



University at Buffalo
The State University of New York
School of Medicine and Biomedical Sciences

DEPARTMENT OF
GYNECOLOGY-OBSTETRICS

Annual
RESIDENT RESEARCH DAY

Visiting Professor:

Douglas W. Laube, MD, MEd
Professor, University of Wisconsin
Immediate Past President,
American College of Obstetricians and Gynecologists

Wednesday, May 28, 2008

8:00am – 11:45am

Lunch – 12:00pm

Gyn-Ob Classroom, 3rd Floor

239 Bryant Street

Buffalo, New York

Resident Research Day Schedule

Wednesday, May 28, 2008

- Moderator:** *Glenna Bett, Ph.D.*
- 8:00 – 8:10 a.m.** “*Indoleamine 2,3 dioxygenase (IDO) and CD8+ T cell infiltration in Endometriosis.*”
Oluseyi Ogunleye, M.B.B.Ch.
Mentors: James Shelton, MS and Drs. Donald Slate and Kunle Odunsi
- 8:15 – 8:25 a.m.** “*Intraperitoneal Chemotherapy*”
Asia Mohsin, M.D.
Mentors: Drs. Shashikant Lele and Pankaj Singhal
- 8:30 – 8:40 a.m.** “*Metronomic Oral Cyclophosphamide or Altretamine for Palliation of Advanced Stage Epithelial Ovarian Cancer*”
Christopher Stevens, M.D.
Mentors: Drs. David Marchetti and Pankaj Singhal
- 8:45 – 8:55 a.m.** “*Postpartum (PP) Depression Patterns for Women Evaluated in the ACOG Depression Screening Project*”
Fatima Hina, M.D.
Mentors: Drs. Gennadiy Ivanov and Lani Burkman and Donna Slawek, NRP
- 9:00 – 9:10 a.m.** “*Prevalence of antepartum depression in Buffalo: early data from the NY State ACOG Perinatal Depression Project (2007- 08)*”
Gennadiy Ivanov, M.D.
Mentors: Drs. Fatima Hina and Lani Burkman and Donna Slawek, NRP
- 9:15 – 9:25 a.m.** “*Micrometastasis on Permanent Section after Diagnosis of Negative Frozen Section of Sentinel Lymph Node Biopsy: Effect on Recurrence Rate in Breast Carcinoma*”
Joseph A. DeNagy, D.O.
Mentor: Dr. Ronald Bauer
- 9:30 – 9:45 a.m.** **BREAK**
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- Moderator:** *Armando Arroyo, M.D.*
- 9:45 – 9:55 a.m.** “*Comparing the Efficacy of Foley and Pitocin against Cervidil for Induction in the Community Hospital Setting: A Retrospective Study*”
John Nguyen, M.D.
Mentor: Dr. Anthony R. Pivarunas
- 10:00 – 10:10 a.m.** “*Ovarian Vein Thrombosis After Hysterectomy*”
Seshadri Kasturi, M.B.B.S.
Mentor: Dr. Ali Ghomi
- 10:15 – 10:25 a.m.** “*C-Reactive Protein and its association with Pre-term Premature Rupture of Membranes*”
Rachel George, M.B.B.Ch.
Mentors: Drs. Kofi Amankwah and Lani Burkman

- 10:30 – 10:40 a.m.** “Colocalization of Synaptophysin in GnRH Neurons”
Bogdan Orasanu, M.D.
Mentor: Dr. Armando Arroyo
- 10:45 – 10:55 a.m.** “Cav1.2, Cav1.3 and Cav1.4 Channels are not Present in Rat GnRH Neurons”
Majid Shaman, M.D.
Mentor: Dr. Armando Arroyo
- 11:00 – 11:30am** “Ob/Gyn Resident Education”
Douglas W. Laube, MD, MEd
Professor, University of Wisconsin
President, American College of Obstetricians
and Gynecologists
- 11:45 a.m.** Presentation of Research Awards By
Dr. Douglas W. Laube
- 12:00 p.m.** Lunch in Gyn-Ob Library / Classroom

ABSTRACTS

“Indoleamine 2,3 dioxygenase (IDO) and CD8+ T cell infiltration in Endometriosis.”
Oluseyi Ogunleye, M.B.B.Ch.
Mentors: James Shelton, M.S., Donald Slate, M.D., and Kunle Odunsi, M.D., Ph.D.

Background: The immunoregulatory enzyme, indoleamine 2,3 dioxygenase (IDO), catalyzes tryptophan metabolism and plays a key role in the immune tolerance of the semi-allogeneic fetus. Since retrograde menstruation is common in reproductive women, but only a fraction develop endometriosis, we hypothesize that a diminished T cell response against ectopic endometrium may facilitate endometriosis. We also sought to determine whether the blunted immune response could be related to IDO mediated arrest of T cell proliferation.

Objective: To compare CD8+ T cell infiltration and IDO expression in patients with and without endometriosis.

Methods: Thirty-three cases (ages 20-45yrs) with laparoscopy proven endometriosis were selected and compared with thirteen controls with no previous documentation of endometriosis. We evaluated archived endometriosis samples, together with eutopic endometrial samples from both cases and controls (between 2006-2007). Tissue blocks from both cases and controls were sectioned and stained with CD8 and IDO antibodies. Each section was evaluated for CD8+ T cells by using a x40 objective lens. Twenty independent areas with the most abundant T cells were selected and scored. The count was performed twice for each photograph without knowledge of earlier results, and the average of the total counts for each patient was used for statistical analysis. Following antibody staining, the extent of immunohistochemical reactivity was graded as number of positive cells per 10 High powered (x40) fields. Positive and negative control slides were included in all assays. Statistical analysis was carried out using the Student's t-test and Pearson correlation (SPSS, Version 11).

Results: For the endometriosis group, CD8+ T cell infiltration was significantly less when compared to controls ($p < 0.01$). IDO expression, on the other hand, did not show a significant difference between cases and controls ($p = 0.464$). A positive correlation was found between the CD8+ T cell and IDO expression, with a Pearson coefficient of 0.381 ($p < 0.01$).

Conclusion: CD 8+ T cell infiltration is diminished in endometriosis and may imply an impairment of effective immunologic responses in the setting of retrograde menstruation. Although our sample size is limited, there appears to be no relationship between IDO expression and levels of CD8+ T cell infiltration. While this will suggest non-IDO related mechanisms for the diminished CD8+ T cell infiltration, additional studies are warranted to examine the relationship between IDO and ratio of CD8+T cells: to regulatory T cells.

“Intraperitoneal Chemotherapy”

Asia Mohsin, M.D.

Mentors: Shashikant Lele, M.D. and Pankaj Singhal, M.D., M.S.

NO ABSTRACT

“Metronomic Oral Cyclophosphamide or Altretamine for Palliation of Advanced Stage Epithelial Ovarian Cancer”

Christopher Stevens, M.D.

Mentors: David Marchetti, M.D. and Pankaj Singhal, M.D., M.S.

NO ABSTRACT

“Postpartum (PP) Depression Patterns for Women Evaluated in the ACOG Depression Screening Project”

Fatima Hina, M.D.

Mentors: Gennadiy Ivanov, M.D., Donna Slawek, N.R.P. and Lani Burkman, Ph.D.

Objectives & Background: To evaluate the usefulness of a modified Edinburgh post partum (PP) questionnaire (EPDS) as an effective screening tool for postnatal depression in an at-risk population. The American College of Obstetricians and Gynecologists advocates addressing psychosocial issues faced by women and their families during the childbearing years. There has been controversy concerning the use of screening questionnaires to identify women at risk for depression during pregnancy and after birth --- with no one screening tool accepted as the gold standard. In this ACOG study (currently ongoing), we used the ACOG tool with our patient population to evaluate its validity. Specifically, we sought to review the optimal cut-off score (≥ 10 is the commonly accepted standard), assess changes in the depression status of women who were scored twice (antepartum and postpartum), and track trends in medical intervention that were cued by the questionnaire responses.

Methods: The preliminary database for this prospective study included 521 patients seen at WCHOB within the Women’s Health Clinic. Most were from lower socioeconomic areas. Subjects were given the ACOG tool (10 questions, score of 0 to 30) at their prenatal visit (≥ 28 weeks), and were asked to return at 6 weeks postpartum. Intervention was offered by the medical staff (simple, intermediate, or extensive) if they appeared to require depression support. Group means or percentages were compared statistically using Student’s t-test or chi-squared calculations.

Results: Out of the 521 patients entered into the database, 184 were listed as completing the postpartum questionnaire (score = QS), including 110 women who filled out both the antepartum and postpartum assessments. For the 184 PP responses, the mean QS (\pm SD) was 6.0 ± 5.4 . For those women with QS = 10, their mean score was 13.8 ± 3.7 --- essentially the same as the matching antepartum data ($p > 0.05$). For those with a response above zero, the mode is 15 for QS, and the scores are not normally distributed. The frequency of women having QS scores of 8 through 12 was essentially the same ($n = 8, 6, 7, 10$, respectively), thus speaking against the use of 10 as a cut-off. Nevertheless, the clinic staff appeared to rely on the QS 10 value, since 22% of the subjects with QS 10-15 received assistance: counseling, telephone calls, medications, etc. In contrast, only 4% of women scoring 0 to 9 received intervention ($p < 0.01$). Suicidal ideation increased somewhat in the postpartum data (22 out of 150 = 15% for antepartum, compared to 14 out of 49 = 20% for the postnatal evaluation). However, due to small numbers, this trend could not be confirmed within the set of patients who had all questions completed.

Conclusions: Despite the lack of complete data sets, this preliminary overview of the Buffalo data clearly indicate a problem with reliance on the standard score of 10 for a postpartum or antepartum questionnaire for depression. These preliminary data could suggest that the cut-off used to determine depression intervention should be lower than 10. We note also that 60% of the postpartum questionnaires were filled out by patients who fell in the QS range of 10 or above. It is possible that patients receiving medical interventions for depression support are also more likely to return and complete the PP segment. The data from this ACOG study, when fully completed, may contribute to improvements in future behavioral health interventions for perinatal depression.

“Prevalence of antepartum depression in Buffalo: early data from the NY State ACOG Perinatal Depression Project (2007-08)”

Gennadiy Ivanov, M.D.

Mentors: Fatima Hina, M.D., Donna Slawek, N.R.P., and Lani Burkman, Ph.D.

Objectives & Background: Prevalence rates for adult depression are estimated at 17 % in the U.S., with women having twice as much depression as men. Approximately 10-16% of pregnant women are diagnosed with antepartum depression, which may continue into the postpartum period if left untreated. The Women’s Health Clinic of WCHOB is participating in the NY state ACOG study, which aims to estimate both prevalence and risk factors for ante- and postpartum depression; document patient intervention; and assess the usefulness of a patient-screening questionnaire.

Methods: This study enrolled 521 antepartum volunteers, many from lower socioeconomic areas, who were in their third trimester of pregnancy. The ACOG screening questionnaire was based on the Edinburgh Postnatal Depression Scale (EPDS) and was completed by patients in order to identify signs of depression or suicidal ideation. The study is ongoing and this preliminary data analysis focused on: the total questionnaire score (QS); patient age; response to a question on suicidal ideation (SI); intervention from the medical staff; and residence in a low socioeconomic area. The final mean values, or group percentages, were compared statistically using Students T-test and Chi-Squared calculations.

Results: Among these women, 150 (28.8%) were considered to have depression (QS cut-off = 10), and 22 of them (4.2%) indicated suicidal ideation which is about 1/7 of the total depression group. Those women with QS of 0 to 9 were not considered depressed ($n = 371$). Within the antepartum depression group, the median QS was 13 (range of 10-30) and the average age was 23 ± 4.7 years. Among all women who were depressed, the peak age group was 20 to 24, accounting for 42 % of all depressed patients. The highest rates of suicidal ideation also occurred for women aged 20 to 24 (26.6% of the SI group). Analysis showed a clear association between low socioeconomic areas of Buffalo and higher depression rates. Patients in three contiguous (low-income) zip code areas accounted for 27% of the entire depressed group. By comparison, only 6 % of women in two moderate-

income areas were depressed (chi-squared = 23.1; $p < 0.001$). Finally, as the depression score (QS) increased above 9, the percentage of women receiving staff assistance for depression also increased.

Conclusions: These data indicate that screening with a questionnaire, such as the EPDS, could be useful for antepartum depression screening. Age and socioeconomic status are risk factors for development of depression. Adequate medical and social resources in our community should be available to identify and treat depression in pregnancy.

“Micrometastasis on Permanent Section after Diagnosis of Negative Frozen Section of Sentinel Lymph Node Biopsy: Effect or Recurrence Rate in Breast Carcinoma”

Joseph A. DeNagy, D.O.

Mentor: Ronald Bauer, M.D.

Objective: Our goal was to determine if micrometastasis to the sentinel lymph node, undiagnosed on frozen-section but subsequently diagnosed on permanent section, is associated with a worse prognosis in patient undergoing lumpectomy for breast malignancy

Methodology: The Cancer Registry of Sisters Hospital was searched for all patients staged N1mi and pN0i+. Inclusion criteria were: five years follow-up from original surgery, frozen section diagnosis of sentinel lymph node being negative for malignancy, lumpectomy as primary surgery, margins negative for malignancy at original surgery.

Results: Ten cases were identified meeting these criteria, and ten controls were matched based on age, histology and grade of tumor. No recurrences were found in either group.

Conclusion: Our study suggests that there is no worsening prognosis if micrometastasis is diagnosed postoperatively.

“Comparing the Efficacy of Foley and Pitocin against Cervidil for Induction in the Community Hospital Setting: A Retrospective Study”

John Nguyen, M.D.

Mentor: Anthony R. Pivarunas, D.O.

Objective: Determine the efficacy of combination intrauterine foley balloon and concomitant Pitocin for labor induction versus Prostaglandin E2 (Cervidil) in the induction of women with unfavorable cervix. An extensive literature search of induction methods used in the past to facilitate labor induction yielded time of induction to delivery of 17 hrs +/- 10 for Foley balloon and concomitant Pitocin. Whereas trials using Prostaglandin E1, Prostaglandin E2, yielded starting time from induction to delivery to be under 24 hrs. **6,8** At the time of this article, there were no head to head studies comparing the efficacy of mechanical foley balloon with Pitocin induction to that of Prostaglandin E2 (Cervidil) on Ovid or Medline databases. Presently, at Sisters of Charity Hospital in Buffalo both of these methods were employed in labor induction. We intend to provide data to show that mechanical Foley balloon with Pitocin induction is safer, more rapid, and more cost effective in facilitating delivery of normal uncomplicated pregnancies than induction with Prostaglandin E2 alone.

Methodology: Women who presented for induction of labor at Sisters of Charity Hospital with Bishop score less than 6 were assigned to receive either foley balloon and concomitant Pitocin protocol or Prostaglandin E2 protocol.

Results: Primary outcome was time from start of induction to vaginal delivery. Secondary outcomes were cesarean delivery rates, incidence of chorioamnionitis, Apgar scores at 1 and 5 minutes, and tachystole, hyperstimulation, use of epidural, meconium, endometritis, NICU admission, and finally the total financial cost of an induced delivery.

Conclusion: Pending

“Ovarian Vein Thrombosis After Hysterectomy”

Seshadri Kasturi, M.B.B.S.

Mentor: Ali Ghomi, M.D.

Objective: To determine the frequency of ovarian vein thrombosis (OVT) in patients who have undergone total abdominal hysterectomy (TAH) with and without bilateral salpingoophorectomy (BSO).

Methods: The medical records of all patients who underwent TAH with or without BSO for benign indications between 2001 to 2003 were reviewed. The primary outcome measure was the incidental finding of OVT based on the CT scan of the pelvis done within 3 months of surgery.

Result: 200 cases of TAH were identified during this period, 20 of which had CT scans of the pelvis within 3 months of surgery: 9 patients had TAH, 3 patients had TAH with unilateral adnexectomy, and 8 patients had TAH BSO. 17/20 CT scans were performed with intravenous contrast enhancement. 1 case of OVT was identified.

Conclusion: The incidence of OVT after TAH, when done for benign indications, is 5% according to this pilot study. This is in sharp contrast to the incidence of OVT after TAH/BSO/staging when done for gynecologic cancer.

“C-Reactive Protein and its association with Pre-term Premature Rupture of Membranes”

Rachel George, M.B.B.Ch.

Mentors: Kofi Amankwah, M.D. and Lani Burkman, Ph.D.

Objective: To re-evaluate the association of C-reactive protein (CRP) in pre-term premature rupture of membranes (PPROM), a long debated subject in obstetrics. We hypothesized that WCC (white cell count) might be a better indicator for chorioamnionitis than CRP.

Methods: We performed a chart review of 100 women who were managed at Women and Children's Hospital of Buffalo with PPRM (2006 cases). The women were 24 to 36 6/7 weeks of gestation. Clinical data collection included temperature on admission, as well as WCC and CRP prior to deliver. We then collected the results of the final placental pathology of each of the 100 patients. We defined abnormal criteria as follows, CRP >4, WCC > 15,000 and Temperature > 99.0. Pathology was classified as chorioamnionitis with funisitis (1), chorioamnionitis (2), deciduitis (3), focal necrosis with deciduitis (4), chorionitis (5), and mild inflammatory changes(6).

Results: Only 29 patients had CRPs (CRP Group) drawn on admission. There were 31 cases of infected placentas found on pathology. Two thirds of the CRP Group had values greater than four. Of these cases, seven were noted to have abnormal placental pathology. With regards to the WCC data, more than 50% of those with abnormal WCC were found to have infected placental pathology. In contrast less than half (43%) of the 14 patient with temperatures > 99.0 had abnormal placental pathology. There was a linear correlation between the pathology code and the incidence for each type of pathology (-0.79, $p < 0.05$). Within the subset of women who had data for all three indicators (CRP, WCC, Temp), the Pearson correlation was negatively and significantly related to pathology ($r = -0.78$; $r = 0.77$; $r = 0.80$, respectively, $p < 0.05$). The current cut-off value for CRP is 4, however, there were two cases of chorioamnionitis with funisitis found in the group below the cut-off point (CRP 0 to 3). Interestingly for those cases with the highest range of CRP (22 to 71), five out of the six cases (83%) had infected pathology. Of these five cases three had chorioamnionitis with funisitis, one had chorioamnionitis alone and one had chorionitis.

Conclusion: This preliminary study is a small sample size however although CRP can be used as a useful component in the evaluation of chorioamnionitis, it was to be a poor indicator of disease. Also, within our institution the collection of CRP was inconsistent. It may also be useful to reconsidering the robustness the cut-off of 4 for CRP. This data could recommend raising the level of CRP cut-off. Also, white cell count may be a better indicator of imminent infection with chorioamnionitis.

"Colocalization of Synaptophysin in GnRH Neurons"

Bogdan Orasanu, M.D.

Mentor: Armando Arroyo, M.D.

Objective: Gonadotropin-releasing hormone (GnRH) neurons are neurosecretory cells that control the reproductive system. GnRH is stored in vesicles and released from axon terminals into the portal vessels in the median eminence (ME) in a pulsatile fashion. GnRH is released by calcium mediated exocytosis. The exact mechanism regulating GnRH vesicle exocytosis is not known. Much evidence indicates that proteins associated with synaptic vesicles are key elements in the mechanism that controls neurotransmitter release. There are currently nine families of synaptic vesicle proteins: synaptophysins, synaptobrevins, synaptogamin, synapsins, CSP, synaptogyrin, SCAMPs, SVOP, and SV2. Whether synaptic vesicle proteins are involved in GnRH release is not known. The aim of our study is to determine whether synaptophysin is present in GnRH neurons in the rat hypothalamus.

Materials and Methods: Double Label Fluorescence Immunohistochemistry technique was used for this study. Rat brains from female Sprague Dawley rats were removed after pericardial perfusion with 4% paraformaldehyde, fixed overnight, and sliced (20 μ m) with a cryostat. Immunostaining was performed using a free-floating technique. Slices were incubated overnight with a mixture of primary antibodies mouse anti-GnRH at 1:200 (Chemicon, Temecula, CA), and rabbit-anti synaptophysin at 1:100 (Santa Cruz Biotechnology, Santa Cruz, CA). It was followed by a mixture of secondary antibodies: Alexa Fluor 488, goat anti-mouse IgG at 1:1000 and Alexa Fluor 594, goat anti-rabbit IgG at 1:1000 (Molecular Probes Inc. Eugene, OR) for 2 hours. Slices were analyzed using a light (Nikon E400) and confocal microscope (Zeiss LSM-510).

Results: 86 brain slices from 3 rats were analyzed and 167 GnRH neurons were identified (Figure 1). 89.71% of GnRH neurons colocalized with synaptophysin (Figures 2 and 3).

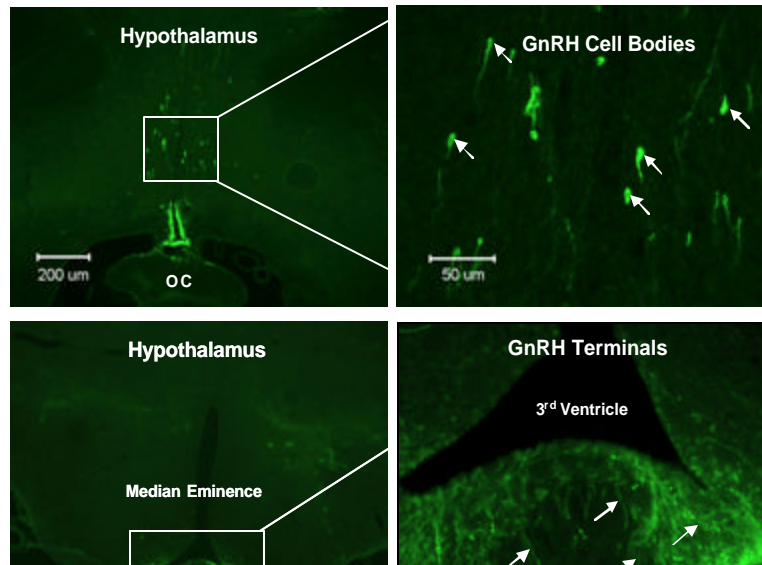
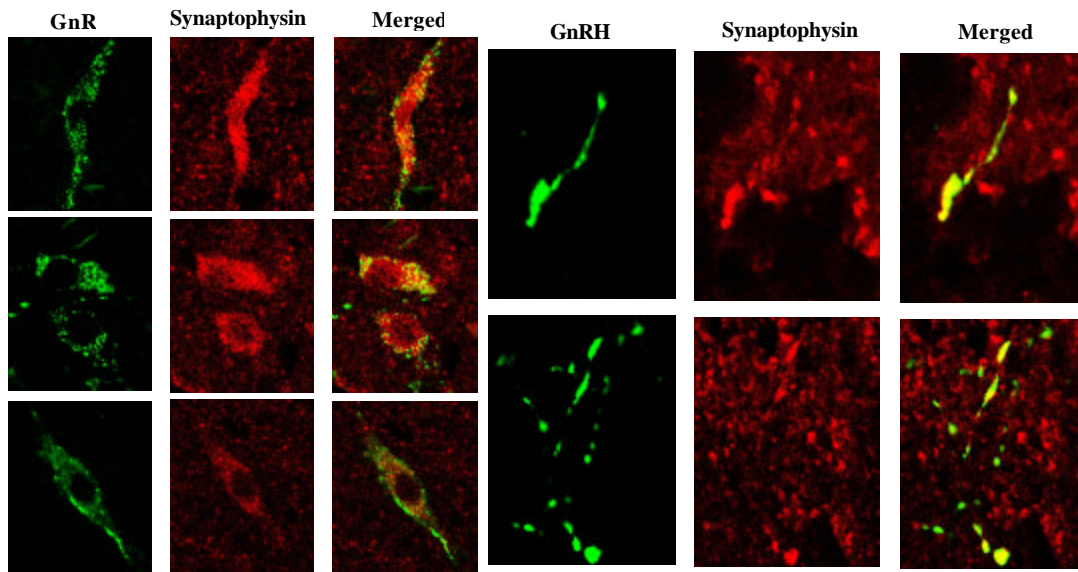


Figure 1. Coronal sections through the hypothalamus at the level of medial preoptic area (above) and median eminence (below). Optic microscopy 4x on the left and 20x on the right.



Confocal images

Figure 2. Colocalization of synaptophysin and GnRH cell bodies in the medial preoptic area. On the left, in green, GnRH cell bodies, in the middle, in red, synaptophysin, and on the right, in yellow, merged images (confocal microscopy, 60x).

Confocal images

Figure 3. Colocalization of synaptophysin in GnRH axon terminals in the median eminence. On the left, in green, GnRH axons, in the middle, in red, synaptophysin, and on the right, in yellow, merged images (confocal microscopy, 60x).

Conclusions:

1. Synaptophysin is present in GnRH cell bodies in the medial preoptic area and GnRH axon terminals in the median eminence in the rat hypothalamus.
2. These findings suggest that synaptophysin and possibly other synaptic vesicle proteins are involved in GnRH vesicle exocytosis.

“Cav1.2, Cav1.3 and Cav1.4 Channels are not Present in Rat GnRH Neurons”

Majid Shaman, M.D.

Mentors: Beomsu Kim, Ph.D., Amanda Biehl, Armando Arroyo, M.D., John Yeh, M.D.

Background: Little is known about the cellular mechanisms that underlie GnRH bursting and GnRH release. Calcium oscillations have been observed in GnRH neurons. Calcium channels may be involved in these cellular processes. L, N, R, P/Q and T type Ca currents are present in GnRH neurons. The L type Ca current is the most common Ca current identified in GnRH neurons. Ca_v1.1, Ca_v1.2, Ca_v1.3 and Ca_v1.4 underlie the L type Ca current.

Objective: The aim of this study is to determine whether Ca_v1.2, Ca_v1.3 and Ca_v1.4 channels are present in rat GnRH neurons.

Method: We used double-label fluorescence immunohistochemistry on rat brain hypothalamic slices. Brain slices were analyzed using light and confocal microscopy.

Results: We found that both Ca_v1.2 and Ca_v1.4 channels were not present at the GnRH neuron axon terminals at the median eminence. Both Ca_v1.2 and Ca_v1.4 channels were absent in GnRH neuron cell bodies at the level of the preoptic area. Ca_v1.3 was not expressed in the rat hypothalamus.

Conclusions: We conclude that Ca_v1.2, Ca_v1.3 and Ca_v1.4 are not present in rat GnRH neurons. Thus they probably do not underlie L-Type Ca currents in GnRH neurons.

