

*The Sahlgrenska Academy at the University of Gothenburg, Institute of Clinical Sciences,
Department of Anesthesiology and Intensive Care: doctoral thesis*

Neurochemical and neuroendocrine reactions during non-neurological surgery



GÖTEBORGS UNIVERSITET
SAHLGRENSKA AKADEMIN

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Göteborg 2010

Cover drawing by Pia Moberg
ISBN 978-91-628-7849-8
Printed in Sweden by Chalmers Reproservice

*The birds they sang
at the break of day
Start again
I heard them say
Don't dwell on what
has passed away
or what is yet to be*

*The wars they will
be fought again
The holy dove
be caught again
bought and sold
and bought again
the dove is never free*

*You can add up the parts
but you won't have the sum
You can strike up the march
there is no drum
Every heart
to love will come
but like a refugee*

Ring the bells that still can ring
Forget your perfect offering
There is a crack in everything
That's how the light gets in

L Cohen

To Henrik

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Abstract

Objective: To study changes in serum and cerebrospinal fluid (CSF) proteins, monoamine metabolites and hormones during non-neurological surgery. *Subjects and methods:* Thirty-five patients without neurological or psychiatric disorders undergoing knee replacements had CSF and serum samples drawn from spinal and arterial catheters before, three hours after and on the morning after surgery. *Results:* The CSF/serum albumin ratios decreased significantly during the study period, especially after the interventions. In contrast, CSF concentrations of beta-2-microglobulin (β 2M) increased significantly during surgery and remained high. The CSF concentrations of beta-trace protein (β TP) remained unchanged. The dopamine metabolite homovanillic acid (HVA) and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) increased sharply in the CSF during surgery and reached 188% and 166% of their initial concentrations on the morning after the intervention. The CSF concentrations of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglucol (MHPG) increased modestly (non-significantly) during and after surgery. The HVA/5-HIAA ratios initially increased but returned almost to their initial level during the night after surgery. During and after surgery, serum thyroid hormones and the T_3/T_4 ratio decreased, while the CSF T_3/T_4 ratio instead increased. At baseline, the CSF MHPG concentrations were significantly correlated to the serum T_3/T_4 ratios. The base-line CSF thyroid hormones were strongly correlated with the subsequent changes in monoamine metabolite concentrations during and after surgery. Serum insulin concentrations first decreased modestly but then increased sharply after surgery with a wide interpersonal variation, while the CSF insulin concentrations changed in the same directions, albeit with smaller amplitudes. Due to the increase in serum insulin, the CSF/serum insulin ratios decreased. *Conclusions:* Central nervous system protein reactions to a non-neurological surgical intervention include sharply decreased permeability of albumin into the CSF and signs of intrathecal inflammatory activity. There was a strong increase in serotonergic and dopaminergic neurotransmission during surgery, with a comparatively stable relationship between the metabolites from these systems. Changes in thyroid hormone and insulin metabolism during surgery are not similar peripherally and in the central nervous system. Thyroid hormone activity may influence brain monoaminergic neurotransmission. No correlations between the CSF/serum ratios of albumin, β TP, insulin and T_4 were found, consistent with separate transport mechanisms from the blood into the CSF for these substances in humans *in vivo*.

Key-words: albumin, beta-2-microglobuline, blood brain barrier, cerebrospinal fluid, inflammation, spinal anesthesia, stress

Acknowledgements

First, I wish to express my sincere gratitude to all the patients who contributed to the Surgical Stress Study.

This work has been supported by numerous persons and institutions. I have had the privilege to have the best of tutors. My main supervisor, Associate Professor Henrik Zetterberg, has shared his invariably sparkling enthusiasm, creative thinking and positive criticism over the years and also helped me practically at a difficult moment during this work. My co-supervisor Professor Kaj Blennow has always been there to give cool advice, humorous remarks and professional support, and helped design the project from start. My other co-supervisor Associate Professor Elisabet Wennberg has been a director and teacher never failing in support for a scientific approach to our common clinical work as anesthesiologists, and helped with the most accurate critical reading of the thesis.

Professor Henrik Anckarsäter, my partner and colleague, initially popped the idea of the Surgical Stress Study at our kitchen table and has ever since supported my work with his never-failing curiosity, vivid scientific imagination and vast knowledge of everything from molecules and the brain to the mysteries of the human.

Many thanks also go to my co-authors, Associate Professor Marianne Kristiansson (always the first to answer any e-mailed inquiry), Dr Lena Jidéus, Associate Professor Jan-Eric Månsson, Dr Sara Bromander, Professor Bo Ahrén, Professor Agneta Holmäng and Dr Caroline Wass (who finally brought all analyses together). Without your help, this project would not have been possible to carry through.

Invaluable assistance and support have come from my colleagues and all the staff at the Anesthesiology and Intensive Care Clinic at Kungälv Hospital, especially from research nurse Anita Klasson. I received fantastic support and never-failing encouragement from research secretaries Monika Montell and Agneta Brimse, who have become dear friends over the years. Psychology student Fredrik Ljungqvist and Psychologist Ola Ståhlberg provided emergency computer help when most needed and biostatisticians Gunnar Ekeröth, Aldina Pivodic and Nils-Gunnar Pehrsson excellent statistical guidance. Professors Sven-Erik Ricksten, Björn Biber and Dick Delbro read the thesis with great care and gave constructive criticism. With all this expert support, it is important to point out that any mistake that remains in the final thesis is all my own.

Institutional support and funding for this project came from the Västra Götaland region, Kungälv Hospital, the Swedish Medical Society, and the Göteborg Medical Society. I am very proud if this study has contributed to scientific collaboration between our countryside hospital and the leading neuroscience laboratories at the University of Gothenburg.

Very special thanks are due to my colleague and friend, Dr Nada Vasic, who contributed to this study in many ways, including collecting samples and clinical data, and who always was there to support me, share her good humor and spread her characteristic humanity in everyday clinical work. Dr Pia Moberg not only creates fantastic medical imagery but is also a long-time friend.

Throughout my life, I have had the gift of extraordinary support from my parents, Doris and Roland. They encouraged my studies, helped in every possible way and always gave me love, trust and joy, as did my parents-in-law, Kerstin and Bertil, and all my friends, especially Malin, Morgan, Thomas, Pontus (who also volunteered for a previous study), Agneta and Emmanuelle. Henrik you always were and remain a light in darkness.

Papers in the thesis

- I. Anckarsäter R, Vasic N, Jidéus L, Kristiansson M, Zetterberg H, Blennow K, Anckarsäter H. Cerebrospinal fluid protein reactions during non-neurological surgery. *Acta Neurol Scand.* 2007 Apr;115(4):254-259.
- II. Anckarsäter R, Zetterberg H, Månsson JE, Blennow K, Anckarsäter H. Non-neurological surgery results in a neurochemical stress response. *J Neural Transm.* 2008 Mar;115(3):397-399.
- III. Anckarsäter R, Zetterberg H, Blennow K, Anckarsäter H. Association between thyroid hormone levels and monoaminergic neurotransmission during surgery. *Psychoneuroendocrinology.* 2007 Sep-Nov;32(8-10):1138-1143.
- IV. Bromander S, Anckarsäter R, Ahrén B, Kristiansson M, Blennow K, Holmäng A, Zetterberg H, Anckarsäter H, Wass C. Cerebrospinal fluid insulin during non-neurological surgery. *Manuscript submitted for publication.*

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Abbreviations

A	Samples drawn before surgery
B	Samples drawn three hours after the end of surgery
C	Samples drawn at 8 am in the morning after surgery
BIS	Bispectral index
βTP	Beta-trace-protein
β2M	Beta-2-microglobulin
CNS	Central nervous system
CSF	Cerebrospinal fluid
HPLC	High-performance liquid chromatography
HVA	Homovanillic acid
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MAO	Monoamine-oxidase
MHPG	3-methoxy-4-hydroxyphenylglucol
TSH	Thyroid stimulating hormone
T₃	Triiodothyronine
T₄	Thyroxine
5-HIAA	5-hydroxyindoleacetic acid

Introduction

Study design

Neuro-chemical changes in the human central nervous system (CNS) in response to stressful events and trauma are but sparingly investigated, not least compared to our knowledge about peripheral changes in the humoral and metabolic systems, which is far more advanced (Desborough, 2000). One possible model for the study of such CNS processes is to draw samples of cerebrospinal fluid (CSF) and serum from indwelling catheters before, during, and after surgery. The CSF contains e.g. proteins, transmitter metabolites and hormones thought to reflect chemical processes in the CNS, such as barrier function (to protect the nervous system from substances in the blood), inflammation, neurotransmission, and hormonal regulation of metabolism (Felgenhauer, 1974, Saunders et al., 1999). The present investigation is a pilot study on thirty-five neurologically healthy persons undergoing knee arthroplasties, which is a relatively standardized surgical intervention. The patients were planned for spinal anesthesia with bupivacaine and general sedation with intravenous propofol. An intrathecal catheter for spinal anesthesia and an arterial catheter were inserted, and samples were systematically drawn from 35 subjects before, three hours after, and in the morning after surgery. Patterns of protein markers, neurotransmitters, and hormones in the three samples obtained from each subject were analyzed and compared for changes during and after the interventions. This kind of orthopaedic intervention represents a stressful event for the whole organism in spite of the spinal blockade, as shown by possible bradycardia during cementation of prostheses (Tarkkila and Kaukinen, 1991). The study design provides the opportunity to assess chemical processes in humans undergoing a relatively conform series of events *in vivo*. At the same time, various confounding factors of the anesthetic and surgical proceedings on specific aspects of the findings have to be taken into account. Considering the findings in relation to each other across the types of markers under analysis may clarify at least some possible confounding factors, such as CSF circulation.

The CSF

The CSF is produced in the CNS by modified ependymal cells in the choroid plexus in the ventricle system of the cerebrum (about two thirds) and from the brain interstitial fluid (about one third), but may also be secreted from the meninges (Thompson, 2005, page 53). It then circulates through the foramina of Monroe into the third ventricle, and from there through the cerebral aqueduct into the fourth ventricle, from which it exits through two lateral apertures (foramina of Luschka) and one median aperture (foramen of Magendie) to the spinal cord and to the surface of the cerebral hemispheres. Caudally, the spinal dura forms a “sack” below

the level of the spinal chord. About 500 mL of CSF is produced a day, which corresponds to approximately 0.34 mL per minute. As the brain can only contain about 135 to 150 mL of CSF, large amounts are drained primarily into the venous blood through arachnoid granulations in the superior sagittal sinus. The CSF may also flow along the cranial nerves and spinal nerve roots, where arachnoid granulations are also located. The caudal meningeal “sack” (from which lumbar puncture samples are drawn) thus plays a substantial role in the reabsorption of the CSF (with an estimated lumbar reabsorption between 0.11 and 0.23 mL per minute depending on the level of activity (Edsbacke et al., 2004). The role of lymphatic drainage in the elimination of the CSF has been demonstrated in animals (e.g. newborn sheep) but remains contested in humans (Zakharov, 2003). The CSF pressure ranges from 10 to 15 cm H₂O, with most variations due to coughing or compression of internal jugular veins in the neck.

The barriers of the CNS

Three barriers limit and regulate molecular exchange between the blood and the neural tissue or its fluid spaces. The most important is the blood-brain barrier (BBB), consisting of tight junctions between the capillary endothelial cells that reduce the exchange between the blood, the CSF, and the brain tissue. The two other barriers are the choroid plexus epithelium and the arachnoid epithelium between the blood vessels and the subarachnoid CSF (Segal, 2000). The CNS is thus protected from free exchange with the blood, meaning that all molecules have to be absorbed through the endothelial cells into the CNS by specific and unspecific mechanisms (Abbott et al., 2006). Water and small gaseous molecules, such as O₂ and CO₂, can diffuse freely through the tight junctions between the endothelial cells. Small lipophilic agents, including drugs such as barbiturates and ethanol, have a route of entry through the lipid membranes of the endothelial cells. Besides these two pathways, hydrophilic molecules, which cannot diffuse freely over the BBB, may cross by one of the remaining three pathways, i.e. active carriers (e.g., glucose, purin bases, amino acids), receptor-mediated transcytosis (certain proteins, e.g., insulin, transferrin and thyroxine) or the less specific adsorptive-mediated transcytosis (e.g., albumin and other plasma proteins, Abbot et al., 2006, Brasnjevic, 2009). Several transport systems (e.g., the large neutral amino acid transporter) provide transport of a proportion of the serum content of a given protein or amino acid unless it is saturated by a rivaling substance using the same transport system (Boado et al., 1999).

Main properties of the CSF

The ionic composition of the CSF is similar to that of plasma (Segal, 2000). Normally, the CSF should contain virtually no blood cells, but lymphocytes may be detected as a sign of inflammation, just as erythrocytes may signal intrathecal bleeding. Overall, the CSF contains about 0.5% of the plasma proteins, or about 0.35 g/L, depending on sampling site, and the relative concentrations of the majority of proteins are similar

to those found in plasma (Felgenhauer, 1974). Notable exceptions include that immunoglobulines, in the normal state, are proportionally under-represented in the CSF while insulin, for example, is over-represented as an expression of the active transport, probably reflecting the high glucose metabolic needs of the nervous tissues (Poduslo et al., 1994). CSF proteins also come from the normal CNS metabolism or are specifically formed in reaction to infections or inflammation. Finally, a combination of intracellular and extracellular enzymes provides a metabolic barrier, which can inactivate neuroactive and toxic substances (Brasnjevic, 2009). The CSF thus also serves to eliminate transmitters and hormones from the CNS, often in degraded, inactive forms, such as the metabolites of the monoamine neurotransmitters.

Protein markers for CNS integrity

The CSF thus contains major protein fractions, such as albumin, originating from the blood plasma, and, in addition, minor protein fractions derived from the CNS tissues. The beta trace protein (β TP) is identical with prostaglandin D₂-producing enzyme (Watanabe, 1994) and is also a retinoid. The β TP may be used as a general marker for CSF proteins, as it is mainly synthesized in the CNS in the dura mater and choroid plexus (Blodorn et al., 1999), besides minute quantities peripherally (e.g. in the male genital system and the heart, Urade, 2000). Changes in protein concentrations may be due not only to changes in protein synthesis or the macromolecular permeability of the BBB, but also to changes in the rate of CSF production and/or changes in drainage resistance (Edsbacke et al., 2004).

The integrity of the barrier systems is routinely assessed by the CSF/serum albumin ratio. Normal reference values for comparisons have been established among healthy volunteers (Blennow et al., 1993). Increased CSF/serum albumin ratios have been reported in patients with CNS lesions, traumas, and tumors (Rapoport 1976), but also in psychiatric conditions such as psychoses (Axelsson et al., 1982, Bauer and Kornhuber 1987), suicide attempters (Bayard-Burfield et al., 1996), and violent offenders (Anckarsäter et al., 2005). The BBB permeability is increased by several inflammatory mediators, such as bradykinin, histamine (by H₂-receptor activation), and arachidonic acid (Abbott 2000). In an assessment of CSF proteins in neurosurgical interventions, de Vries and coworkers (2001) found that albumin ratios and other protein markers, such as the S-100 protein and myelin basic protein, were raised in subjects with various types of neurological diseases.

Inflammatory reactions in the CNS

Inflammatory reactions in the CNS may be assessed by some of the minor protein fractions in electrophoresis, such as immunoglobulins, β -2-microglobulin (β 2M) and other proteins associated with inflammation. Increased IgM indicates an acute infectious process, while IgG is elevated in chronic inflammatory states. The β 2M is

found both in serum and in the CSF. It is a membrane-associated small protein forming a part of the major histocompatibility complex essential for immunological activation (Rosano 2005). In the CSF, it is thought to reflect the turn-over of cell membranes and the activation of lymphocytes, both of the B and T type. Increased CSF concentrations of $\beta 2M$ have been found in CNS infections such as meningitis or encephalitis (both viral and bacterial, Ibsen 1980), CNS neoplasms such as leukemia or lymphomas (Mavlight et al., 1980), and in AIDS-related CNS involvement (Lazzarin et al., 1992).

Neurotransmitter-specific brain systems

The monoaminergic brain systems (using serotonin, dopamine and norepinephrine as neurotransmitters) are phylogenetically old and of core importance for CNS functionality. The activity in these systems may be approximated by the CSF concentrations of their main metabolites 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). These systems influence vast parts of the brain, especially functions that are involved in coping and survival. Other transmitter systems may also be assessed through CSF markers, such as metabolites of GABA and glutamate.

Serotonin

Serotonin (5-hydroxytryptamine) is mainly synthesized in the raphe nuclei, which are centered in the brainstem reticular formation, from the amino acid tryptophan by two enzymatic steps catalyzed by tryptophan hydroxylase and amino acid decarboxylase. Axons from the raphe nuclei terminate in widespread areas of the brain, among others in the thalamus, limbic system (hypothalamus, hippocampus, amygdala, and the striatum including the accumbens nuclei), certain areas of the cortex (such as the cingulate gyri), the cerebellum (the deep cerebellar nuclei and the cortex), and the spinal cord. Serotonin is involved in a vast number of different brain functions, such as appetite, mood control, sleep, memory, learning and muscular tone. Peripherally, serotonin is important for e.g. blood clotting (platelet aggregation) and for intestinal movements.

Serotonin activates several classes of receptors on the cell bodies, pre- and postsynaptic terminals, and dendrites of the neurons. Except for the 5-HT₃ receptor, which is a ligand-gated ion channel, all serotonin receptors are G protein-coupled transmembrane receptors, which activate an intracellular second messenger cascade (Nichols et al., 2008). Serotonin is inactivated by a specific reuptake transporter on the presynaptic neuron or by degradation through the enzyme monoamine-oxidase (MAO) in the synapses or perisynaptic neurons. Drugs that increase serotonin neurotransmission include reuptake inhibitors (e.g. selective serotonin reuptake inhibitors, SSRI) and blockers of enzymatic degradation (e.g. MAO inhibitors) used

to treat depression and anxiety in psychiatry. Specific serotonin receptor antagonists (for the 5-HT₃ receptor) are powerful antiemetics used to treat nausea. One of the major serotonin metabolites is the 5-HIAA, which is excreted in the urine and can be measured in blood and CSF samples.

Dopamine

Dopamine is both a hormone and a neurotransmitter. Dopaminergic neurons form different neurotransmitter systems, originating in the pars compacta of the substantia nigra, in the ventral tegmental area, and in the hypothalamus (especially the arcuate nucleus). Axons from these nuclei project to large areas of the brain through four major pathways: the nigrostriatal (motor control), the mesocortical (cognition, memory, learning), the mesolimbic (motivation, pleasure), and the tubero-infundibular pathways. In the latter, dopamine is released as a hormone from the hypothalamus to inhibit the release of prolactin from the pituitary.

Dopamine is synthesized from the amino acid L-tyrosine by tyrosine hydroxylase to L-DOPA, and subsequently by dopa-decarboxylase to dopamine. Dopamine receptors are all G protein-coupled receptors (Missale et al., 1998). Dopamine is inactivated by reuptake in the presynaptic neuron (by the dopamine transporter) or enzymatic breakdown by catechol-O-methyl transferase or MAO. The HVA is its main metabolite, and can be measured in CSF and urine. As a drug, dopamine increases the heart rate and blood pressure, but as it cannot cross the BBB, it cannot affect the nervous system when administered systemically. Therefore, in order to treat CNS dopamine deficiency (in for example Parkinson's disease), the precursor L-DOPA has instead been developed as a pharmaceutical. Dopamine receptors are also the targets of several other types of drugs, such as antipsychotics, which are generally dopamine receptor antagonists, and psychostimulants, which are typically indirect agonists of dopamine receptors or increase dopaminergic transmission through presynaptic release or diminished re-uptake. The unselective MAO inhibitors used to treat depression also increase dopaminergic neurotransmission, as does the selective MAO-B inhibitor selegiline.

A strong co-variation between CSF HVA and 5-HIAA has been noted in many studies (e.g. Ågren et al., 1986), consistent with the notion that the serotonergic system exerts a modulatory regulation of the dopaminergic activity (Geraciotti et al., 1998).

Norepinephrine

The norepinephrinergic brain systems originate from an area of the brain stem called the locus coeruleus. Norepinephrinergic neurons project bilaterally from this area along distinct pathways to many parts of the CNS, including the cerebral cortex, limbic system, and the spinal cord. This system enhances alertness and cognition

globally in the CNS. Norepinephrine neurons also originate in the lateral tegmental field. Norepinephrine is released from postganglionic neurons of the sympathetic nervous system in response to stress. The adrenal medulla is also a form of postganglionic nerve cells, although they release norepinephrine into the blood as a hormone. Norepinephrine is thus both a neurotransmitter in the CNS and the sympathetic nervous system, and a hormone.

Norepinephrine is synthesized from dopamine by dopamine beta-hydroxylase. The actions of norepinephrine are carried out via the binding to adrenergic receptors, which are also G protein-coupled molecules (Zill et al., 2003) characterized as subtypes of either alpha or beta receptors.

Along with epinephrine, norepinephrine underlies the fight-or-flight response, directly increasing heart rate, triggering the release of glucose from energy stores, and increasing blood flow to skeletal muscles.

Neuroendocrine markers

Thyroid hormones

Thyroid hormones are secreted from the thyroid gland into the blood and are essential for the adaptation of metabolic processes to the different demands that face an individual across life situations. Their effects are dependent not only on the pituitary thyroid stimulating hormone (TSH) and the subsequent synthesis of thyroxine (T_4) and triiodothyronine (T_3) by the thyroid gland, but also on a number of factors in the transport (protein-binding to albumin, transthyretin and thyroid-hormone binding globulin), metabolism (de-iodination of T_4 to form the more active T_3 and the inactive rT_3), and binding of the hormone to intra-cellular receptors. The circulating hormones cannot pass the BBB freely, even if free T_4 to some extent may cross the choroid plexus (Southwell et al., 1993). T_4 is actively transported into the brain by the organic-anion-transporting polypeptide 1c1 (OATP1c1) (Friesema et al., 2005) and transthyretin (Schreiber 2002) and is subsequently de-iodinated by deiodinases (type 2 and type 3). These enzymes contribute to the control of thyroid hormone action in the nervous system by regulating the local concentrations of T_3 , the main active thyroid hormone. The regulation of the deiodinases by many factors in addition to the thyroid hormones indicates that their role is not limited to mitigate the fluctuations in plasma T_4 and T_3 (Courtin et al., 2005). The transport proteins may also play a role in the distribution of T_4 from the CSF into the brain (Kassem et al., 2006). Thyroid hormones increase metabolism by binding to intracellular receptors to influence the DNA transcription. Deficiency of thyroid hormones leads to severe dysfunctions of all organs in the body, especially the brain, and may cause irreversible brain damage under development (cretinism) and a broad range of psychiatric and cognitive problems in developed persons.

Animal studies have pointed to possible interactions between the hypothalamo-pituitary-thyroid axis and the activity in the central nervous monoaminergic systems. In rats, T₄ increased the synthesis of monoaminergic neurotransmitters (Claustre et al., 1996), and stress increased the secretion of both thyroid hormones and catecholamines (Fukuhara et al., 1996). Neonatal treatment with triiodothyronine evoked persisting increases in β -adrenergic receptor binding (Lau et al., 1985), and, conversely, early postnatal administration of drugs antagonistic to the dopaminergic or the serotonergic systems resulted in a persistently high basal secretion of TSH (Mess et al., 1989). Conflicting results from studies of serotonergic effects on thyroid hormone synthesis have been presented, reporting increased TSH and T₄ in combination with decreased T₃ (Morley et al., 1980). In birds, the dopamine precursor L-DOPA inhibited thyroid activity, while α -adrenergic stimulation had the opposite effect (Hordiienko et al., 1995).

Insulin

Insulin is secreted from the Langerhan's islets in the pancreas into the blood in reaction to metabolic needs. The role of peripheral insulin as a regulatory hormone for metabolic processes has been extensively studied in humans. Not until recently, however, has the role of insulin as a neuromodulator in the CNS attracted interest. Insulin controls neuronal glucose metabolism, promotes synthesis of acetylcholine (Hoyer 2003), and influences the activity in the dopamine and noradrenalin systems (Kwok and Juorio 1988, Montefusco et al., 1983). Insulin may function as a transmitter in the CNS via effects on membrane-bound insulin receptors, facilitating potassium channel permeability (Plum et al., 2005). As the brain probably does not synthesize insulin itself, it has to be taken up across the BBB (Banks 2004). The transport system for insulin is a receptor-mediated transcytosis that seems to be saturable, meaning that increased serum levels of insulin are not followed by proportional increases in the CSF (Banks et al, 1997a, Israel et al., 1993). Conditions reported to affect insulin transport across the BBB include obesity (Kaiyala et al., 2000), starvation (Urayama and Banks 2008), ageing (Pirola et al., 2004), inflammation (Banks et al., 2008; Xaio et al., 2001), and dexamethasone treatment (Baura et al., 1996). Increased insulin transport into the CNS is observed in diabetes mellitus (Banks et al., 1997b). In obese mice, reduced levels of brain insulin are thought to be caused by a defect in insulin transport across the BBB, and CNS insulin is thought both to be influenced by nutrition and to be a mediator of appetite (Strubbe et al., 1988; Urayama and Banks 2008).

Aims of the thesis

This thesis is based on the following aims, which were formulated pre-hoc and detailed in the ethical review board application:

1. to assess whether a surgical intervention is associated with an increased BBB permeability
2. to assess whether CSF concentrations of monoamine metabolites are associated with hormonal reactions to surgery
3. to assess whether peripheral thyroid hormone concentrations are associated with intrathecal thyroid hormone concentrations
4. to describe overall changes in monoamine neurotransmitters and hormones during a surgical intervention

Further pre-study aims included psychiatric and personality related assessments and their relations to the changes in neurotransmitters and hormones during surgery. These results will be published elsewhere. The study group assembled here has also been used as controls in studies on for instance multiple sclerosis and neuroborreliosis. At the time of writing this thesis, another three papers from the project are published or submitted for publication, and several are being written up.

Subjects and Methods

Subjects

This study involves 35 psychiatrically and neurologically healthy patients undergoing knee prosthesis surgery (20 men, 15 women, aged 51-82 years, median 73). One patient had bilateral surgery in the same session, while the others were unilateral interventions. The subjects were chosen to have as few complicating medical disorders as possible. Given the age range of the patients, it was not possible to include overall "healthy" patients. Exclusion criteria were: 1. psychotropic, 2. anti-Parkinson, 3. systemic corticosteroid, or 4. anticoagulant treatment. Twenty-one patients had hypertension and seven had diabetes mellitus, with the following ongoing treatments: beta-blockers (11 cases), ACE-blocker (one case), angiotensin-2 antagonists (two cases), diuretics (two cases), and calcium-blockers (two cases). Seven patients had per-oral antidiabetics. One patient had a long-term medication with 10 mg of the serotonin reuptake inhibitor citalopram, which was not detected before the final analyses, as a generic substitution was used. Three patients had an as-needed prescription of weak opioid pain killers. All subjects had been on these medications for a long and stable period. Non-steroid anti-inflammatory drugs were routinely discontinued well before surgery in order to have normal coagulation at lumbar puncture. No other abrupt wash-out was performed for the purpose of the study. No per-oral medication besides beta-blockers was given on the day of surgery.

Routine pre-operative blood tests were overall within normal reference values, apart from three subjects with diabetic nephropathy who had creatinine concentrations $>100 \mu\text{mol/L}$. All subjects had per-oral pre-medication with paracetamol 1 gram, and an intraoperative 1 gram of tranexamic acid treatment. Before onset of anesthesia, all patients had a BD™ 1.10x45 millimeter arterial catheter inserted in the right or left radial artery. After a subcutaneous local anesthesia with 10 mL of mepivacaine 5mg/mL, a lumbar puncture was performed with an 18-Gauge Portex™ epidural needle in the L3-L4 interspace and 12 mL of CSF was sampled after disposing of 2-3 mL to avoid admixture of puncture bleeding. In one case with a visible puncture bleeding, CSF was let flowing until all visual signs of blood admixture had disappeared. After the sampling, the catheter was inserted, and an initial spinal anesthesia was administered with 3 mL of bupivacain 5mg/mL. Fifteen mL of blood for serum analyses, 20 mL of EDTA blood for plasma analyses, and 20 mL of EDTA blood for possible later genetic analyses were simultaneously sampled. The blood for genetic analyses was stored in -70°C without any further treatment, while the CSF and blood for serum and plasma analyses were centrifuged at 2000 g for 10 minutes to eliminate cells and other insoluble material and pipetted in new tubes for transport to the neurochemistry lab. Aliquots were then stored at -80°C until biochemical analyses. The first set of samples was labeled the "A-samples".

After surgery, further pain control was given as needed by intrathecal bupivacain infusion. Three hours after the completion of surgery, "B-samples" were drawn (after 2 mL that were disposed to avoid any admixture of ongoing infusions), and at 8 am on the morning after surgery, "C-samples" were drawn by the same procedure.

During surgery, the following medications were administered: propofol as a continuous infusion to all patients using the bispectral index (BIS) to titrate the dosage for an optimal sedation of BIS 70, yielding a total dosage of propofol ranging from 102 to 1423 mg, with a mean of 392 mg (standard deviation 228). The BIS was measured continuously and noted every 15 min (between 6 and 15 times in the patients depending on the duration of the intervention). Mean BIS in the patients ranged from 56 to 84, with a mean of 75 (standard deviation 6). Because of technical problems, the BIS could not be measured in four patients and the propofol administration had to be guided by clinical assessments. The following drugs were administered: between the A and B samplings, i.e. during the intervention and the first hours after, phenylefrine (20 cases), ephedrine (4 cases), atropine (8 cases), metoprolol (1 case), droperidol (2 cases), morphine and petidine chloride (1 case), ketobemidone (1 case), fentanyl (1 case), diazepam (1 case), and ondansetrone (1 case), and between the B and C samplings, ketobemidone (10 cases), morphine (7 cases), fentanyl (2 cases), ondansetrone (4 cases), atropine (4 cases), dixyrazine (6 cases), ephedrine (1 case), phenylefrine (4 cases), diazepam (3 cases), bethametazone (1 case), droperidol (4 cases), and zopiclone or zolpidem in normal sleeping dosage (14 cases).

Bupivacaine was administered intrathecally as a 5 mg/mL (0.5%) solution in natrium chloride, between the A and B samplings with a mean dosage of 3.7 mL (range 3–7.4 mL, standard deviation 0.84, 3 mL of which constituted the initial spinal anesthetic dose, which was repeated in the patient who had bilateral surgery) and between B and C with a mean dosage of 6.19 mL (range 2.80–11.40 ml, standard deviation 2.46). Three patients had a local instillation of ropivacaine (200 mg), ketorolac (30 mg) and epinephrine (0.5 mg) in the knee before termination of the operation according to another research protocol.

Methods

Laboratory methods

Albumin was analyzed by nephelometry on the Immage instrument (Beckman Coulter). β 2M and β TP were analyzed by nephelometry on the BNProSpec instrument (Dade Behring), using the NLatex β ₂-microglubulin kit, and the NLatex β TP kit, respectively. There were technical problems with the analyses of CSF β 2M and β TP in one patient at the B sampling and in another at the C sampling.

Monoamine metabolites were assessed by high-performance liquid chromatography (HPLC) with electrochemical detection, as described by Blennow and coworkers (1993). The intra-assay coefficient of variation was <5% for all metabolites.

Thyroid hormone concentrations in serum and CSF were determined by biochip array technique (Fitzgerald et al., 2005), on the Evidence Investigator (Randox, Crumlin, UK) according to the manufacturer's instructions, using the total thyroid array for TSH, total T₃ and total T₄. The array is a solid substrate device containing discrete regions of immobilised antibodies specific for the different thyroid hormones, which allows for simultaneous quantification of the different analytes from a single patient. Insulin was analyzed with a double antibody radioimmunoassay (Linco Research, St Charles, MO).

Missing results

The numbers of subject included in the different analyses varied to some extent. First, CSF could not be had from one patient at the B and C samplings and from another at C samplings, as there was no backflow in the CSF catheter. The patient with two missing samplings was omitted from all analyses, while the other was kept for the analyses of the results at the A and B samplings. Five patients were excluded from the analyses of proteins presented in Paper I as they had signs of a pathological BBB functioning at baseline or an initial bleeding at the lumbar puncture. In the laboratory, all analyses of albumin and monoamine metabolites were successful, while small numbers of subjects were lost for technical reasons in the other analyses (Table 1). Height and weight corrected monoamine metabolites could not be calculated in three subjects, as the information on height and weight could not be retrieved.

Table 1. Numbers of subjects remaining after various problems during the study. The whole study group contained 35 patients. One patient was excluded from all analyses as neither B nor C samples could be obtained, and another was available for the A and B samplings only. Thirty-four patients thus remained for the A and B samplings and 33 patients for the C samplings. Further missing data were due to either laboratory or clinical problems as described below, giving the final numbers for each analysis in the column on the far right. If not indicated otherwise, the results presented in this thesis are based on the groups as specified in this Table.

Paper	Back-flow problems	Lab problems	Clinical considerations	Remaining Numbers
I. Proteins: albumin	1: no back-flow at B & C (excluded from all analyses) 1: no back-flow at C		4: pathological albumin ratios at A 1: outlier at B with initial bleeding	29 at A & B 28 at C
I. Proteins: β TP & β 2MG	1: no back-flow at B & C (excluded from all analyses) 1: no back-flow at C	1: at B for CSF 2: at C for CSF	4: pathological albumin ratios at A 1: outlier at B with initial bleeding	29 at A 29/28 at B 29/27 at C (serum/CSF)
II. Monoamine metabolites	1: no back-flow at B & C (excluded from all analyses) 1: no back-flow at C	3: height unknown		34 at A & B 33 at C
III. Thyroid hormones	1: no back-flow at B & C (excluded from all analyses) 1: no back-flow at C	2: at B for CSF		34 at A 34/32 at B 34/33 at C (serum/CSF)
IV. Insulin ¹	1: no back-flow at B & C (excluded from all analyses) 1: no back-flow at C	1: at B and C for serum, 1: at A and B, and 6 at C for CSF	1: outlier with initial bleeding.	33/32 at A 32/31 at B 31/26 at C (serum/CSF)

¹ In paper IV, analyses of albumin, β TP and T_4 were recalculated for the subjects who were available with measures from all three samplings and therefore could be included in the ANOVAs, leading to slightly different figures as compared to the previous publications.

Data administration

All data were reported to the research team, who entered laboratory results into an SPSS database together with structured information from the medical records. All medical files from the surgical interventions were available to the research team throughout the study. All computerized information and laboratory analyses were

made on coded, anonymized data while a paper code was kept in a safe as research documentation.

Statistical analyses

For each type of neurobiological measure (proteins, monoamine metabolites and hormones), concentrations in serum and CSF at the three samplings were first plotted. To analyze whether there were significant differences between the samplings, two-way ANOVAs were also performed with concentrations as dependent variable, time periods as main effects and subjects as block effects for each concentration. The p-values derived from the ANOVAs were adjusted for multiple comparisons (the number of analyses presented in each paper, not recalculated for the total number of analyses in the thesis) with Tukey's post-hoc tests. In some of the papers, other types of statistical analyses were used (e.g. paired samples t-tests, ANOVAs with individual p-values between the samplings and Bonferroni corrections), but these were recalculated by the described ANOVAs (with one overall assessment of probability) in order to facilitate comparisons of results and to enhance the readability of the thesis. The means and standard deviations provided in the thesis are based on all subjects included in the analyses as detailed in Table 1. In Paper I and III, they were instead based on those subjects included in the t-tests or ANOVAs, which accounts for some minor discrepancies between figures.

The second analytical step performed for each measure was the stability assessed as Pearson correlations (or Spearman for thyroid hormones) between the results at A, B and C. A high correlation coefficient indicates covariance between high vs. low individuals at the different samplings. Negative correlations between these measures were not expected.

In the third step, for the proteins and hormones that were assessed both in serum and CSF, ratios between the CSF concentrations over the serum concentrations were plotted at the three samplings. These ratios were subsequently compared by ANOVAs, just as described for the individual concentrations.

In the fourth step, co-variance between CSF and serum concentrations was assessed by Pearson's correlations (or Spearman for thyroid hormones), where a high correlation will indicate that subjects with high CSF concentrations also have high serum concentrations and vice-versa, meaning that the CSF concentrations reflect those found in serum. A negative correlation would indicate the opposite, albeit improbable, pattern.

In the fifth step, co-variance between different proteins, neurotransmitters and hormones was assessed by correlations.

Finally, co-variance between the different measures and possible confounders was assessed by correlations according to the principles described above.

Study parameters were assessed to be overall normally distributed, and group comparisons were therefore made by ANOVAs and correlations by Pearson's model, while the non-parametric Spearman rank correlations were used for analyses of co-variance with thyroid hormones, some of which had skewed distributions. For correlations, a lower significance level of 1% was chosen instead of corrections of p-values in view of the large number of comparisons performed and the comparatively limited power of the study (for further discussion of the problem of mass significance and power, see below and in the Discussion part of the thesis).

All statistical analyses were performed using the SPSS 12.0, 14.0 or 17.0, the Sample Power 2.0 or the SAS 8.2 soft-wares (further references omitted). All p-values are two-tailed.

Statistical power

Pre-study power analyses as submitted to the ethical review board arrived at an approximate sample size of 50 in order to have 60% power to detect a correlation at $r \geq 0.30$, i.e. with an explanatory value of about 10% (the strength of co-variance may be assessed by the squared correlation coefficients, r^2 , which is an estimate of the proportion of the variance in one of the variables that may be statistically referred to the variance in the other). This may be regarded as a lower level for associations between parameters to be considered biologically meaningful. In descriptive studies with a large number of measures under study, power analyses are, however, difficult to carry out pre-hoc. During the study period, determinations of albumin and monoamine metabolites were available to the authors, while thyroid hormones and insulin concentrations were analyzed after the completion of the data collection. We performed no statistical analyses during the data collection but saw that large changes in both albumin and monoamine metabolite concentrations were present in a vast majority of cases. In view of this finding, we stopped data collection at 35 subjects to be able to analyze this as a pilot study, which is presented here and will be used to design further studies, for which we will have access to data that will permit more exact pre-hoc power analyses for the variables studied here (using the effect size calculated by the mean change in the parameter from A to C and the common standard deviation, calculated by the standard deviations at A and C and the correlation between the measures in the Sample Power 2.0 soft-ware).

Once significant differences have been demonstrated, it may be argued that the need for power analyses has been satisfied. In contrast, it is crucial to keep a lack of pre-hoc power analyses in mind when interpreting negative findings. In order for negative findings to be taken as a true lack of correlation or difference, the power of the study has to be considered. With the present sample size, we had an 82% power to detect differences between samplings with an effect size of 0.5 (i.e. the difference between the means/the common standard deviation), or a 71% power to detect correlations at $r \geq 0.40$ (with a value of explanation of about 15%). Negative findings can therefore be interpreted as an argument against the existence of larger differences or stronger associations than these, but not for differences or correlations with smaller effect sizes.

Multiple comparisons

Power analyses refer to the risk of type II errors (or “false negative errors”), i.e. assuming that there is no difference or association when there in fact is one. Mass significance refers to the opposite type of errors (type I or “false positive errors”), namely to assume that there is a difference or association when in fact there is none. With an alpha at 0.05, i.e. the level of significance customarily set at 5%, one analysis in every twenty will be positive by chance rather than by fact. This means that the more analyses made in a study, the more likely it will be that random differences are interpreted as real. To counteract this risk, a number of corrections of the p-values have been developed. It has to be remembered that such corrections also reduce the power of the study, meaning that power and corrections for mass significance have to be weighted against each other. We have corrected the results of the overall ANOVAs used to identify statistically significant differences between samplings by Tukey’s method in order to have probability estimates that have taken the number of analyses in each section into account. For correlations, the level of significance was lowered to 1% in view of the large number of correlations assessed. The most important factor to consider in the interpretation of biological measures is, however, the raw data. When over 80% of patients have large changes in, for example, CSF albumin or monoamine metabolite concentrations, it may be safely assumed that these changes are neither random nor caused by any confounder present only in a subgroup of the subjects, such as diabetes mellitus. In contrast, it remains an open question whether they are caused by the stressful surgical event directly or by some confounder that is present across the whole sample, such as propofol administration or spinal anesthesia.

Ethical considerations

All potential subjects were given written information about the study and opportunities for questions and discussions. No complications were noted. One subject even had a note to the editor published in the local newspaper, where he expressed his appreciation of how well he had been treated and of being part of a research initiative in a “countryside” hospital. The subjects earned no material compensation for their participation.

The project was approved by the Research Ethics Committee at the University of Gothenburg (Dnr 476-02).

Results

Proteins: Paper I

Proteins

Both serum and CSF concentrations of albumin decreased significantly during surgery (between the A and B samplings), and the serum concentrations remained low, while the CSF concentrations of albumin continued to decrease significantly also between the B and C samplings (Table 2). All but one subject (96%) had decreased CSF concentration of albumin and all but two (93%) had decreased serum albumin concentrations, while the other had concentrations that remained unchanged.

Table 2. Mean \pm standard deviations of protein concentrations at the different samplings and *p*-values from two-way ANOVAs with post-hoc Tukey's corrections for multiple comparisons. Means and standard deviations are calculated on all subjects included in the analyses (while those with one missing value are excluded from the statistical group comparisons), explaining small differences with the corresponding Table in Paper I).

	A samplings	B samplings	C samplings	ANOVA
Serum albumin g/L	37.48 \pm 3.59	32.03 \pm 2.54	32.25 \pm 2.94	F=53.36 p<0.001
CSF albumin mg/L	246.59 \pm 68.35	204.48 \pm 91.24	163.61 \pm 51.53	F=22.24 p<0.001
Serum β TP mg/L	0.66 \pm 0.21	0.63 \pm 0.23	0.63 \pm 0.27	F=1.64 p=0.20
CSF β TP mg/L	16.16 \pm 6.32	15.75 \pm 6.97	15.50 \pm 6.81	F=0.56 p=0.57
Serum β 2M mg/L	1.71 \pm 0.58	1.73 \pm 0.56	1.79 \pm 0.64	F=1.22 p=0.30
CSF β 2M mg/L	1.03 \pm 0.40	1.29 \pm 0.37	1.28 \pm 0.36	F=28.04 p<0.001

Serum and CSF concentrations of β TP did not change during or after surgery. The serum concentrations of β 2M remained unchanged, while the CSF concentrations of β 2M increased significantly during surgery and remained high. Increased CSF concentrations of β 2M during surgery were noted in 86% of patients.

Correlations between protein concentrations at the A, B and C samplings

Strong correlations between the three samplings were noted for albumin, β 2M and β TP both in serum and CSF (data not shown).

CSF/serum ratios of proteins

The CSF/serum albumin ratios, which remained unchanged from the A to the B samplings as both the CSF and serum concentrations decreased in parallel, decreased significantly from the B to the C samplings, as the CSF concentrations of albumin continued to decrease while the serum concentrations remained unchanged (Table 3). A decreased CSF/serum albumin ratio was noted in 85% of study subjects. β TP concentrations remained unchanged both in the CSF and the serum during surgery and their ratio was unchanged across the three samplings.

Table 3. CSF/serum ratios of protein markers at the A, B and C samplings with *p*-values from two-way ANOVAs with post-hoc Tukey's corrections for multiple comparisons (NB that the albumin ratio compares mg/L in CSF with g/L in serum and therefore is a 1000-fold smaller than the other ratios)

	A samplings	B samplings	C samplings	ANOVA
CSF/serum albumin ratio	6.60±1.78	6.41±2.82	5.09±1.55	F=8.22 p<0.001
CSF/serum β TP ratio	25.26±8.43	26.09±12.93	26.14±11.42	F=0.32 p=0.72
CSF/serum β 2M ratio	0.63±0.22	0.76±0.21	0.75±0.19	F=15.90 p<0.001

Correlations between serum and CSF concentrations of proteins

CSF concentrations of albumin did not correlate with serum albumin concentrations in any of the samplings (Table 4). Highly significant correlations were noted between the serum and CSF concentrations of β 2M at all samplings. For the β TP, the corresponding correlations between serum and CSF concentrations were significant at the A samplings but not at B or C.

Table 4. Correlations between serum and CSF concentrations of the proteins at the A, B or C sampling as specified in the rows (e.g., the correlation coefficient between serum albumin A and CSF albumin refers to the A samplings for both measures, while the correlation coefficient between the serum albumin B and CSF albumin refers to the B samplings for both measures etc.)

	CSF albumin A	CSF β 2M A	CSF β TP A
Serum albumin A	0.21 p=0.27	0.10 p=0.60	0.10 p=0.59
Serum albumin B	0.05 p=0.79	-0.04 p=0.83	-0.16 p=0.42
Serum albumin C	0.10 p=0.36	-0.03 p=0.62	0.02 p=0.48
Serum β 2M A	0.34 p=0.071	0.57** p=0.001	0.46 p=0.012
Serum β 2M B	0.19 p=0.16	0.52** p=0.004	0.19 p=0.34
Serum β 2M C	0.54** p=0.003	0.55** p=0.003	0.17 p=0.39
Serum β TP A	0.39 p=0.035	0.42 p=0.023	0.47 p=0.010
Serum β TP B	0.05 p=0.79	0.29 p=0.13	0.17 p=0.38
Serum β TP C	0.45 p=0.016	0.38 p=0.054	0.17 p=0.400

** Correlation is significant at the 0.01 level (2-tailed).

The CSF concentrations of albumin correlated significantly with the CSF concentrations of β TP and β 2M at all samplings (Table 5). The CSF/serum albumin ratios were significantly correlated with the CSF concentrations of β 2M at the B and C samplings. In contrast, the CSF/serum albumin ratios did not correlate with the corresponding CSF/serum ratios for β TP and β 2M, except at the B samplings, when the association between the CSF/serum ratios of albumin and β TP was significant. In addition (not included in the Table), the serum β TP tended to correlate with the CSF/serum albumin ratios at the A samplings ($r=0.43$, $p=0.019$) and correlated significantly at the C samplings ($r=0.50$, $p=0.007$).

Table 5. Correlations between the CSF albumin concentrations and the CSF β 2M and β TP concentrations, or between the CSF/serum albumin ratios and the CSF/serum ratios of β 2M and β TP (e.g. the CSF albumin concentration was correlated with the CSF β TP concentrations while the CSF/serum albumin ratio to the CSF/serum β TP ratio) at the samplings specified in the rows.

	CSF β 2M	CSF /serum β 2M ratio	CSF β TP	CSF/serum β TP ratio
CSF albumin A or CSF/serum albumin ratio A	0.50** p=0.006	0.20 p=0.30	0.68*** p<0.001	0.36 p=0.059
CSF albumin B or CSF/serum albumin ratio B	0.52** p=0.004	0.45 p=0.016	0.54** p=0.003	0.62*** p<0.001
CSF albumin C or CSF/serum albumin ratio C	0.76*** p<0.001	-0.12 p=0.56	0.57** p=0.003	0.11 p=0.59

** Correlation is significant at the 0.01 level (2-tailed).

*** Correlation is significant at the 0.001 level (2-tailed).

Proteins and confounding factors

No significant correlations were noted between any of the proteins, their relative changes and sex or age, or between their changes and the administered volumes of intrathecal or systemic fluids.

Monoamine neurotransmitters: Paper II

Monoamine metabolites and HVA/5-HIAA ratios

CSF HVA and CSF 5-HIAA increased in 32/33 subjects (97%) during the day and night following surgery. Their ratio also increased significantly during surgery but returned to nearly the initial value at the C samplings (the HVA/5-HIAA ratios at A did not differ significantly from those at C). The norepinephrine metabolite increased, but to a much lesser degree, during surgery and remained unchanged thereafter (Table 6).

Table 6. Mean \pm standard deviations of concentrations in nmol/L of studied parameters at the different samplings, and *p*-values from two-way ANOVAs with post-hoc Tukey's corrections for multiple comparisons.

	A samplings	B samplings	C samplings	ANOVA
CSF HVA nmol/L	259.24 \pm 113.93	386.97 \pm 161.81	446.45 \pm 208.15	F=43.65 p<0.001
CSF-5-HIAA nmol/L	174.09 \pm 55.02	229.62 \pm 71.75	281.45 \pm 76.51	F=86.13 p<0.001
CSF MHPG nmol/L	43.09 \pm 10.68	45.59 \pm 9.50	46.48 \pm 8.48	F=3.00 p=0.057
HVA/5-HIAA	1.52 \pm 0.54	1.73 \pm 0.58	1.59 \pm 0.55	F=6.33 p=0.0031

Correlations between monoamine metabolites at the A, B and C samplings

Strong correlations between the three samplings were noted for all the monoamine metabolite concentrations (data not shown).

Monoamines and confounding factors

No significant correlations were seen between age, sex, and any of the monoamine metabolite concentrations. There were no significant correlations between any of the changes in monoaminergic metabolite concentrations and administered doses of bupivacain, propofol, phenylephrine, atropine, ketobemidon or morphine, besides a positive correlation between atropine during surgery and the CSF HVA/5-HIAA ratio.

Thyroid hormones and insulin: Papers III & IV

Thyroid hormones (including the T₃/T₄ ratios) and insulin

During the study period, the availability of activated T₃ in serum decreased significantly, both in absolute figures and as the relative fraction compared to the precursor form T₄ (assessed as the serum T₃/T₄ ratio). Also the serum T₄ and TSH concentrations decreased. In the CSF, T₃ increased non-significantly and T₄ decreased, thus leading to a significant increase in the CSF T₃/T₄ ratio, opposite from that in the periphery, indicating that the availability of activated thyroid hormone in the CNS increases during a surgical intervention (Table 7).

Serum insulin levels were lower directly after surgery as compared to before, but they were very much higher on the morning after surgery than at any of the previous samplings, although with a wide variation (the standard being half of the mean concentration). CSF insulin concentrations showed considerably smaller changes with less variation, they decreased, like serum insulin, significantly between the A and B samplings, but did not change significantly between A and C, even if there was a trend towards a change in the same direction as the serum insulin.

Table 7. Mean \pm standard deviations of hormone concentrations at the different samplings with *p*-values from two-way ANOVAs with post-hoc Tukey's corrections for multiple comparisons. Means and standard deviations are calculated on all subjects (while those with one or two missing values are excluded from the ANOVAs), explaining small differences with the corresponding Table in Paper III).

	A samplings	B samplings	C samplings	ANOVA
Serum TSH (μ IU/mL)	1.76 \pm 0.96	1.19 \pm 0.62	1.45 \pm 1.53	F=6.74 p=0.0022
Serum T ₃ (ng/mL)	1.40 \pm 0.20	1.24 \pm 0.18	1.09 \pm 0.21	F=47.79 p<0.001
Serum T ₄ (μ g/dL)	8.03 \pm 1.43	7.75 \pm 1.35	7.64 \pm 1.42	F=5.14 p=0.0084
Serum T ₃ /T ₄	0.18 \pm 0.080	0.17 \pm 0.074	0.15 \pm 0.053	F=25.33 p<0.001

CSF TSH ($\mu\text{IU}/\text{mL}$)	0.019 \pm 0.018	0.020 \pm 0.022	0.016 \pm 0.017	F=0.07 p=0.93
CSF T ₃ (ng/mL)	0.92 \pm 0.096	0.90 \pm 0.12	0.93 \pm 0.11	F=0.55 p=0.58
CSF T ₄ ($\mu\text{g}/\text{dL}$)	1.13 \pm 0.20	1.11 \pm 0.35	1.02 \pm 0.22	F=3.70 p=0.030
CSF T ₃ /T ₄	0.83 \pm 0.13	0.87 \pm 0.23	0.95 \pm 0.21	F=7.81 p<0.001
Serum insulin (pmol/L)	74.45 \pm 37.43	64.16 \pm 41.05	232.65 \pm 189.38	F=28.78 p<0.001
CSF insulin (pmol/L)	5.77 \pm 0.58	5.24 \pm 0.72	5.47 \pm 0.95	F=5.79 p=0.0053

Correlations between hormone concentrations at the A, B and C samplings

Strong inter-individual correlations were noted for T₄ and TSH between the A, B, and C samplings, while those for T₃ were all positive but not statistically significant (data not shown). For insulin in serum and CSF, the corresponding correlations were all significant.

CSF/serum ratios of thyroid hormones and insulin

The CSF/serum ratios of T₃ and TSH increased, while the same ratios for T₄ remained unchanged (Table 8). The CSF/serum ratios of insulin first increased from the A to the B samplings but decreased sharply between the B and C samplings.

Table 8. CSF/serum ratios of hormones at the A, B and C samplings with p-values from two-way ANOVAs with post-hoc Tukey's corrections for multiple comparisons.

	A samplings	B samplings	C samplings	ANOVA
CSF/serum T ₃	0.67 \pm 0.13	0.74 \pm 0.13	0.89 \pm 0.19	F=41.69 p<0.001
CSF/serum T ₄	0.15 \pm 0.059	0.15 \pm 0.062	0.14 \pm 0.045	F=2.20 p=0.12
CSF/serum TSH	0.011 \pm 0.0073	0.017 \pm 0.018	0.015 \pm 0.011	F=5.19 p=0.082
CSF/serum insulin	0.095 \pm 0.045	0.11 \pm 0.056	0.047 \pm 0.042	F=29.11 p<0.001

Correlations between serum and CSF hormone concentrations

No correlations between serum and CSF concentrations of T₃ and T₄ were observed at any of the samplings. Serum and CSF concentrations of TSH were positively correlated (Table 9).

Table 9. Spearman rank correlations between serum thyroid hormones and the CSF hormones at each of the samplings as specified in the rows.

	CSF T ₃	CSF T ₄	CSF TSH
Serum T ₃ A	-0.11 p=0.56	-0.01 p=0.96	-0.00 p=0.99
Serum T ₃ B	0.07 p=0.71	-0.11 p=0.54	0.07 p=0.72
Serum T ₃ C	-0.01 p=0.97	0.02 p=0.94	0.13 p=0.48
Serum T ₄ A	-0.10 p=0.56	0.23 p=0.20	0.03 p=0.88
Serum T ₄ B	0.20 p=0.26	0.35 p=0.049	0.08 p=0.66
Serum T ₄ C	-0.02 p=0.92	0.27 p=0.12	0.09 p=0.63
Serum TSH A	-0.27 p=0.13	-0.44** p=0.009	0.56** p=0.001
Serum TSH B	-0.31 p=0.089	-0.29 p=0.11	0.45** p=0.009
Serum TSH C	0.17 p=0.34	-0.20 p=0.27	0.34 p=0.051

** Correlation is significant at the 0.01 level (2-tailed).

Concentrations of insulin in serum and CSF did not correlate at either A or B ($r=0.31$, $p=0.084$ and $r=-0.17$, $p=0.36$, respectively), while there was a significant correlation between the two at C ($r=0.58$, $p=0.002$).

Correlations across thyroid hormones

Serum TSH was initially negatively correlated with the CSF T₄ (A rho=-0.44, p=0.009), but not with serum concentrations of T₃, T₄ or TSH. CSF TSH was uncorrelated with other thyroid hormone concentrations in the CSF or serum. Serum T₃ was uncorrelated with serum T₄. CSF T₃ and T₄ were strongly correlated at the A samplings (rho=0.56, p=0.001) but this association was lost at the B and C samplings (rho=0.09 and 0.28, respectively).

Monoamine metabolites and thyroid hormones

The previously reported correlation between CSF MHPG and the serum T₃/ T₄ ratio was replicated in this study. It became even stronger if height and weight corrected MHPG values were used (rho=0.51, p=0.004, n=31). Baseline CSF monoamine metabolite concentrations were not related to thyroid hormones during surgery, while the baseline CSF thyroid hormone concentrations were strongly correlated to the subsequent changes in monoamine metabolite concentrations during and after surgery (data not shown, please see Paper III for details), indicating that pre-operative thyroid hormone activity in the CNS co-varies with changes in the brain monoaminergic activity during a surgical intervention. There were no significant correlations between serum and/or CSF insulin concentrations and CSF monoamine metabolite concentrations that would have survived any reasonable testing for multiple comparisons.

Insulin and BMI

The BMI was significantly correlated with the serum insulin concentrations at the A (r=0.53, p=0.002) and B (r=0.35, p=0.053), but not at the C samplings. There were no correlations between the BMI and CSF insulin concentrations at either point, and no correlations between any of the albumin concentrations and BMI (data not shown). Significant correlations between the CSF/serum insulin and BMI in the predicted negative directions were thus due to the increases in serum insulin concentrations that were not followed by similar increases in CSF insulin concentrations among those with higher BMI.

CSF/serum ratios of proteins and hormones

As we have seen, CSF/serum albumin ratios decreased significantly after surgery, while the corresponding ratios for βTP remained unchanged. In contrast, the CSF/serum ratios of T₃ increased, and the CSF/serum insulin ratios first increased and then decreased. There were no correlations between the CSF/serum ratios of albumin, T₃ and insulin at the A, B, or C samplings, or between the *changes* of these ratios from the A to B samplings, from the B to C samplings, or from the A to C samplings (data not shown).

Hormones and confounding factors

There were no significant correlations between any of the hormone measures and age, sex, total administered volumes of propofol, bupivacain, fluids or glucose, or time/duration of the interventions besides between T_4 and age ($\rho=0.46$ at the A and 0.47 at the C samplings, both $p=0.006$).

Summary of findings

Paper I

Contrary to the first pre-hoc hypothesis, CSF albumin concentrations *decreased* during and after surgery both absolutely and, after surgery, also in relation to the serum albumin concentrations. The CSF β -2-microglobulin concentrations and their ratios over the serum concentrations increased. These findings are consistent with a decreased permeability of macromolecules such as albumin from the blood into the CSF after a surgical intervention (an “oyster” reaction of the CNS), and with an increased intrathecal concentration of the inflammatory marker β 2M during non-neurological surgery with spinal anesthesia. B-trace protein concentrations remained unchanged across the three samplings both in serum and the CSF, indicating that the findings were not due to dilution or concentration effects in the CSF compartment.

Paper II

The CSF concentrations of the dopamine metabolite homovanillic acid (HVA) and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), which are related to the activity of the dopaminergic and serotonergic systems of the brain, increased sharply during surgery and after the interventions. The CSF concentrations of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglucol (MHPG) tended to increase during and after surgery. The HVA/5-HIAA ratios initially increased but returned almost to the baseline level during the night after surgery. These findings suggest that there is an increased serotonergic and dopaminergic neurotransmission during and after surgery, and that these systems are interconnected. The MHPG results are more difficult to interpret as a substantial proportion of CSF MHPG is derived from the sympathetic nerve system ganglia, which are blocked to a high thoracic level by the spinal anesthesia.

Papers III & IV

Serum concentrations of the active thyroid hormone, triiodothyronine (T_3), its precursor hormone thyroxine (T_4), and the pituitary thyroid stimulating hormone (TSH) all decreased during and after surgery. The CSF concentrations of T_3 instead remained unchanged, and the T_3/T_4 ratios, thought to reflect the activation of thyroid hormones, increased significantly in the CSF while it decreased peripherally. This may, however, only reflect decreasing CSF T_4 concentrations due to a reduced transport over the BBB. The third pre-hoc aim of the study was to assess correlations between serum and CSF levels of thyroid hormones. No significant correlations between these measures were found, besides the pituitary TSH. Serum insulin

decreased during surgery but increased dramatically after the interventions, while the CSF insulin concentrations remained unchanged during surgery. There was an overall correlation between serum and CSF insulin concentrations, but the increase in CSF insulin was not proportionate to that in serum. The CSF/serum ratios for thyroid hormones and insulin did not correlate with each other, which is consistent with specific, independent transport mechanisms for T₄ and insulin over the BBB. Generally, intrathecal variations in hormone concentrations were much smaller than the corresponding changes in their serum concentrations, again indicating that the CNS may be protected from strong fluctuations in peripheral hormones due to trauma, and that the transport mechanisms are saturable. The second hypothesis concerned the association between the monoaminergic activity and the thyroid hormone metabolism. We could replicate a previous finding of a correlation at baseline between the norepinephrinergic metabolite MHPG in the CSF and the serum T₃/T₄ ratio, but during surgery, instead of correlations indicating that the CNS monoamine systems influenced the intrathecal metabolism of thyroid hormones, there were strong correlations between the baseline CSF thyroid hormone concentrations and subsequent the CSF concentrations of monoamine metabolites, including their changes during surgery.

Discussion

General limitations

The limitations of the present study are obvious. The study sample is small and heterogeneous regarding concomitant medical disorders and long-term medications. Since the operation, knee prosthesis surgery, is generally performed on older people, several subjects had concomitant medical disorders and long-term medications. Seven of the patients had diabetes mellitus, a condition known to affect the levels of CNS insulin, and 19 were overweight with a BMI of over 25. The subjects were also exposed to both a significant stressor in the form of a surgical intervention and to anesthetic drugs (Desborough, 2000). We were unable to have continuous CSF samplings and therefore chose to collect samples three hours after termination of the intervention to pinpoint the chemical reactions during the surgical intervention considering the absorption time for the CSF (Edsbacke et al., 2004), and on the morning after surgery to assess the continued neurobiological activity during the night. We have considered possible signs of dilution or dynamic changes in the CSF as possible mediators behind the findings, and although we found no indications pointing in this direction, such possibilities obviously have to be further investigated, by, for example, radioligand methods, before they can be ruled out. Furthermore, it would have been advantageous to continue samplings for a few more days to study the return to normal, but this was judged unfeasible as the patients no longer had any need of intrathecal or arterial catheters.

As the study design did not include a non-surgery control group, confounding by factors across the majority or all subjects cannot be discarded. Propofol or bupivacaine may, for instance, be involved in one or several of the main findings. In view of these limitations, the study should be regarded as descriptive on the basis of correlations and observations: it includes no formal testing of causation behind the changes described in the patient group.

Interpretation of results

Large changes in CSF neurochemistry were noted during and after a non-neurological surgical intervention with spinal anesthesia. The analyses of proteins contained unexpected results. The CSF/serum albumin ratios decreased rather than increased. Based on the literature on BBB permeability and albumin ratios in various forms of pathological states, we had hypothesized that a surgical intervention would lead to increased BBB permeability. In contrast, the study showed that the CSF albumin concentrations actually decreased very significantly during these non-neurological surgical interventions and during the following night, as the serum albumin stabilized after surgery, the CSF/serum albumin ratios continued to decrease. As the CSF and serum β TTP concentrations (as well as the CSF/serum β TTP ratios) remained unchanged, dilution or any other change in CSF production or re-

absorption is not indicated as a main cause behind the changes in CSF albumin, which were also seen in over 90% of the patients. Nor did any of the proteins, or their changes, correlate with the administered volumes of venous or intrathecal fluids, providing a further argument against dilution as a major cause behind the noted changes. Further, as the changes were noted in almost all patients, the various co-existing medical conditions and pharmacological treatments given in subgroups were not implicated as confounding factors of general importance. It thus seems that a possible physiological mechanism behind the finding is that the BBB becomes less permeable for circulating macromolecules during a stressful event in an “oyster-like” fashion.

There was also an increase in the CSF concentrations of β 2M, the explanation of which is far from obvious. The CSF β 2M concentrations were significantly correlated with CSF albumin and CSF/serum albumin ratios, meaning that the CSF concentrations of β 2M were higher in the subjects who had higher CSF albumin concentrations and CSF/serum albumin ratios. As there were no changes in serum concentrations of β 2M, the increase in CSF β 2M does not seem to be related to peripheral inflammatory activity or changes in the BBB permeability. The most obvious explanation for this finding would be the lumbar puncture and the intrathecal catheter. There are, however, some circumstances that do not intuitively support this interpretation. The CSF concentration of β TP, which is more specific for the meninges than the β 2M, did not change. A local reaction to the catheter insertion would hardly lead to an increase in β 2M by more than 20% in the whole CSF compartment, even if there may, of course, be a gradient effect that gives an artefactually high local concentration in the lumbar CSF. Given these reservations, the results presented indicate an increased cellular turnover and immunological activation in the CNS during a non-neurological surgical intervention. Judging from our review of the literature, none of the administered drugs has been associated with changes in the β 2M or intrathecal inflammatory activity. It would be interesting to assess CSF β 2M and other inflammatory markers in a design that controls for the various steps during lumbar puncture, catheter insertion, spinal anesthesia and surgery. Bupivacain in higher concentrations than those used in this study has been shown to be neurotoxic (Takenami et al., 2005), and it would therefore seem especially important to study a possible association between the increase in β 2M and bupivacain in various concentrations used for spinal anesthesia.

Stressful events may influence the concentrations of monoamine metabolites in the CSF and carry a risk of neuropsychiatric reactions such as confusion. In healthy volunteers, CSF HVA and 5-HIAA concentrations almost doubled between lumbar punctures and samples taken after a rest of between three and nine hours (Hill et al., 1999). An older study measuring blood concentrations of monoamine metabolites after cholecystectomies also showed increases in all metabolite concentrations after

the intervention (Håkanson et al. 1984). The mechanisms behind such changes are not fully elucidated. Increased monoaminergic neurotransmission during stressful events seems to be the most probable explanation, but the diffusion time of metabolites along the spinal channel has to be considered, and so should administered drugs. A possible confounder in the present study is the sedating drug given to all subjects. Propofol sedation increased dopaminergic and serotonergic transmission in the somatosensory cortex in a microdialysis study on rats (Shyr et al., 1997). Propofol is thought to exert its effects by a non-specific binding to membranes. Propofol has a rapid distribution half-life of about two to four minutes, and a slower elimination phase with a half-life of about 144 min (Wessen et al., 1994). The increase in the CSF concentrations of HVA and 5-HIAA noted in our study did not correlate with the propofol dosage, and the metabolites remained high even on the day after surgery, which makes propofol an unlikely sole agent behind the increased neurotransmission, as it was only administered during surgery. Still, a propofol-induced process that remains active after the discontinuation of the administration itself might explain the continued increase. The CSF MHPG concentrations increased to a much smaller extent, but, in contrast to CSF HVA and 5-HIAA, MHPG is to a considerable extent derived from the spinal cord, which were blocked up to approximately the fourth thoracic segment by the spinal anesthesia. It is somewhat surprising that the CSF MHPG did not increase after the intervention. Even if the exact mechanisms cannot be identified in a non-experimental study like this, it may be concluded that the heterogeneous challenges during this kind of surgery seem to be associated with an increased activity in at least parts of the dopaminergic and serotonergic systems in the brain, but that the balance between the two transmitter systems is maintained through the changes in activity. Further studies are needed to clarify if the magnitude of these changes might help to predict psychiatric complications, such as confusion, in patients undergoing surgical interventions.

Serum thyroid hormones decreased considerably during the surgical interventions and during the following night. A significant decrease in serum TSH indicated that the decreased thyroid activity might be due to a down-regulation exerted from the pituitary under hypothalamic control. This did not reflect the spontaneous diurnal fluctuation in TSH secretion that normally gives the highest TSH levels during the night (Kok et al., 2005). Also the peripheral T_3/T_4 ratio decreased, which might reflect a reduced deiodination in the liver and kidneys serving to reduce the thyroid hormone activity during stress, or a consumption of T_3 in excess to the metabolic capacity. In contrast, the CSF T_3/T_4 ratio increased during surgery, reflecting an increased metabolism of thyroid hormones to increase their activity or a reduced transport of T_4 into the CNS. As the thyroid hormones increase metabolism, it may be physiologically beneficial to reduce their activity in acute stress, but it also seems obvious that they may play an important role in the adaptation of an individual to

situations where a higher demand is placed on the metabolic activity in order to adapt and survive. The data presented here may reflect an increased thyroid support of brain activity during a stressful event, which may be both necessary for the individual and physiologically possible—as the metabolic demands on the CNS are a mere fraction of those in the peripheral organs and muscles, in combination with decreased serum thyroid hormones and a metabolic down-regulation in acute stress. Separate mechanisms involved in the thyroid hormone metabolism in the CNS and peripherally are also supported by the lack of correlations between CSF and serum concentrations of thyroid hormones throughout the study. In the baseline samples, serum TSH was negatively correlated with the CSF T₄ concentrations, consistent with the inhibitory feed-back role of T₄, but this association was lost during the intervention. In contrast, the high correlations between the various samplings of the individual hormones indicated interindividual stability of thyroid hormone activity.

In the present study, we could replicate our previous finding of a positive correlation between the CSF concentrations of MHPG and the serum T₃/T₄ ratio at baseline (Yhede et al., 2003). This finding was initially interpreted as an indication that thyroid hormone metabolism in the liver and kidneys is under catecholaminergic control from the sympathetic nerve system. As the present study made it possible to assess CSF thyroid hormone concentrations, we also attempted to broaden this hypothesis to the CNS this time by comparing the baseline CSF concentrations of catecholaminergic metabolites to the subsequent changes in the thyroid hormone metabolism during the study period. The expected correlations did not emerge, however. To pursue this track, we tried the opposite analyses and compared baseline thyroid hormone activity with subsequent changes in monoaminergic neurotransmission. Strong correlations then emerged between baseline CSF thyroid hormone activity and the degree of increase in dopaminergic and serotonergic neurotransmission during surgery. This association suggests that the basal thyroid hormone availability in the CNS plays a role for the ability to react to stress by an increased monoaminergic neurotransmission.

The CSF insulin concentrations first decreased during surgery and then remained stable, while the serum insulin levels varied substantially both three hours after surgery and in the morning following surgery. As the mean serum insulin concentration more than tripled during the study, the mean CSF insulin concentration did not increase from baseline. It thus seems as if the CNS is protected from strong fluctuations in peripheral insulin levels and the transport over the BBB is saturable, even if the present study replicated, in humans, previous animal findings of correlations between serum and CSF insulin levels (Baura et al., 1993). The transport of insulin across the BBB is a specific receptor-mediated transcytosis distinct from the adsorptive transcytosis of albumin (Poduslo et al., 1994). The findings presented here indicate that the transport systems for albumin and insulin

are independent in humans, as the ratios for CSF/serum insulin and albumin were not correlated either at baseline or after surgery. The BMIs were strongly correlated with serum insulin concentration at the A samplings. However, there were no correlations between the BMIs and the CNS insulin concentrations. The negative correlations between the BMI and the CSF/serum insulin ratios could be said to be in accordance with the animal findings that the insulin BBB permeability is decreased in obesity, but we would rather argue that this association depends on the strong relationship between BMI and serum insulin concentrations, while the CNS insulin concentrations stood out as independent of the proposed predictors.

Future research directions

The results presented here call for further studies. Overall, there is a stunning lack of studies on intrathecal hormone metabolism and barrier functions in humans. If confirmed, the findings of decreased BBB permeability and changes in monoaminergic neurotransmission and intrathecal hormone metabolism may have implications for pharmacotherapy and hormone substitution in the perioperative care. For this purpose, causation and specific associations between the changes described here and the various stages of a surgical intervention have to be formally tested in quasi-experimental clinical studies and in animal models.

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