par immunocytochimie que nous avons étudié la SGP-2 dans l'hypothalamus de rats normeaux ou injectés de VA. Chez les rats normeaux une réaction positive aux anticorps de la SGP-2 a été observée dans le périkaryon de neurones dont la distribution très précise dans l'hypothalamus est la suivante: dans l'aire préoptique médiane (MPOA), le noyau supraoptique (SON), le noyau paraventriculaire (PVN), le noyau dorsomédian (DMN), l'aire périfornicale (PA). La distribution de la réaction est la même chez le mâle et la femelle. Par ailleurs le neuropil et les petites cellules gliales sont négatifs. Chez les rats traités au VA, il y a deux fois plus de neurones immunoréactifs dans le MPOA alors que dans les autres aires où noyaux, il n'y a pas de changements notables. Toutefois chez ces animaux, les petites cellules sont immunoréactives dans le MPOA exclusivement alors que le neuropile est réactif dans le PVN, PA et MPOA. La vitamine E, qui inhibe l'effet neurotoxique du VA, annule l'effet de cette substance sur la distribution de la SGP-2 immunoréactive. Il semble donc que l'augmentation du nombre de neurones SGP-2 positifs, ainsi que l'augmentation de la réactivité du neuropile puisse être le résultat de la déafférentation ou déconnection produite par le VA plutôt qu'un effet direct de l'hormone sur le périkaryon des neurones. Le rôle exact de la SGP-2 dans

l'hypothalamus reste à préciser. Nous pouvons toutefois suggérer que la SGP-2 joue un rôle de médiateur dans la synaptogénèse aussi bien chez l'animal normal que chez les animaux lésés. Nous concluons donc que l'augmentation de la réactivité du MPOA est vraisemblablement reliée à la régulation sélective des récepteurs opioides suite à une déafférentation induite par le VA.

To Lizzie and my family

ACKNOWLEDGEMENTS

It is with immense gratitude and respect that I wish to acknowledge my thesis supervisor, Dr. James Brawer, without whose guidance and constant encouragement I could not have completed this project.

I am grateful to Dr. Carlos Morales for technical and analytical advice with the immunohistochemical experiments. I would also like to thank Dr. Norman Nadler for helpful suggestions regarding the statistical analysis and computer usage. In addition, I wish to acknowledge Dr. Suleiman Igdoura for his critical discussions and Dr. Yves Clermont for help in translating the French abstract. Thanks to Dr. Michael Griswold of Washington State University (Pullman, WA) for supply of the rabbit anti-SGP-2 polyclonal antibody.

My gratitude is also extended to Dalia Piccioni-Chen for her excellent technical laboratory assistance and help with all phases of the animal work. Her expert assistance has been invaluable. I wish to acknowledge Sonia Bujold and Robert Hodge for their assistance with the photographic work.

Finally, it is with my most heartfelt thanks that I wish to acknowledge my family for their continuing love and support throughout all my endeavours.

This work has been supported by a grant to Dr. J.R. Brawer from the Medical Research Council of Canada.

TABLE OF CONTENTS

ABSTRACT iii
RÉSUMÉ v
DEDICATION vii
ACKNOWLEDGEMENTS viii
TABLE OF CONTENTS ix
FIGURE LISTING xi
ABBREVIATIONS xii

INTRODUCTION 1

Hypothalamic control over the reproductive cycle 1
LHRH signal modulation 2
Opioid regulation of LHRH release 3
Estradiol effect on the reproductive cycle 4

Estradiol mediated pathology 5

Sulfated glycoprotein-2 8
Neural expression of sulfated glycoprotein-2 10

Rationale 11

MATERIALS AND METHODS 13

Animals 13

Tissue preparation for immunohistochemistry 14

Immunostaining 14

Quantitative analysis 16

Statistics 16

RESULTS 18

Effects of vitamin E and estradiol on cyclicity and animal body weight . . . 18

Regional distribution of SGP-2 within the hypothalamus of normal female and male rats 18

Effects of estradiol-valerate-induced destruction of ß-endorphin neurons on hypothalamic regional distribution of SGP-2 20

Effects of vitamin E treatment on SGP-2 distribution in the normal and estradiol-lesioned hypothalamus 21

DISCUSSION 23

The distribution of SGP-2 in the normal hypothalamus 23

The distribution of SGP-2 in the EV-treated hypothalamus 24

SGP-2 as a mediator of synaptic remodelling 26

SGP-2 as a response to neuronal injury 27

CONCLUSION 29

CONTRIBUTIONS TO ORIGINAL RESEARCH 30

REFERENCES 32

FIGURES 45

FIGURE LISTING

Figure 1. The effect of estradiol-valerate (EV) treatment and vitamin E on body weight 46

Figure 2. SGP-2 labelling in the medial preoptic area 48

Figure 3. SGP-2 labelling in the supraoptic nucleus 50

Figure 4. SGP-2 labelling in the paraventricular nucleus 52

Figure 5. SGP-2 labelling in the dorsomedial nucleus and perifornical area 54

Figure 6. Percent SGP-2-positive cells in the hypothalamic nuclei of different treatment groups 56

Figure 7. Comparison of SGP-2-immunopositive cells in the medial preoptic area of normal and EV-treated animals 58

Figure 8. SGP-2-immunoreactivity in the MPOA of the EV-treated rat 60

ABBREVIATIONS

APO J	apolipoprotein J
ARC	arcuate nucleus
CNS	central nervous system
CRF	corticotropin-releasing factor
DMN	dorsomedial nucleus
EV	estradiol-valerate
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
LHA	lateral hypothalamic area
LH	luteinizing hormone
LHRH	luteinizing-hormone releasing hormone
MPOA	medial preoptic area
NMDA	N-methyl-D-aspartate
PA	perifornical area
POMC	pro-opiomelanocortin
PVN	paraventricular nucleus
SGP-2	sulfated glycoprotein-2
SON	supraoptic nucleus
SP-40,40	serum protein 40,40
TBS	tris-buffered saline
Vit.E	vitamin E
VMN	ventromedial nucleus

INTRODUCTION

Hypothalamic control over the reproductive cycle

The hypothalamus represents only a very small portion of the diencephalon but is critically important as a nodal point in pathways concerned with autonomic, endocrine, emotional and somatic functions (Morgane and Panskepp, 1980). One of its endocrine functions involves the maintenance and regulation of the reproductive cycle. The hypothalamic-pituitary-ovarian axis is the major circuit in mammalian control of the female reproductive cycle. Proper signalling within this loop ensures that the stages representing oocyte maturation, ovulation, and uterine implantation succeed in a highly regulated and cyclic fashion. Hypothalamic control over reproductive function mainly resides in its ability to release luteinizing-hormone releasing hormone (LHRH). The LHRH decapeptide is synthesized by a small number of neurons located in the septal, preoptic and anterior regions of the hypothalamus (Sternberger and Hoffman, 1978). Secreted in pulsatile fashion into the hypophyseal portal system, LHRH stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from pituitary gonadotrophs. LHRH has been shown to stimulate the synthesis of both mRNAs specific to LH and FSH (Gharib et al., 1990). Chronic cannulation experiments have shown that each pulse of plasma LH coincides with an episode of LHRH (Levine and Ramirez, 1982). Furthermore, animals treated with antibodies against LHRH showed a marked decrease in both LH and FSH concentrations (Knobil, 1974). Secretion of FSH is also regulated by a number of ovarian peptides, including inhibin and activin suggesting that FSH secretion, unlike LH, is not entirely dependent on LHRH stimulation (Kotsuji et al., 1988). It is important to realize that not only the amplitude

of the LHRH pulse governs gonadotropin release from the pituitary, but the pattern as well. Exogenous LHRH administered in pulsatile fashion maintained normal patterns of gonadotropin release and ovulatory cyclicity in monkeys with hypothalamic ablation. However, continuous administration of exogenous LHRH resulted only in an initial rise of both LH and FSH but then levels always declined (Belchetz et al., 1978). Finally, in addition to regulating LH secretion, biosynthesis and glycosylation, pulsatile LHRH also controls the number of LHRH receptors on pituitary gonadotropes (Loumaye and Catt, 1983).

LHRH signal modulation

The medial preoptic area of the hypothalamus which has been shown to contain the majority of LHRH neurons, receives diverse stimuli from a number of hypothalamic, limbic and mesencephalic structures. The complex interaction between various neurotransmitter systems and LHRH releasing neurons results in regulated LH release. Of the neurotransmitters, noradrenaline from medullary A1 and A2 noradrenergic neurons has been shown to play a critical role in the stimulation of LHRH neurons (Barraclough and Wise, 1982; Moore and Bloom, 1979). However, electrical stimulation of A1 neurons failed to alter basal plasma LH concentrations (Barraclough, 1992) suggesting that other cell types also play modulating roles in LHRH release. Local GABAergic interneurons have been shown to inhibit LHRH release. GABA receptor agonists suppress LH surges in steroid treated, ovariectomized rats (Adler and Crowley, 1986). Furthermore, at the LH surge, push-pull cannulae experiments have shown GABA release rates decline and noradrenaline secretion increases (Demling et al., 1985). Another group of LHRH modulators includes the excitatory amino acids, most notably N-methyl-D-aspartate (NMDA). NMDA has been shown to act at the level of the hypothalamus to rapidly induce both LHRH mRNA and peptide production (Zanisi and

Messi, 1991). Furthermore, DL-2-amino-phosphopentanoic acid, a specific NMDA receptor antagonist has been shown to suppress pulsatile LH release in the rat (Arslan et al., 1988). Whether this drug acts directly on LHRH or other local modulators remains unknown. Morphological evidence also supports further modulation of LHRH cells by dopamine and serotonin. Tyrosine-hydroxylase neurons have been shown to terminate on the perikarya and dendrites of LHRH cells (Leranth et al., 1986). Likewise, serotinergic afferents from the dorsal and median raphe form projections to the preoptic LHRH neurons (Kiss and Halasz, 1985; Hokfelt et al., 1984). Furthermore, LHRH neurons were shown to receive direct synaptic input from ß-endorphin/ACTH containing neurons (Leranth et al., 1986; Chen et al., 1989). These inputs are likely to arise from the ß-endorphin neurons located in the arcuate nucleus (Bloom et al., 1978).

Opioid regulation of LHRH release

It is well established that endogenous opioids suppress LH release (Kalra and Kalra, 1983; Kalra, 1983). Intraventricular injections of ß-endorphin inhibited LH release to a degree greater than met-enkephalin or dynorphin opioids (Cox et al., 1982; Wood, 1982), suggesting that ß-endorphin is the major negative regulator of LH release (Kalra et al., 1983). However, direct action of ß-endorphin on pituitary gonadotrophs seems unlikely. In vitro studies using hemipituitary preparations treated with the opioid antagonist naloxone failed to increase LH release (Gabriel et al., 1983). Furthermore, the anterior pituitary has only been shown to have scant levels of the mu, delta or kappa opioid receptor (Rotten et al., 1986).

Most of the evidence points to the hypothalamus as the site for opioid modulation of LH through action on LHRH neurons (Adler and Crowley, 1984; Kalra et al., 1987). Endogenous opioids have been shown to inhibit the release of LHRH from hypothalamic explants (Wilkes and yen, 1980) and have been shown to diminish accumulation and

efflux of LHRH from median eminence terminals (Kalra and simpkins, 1981; Ching, 1982). The fact that ß-endorphin terminals contact LHRH neuronal cell bodies also suggests direct modulation of ß-endorphin on synthesis, storage and/or release of LHRH (Leranth et al., 1986). However, naloxone had no effect on LHRH release when noradrenaline was infused intraventricularly (Hartman et al., 1991). Kalra and Kalra (1983) have provided evidence which suggests that the inhibitory effects of opioids on LHRH secretion are mediated via presynaptic inhibition of the stimulatory noradrenergic system. Alternatively, evidence has been shown that adrenergic agents block opioid inhibition of LH release via interference with the opioid receptor on LHRH neurons and not by presynaptic inhibition of noradrenergic neurons (Blank et al., 1983). Although it remains unclear how adrenergic and opioid compounds influence LHRH release, a synergistic interaction between the two systems has recently been proposed (Clough et al., 1990). The precise site of action of the opioid regulators has also been investigated. Direct intracerebral infusion of ß-endorphin to various hypothalamic regions including, the septal area, anterior area or diagonal band of Broca failed to significantly alter LH release. However infusions near the arcuate or medial preoptic area completely inhibited LH release (Wiesner et al., 1984). Conversely, naloxone treatment caused significant enhancement of LH release when implanted at the arcuate or medial preoptic area but not at other hypothalamic sites (Kalra, 1981).

Estradiol effect on the reproductive cycle

It is well documented that ovarian estradiol feeds back on both the pituitary and hypothalamus to exert a negative influence on synthesis, storage and release of LH and FSH (Schall et al., 1972; Knobil, 1974). Removal of such influence during ovariectomy causes instant increases in both LH and FSH release (Wise et al., 1981). Conversely, estradiol has also been described as a potent stimulator of LH and FSH during the LH

surge (Schall et al., 1972; Knobil, 1974). The site and action of estradiol remains controversial as both the hypothalamus and pituitary gonadotrophs have estradiol receptors (Simantov and Snyder, 1977; Pfaff, 1968). It is likely that estradiol effects on LH and FSH secretion are mediated in multiple loci within the hypothalamic-hypophyseal system. However, hypothalamic effects are likely via indirect neuromodulatory inputs because LHRH neurons have also been shown to be devoid of estradiol receptors (Shivers et al., 1983; Kalra and Kalra, 1983; Kalra, 1983).

One of the documented effects of estradiol has been on the opioid system. Treatment with estradiol for 3 weeks decreased hypothalamic ß-endorphin levels in ovariectomized rats relative to controls (Wardlaw et al., 1982). The suppression of ß-endorphin by estradiol has been confirmed by RNA dot blot quantification. Estradiol implantation for 3 days caused a 40% decrease in the POMC transcript (Wilcox and Roberts, 1985). In addition to having a pronounced effect on ß-endorphin levels, estradiol has been shown to significantly alter hypothalamic opioid receptor binding. During the rat estrus cycle ^{3}H-naloxone binding in the medial preoptic area was lowest at proestrus and highest at estrus phase paralleling estrogen levels (Hammer and Bridges, 1987).

Estradiol-mediated pathology

One of the effects of estradiol which has not been greatly appreciated is its pathologic effect. A single 2.0mg intramuscular injection of estradiol-valerate (EV) given to normally cycling female adult rats produces, within four weeks, chronic anovulation, persistent vaginal cornification and polycystic ovaries (Brawer et al., 1978, 1980, 1986; Hemmings et al., 1983; Schulster et al., 1984). In addition to the ovarian disturbance, a progressive multifocal lesion develops, concurrently, throughout the hypothalamic

arcuate nucleus (Brawer et al., 1978, 1980). This lesion is characterized by degenerating neuronal elements as well as reactive microglial cells and astrocytes (Brawer et al., 1978). The pathological changes associated with the estradiol-valerate injection have also been shown to develop in time due to circulating physiological levels of estrogen (Brawer et al., 1981, 1983). Ovariectomized, EV-injected rats do not develop the lesion, indicating that the hypothalamic and ovarian pathology develops due to the unopposed physiological concentration of ovarian estradiol caused by the initial EV insult to the neuroendocrine balance (Brawer et al., 1980, 1983).

Although several neuronal populations exist in the arcuate nucleus, including neuropeptide Y (Kalra et al., 1990, 1988), neurotensin (Akema et al., 1987; Alexander et al., 1989), somatostatin (Kalra et al., 1988; Ishikawa et al., 1987), tyrosine-hydroxylase (Piotte et al., 1988), met-enkephalin and ß-endorphin neurons (Bicknell, 1985), estradiol-valerate induces a lesion specific to the ß-endorphin immunoreactive cells (Desjardins et al., 1993). The lesion results in a 60% decrease in the total number of arcuate ß-endorphin neurons as compared to normal intact control animals (Desjardins et al., 1993). Compromise of the ß-endorphin system produces a compensatory up-regulation of opioid receptor concentration in the anterior hypothalamus (Wilkinson et al., 1983). Radioautographic studies using specific opioid ligands have demonstrated the increase in opioid binding is a result of selective increase in mu- but not delta- or kappa-receptors and that it was confined to a region of the medial preoptic area densely populated with LHRH neurons (Desjardins et al., 1990). Further support that the mu-opioid receptor increase is a compensatory response to deafferentation in the MPOA comes from evidence in monosodium-glutamate treated neonates, in which the arcuate nucleus is selectively damaged resulting in a significant loss in ß-endorphin neurons. These animals also exhibit a marked increase in mu-opioid binding (Desjardins et al.,

1992). The cells which exhibit this increase in mu-opioid binding are unknown. A likely candidate may be the LHRH cells themselves. Direct synapses have been shown between ß-endorphin and LHRH neurons and may account for as much as 10% of the total synaptic input (Chen et al., 1989). It has been hypothesized that it is this subsequent hypersensitivity of the MPOA to residual opioid that elicits the cascade of neuroendocrine aberrations which ultimately results in the arrested ovulatory cycle and the multicystic ovaries (Brawer et al., 1993).

Until recently, it has been unclear as to how and why estradiol is selectively pathogenic to the arcuate nucleus. However, a unique population of astrocytes found in the periventricular and arcuate regions of the hypothalamus have been suggested as a possible mediator of the neural lesion (Schipper et al., 1990). These astrocytes possess dense, Gomori-positive inclusions which have been shown to increase in both number and size in response to the estradiol lesion (Brawer et al., 1978, 1980). The membrane-bound Gomori inclusions exhibit orange-red auto-florescence and non-enzymatic peroxidase activity (Schipper et al., 1991). A suggested mechanism underlying the estradiol induced damage involves the conversion of estradiol to catechol estrogen and subsequent oxidation to cytotoxic o-semiquinone free radicals within the peroxidase-positive astrocytes (Liehr and Roy, 1990; Schipper et al., 1991). 5α-reduced androgens, a class of steroids which block the enzymatic conversion of estradiol to catechol estrogens has been shown to impede the arcuate lesion (Brueggemeier and Bannon, 1986; Brawer et al., 1983). Vitamin E (α-tocopherol), a potent anti-oxidant, when given concomitantly with the EV-injection, has been shown to prevent the estradiol-induced selective loss of ß-endorphin neurons and the anovulatory condition (Desjardins et al., 1992). Furthermore, 21-aminosteroid (U74389F), an anti-oxidant chemically unrelated

to vitamin E, has confirmed anti-oxidant ability to block the pathologic effects of estradiol (Schipper et al., 1994).

Sulfated glycoprotein-2

Over the last decade sulfated glycoprotein-2 (SGP-2) has been isolated and cloned by a number of different researchers all investigating distantly related questions. SGP-2 was first isolated by Griswold as a major secretory product of rat Sertoli cells which is also bound to spermatozoa (Griswold, 1988; Kissinger et al., 1982; Sylvester et al., 1984). SGP-2 is now endowed with over a dozen different acronyms based on the discovery of homologous forms of the glycoprotein from divergent sources (May and Finch, 1992). SGP-2 and related forms have been discovered in a number of mammalian species in a variety of biological fluids and a variety of tissues, both neural and non-neural (May and Finch, 1992; Michel et al., 1992). It thus appears that SGP-2 is a multifunctional glycoprotein. The physiological role, or roles, of SGP-2 have yet to be clearly elucidated. However, the protein has been implicated in a variety of diverse functions including cell death (Danik et al., 1991), tissue response to injury (Pasinetti et al., 1991; Lampert-Etchells et al., 1991), lipid transport (de Silva et al., 1989), spermatozoa maturation (Sylvester et al., 1991), secretion (Fischer-Colbrie et al., 1984; Hartmann et al., 1991) and complement regulation (Kirszbaum et al., 1989; Jenne and Tschopp, 1989).

Structural analysis has shown SGP-2 to be a heterodimer composed of two disulfide-linked subunits whose reduced forms migrate on SDS-polyacrylamide gels with mobilities of approximately 34 and 47 kDa respectively (Sylvester et al., 1984). SGP-2, previously known as 'dimeric acidic glycoprotein' contains 23.7% carbohydrate and its N-linked oligosaccharides are extensively sulfated (Griswold et al., 1982). Computer

sequence analysis has confirmed a common evolutionary relationship between SGP-2 homologues (Tsuruta et al., 1990). The multiple physiologic functions associated with SGP-2 is consistent with the numerous distinct functional domains (Jenne and Tschopp, 1989).

Although no common function between disparate forms of SGP-2 has been ascribed, high levels of percent amino acid homology suggest a common link. Clusterin, a glycoprotein isolated from ram rete testis fluid, which elicits aggregation of a wide variety of cells (Fritz et al., 1983; Fritz and Murphy, 1993; Blaschuk et al., 1983) exhibits 54% amino acid homology with rat SGP-2 (Fritz et al., 1983). Serum protein 40,40 (SP-40,40), a member of the human complement system capable of modulating the terminal complement pathway shows 77% homology (Kirszbaum et al., 1989). Likewise, Complement lysis inhibitor (CLI), a membrane attack complex cytolytic inhibitor, and Apolipoprotein J (Apo J), a human plasma lipid transporter both show 77% identity to rat SGP-2 (Jenne and Tschopp, 1989; de Silva et al., 1990a, 1990b). Bovine glycoprotein III (GpIII), secreted from adrenal chromaffin cells under cholinergic stimulation (Fisher-Colbrie et al., 1984) and canine glycoprotein (Gp80) associated with constitutive secretion of kidney epithelial cells (Hartmann et al., 1991) exhibit 68% and 72% homology respectively (Palmer and Christie, 1990; Hartmann et al., 1991). Another functionality associated with SGP-2 is involvement with the pathologic state. Testosterone repressed prostate message 2 (TRPM-2), isolated from the rat prostate, which is involved in necrotic interdigital tissue and apoptosis in regressing prostate shows (Buttyan et al., 1989) 77% homology to rat SGP-2 (Leger et al., 1987). Similarly, pADHC-9 associated with the Alzheimer diseased hippocampus (May et al., 1989, 1990) and TB16 associated with human gliomas (Danik et al. 1991) exhibit 77% amino acid sequence homology with rat SGP-2.

Neural expression of sulfated glycoprotein-2

In the past, most of the research on neural sulfated glycoprotein-2 (SGP-2) has focused on its involvement in high injury states associated with apoptosis and necrosis in the central nervous system (CNS) (Michel et al., 1992). The SGP-2 message has been independently identified in numerous neuropathological conditions including scrapie-infected hamster brain and Pick's disease (Duguid et al., 1989). Likewise, the human cognate of SGP-2 was found to be elevated in hippocampal tissue from the Alzheimer diseased brain (May et al., 1989, 1990). Cases of astrocytoma and various epileptic foci have also produced increased transcription of the SGP-2 message (Danik et al., 1991). SGP-2 expression was shown to be elevated in astrocytes in response to transient ischemic damage (May et al., 1992), neuronal deafferentation (Lampert-Etchells et al., 1991), excitotoxic lesions (May et al., 1990) and kainate acid-induced seizures (Schreiber et al., 1993). Neurons have also been shown to elevate SGP-2 production in cases of injury. Hippocampal tissue demonstrates an increased number of SGP-2-immunopositive neurons following kainate lesion (May et al., 1990). It is thus clear that the overexpression of the SGP-2 gene is associated with CNS disorders and injuries, however, the exact role SGP-2 plays in these conditions remains unclear. Speculative functions for SGP-2 after injury include neuronal protection against further complement-mediated cytolytic damage (May, 1993), neuronal clearance of cellular debris (May, 1993) or possible aiding in synaptic repair or remodelling of damaged neurons (May et al., 1992).

It must be clear however, that SGP-2 gene expression is not restricted to the pathological CNS and to degenerating neurons in general. Recently, using dot blot hybridizations, investigators have detected significant quantities of SGP-2 mRNA in a

wide range of neurons and astrocytes in normal brain tissue (Danik et al., 1993). Using immunocytochemical techniques investigators have begun to compile a map of SGP-2 distribution throughout the normal brain (Senut et al., 1992; Pasinetti et al., 1994). SGP-2-immunopositive structures have been localized to a number of areas including midbrain tegmentum, red nucleus, cerebellar Purkinje cells and pyramidal neurons of the hippocampus (Pasinetti et al., 1994). By immunocytochemistry, no SGP-2-immunopositive glial cells have been detected in any region of the normal rat brain (Senut et al., 1992; Pasinetti et al., 1994). The high levels of SGP-2 transcripts along with its corresponding protein found in normal mammalian brain is clearly suggestive of a functional role for SGP-2 in the normal nervous system.

Rationale

It is clear that sulfated glycoprotein-2 has been repeatedly associated with models of apoptosis and neuropathology (May and Finch, 1992). In as much as SGP-2 is expressed in degenerating neurons and reactive astrocytes, it seemed an excellent marker for the molecular changes occurring in the hypothalamic circuitry as a result of the estradiol-valerate induced pathology. This thesis attempts to confirm this supposition by examining SGP-2-immunoreactivity in the EV-lesioned hypothalamus. Furthermore, using the SGP-2 marker, this project attempts to examine the anti-oxidizing potential of vitamin E against the neurotoxic action of estradiol.

Recent studies have produced a detailed map of the regional expression of SGP-2 throughout the normal adult rat brain (Senut at al., 1992; Pasinetti et al., 1994). These studies, though compelling, have only produced an incomplete picture of SGP-2 distribution. Only *in situ* hybridization assays have detailed SGP-2 distribution throughout the hypothalamus (Danik et al., 1993). This thesis attempts to complete this

picture by providing a detailed immunohistological examination of SGP-2 throughout the normal rat hypothalamus. Given the dimorphic patterns of hypothalamic development and wiring, both female and male SGP-2 distributions were considered (Arnold and Gorski, 1984).

Our results demonstrate for the first time that SGP-2 expression occurs in a highly selective manner throughout the normal rat hypothalamus. The overall pattern includes localized expression in neurons of the medial preoptic area (MPOA), lateral, supraoptic, paraventricular and dorsomedial nuclei. Other regions including the arcuate nucleus, suprachiasmatic, ventromedial and mammillary nuclei did not show any SGP-2-immunoreactivity. Both male and female rats exhibited identical patterns of SGP-2 localization indicating that SGP-2 distribution is not sexually dimorphic. The estradiol-valerate induced destruction of ß-endorphin cells produced a greater than two-fold increase the number of SGP-2-immunoreactive cells only within the medial preoptic area. Other populations of SGP-2-positive neurons remained unchanged. Furthermore, vitamin E protection of ß-endorphin neurons against the estradiol lesion maintained SGP-2-immunoreactivity at normal levels.

MATERIALS AND METHODS

Animals

Animals in this study were treated according to the practices and procedures approved by McGill University's Policy on the Handling and Treatment of Laboratory Animals. Male and female Wistar rats, 4 weeks of age were obtained from Charles River Breeding Company (St. Constant, Quebec). At the onset of the experiment all groups were weight matched. All animals were housed in groups of 3 and maintained under controlled light (12L:12D) and temperature (20°C). Food and water, unless otherwise noted, were provided *ad libitum*. After acclimatization, female ovulatory cyclicity was monitored by daily light microscopic examination of vaginal smears. Once it was ascertained that all females were exhibiting normal estrus cycles, they were divided into four separate groups (n=6). The first group was subjected to a single 2.0mg intramuscular injection of estradiol-valerate (EV; Delestrogen, Sqibb Laboratories, Montreal, QC) dissolved in sesame oil (Brawer et al., 1978). The second group was supplied with vitamin E, d-alpha-tocopherol (Sigma Chemical Co., St-Louis, MO) via daily ingestion through their diet (25mg d-alpha-tocopherol per kg powdered Purina rat chow) (Desjardins et al., 1992). After 2 weeks of the enriched vitamin E diet these animals were injected with estradiol-valerate. The third group was treated with the enriched vitamin E diet only and the fourth consisted with aged matched normally cycling female control animals. Finally, a group of normal males (n=6) was also used for comparative purposes.

Group	Sex	n	Treatment
EV	♀	6	normal diet; estradiol-valerate injected
Vit.E/EV	♀	6	enriched vitamin E diet; estradiol-valerate injected
Vit.E	♀	6	enriched vitamin E diet; no injection (vehicle)
Normal ♀	♀	6	normal diet; no injection (vehicle)
Normal ♂	♂	6	normal diet; no injection

Tissue Preparation for Immunohistochemistry

Four weeks following initiation of treatment (six weeks of age) all groups of animals were sacrificed. The animals were first weighed, then anaesthetized with an intraperitoneal injection of 15% urethane (0.9cc/100g body weight). The brains were first washed with lactated Ringer's solution for 1 minute (50ml) and then fixed by intracardiac perfusion with Bouin's fixative for 15 minutes (250ml). Following perfusion, the brains were removed and bathed in Bouin's fixative for another 24 hours, after which they were immersed once daily for two consecutive days in fresh 70% ethanol. After fixation, hypothalami were isolated, dehydrated and embedded in paraffin. Serial coronal tissue sections 5 μm thick were cut from the anterior preoptic limit to the posterior mammillary limit. Tissue was mounted on glass slides and maintained at 42-45°C for three nights.

Immunostaining

The glass slides were then immersed in xylene and graded ethanol solutions (5 minutes each) to rehydrate and deparaffinize the tissue. During hydration, any residual picric acid from the Bouin's fixative was inactivated by immersing the sections for 5 minutes in 70% ethanol containing 1% lithium carbonate. During this process the sections were

also incubated for 5 minutes in 70% ethanol containing 1% v/v hydrogen peroxide to inactivate any endogenous peroxidase activity in the tissue. Following hydration, the sections were washed for 5 minutes in 300mM glycine to block any free aldehyde groups. Prior to the immunolabelling process, tissue sections were incubated for 20 minutes with tris-buffered saline (TBS), pH 7.4, containing 10% goat serum. This procedure ensured blocking of nonspecific primary antibody attachment. Two different stocks of anti-sulfated glycoprotein-2 antibody were tested as the primary antibody. Polyclonal rabbit-anti-rat-SGP-2 antibody, provided by Dr. M.D. Griswold (Washington State University, Pullman, WA), was used at a concentration of 1:100 in tris-buffered saline and polyclonal sheep anti-rat-SGP-2 antibody (Quidel Corp., San Diego, CA) was used at a concentration of 1:100 in tris-buffered saline. The rabbit anti-rat-SGP-2 antibody proved to be more effective. After incubation with 60μl of the primary antibody for 2.0 hours at 37°c, sections were washed four times in sequential solutions (2 minutes each) of TBS containing 0.1% Tween-20. The sections were then incubated again with 10% goat serum in TBS for 15 minutes to block nonspecific binding of the secondary antibody. The tissue was then incubated for 45 min. at 37°c with either goat anti-rabbit IgG conjugated to peroxidase (Sigma Chemical Co., St. Louis, MO) or donkey anti-sheep IgG conjugated to peroxidase (Sigma Chemical Co., St. Louis, MO) both at a concentration of 1:250 in tris-buffered saline. Sections then underwent thorough washing with TBS containing 0.1% Tween-20 (4x2 minutes each). Finally sections were incubated in 250ml of TBS containing 0.03% hydrogen peroxide, 0.1M imidazole and 0.05% diaminobenzidine tetrachloride (pH 7.6). Staining occurred during a 10 minute incubatory time after which time the slides were washed in distilled water for 10 minutes before being exposed for 5 minutes to 0.01% methylene blue counterstain. Finally, the sections were dehydrated in graded ethanol and xylene (3 minutes each) and

cover slips were mounted with Eukitt (Kindler, Germany). Control sections were treated as mentioned above except that primary antibodies were replaced with normal serum at a dilution of 1:100 in TBS. This pre-immune serum served as a negative control for the experimental sections.

Quantitative Analysis

Using the light microscope, a quantitative evaluation of the density of SGP-2-positive cells was performed in each group of rats. The only regions of the hypothalamus that contained definitive SGP-2-positive neurons were the medial preoptic area, supraoptic nucleus, paraventricular nucleus, dorsomedial nucleus and lateral hypothalamus. All cells (SGP-2-positive and negative) were counted unilaterally on five sections from each hypothalamic area in each animal. In the case of the lateral hypothalamic area, an area lacking distinct borders, counts were performed with the aid of a 0.05 cm^2 ocular grid. Lateral hypothalamic staining was quantified using a magnification of x100 with the fornix set at the bottom corner of the ocular grid with the area of measurement extending laterally away from the ventricle. Each section's labelled count was divided by the total number of cells (labelled + unlabelled) in the grid to produce the percentage of labelled cells. All percentages were then averaged to determine the mean percentage of SGP-2-labelled cells in each hypothalamic area. The degree of cellular immunoreactivity was not considered. All quantitation was performed while unaware of the treatment group.

Statistics

The mean weights of the groups of treated animals versus controls were compared by analysis of variance. The unpaired Student's t-test (2-tailed) was used to determine

significance between groups at the $p<0.05$ level. The chi-square contingency test was performed to determine significance ($p<0.05$) in the counts of labelled cells associated with each hypothalamic area in the various treated groups. All statistical analysis was performed with the aid of Statview 4.0 (Abacus Concepts Inc.) for the Apple Macintosh computer.

RESULTS

Effects of vitamin E and estradiol on cyclicity and animal body weight

After having normal cycles before estradiol-valerate treatment (EV), EV animals exhibited long stretches of persistent vaginal estrus interrupted by occasional di- or met-estrus stages during the four weeks following injection. Animals given enriched vitamin E diets and treated with EV (Vit E/EV) as well as those only given vitamin E (Vit E) exhibited normal four day estrus cycles throughout the six week duration of the experiment. Four weeks following estradiol injection, EV-treated animals weighed significantly less than untreated animals ($p<0.01$) (Fig. 1). Vit E/EV animals weighed the same as normal cyclic controls, as did animals receiving only the vitamin E enriched diet.

Regional distribution of SGP-2 within the hypothalamus of normal female and male rats

Polyclonal anti-SGP-2 antibodies obtained from Dr. MD Griswold and from Quidel corp. (San Diego, CA) were used for immunostaining of the normal male and female hypothalamus. SGP-2-immunoreactivity in serial coronal sections cut from the preoptic area to the mammillary region of normal hypothalamus was distributed in a highly specific manner. The medial preoptic area, supraoptic nucleus, paraventricular nucleus, lateral hypothalamus and dorsomedial nucleus all contained neurons that labelled for SGP-2. Whereas the suprachiasmatic, arcuate, ventromedial, posterior and mammillary nuclei were devoid of labelling. The distribution of immunopositive neurons was the same for both antibodies in both male and female.

The number of labelled cells in the medial preoptic area (MPOA) was greatest most medially and diminished in the more lateral region of the nucleus (Fig. 2a). 15% of neuronal perikarya, as determined by their large size, displayed SGP-2-reactivity. Smaller cell profiles (neuronal or glial) were unlabelled within the normal MPOA. Higher magnification of the area revealed dark immunopositive granules within SGP-2-labelled cells (Fig. 2b). Occasional perikarya exhibited a conspicuous perinuclear ring of large SGP-2-positive granules. At the level of the MPOA and throughout the hypothalamus, the ciliated ependymal cells lining the third ventricle displayed considerable variety in SGP-2-immunoreactivity. The intensity of ependymal cell labelling varied directly with the degree of background staining. However, consistent SGP-2-immunopositive granules were associated with the apical border of the ependymal lining (Fig. 2c).

Situated above the lateral margin of the optic chiasm, the supraoptic nucleus (SON) exhibited numerous SGP-2-positive cells (Fig. 3a). Quantitation revealed that approximately 90% of cells within the normal female rat's supraoptic nucleus were reactive. Observation at higher magnification revealed that most if not all perikarya displayed a uniform diffuse background of staining upon which was superimposed darker SGP-2-positive granules (Fig. 3b). Occasional perikarya of the supraoptic nucleus exhibited a perinuclear ring of SGP-2-labelling. Smaller neuronal or glial profiles showed little if any staining. Although clearly defined staining was associated with many of the perikarya a degree of non-specific staining was associated with this area.

The paraventricular nucleus (PVN) displayed SGP-2-positive intermingled with SGP-2-negative cells. Immunopositive cells were, however, restricted to a medial region of the nucleus (Fig. 4a). Quantitation showed approximately 25% of the cells within the PVN to be labelled. As in the case of the MPOA, only larger perikarya were SGP-2-

positive. No SGP-2-positive small profiles consistent with glia could be detected amongst the large immunopositive perikarya of the PVN. The intracellular pattern of labelling was similar to that for cells in the MPOA and SON (Fig. 4b).

In the tuberal region of the hypothalamus, the lateral hypothalamic area (LHA) and dorsomedial nucleus (DMN) contained neurons that exhibited SGP-2-immunoreactivity. The labelled large cells formed a loose continuum extending laterally from the DMN to the perifornical area (Fig. 5a). The labelled cells were interspersed among a majority of non-labelled profiles. Staining was restricted to large sized perikarya and consistently no immunopositive glia were observed. SGP-2-positive neurons displayed dark cytoplasmic immunoreactive granules and as in the case of the SON or MPOA, were occasionally organized as a perinuclear ring (Fig. 5b).

Also, in addition to the well delineated clusters of SGP-2-immunoreactive cells described above, occasional random darkly stained perikarya could also be observed throughout the entire hypothalamus. However, in all sections examined throughout the normal hypothalamus, label was never detected in the neuropil or in small cell profiles.

Immunostaining using Griswold's rabbit-anti-rat-SGP-2 polyclonal antibody or Quidel corp.'s sheep-anti-rat-SGP-2 polyclonal antibody both exhibited identical distributions of SGP-2-positive cells. No differences were noted in the quantitative aspect of the staining. However, the intensity of labelling within reactive perikarya using Quidel's sheep-raised antibody was less intense than that using Griswold's rabbit-raised antibody. The dark granulations seen when using Dr. Griswold's anti-SGP-2 antibody could no longer be detected when using Quidel's antibody. Only a homogeneous diffuse staining was detected within reactive cells.

Effects of estradiol-valerate-induced destruction of ß-endorphin neurons on hypothalamic regional distribution of SGP-2

Estradiol-valerate treatment had marked selective effect on SGP-2 expression. Although the treatment had no effect on the distribution and number of SGP-2-positive cells in the supraoptic, paraventricular, dorsomedial or lateral hypothalamic region, it resulted in a marked increase in the number of SGP-2-positive cells in the medial preoptic area. The percent of SGP-2-positive cells in the MPOA of EV-treated rats was approximately 2.5 times greater than in control animals (Fig. 6). The distribution of labelled cells was similar to that for untreated controls (Fig. 7a). Most positive cells occurred medially diminishing towards the lateral portion (Fig. 7b). Both large and smaller cells were SGP-2-positive within the EV-treated MPOA. Although no glial acidic fibrillary protein (GFAP) double labelling was performed, based on their small size, glial cells within the MPOA of EV-treated animals now appeared to be labelled (Fig. 8). Neuronal staining appeared to be more intense with numerous dark immunopositive granules filling the stained cells. Generally speaking, there appeared to be no additional non-specific staining associated with the EV-treatment as compared to the normal untreated group. Within the neuropil of the MPOA punctate SGP-2-immunodeposits could also now be detected not associated with the stained perikarya (Fig. 8). Areas, other than the MPOA showed no significant alteration in density or distribution of staining as compared to normal untreated controls. However, a few small cell profiles and punctate SGP-2-deposits could both be found in the paraventricular nucleus and perifornical area. Although EV-lesioning specifically destroys cells of the arcuate nucleus no SGP-2-reactivity could be detected within any elements of this region.

Effects of vitamin E treatment on SGP-2 distribution in the normal and estradiol-lesioned hypothalamus

Animals injected with estradiol-valerate and treated with vitamin E (α-tocopherol), showed no difference in the number or distribution of SGP-2-positive cells when compared to normal controls ($p<0.01$) (Fig. 6). The punctate immunodeposits that appeared in the medial preoptic area, paraventricular nucleus and perifornical area of EV-treated animals were not present in the Vit E/EV animals. The number and distribution of SGP-2-positive cells in animals receiving the vitamin E enriched diet alone were indistinguishable from the untreated controls.

DISCUSSION

SGP-2 distribution in the normal hypothalamus

The present immunocytochemical study shows that sulfated glycoprotein-2 (SGP-2) is well localized to neurons within specific areas of the normal rat hypothalamus. Similar distribution of SGP-2-immunoreactivity was found in both the normal male and female hypothalamus indicating that hypothalamic SGP-2 expression is not sexually dimorphic. This study is the first to show the presence of SGP-2-positive neurons within the medial preoptic area (MPOA), supraoptic nucleus (SON), and paraventricular nucleus (PVN). Previous studies, using antibodies directed against the SGP-2 homologue, SP-40,40, have shown the presence of immunopositive neurons in the perifornical area (PA) (Senut et al., 1992). However, this study also found numerous strongly immunoreactive perikarya in the arcuate, mammillary and supramammillary nuclei, all of which were negative in the present study. The different distributions likely reflect sequence differences between the homologues. Recent *in situ* hybridization results substantiate our immunocytochemical data. SGP-2 homologous transcripts were detected in multiple brain structures including the MPOA, PVN, PA (Danik et al., 1993). However, only weak hybridization signals were detected in the SON, an area shown in this study, to possess the highest percentage of SGP-2-positive cells. Furthermore, *in situ* studies also detected SGP-2 mRNA hybridization signals from the arcuate, ventromedial and suprachiasmatic nuclei indicating that the transcription of SGP-2 and the actual production of the molecule often do not coincide. This suggests that SGP-2 may be differentially regulated within the hypothalamus (Danik et al., 1993). The absence of small SGP-2-immunopositive profiles in the normal hypothalamus is consistent with

previous studies which have found glia to possess SGP-2 mRNA but lack the glycoprotein (Pasinetti at al., 1994).

SGP-2 distribution in the EV-treated hypothalamus

Estradiol-valerate (EV) given to normally cycling female rats is known to produce persistent vaginal cornification, polycystic ovaries and acyclicity (Brawer et al., 1978, 1980, 1986; Hemmings et al., 1983; Schulster et al., 1984). The estradiol treatment has been shown to selectively destroy approximately 60% of the ß-endorphin neurons in the hypothalamic arcuate nucleus (Desjardins et al., 1993). Since the MPOA is a major target of ß-endorphin afferents, the EV pathology results in a major deafferentation of this nucleus.

In this study, EV-treatment produced three marked effects on hypothalamic SGP-2-immunoreactivity. Firstly, the percentage of SGP-2-immunopositive cells only within the MPOA was greater then two-fold that of control animals. The increase in SGP-2-positive neurons has been consistently associated with cases of neuronal deafferentation. Kainic acid induced destruction of hippocampal pyramidal neurons caused a 2-3 fold increase in SGP-2 RNA and protein levels (May et al., 1990). Likewise, experimental deafferentation of the rat hippocampus analogous to the lesion associated with the Alzheimer's neuropathology (Lampert-Etchells et al., 1991) and striatal deafferentation (Pasinetti et al., 1993) both resulted in an increased expression of SGP-2 mRNA and protein. The second effect observed following the EV-treatment was the presence of small SGP-2 cell profiles mainly associated with the MPOA. The presence of smaller profile SGP-2-positive cells within the MPOA is likely the result of recruitment of astrocytes in response to the deafferentation. *In situ* hybridization performed following numerous lesion paradigms including entorhinal cortex lesioning or cortical aspiration

have localized much of the SGP-2 induction to reactive astrocytes (Lampert-Etchells et al., 1991; Pasinetti et al., 1993). The third observation made following EV-treatment was the presence of SGP-2-immunodeposits in the neuropil of the PVN, PA and MPOA. Extracellular deposits immunoreactive for SGP-2 have been repeatedly observed in various neuropathologies and in aging rat brains (Senut et al., 1992). Lesion studies have shown early induction of the SGP-2 was shown to occur in reactive astrocytes, but at later intervals, SGP-2-immunoreactive deposits appeared in the neuropil (Lampert-Etchells et al., 1991). Furthermore, cultured astrocytes have the ability to secrete SGP-2, whereas cultured neurons do not, supporting our interpretation that the extracellular SGP-2-positive deposits represent astrocytic secretions (Pasinetti et al., 1994). We interpret the elevation in SGP-2-positive neurons in the MPOA, the presence of smaller profile immunopositive cells and the neuropil deposits as a direct consequence of the EV-induced destruction of ß-endorphin neurons in the arcuate nucleus and not due to the direct action of estradiol. This interpretation is supported by the fact that vitamin E treatment in EV-injected animals blocked the increase in SGP-2 containing cells and prevented the neuropil deposits from forming.

It has been established that one of the effects of the ß-endorphin deafferentation engendered by the estradiol lesion to the arcuate nucleus, is a chronic compensatory up-regulation of mu-opioid receptors restricted to the MPOA. Mu-, kappa- and delta-opioid receptor binding elsewhere in the hypothalamus was unaffected (Desjardins et al., 1990). It has been speculated that the up-regulation of mu-binding within the MPOA engendered by the deafferentation causes the suppression of LHRH. Loss of ß-endorphin inputs to other regions of the hypothalamus including the corticotropin-releasing factor (CRF) neurons of the parvocellular PVN results in diminution in opioid influence without any compensatory up-regulation in opioid binding (Suda et al., 1992; Leibowitz and Hor,

1982). The fact that ß-endorphin is inhibitory to CRF (Whitnal et al., 1993) and CRF exerts a powerful anorectic effect (Dagnault et al., 1993), the loss of inhibitory ß-endorphin input to this area should result, in part, in a decrease in body weight. The fact that in the present study vitamin E prevents the anorectic effects of the EV-induced loss of weight, suggests that this decrement in animal body weight is a direct effect of the deafferentation and not some other effect of the estradiol.

Recently, radioautographic binding studies have shown that, SGP-2 homologue, SP-40,40 has the ability to bind ß-endorphin with high affinity and thus inhibit ß-endorphin receptor binding (Choi-Miura et al., 1993). With this in mind, it is possible that the elevation of SGP-2 within cells of the MPOA following the EV lesion may be an attempt by the hypothalamus to realign the LHRH-axis. However, if binding of residual ß-endorphin or inhibition of ß-endorphin receptors by SGP-2 is a compensatory reflex to reestablish normal patterns of LH release, it clearly is insufficient. Following formation of the arcuate pathology only the gonadotropin surge mechanism can be restored by hemiovariectomy (Farookhi et al., 1986; Convery et al., 1990). The chronic suppression of basal plasma LH levels is a result of impairment in the hypothalamic circuitry which has been shown to be intractable (Simard et al., 1987).

SGP-2 as a mediator of synaptic remodelling

The hypothalamus exhibits considerable neuronal plasticity throughout life. Neurons within the hypothalamus undergo irreversible spontaneous degeneration as well as a variety of reversible alterations in dendritic morphology and synaptology (Theodosis and Poulain, 1992). We have already discussed the synaptic degeneration of arcuate nucleus ß-endorphin neurons following estradiol treatment. However, gonadal steroids and other hormones also exert a non-pathological effect on hypothalamic structures. Repetitive

periodic synaptic remodelling has been shown to occur in the arcuate-LHRH axis in phase with the estrus cycle (Naftolin et al., 1990). Plastic alterations in synaptic patterns have also been reported in the paraventricular and supraoptic nuclei in response to dehydration and lactation (Theodosis and Poulain, 1984, 1989).

While it is evident that SGP-2 mRNA and protein is broadly expressed by various cell types in the normal CNS (Danik et al., 1993; Pasinetti et al., 1994), its functional role remains to be elucidated. However, a fundamental aspect of SGP-2 is an ability to interact with lipids of plasma membrane or of circulating origin. SGP-2 has been isolated as an apolipoprotein called apolipoprotein J (apo J), that shares several features with other apolipoproteins including amphipathic α-helices (de Silva et al., 1990a). In the nervous system, SGP-2 may act as such a putative apolipoprotein to ensure lipid recycling for synaptic remodelling linked to neuronal plasticity. In this regard, it is of no surprise that in this study high levels of SGP-2 were discovered in the SON and PVN, areas of normal synaptic turnover (Theodosis and Poulain, 1992). However, both the ventromedial (Frankfurt et al., 1990) and the arcuate nucleus (Naftolin et al., 1990), areas shown to be devoid of SGP-2, undergo considerable synaptic remodelling, suggesting SGP-2 is not exclusively involved in synaptic remodelling.

SGP-2 as a response to neuronal injury

In numerous neural pathologies, neuronal losses are accompanied by increases in SGP-2 expression in neurons and reactive astrocytes. High levels of SGP-2 mRNA have been isolated from various pathological states including cases of human astrocytoma and various epileptic foci (Danik et al., 1991). Likewise, other groups have isolated equivalent clones of SGP-2 mRNA from neuropathological tissue, namely quail Rous sarcoma virus (RSV)-transformed neuroretinal cells (Michel et al., 1989), scrapie

infected hamster brain (Duguid et al., 1989), human retinitis pigmentosa retinas (Jones et al., 1992) and Alzheimer's diseased hippocampus (May et al., 1990). These data clearly suggests that SGP-2 is a key gene associated with cell response to neuronal injury, however, its precise role in CNS injury also remains to be delineated. It is likely that the sprouting of new neurites or repair of damaged ones places a greater demand on neurons for membrane precursors. We have already discussed a possible role for SGP-2 as a putative lipid transporter. However, SGP-2's ability to bind heparan-sulfate proteoglycan via predicted heparin-binding domains may facilitate a neurotrophic mechanism through cell:cell or cell:extracellular matrix interaction (de Silva et al., 1990b). Following entorhinal cortex lesioning, extracellular deposits of SGP-2 were transiently found in the areas of terminal projections at the time of axonal sprouting and reactive synaptogenesis (Lampert-Etchells et al., 1991). Based on this we suggest the possibility that the neuropil deposits are in fact sites of synaptic repair in regions damaged by deafferentation generated by the EV-induced loss of ß-endorphin.

CONCLUSION

In this thesis, we report for the first time, the distribution of SGP-2-immunopositive neurons within the normal hypothalamus. SGP-2-positive neuronal perikarya were found in the medial preoptic area (MPOA), the supraoptic nucleus (SON), the paraventricular nucleus (PVN), the dorsomedial nucleus (DMN) and the perifornical area (PA). Both male and female rats exhibited similar patterns of SGP-2-immunoreactivity, thus leading to the conclusion that hypothalamic SGP-2 distribution is not sexually dimorphic. No small cell profiles or neuropil immunodeposits were detected in the untreated hypothalamus. Estradiol-valerate treatment resulted in a greater than two-fold increase in the percentage of SGP-2-immunopositive cells, exclusively within the MPOA. Other cell populations remained at normal untreated levels. Following EV-treatment, small cell profiles, consistent with glia, as well as neuropil immunodeposits both occurred in the MPOA. Other areas including the PVN, DMN and PA exhibited a few small-immunopositive profiles and punctate immunodeposits. Since vitamin E, which blocks the estradiol-mediated destruction of arcuate nucleus ß-endorphin cells also prevented these EV-induced changes, we conclude the increase of SGP-2-immunopositive cells and the presence of neuropil deposits are a result of the deafferentation engendered by the estradiol-induced lesion rather than a direct effect of estradiol. It is possible that SGP-2 either acts as a mediator of neuronal synaptogenesis or a neurotrophic factor in both the normal and lesioned states. We conclude that the SGP-2 response in the MPOA is possibly the result of the loss of ß-endorphin afferents possibly related to the selective up-regulation of mu-opioid binding sites following the EV-induced deafferentation.

CONTRIBUTIONS TO ORIGINAL RESEARCH

1) We have shown for the first time that animal treatment with an enriched vitamin E diet blocks the estradiol-valerate induced weight-loss.

2) We report for the first time the presence of SGP-2-immunopositive neurons in the medial preoptic area (MPOA), the supraoptic nucleus (SON), the paraventricular nucleus (PVN), the dorsomedial nucleus (DMN) and the perifornical area (PA) of the normal hypothalamus. Other areas including the arcuate nucleus, suprachiasmatic nucleus, ventromedial nucleus or mammillary nucleus did not show SGP-2-immunoreactivity.

3) Neither the distribution nor the patterning of SGP-2-immunoreactivity differed between the female or male hypothalamus.

4) Although many researchers have described a significant increase in SGP-2 following neural-lesioning, we have shown that EV-treatment specifically results in a greater than two-fold increase in the percentage of SGP-2-immunopositive cells strictly within the MPOA. The percent of SGP-2 labelled cells in other areas including the SON, PVN, DMN and PA was not altered by the EV-treatment.

5) We have shown that following EV-treatment small cell profiles, consistent with glia and punctate SGP-2-immunodeposits appear in the MPOA as well as in the PVN, DMN and PA.

6) Vitamin E treatment prevents any of the changes in SGP-2 distribution associated with the neurotoxic effects of estradiol.

REFERENCES

Adler BA, Crowley WR. Modulation of luteinizing hormone release and catecholamine activity by opiates in the female rat. *Neuroendocrinology* 1984; 38:248-253.

Adler BA, Crowley WR. Evidence of gamma-aminobutyric acid modulation of ovarian hormonal effects on luteinizing hormone secretion and hypothalamic catecholamine activity in the female rat. *Endocrinology* 1986; 118:91-97.

Akema TC, Praputpittaya C, Kimura F. Effects of preoptic microinjection of neurotensin on luteinizing hormone secretion in unanesthetized ovariectomized rats with or without estrogen priming. *Neuroendocrinology* 1987; 48:345-349.

Alexander MJ, Mahoney PD, Ferris CF, Carraway RE, Leeman SE. Evidence that neurotensin participates in the central regulation of the preovulatory surge of luteinizing hormone in the rat. *Endocrinology* 1989; 124:783-788.

Arnold AP, Gorski RA. Gonadal steroid induction of structural sex differences in the central nervous system. *Annu Rev Neurosci* 1984; 7:413-432.

Barraclough CA, Wise PM. The role of catecholamines in the regulation of luteinizing hormone and follicle stimulating hormone secretion. *Endocrin Rev* 1982; 3:91-119.

Barraclough CA. Neural control of the synthesis and release of luteinizing hormone-releasing hormone. In: *Functional anatomy of the neuroendocrine hypothalamus* Ciba Foundation Symposium, 1992.

Belchetz PE, Plant TM, Nakai Y, Keough EJ, Knobil E. Hypophyseal responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* 1978; 202:631-632.

Bicknell RJ. Endogenous opioid peptides and hypothalamic neuroendocrine neurons. *J Endocrinol* 1985; 107:437-446.

Blank MS, Diez JA, Roberts DL. Monoaminergic antagonists which block naloxone-induced release of luteinizing hormone bind selectively to hypothalamic opiate receptors. *Brain Res* 1983; 279:152-158.

Blaschuk O, Burdzy K, Fritz IB. Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid. *J Biol Chem* 1983; 258:7714-7720.

Brawer JR, Naftolin F, Martin J, Sonneschein C. Effects of a single injection of estradiol-valerate on the hypothalamic arcuate nucleus and on reproductive function in the female rat. *Endocrinology* 1978; 103:501-512.

Brawer JR, Schipper H, Naftolin F. Ovary-dependant changes in the hypothalamic arcuate nucleus. *Endocrinology* 1980; 107:274-279.

Brawer JR, Schipper H, Robaire B. Effects of long-term androgen and estrogen exposure on the hypothalamus. *Biol Reprod* 1981; 112:194-199.

Brawer JR, Schipper H, Naftolin F. Ovary dependent degeneration in the hypothalamic arcuate nucleus. *Endocrinology* 1983; 103:274-279.

Brawer JR, Munoz M, Farookhi R. Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. *Biol Reprod* 1986; 35:647-655.

Brawer JR, Beaudet A, Desjardins GC, Schipper HM. Pathologic effects of estradiol on the hypothalamus. *Biol Reprod* 1993; 49:647-652.

Brueggemeier RW, Bannan RA. Androgens inhibit the formation of catechol estrogens by estrogen 2-hydroxylase present in the rat microsomal preparations. *J Steroid Biochem* 1986; 25:351-354.

Buttyan R, Olson CA, Pintar J, Chang C, Bandyk M, Ng PU, Sawczuck IS. Induction of the TRPM-2 gene in cells undergoing programmed death. *Mol Cell Biol* 1989; 9:3473-3481.

Chen W, Witkin J, Silverman A. Beta-endorphin and synaptic input to gonadotropin-releasing hormone neurosecretory cells in the male rat. *J comp Neurol* 1989; 286:85-95.

Ching M. Morphine suppresses the proestrus surge of GnRH in pituitary portal plasma of rats. *Endocrinology* 1982; 112:2209-2212.

Choi-Miura NH, Ihara Y, Fakuchi K, Takeda M, Tobe T, Tomita M. SP 40,40 is a constituent of Alzheimer's amyloid. *Acta Neuropathol* 1992; 83:260-264.

Choi-Miura NH, Nakano Y, Tobe T, Mazda T, Tomita M. Beta-endorphin binding activity of SP-40,40. *Biol Pharm Bull* 1993; 16(3):228-231.

Clough RW, Hoffman GE, Sladek CD. Synergistic interaction between opioid receptor blockade and alpha-adrenergic stimulation on luteinizing hormone-releasing hormone (LHRH) secretion in vitro. *Neuroendorcrinology* 1990; 51:131-138.

Convery M, McCarthy GF, Brawer JR. Remission of the polycystic ovarian condition (PCO) in the rat following hemiovariectomy. *Anat Rec* 1990; 226:326-336.

Cotman CW, Nieto-Sampedro M. Cell biology of synaptic plasticity. *Science* 1984; 225:1287-1294.

Dagnault A, Ouergi D, Richard D. Treatment with alpha-helical CRF prevents the anorectic effect of estradiol. *Brain Res Bull* 1993; 32:689-692.

Danik M, Chabot JG, Mercier C, Benabid AL, Chauvin C, Quirion R, Suh M. Human gliomas and epileptic foci express high levels of a mRNA related to rat testicular sulfated glycoprotein 2, a purported marker of cell death. *Proc Natl Acad Sci* USA 1991; 88:8577-8581.

Danik M, Chabot JG, Hassan-Gonzalez D, Suh M, Quirion R. Localization of sulfated glycoprotein-2/clusterin mRNA in the rat brain by in situ hybridization. *J Comp Neurol* 1993; 334:209-227.

de Silva HV, Stuart WD, Duvic CR, Wetterau JR, Ray MJ, Ferguson DG, Albers HW, Smith WR, Harmony JAK. A 70 kDa apolipoprotein designated ApoJ is a marker for subclasses of human plasma high density lipoproteins. *J Biol Chem* 1990a; 265:13240-13247.

de Silva HV, Harmony JAK, Stuart WD, Gil CM, Robbins J. Apolipoprotein J: Structure and tissue distribution. *Biochemistry* 1990b; 29:5380-5389.

Demling J, Fuchs E, Baumert M, Wuttke W. Preoptic catecholamine, GABA and glutamate release in ovariectomized and ovariectomized estrogen treated rats utilizing a push-pull cannula technique. *Neuroendocrinology* 1985; 41:212-218.

Desjardins GC, Beaudet A, Brawer JR. Alteration in opioid parameters in the hypothalamus of rats with estradiol-valerate induced polycystic ovarian disease. *Endocrinology* 1990; 127:2969-2976.

Desjardins GC, Beaudet A, Schipper H, Brawer JR. Vitamin E protects hypothalamic ß-endorphin neurons from estradiol neurotoxicity. *Endocrinology* 1992; 131:2482-2484.

Desjardins GC, Brawer JR, Beaudet A. Estradiol is selectively neurotoxic to hypothalamic ß-endorphin neurons. *Endocrinology* 1993; 132:89-93.

Duguid JR, Bohmont CW, Liu N, Tourtellotte WW. Changes in brain gene expression shared by scrapie and Alzheimer disease. *Proc Natl Acad Sci* USA 1989; 86:7260-7264.

Farookhi R, Hemmings R, Brawer JR. Unilateral ovariectomy restores ovulatory cyclicity in rats with polycystic ovarian condition. *Biol Reprod* 1986; 32:530-540.

Fischer-Colbrie R, Zangerle R, Frischenschlager I, Weber A, Winkler H. Isolation and immunological characterization of a glycoprotein from adrenal chromaffin granules. *J Neurochem* 1984; 42:1008-1016.

Frankfurt M, Gould E, Wooley CS, McEwen BS. Gonadal steroids modify dendritic spine density in ventromedial hypothalamic neurons: a golgi study in the adult rat. *Neuroendocrinology* 1990; 51:530-535.

Fritz IB, Burdzy C, Setchell B, Blaschuk O. Ram rete testis fluid contains a protein (clusterin) which influences cell-cell interactions in vitro. *Biol Reprod* 1983; 28:1173-1188.

Fritz I, Murphy B. Clusterin: Insights into a multifunctional protein. *Trends Metab Endocrinology* 1993; 4:41-45.

Gabriel SM, Simpkins JW, Kalra SP. Modulation of endogenous opioid influence on luteinizing hormone secretion by progesterone and estradiol. *Endocrinology* 1983; 113:1806-1811.

Gharib SD, Wierman ME, Shupnik MA, Chin WW. Molecular biology of the pituitary gonadotropins. *Endocrine Rev* 1990; 11:177-199.

Griswold MD. Protein secretion of Sertoli cells. *Int Rev Cytol* 1988; 110:133-156.

Hammer RP, Bridges RS. Preoptic area opioids and opiate receptors increase during pregnancy and decrease during lactation. *Brain Res* 1987; 308:172-176.

Hartmann K, Rauch J, Urban J, Parczyk K, Diel P, Pilarsky C, Appel D, Haase W, Mann K, Weller A, Koch-Brandt C. Molecular cloning of gp-80, a glycoprotein complex secreted by kidney cells in vitro and in vivo . *J Biol Chem* 1991; 266:9924-9931.

Hemmings R, Farookhi R, Brawer JR. Pituitary and ovarian response to luteinizing hormone releasing hormone in a rat with polycystic ovaries. *Biol Reprod* 1983; 29:239-248.

Ishikawa K, Taniguchi Y, Kurosumi K, Suzuki M, Shinoda M. Immunocytochemichal identification of somatostatin containing neurons projecting to the median eminence of the rat. *Endocrinology* 1987; 121(1):94-97.

Jenne DE, Tschopp J. Clusterin: The intriguing guises of a widely expressed glycoprotein. *Trends Biochem Sci* 1992; 17:154-159.

Jenne DE, Tschopp J. Molecular structure and functional characterization of a human cytolysis inhibitor found in blood and seminal plasma: Identity to sulfated glycoprotein 2, a constituent of rat testis fluid. *Proc Natl Acad Sci* USA 1989; 86:7123-7127.

Jones SE, Meerabux JMA, Yeates DA, Neal MN. Analysis of differentially expressed genes in retinitis pigmentosa retinas. *FEBS Lett* 1992; 300:279-282.

Kalra SP, Simpkins JW. Evidence for noradrenergic mediation of opioid effects on LH secretion. *Endocrinology* 1981a; 109:776-782.

Kalra SP. Neural loci involved in naloxone-induced luteinizing hormone release: effects of a norepinephrine synthesis inhibitor. *Endocrinology* 1981b; 109:1805-1810.

Kalra SP, Kalra PS. Neural regulation of luteinizing hormone secretion in the rat. *Endocrine Rev* 1983a; 4:311-351.

Kalra SP. Opioid peptides-inhibitory neuronal systems in the regulation of gonadotropin secretion. In: *Role of peptides and proteins in control of reproduction* (McCann SM, Dhindsa DS) Elsevier Biomedical, New York : 63, 1983b.

Kalra PS, Crowley WR, Kalra SP. Differential in vitro stimulation by naloxone and of Luteinizing hormone-releasing hormone and catecholamine release from hypothalami of intact and castrated rats. *Endocrinology* 1987; 122:997-1003.

Kalra SP, Allen LG, Sahu A, Kalra PS, Crowley WR. Gonadal steroids and neuropeptide Y-opioid-LHRH axis; interactions and diversities. *J Steroid Biochem* 1988; 27:677-681.

Kalra SP, Sahu A, Kalra PS, Crowley WR. Hypothalamic neuropeptide Y: a circuit in the regulation of gonadotropin secretion and feeding behaviour. *Ann New York Acad Sci* 1990; 611:273-283.

King J, Tobet S, Snavely F, Arimura A. The LHRH system in normal and neonatally androgenized female rats. *Peptides* 1980; 1:85-100.

Kirszbaum K, Sharpe JA, Murphy AJF, d'Apice B, Classon P, Hudson P, Walker D. Molecular characterization of the novel, human complement-associated protein, SP 40,40: A link between the complement and the reproductive systems. *EMBO J* 1989; 8:711-718.

Kissinger C, Skinner MK, Griswold MD. Analysis of Sertoli cell secreted proteins by two-dimensional electrophoresis. *Biol Reprod* 1982; 25:7265-7270.

Knobil E. On the control of gonadotropin secretion in the rhesus monkey. *Recent Prog Horm Res* 1974; 30:1.

Kotsuji F, Winters SJ, Keeping HS, Attardi B, Oshima H, Troen P. Effects of inhibin from primate Sertoli cells on follicle stimulating hormone and luteinizing hormone release by perifused rat pituitary cells. *Endocrinology* 1988; 122:2796-2802.

Lahr G, Mayerhofer A, Bergmann M, Takiyyuddin MA, Gratzl M. Chromogranin A in neurons of the rat cerebellum and spinal cord: Quantification and sites of expression. *J Histochem Cytochem* 1992; 40:993-999.

Lampert-Etchells M, McNeil TH, Laping NJ, Zarow C, Finch CE, May PC. Sulfated glycoprotein 2 is increased in rat hippocampus following entorhinal cortex lesioning. *Brain Res* 1991; 563:101-106.

Leger JG, Montpetit ML, Tenniswood MP. Characterization and cloning of androgen-repressed mRNA's from rat ventral prostate. *Biochem Biophys Res Commun* 1987; 147:196-203.

Leibowitz SF, Hor L. Endorphinergic and alpha-noradrenergic systems in the paraventricular nucleus affects eating behaviour. *Peptides* 1982; 3:421-428.

Leranth C, MacLusky N, Shanaborough A, Naftolin F. Immunohistochemical evidence for synaptic connection between pro-opiomelanocortin-immunoreactive axons and LHRH neurons in the preoptic area of the rat. *Brain Res* 1986; 345:332-336.

Levine JE, Ramirez VD. Luteinizing hormone-releasing hormone release during the rat estrus cycle and after ovariectomy as estimated with push-pull cannulae. *Endocrinology* 1982; 111:1439.

Liehr JG, Roy D. Free radical generation by redox cycling of estrogens. *Free Radical Biol & Med* 1990; 8:415-423.

Loumaye E, Catt KJ. Agonist-induced regulation of pituitary receptors for gonadotropin-releasing hormone. *J Biol Chem* 1983; 258:12002-12009.

Mahata SK, Mahata M, Marksteiner J, Sperk G, Fischer-Colbrie R, Winkler H. Distribution of mRNAs for chromogranins A and B and secretogranin II in rat brain. *Eur J Neurosci* 1991; 3:895-904.

May PC, Lampert-Etchells M, Johnson SA, Poirier J, Masters JN, Finch CE. Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. *Neuron* 1990; 5:831-839.

May PC, Finch CE. Sulfated glycoprotein 2: New relationships of this multifunctional protein to neurodegeneration. *Trends Neurosci* 1992; 15:391-396.

May PC, Johnson SA, Poirier J, Lampert-Etchelles M, Finch CE. Altered gene expression in Alzheimer's disease brain tissue. *Can J Neurol Sci* 1989; 16(*suppl*):473-476.

May PC, Robinson P, Fuson K, Smalstig B, Stephenson D, Clemens JA. Sulfated glycoprotein 2 expression increases in rodent brain after transient global ischemia. Mol *Brain Res* 1992; 15:33-39.

May PC. Sulfated glycoprotein 2: An emerging molecular marker for neurodegeneration. *Ann New York Acad Sci* USA 1993; 679:235-244.

Michel D, Chabot JG, Moyse E, Danik M, Quirion R. Possible functions of a new genetic marker in the central nervous system: The sulfated glycoprotein 2 (SGP-2). *Synapse* 1992; 11:105-111.

Michel D, Gillet G, Volovitch M, Pessac B, Calothy G, Brun G. Expression of a novel gene encoding a 51 kDa precursor protein is induced by different retroviral oncogenes in quail neuroretinal cells. *Oncogene Res* 1989; 4:127-136.

Moore RY, Bloom FE. Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annu Rev Neurosci* 1979; 2:113-168.

Morgane PJ, Panskepp J. *Handbook of the hypothalamus: Physiology of the hypothalamus* (Vol 2) New York: Dekker Inc. 1980.

Naftolin F, Garcia-Segura LM, Keefe D, Leranth C, Maclusky NJ, Brawer JR. Estrogen effects on the synaptology and neural membranes of the rat hypothalamic arcuate nucleus. *Biol Reprod* 1990; 42:21-28.

Palmer DJ, Christie DL. The primary structure of glycoprotein II from bovine adrenal medulla chromaffin granules. *J Biol Chem* 1990;265:6617-6623.

Pasinetti GM, Finch CE. Sulfated glycoprotein 2 (SGP-2) mRNA expressed in rat striatal astrocytes following local ibotenic acid lesions. *Neurosc Lett* 1991; 130:1-4.

Pasinetti GM, Cheng HW, Morgan M, Lampert-Etchells M, McNeil TH, Finch CE. Astrocytic mRNA response to striatal deafferentation in male rat. *Neuroscience* 1993; 53:199-211.

Pasinetti GM, Johnson SA, Oda T, Rozovsky I, Finch CE. Clusterin (SGP-2): A multifunctional glycoprotein with regional expression in astrocytes and neurons of the adult rat brain. *J comp Neurol* 1994; 339:387-400.

Pfaff DW. Uptake of estradiol-17-^{3}H in the female rat brain. An autoradiographic study. *Endocrinology* 1968; 82:1149-1155.

Piotte M, Beaudet A, Brawer JR. Light and electron microscopic study of tyrosine-hydroxylase-immunoreactive neurons within the developing rat arcuate nucleus. *Brain Res* 1988; 439(1-2):127-137.

Rotten B, Leblanc P, Kordon C, Weiner I, Enjelbert A. Interference of endogenous beta-endorphin with opiate binding in the anterior pituitary. *Neuropeptides* 1986; 6:377-392.

Schall AV, Kastin AJ, Arimura A. FSH-releasing hormone and LH releasing hormone. *Vitam Horm* 1972; 30:83.

Schipper HM, Desjardins GC, Beaudet A, Brawer JR. The 21-aminosteroid antioxidant U74389F, prevents estradiol-induced depletion of hypothalamic ß-endorphin in adult female rats. *Brain Res* 1994; 652:161-163.

Schipper HM, Leehan L, Reichlin S. Glial peroxidase activity in the hypothalamic arcuate nucleus: effects of estradiol-valerate induced persistent estrus. *Brain Res* 1990; 507:200-207.

Schipper HM, Kotake Y, Janzen E. Catechol oxidation in peroxidase positive astrocytes in primary culture: an electron spin resonance study. *J Neuroscience* 1991; 11:2170-2176.

Schulster A, Farookhi R, Brawer JR. Polycystic ovarian condition in estradiol valerate-treated rats: spontaneous changes in characteristic endocrine features. *Biol Reprod* 1984; 29:239-248.

Senut MC, Jazat F, Choi NH, Lamour Y. Protein SP 40,40-like immunoreactivity in the rat brain: Progressive increase with age. *Eur J Neurosci* 1992; 4:917-928.

Shivers BD, Harlan RE, Morell JI, Pfaff DW. Absence of estradiol concentration in cell nuclei of LHRH-immunoreactive neurons. *Nature* 1983; 304:345-347.

Shreiber SS, Tocco G, Najm I, Baudry M. Seizure activity causes a rapid increase in sulfated-glycoprotein-2 messenger RNA in the adult but not in neonatal rat brain. *Neurosci Lett* 1993;153:17-20.

Simantov R, Snyder P. Opiate receptor binding in the pituitary gland. *Brain Res* 1977; 124:178-188.

Simard M, Brawer JR, Farookhi R. An intractable ovary-independent impairment in hypothalamo-pituitary function in the estradiol-valerate-induced polycystic ovarian condition in the rat. *Biol Reprod* 1987; 36:1229-1237.

Sternberger LA, Hoffman GE. Immunocytology of luteinizing hormone-releasing hormone. *Neuroendocrinology* 1978; 25:111-128.

Suda T, Sato Y, Sumitomo T, Nakano Y, Tozawa F, Iwai I, Ymada M, Demura H. ß-endorphin inhibits hypoglycemia-induced gene expression of corticotropin-releasing factor in the rat hypothalamus. *Endocrinology* 1992; 130:1325-1330.

Sylvester SR, Skinner MK, Griswold MD. A sulfated glycoprotein synthesized by Sertoli cells and by epididymal cells is a component of the sperm membrane. *Biol Reprod* 1984; 31:1087-1101.

Sylvester SR, Morales C, Oko R, Griswold MD. Localization of sulfated glycoprotein-2 on spermatozoa and in the reproductive tract of the male rat. *Biol Reprod* 1991; 45:195-207.

Theodosis DT, Poulain DA. Evidence for structural plasticity in the supraoptic nucleus of the rat hypothalamus in relation to gestation and lactation. *Neuroscience* 1984; 11:183-193.

Theodosis DT, Poulain DA. Neuronal-glial and synaptic plasticity in the adult rat paraventricular nucleus. *Brain Res* 1989; 484:361-366.

Theodosis DT, Poulain DA. Neuronal-glial and synaptic remodelling in the adult hypothalamus in response to physiological stimuli. In: *Functional anatomy of the neuroendocrine hypothalamus* Ciba Foundation Symposium, 1992.

Tsuruta JK, Wong K, Fritz IB, Griswold MD. Structural analysis of sulfated glycoprotein 2 from amino acid sequence. Relationship to clusterin and serum protein SP 40,40. *Biochem J* 1990; 268:571-578.

Wardlaw SL, Thoron L, Frantz AG. Effects of sex steroids on brain beta-endorphin. *Brain Res* 1982; 245:327-331.

Weisner J, Koenig J, Krulich L, Moss RL. Site of action for beta-endorphin induced changes in plasma luteinizing hormone and prolactin in the ovariectomized rat. *Life Sci* 1984; 34:1463-1473.

Whitnall MH. Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. *Prog Neurobiol* 1993; 40:573-629.

Wilcox JN, Roberts JL. Estrogen decreases rat hypothalamic pro-opiomelanocortin messenger RNA levels. *Endocrinology* 1985; 117:2392-2396.

Wilkinson M, Bhanot D, Wilkinson DA, Brawer JR. Prolonged estrogen treatment induces changes in opiates, benzodiazipine and beta-adrenergic binding sites in female rat hypothalamus. *Brain Res Bull* 1983; 11-279-281.

Wise PM, Rance N, Barraclogh CA. Effects of estradiol and progesterone on catecholamine turnover rates in discrete hypothalamic regions in ovariectomized rats. *Endocrinology* 1981; 108:2186-2193.

Wood PL. Multiple-opiate receptors: A guide to structure and terminology. *Life Sci* 1982; 31:1645.

Zanisi M, Messi E. Sex steroids and the control of LHRH secretion. *J Steroid Biochem* 1991; 40(1-3):155-163.

FIGURES

Figure 1 46
Figure 2 48
Figure 3 50
Figure 4 52
Figure 5 54
Figure 6 56
Figure 7 58
Figure 8 60

Figure 1. The effect of estradiol-valerate (EV) treatment and vitamin E on body weight.

Mean body weights of animals in the various experimental groups are shown in the histogram. Rats treated with EV weigh significantly less (asterisk) than animals in the other three groups ($P<0.01$). There are no other significant differences between treatment groups in animal body weight. ($n=6$ for each group).

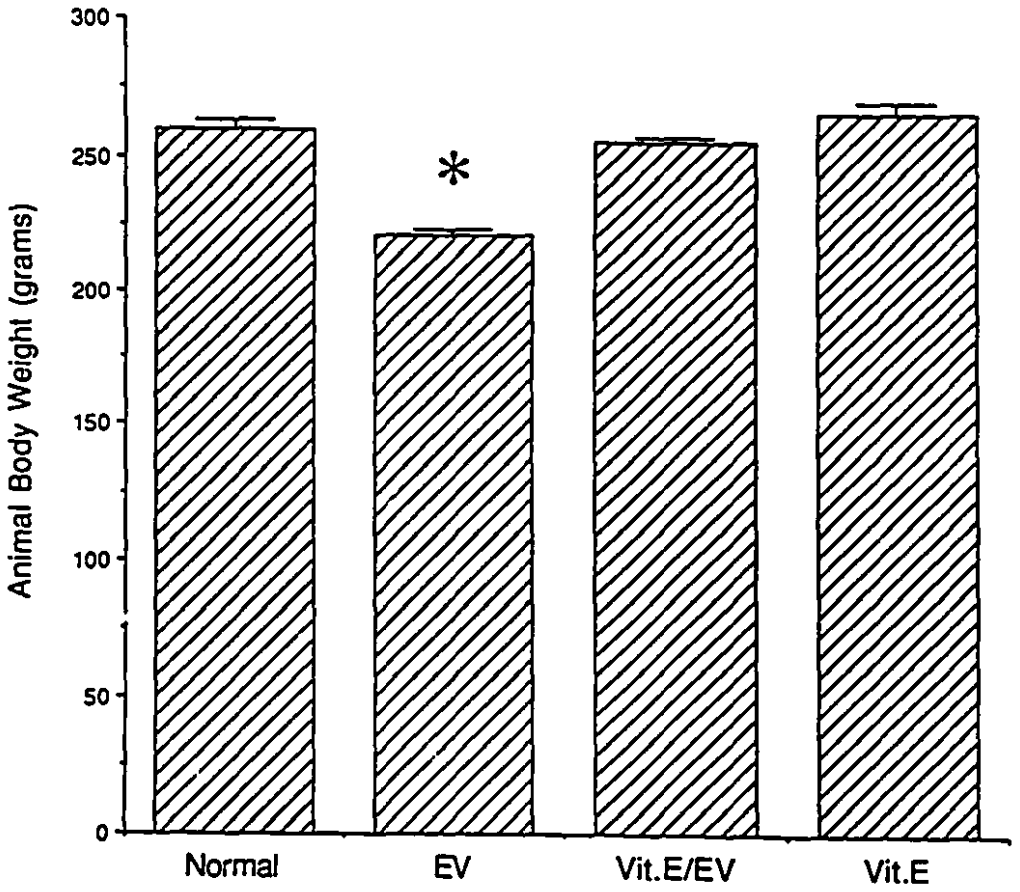
Animal Body Weight (grams)
300
250
200
150
100
50
0
*
Normal
EV
Vit.E/EV
Vit.E

Figure 2. SGP-2 labelling in the medial preoptic area.

(a) SGP-2-immunopositive neuronal perikarya are scattered in the medial region of the MPOA (mag=250x). (b) High power magnification of SGP-2-immunopositive neurons in the MPOA reveal heavily stained neuronal profiles scattered amongst unstained neurons. Only larger cell profiles appear labelled with dark intracellular granules (mag=1000x). (c) Magnification of the labelled ependymal border lining the third ventricle, at the level of the MPOA, reveals small dark SGP-2-immunopositive granules (arrowheads) associated with the apical region of the ependymal cells (mag=1000x).

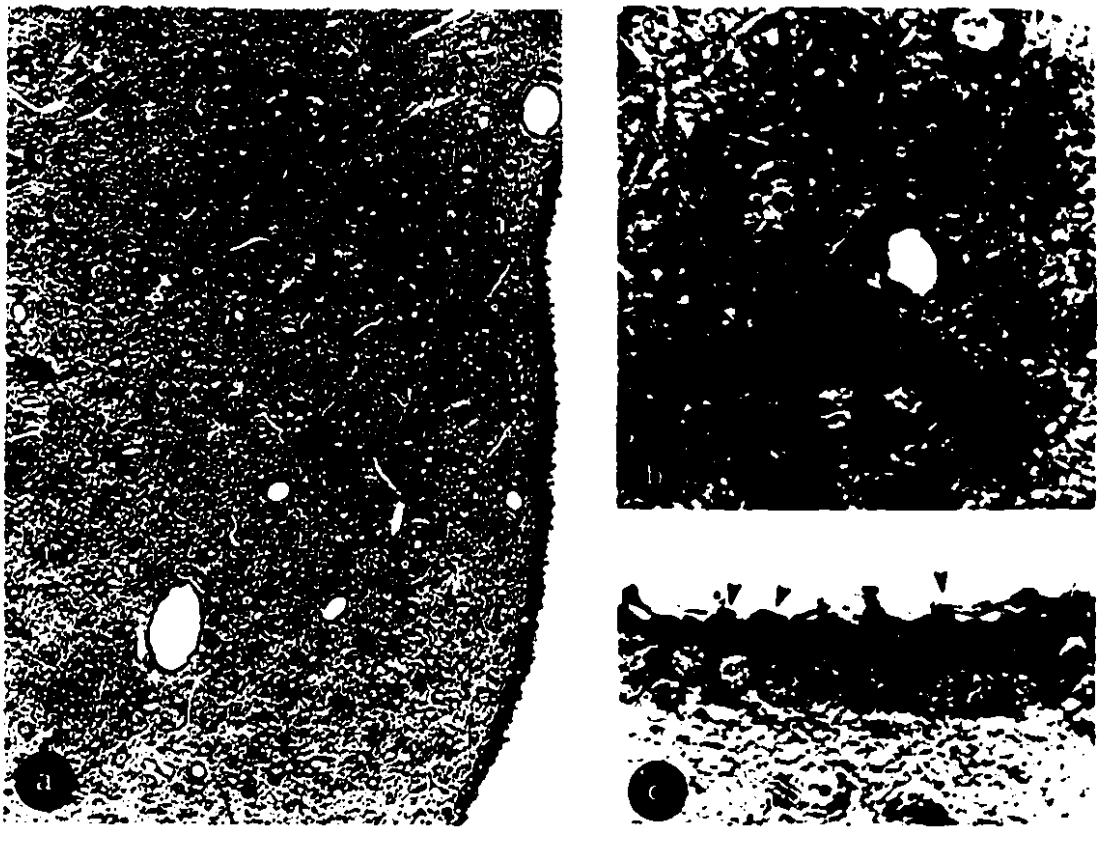
a
b
c

Figure 3. SGP-2 labelling in the supraoptic nucleus.

(a) Set at the lateral border of the optic chiasm (Oc), most of the neuronal profiles of the SON appear SGP-2-positive (mag=400x). (b) Higher magnification of immunopositive neurons in the SON reveals large neuronal profiles which exhibit intracellular SGP-2-immunopositive granules superimposed on a more diffuse labelling (mag=1000x).

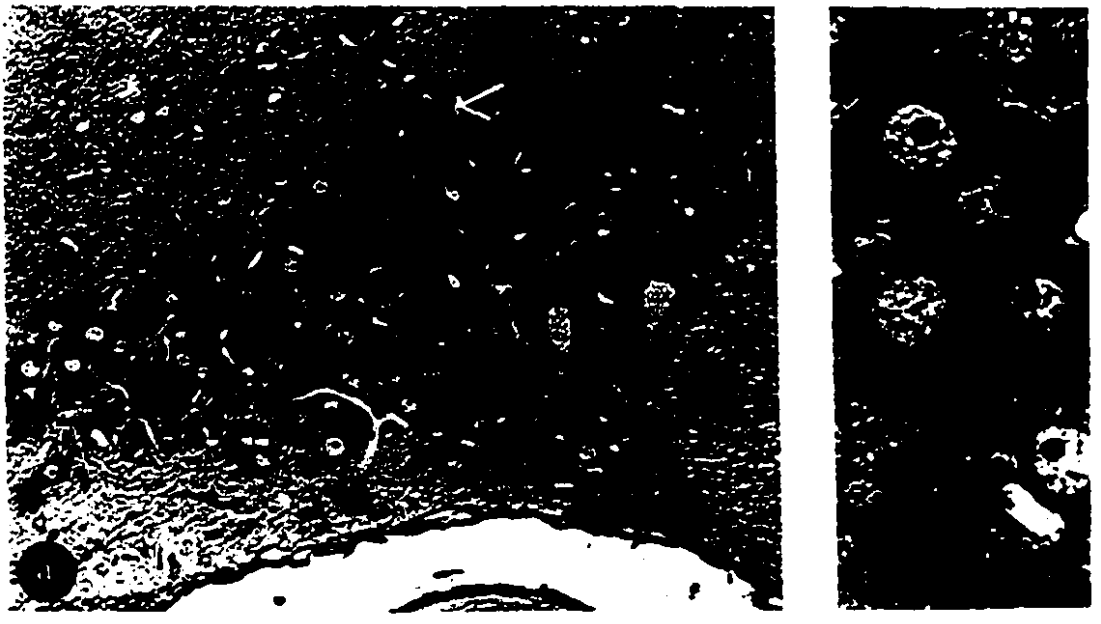

Figure 4. SGP-2 labelling in the paraventricular nucleus.

(a) A tight cluster of SGP-2-immunopositive neurons occupy a small medial region of the PVN (mag-250x). (b) Higher magnification of PVN immunopositive neurons reveals large neuronal profiles with a granular intracellular pattern of labelling (mag=1000x).

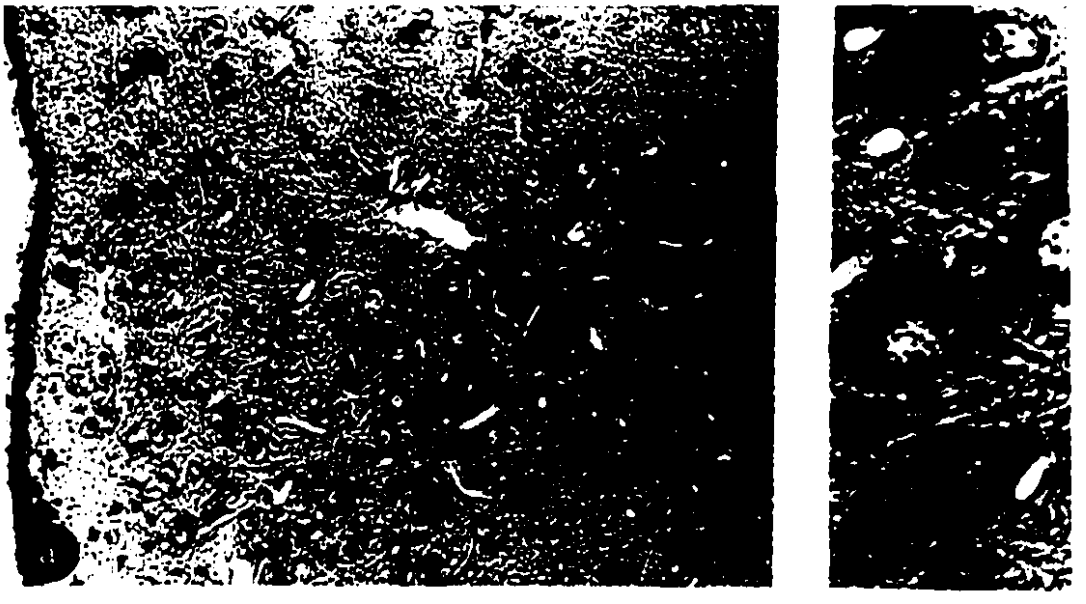

Figure 5. SGP-2 labelling in the dorsomedial nucleus and perifornical area.

(a) Intensely stained neuronal perikarya are loosely scattered from the medial region of the DMN to the lateral hypothalamic area in the region of the fornix (Fx) (mag=150x). (b) Magnified immunopositive neurons of the DMN appear darkly stained with granular SGP-2 labelling concentrated as a perinuclear ring (arrow) (mag=1000x).

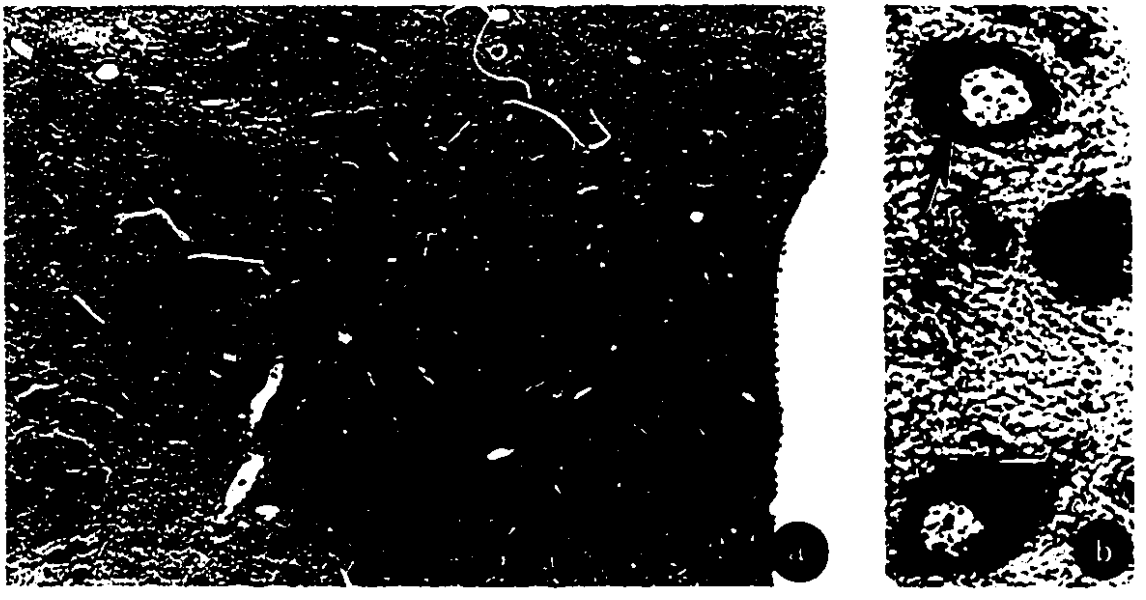
a
b

Figure 6. Percent SGP-2-positive cells in the hypothalamic nuclei of different treatment groups.

Experimental treatments had no effect on the percentage of SGP-2-immunopositive neurons in the supraoptic nucleus (SON), paraventricular nucleus (PVN), dorsomedial nucleus (DMN), or lateral hypothalamic area (LHA). In the medial preoptic area (MPOA) (asterisk), EV-treatment resulted in a greater than two-fold increase in the number of SGP-2 labelled cells ($P<0.01$). High vitamin E diet precluded the effect of EV-treatment and maintained MPOA SGP-2-positive cells at control levels. Vitamin E by itself had no effect on the MPOA.
($n=6$ for each group).

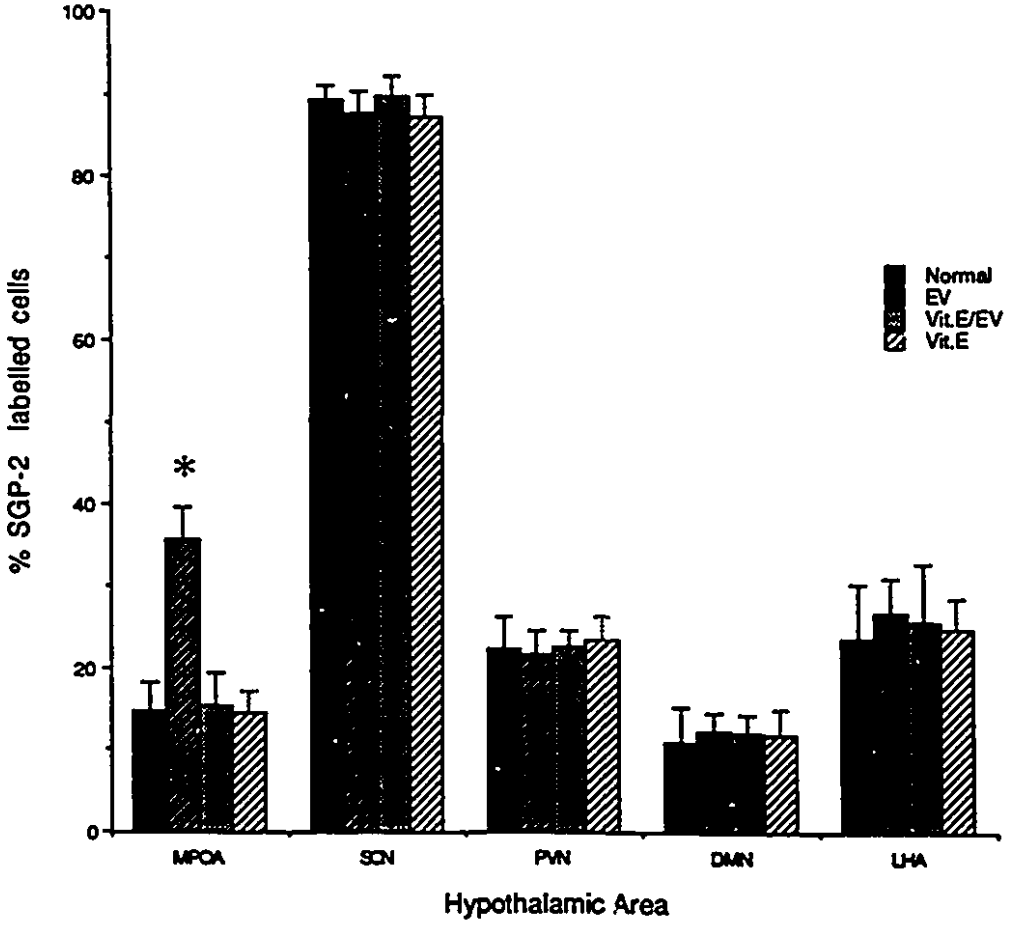
100
80
60
40
20
0
% SGP-2 labelled cells
*
Normal
EV
Vit.E/EV
Vit.E
MPOA
SCN
PVN
DMN
LHA
Hypothalamic Area

Figure 7. Comparison SGP-2-immunopositive cells in the medial preoptic area of normal and EV-treated animals.

(a) In the normal control animal, SGP-2-positive neuronal perikarya are seen scattered in the MPOA. (b) In the EV-treated MPOA, the distribution of SGP-2-positive cells is similar to that in normal untreated controls but the number of labelled cells is considerably greater (mag=250x).

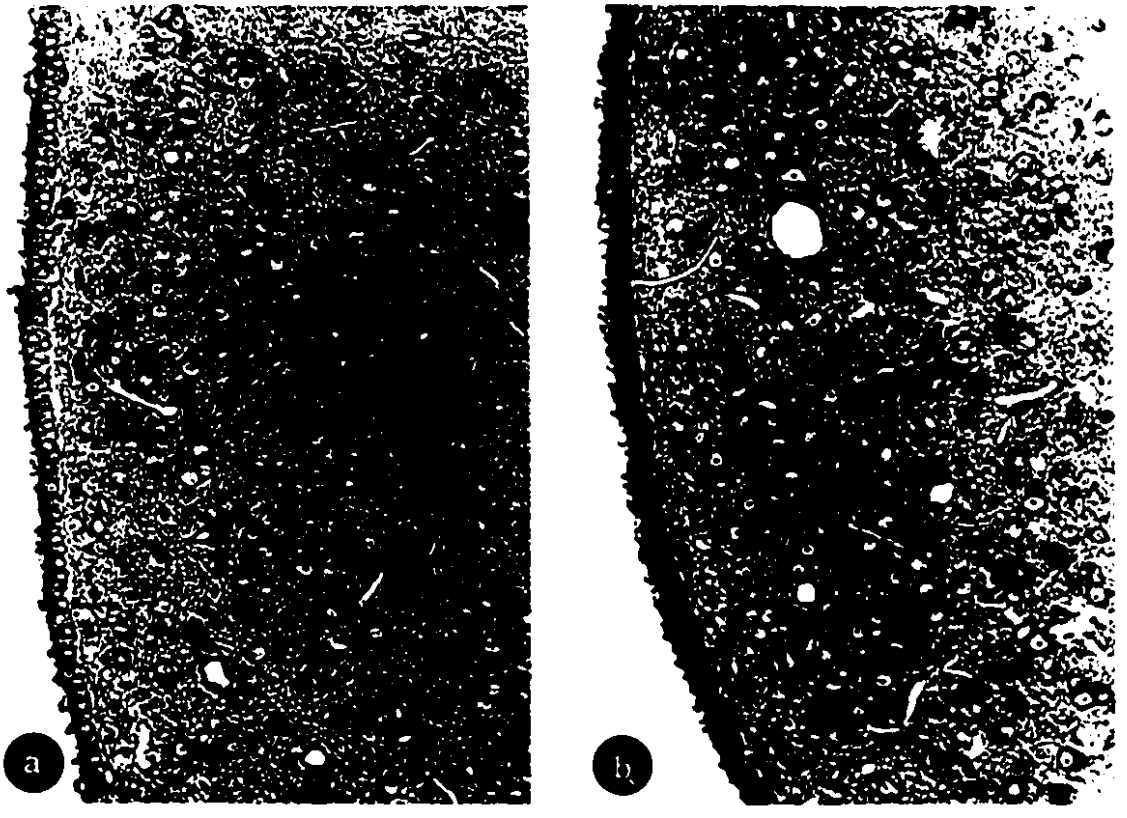
a
b

Figure 8. SGP-2-immunoreactivity in the MPOA of the EV-treated rat.

In addition to the labelled perikarya, small cell profiles, consistent with glia (g) appear labelled. Small punctate accumulations of label appear in the neuropil (arrows) following EV-treatment (mag=1000x).