



Vulvodynia:

Toward Understanding a Pain Syndrome

Proceedings from the Workshop
April 14-15, 2003

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Introduction

The aims of this workshop were to present an overview of the science and epidemiology of vulvodynia and to elucidate the fundamental mechanisms of vulvodynia and related pain syndromes. The ultimate aim is thus to stimulate innovative research approaches to the study of vulvodynia and to develop clinical strategies for appropriate and evidence-based methods of alleviating vulvar pain.

Session I: Overview and Epidemiology

Dr. Maria Turner, National Cancer Institute (NCI)

Dr. Turner opened the workshop by presenting an overview of vulvodynia, focusing on those topics that were discussed at the first Vulvodynia Workshop in 1997, including the recommendations that were made at the meeting and some of the advances that occurred in the field between 1997 and 2003.

Vulvodynia, as defined by the International Society of Study of Vulvar Diseases (ISSVD) in 1993, is chronic vulvar discomfort characterized by the patient's complaint of burning, stinging, irritation, and rawness. Commonly, women complained of pain in the vulvar vestibule. These symptoms have multiple causes.

In 1997, very little knowledge existed regarding vulvodynia; what was known was based on clinical observations. At the first workshop, information was presented indicating that microscopic pathology studies of the vulva demonstrated very non-specific findings. Occasionally, lymphocytic infiltration surrounding the vestibular gland opening was noted, but at other times there was only a very small infiltration just below the dermal mucosa. In vulvodynia patients, the bacteria observed in the lower genital tract were abnormal, with a high prevalence of candida. A survey report from the National Vulvodynia Association showed that 30 percent of women with vulvodynia also had fibromyalgia.

Although the first workshop included presentations of pelvic neurophysiology and disorders of the pelvic floor, no information was presented on the epidemiology of vulvodynia because there was no information available at that time. A highlight of the workshop, however, was the recognition that vulvodynia is a chronic pain syndrome. Generalized pain mechanisms, especially those of visceral pain, such as altered central processing, could be maintained by stimulation of peripheral nerves. The genetics of pain were discussed, as was gender-specific pain perception. The myth that vulvodynia was solely psychosomatic was dispelled.

Several research recommendations were made at the first workshop including the need for: an exact definition of vulvodynia, multicentered clinical trials, well-conducted epidemiological studies to identify those at risk for the vulvodynia, studies to explore the relationship of psychosexual history to the disorder, and an examination of the role of infections such as Herpes Simplex Virus (HSV), Human Papilloma Virus (HPV), and candida as initiators of vulvodynia. It was also recommended that basic scientists be stimulated to develop animal models for neuropathic conditions of the pelvic floor.

In 2003, clinicians and scientists were still having difficulty using standard terminology, although most accepted a definition that excluded the dermatoses and nerve damage. In the interval between the first and the current workshop, a population-based prevalence study was initiated and a preliminary report of some of the findings would be presented at this workshop. In addition, six other studies had been funded by the NICHD.

Dr. Barbara Reed, University of Michigan

Dr. Reed, a discussant, remarked on research on vulvodynia—that is, where it is and where it should be. Half of all the vulvodynia-related publications were published since 1997. No longer was the disease thought to be psychological in origin. In the future, physician awareness of vulvodynia should increase so that women suffering from the disease would be diagnosed (and treated) in a timely fashion. Patients who currently had vulvodynia hoped that this workshop would lead to cures and treatments for the condition. However, one of their most urgent requests was to spread the word about vulvodynia so that women may receive the help that they need now.

An e-mail survey was conducted with 3,000 women, ages 18 to 78, resulting in the response of 1,025 women. One-half stated that they had pain with intercourse, and 28 percent said that they had pain at the introitus at some point in time. Of those, 8 percent stated that this pain had been felt within the six months prior to the survey, while 3 percent had had pain for more than three months. At the time of the survey, 1.7 percent had current pain that had lasted more than three months, the equivalent to 15 women in each family physician's practice, and double that in each gynecology practice. This number represents more than 2.4 million women, when the information is extrapolated to the general population.

Based on this survey, the average time from pain onset to diagnosis was 5.3 years and ranged from less than one to 29 years. About 50 percent of the women had seen more than two physicians, while 25 percent had seen four doctors; 80 percent of the patients had seen a gynecologist, and more than 50 percent had seen a family physician.

In addition, women with vulvodynia were not sexually averse, but were sexually active and interested, and were no more likely to have been physically or sexually abused. More than 50 percent had had sexual intercourse in the year prior to the survey.

This workshop was intended to provide a forum where the newest science could be discussed, ideas traded, and perceptions challenged. As what has been learned in the past six years was

evaluated and the future research agenda was discussed, Dr. Reed urged participants to remember the women who have vulvodynia and what they have to say about this condition.

Dr. Bernard Harlow, Harvard University

Dr. Harlow described NICHD-funded, population-based studies of vulvar dysesthesias. The goal of this research was to estimate the prevalence of these chronic, unexplained lower-genital-tract disorders, and to identify cases and match them to population-based controls to assess factors, such as perineal injuries, environmental irritants, abrasives, and other antecedent exposures to the first onset of genital symptoms.

The prevalence study was conducted with access to the Massachusetts Town Books, a set of state-mandated annual census directories. Approximately 16,000 women from the general population are screened by a one-page, optically scannable questionnaire, with 450 subjects per month being accessed into the study. Participants are separated into groups of those who had past vulvar symptoms, those experiencing current vulvar symptoms, and those with no history of such symptoms. So far, 8,700 women have been screened, and 7,000 women were given the opportunity to participate in the first phase of the screening questionnaire. There has been an overall 67 percent response rate to this invitation to participate. Of all the women, 16 percent stated that, at some point in their lives, they had chronic burning pain on contact, or pain that was “knife-like” and sharp for three months or longer. When women with endometriosis, uterine fibroids, and polycystic ovary syndrome were removed from the calculations, 14 percent of women experienced a chronic vulvar pain disorder. Of this percentage, three-fourths had pain on contact, and one-fourth had burning or knife-like pain. The cumulative incidence was similar in African American and Caucasian women. Women who identified themselves as Hispanic were 70 percent more likely to report a history of chronic vulvar pain (21.4 percent) and Asian American women were somewhat less likely (10 percent).

Within the 16 percent prevalence estimate, 40 percent of the women never sought treatment. Of those who did seek treatment, 30 percent had to see five or more physicians prior to diagnosis, while 40 percent of those who sought treatment did not receive a diagnosis. Only 9 percent were diagnosed with a chronic vulvar pain disorder. Thus, women seeking medical care are forced to see several clinicians and often receive an inaccurate diagnosis. In the study so far, women who reported great pain or difficulty with first tampon use were six to seven times more likely to report a chronic vulvar pain disorder. Women with a history of burning or sharp-contact pain were much more likely to self-report recurring yeast infections, as well as bacterial vaginosis, when compared to women with no chronic pain.

The case-controlled study was conducted by a telephone questionnaire administered to the women identified as having a current vulvar pain disorder in the prevalence study. Stringent criteria were used to classify the pain disorders and to avoid including women with pelvic pain. There was an excellent correlation of diagnosis as evaluated by phone and in actual physical examination of the subjects. Of those women selected as cases and evaluated clinically, 80 percent were more likely than not to meet ISSVD criteria for vulvar dysesthesia. Cases were matched by age and community of residence to women with no history of vulvar pain, and a reference age was used for analysis. For instance, if vulvar pain in a 35-year-old subject began

at age 25, information regarding potential risk factors was collected from age 25 and prior years in both cases and controls. So far, the case-control study findings with respect to first tampon use replicated the findings from the screening questionnaire in the prevalence study. Interestingly, this finding of pain with first tampon use in the study was associated with childhood victimization prior to age 11. Women who never or rarely received support from their families were three times more likely to develop a vulvar pain disorder in adult life. Women who were physically harmed and lived in fear of physical abuse were five times more likely to develop a vulvar pain disorder. In particular, women who were harmed by a primary family member during childhood were much more likely to develop such a disorder when compared to those harmed by a non-family member. No strong association with sexual abuse and later vulvar pain was found. However, women who had been sexually abused and also lived in fear of further abuse were 2.6 times more likely to develop vulvar pain. This association was greatest in women who had been abused by a family member and was attenuated when both physical abuse and sexual abuse were analyzed, suggesting that the association with abuse was due mostly to physical abuse.

A two-fold increase in risk for vulvar disease occurred in women with symptoms of depression, when those symptoms occurred before the onset of vulvar pain. Furthermore, almost all of the association with major depression was noted in women who were never physically abused, while the impact of depression due to childhood physical abuse had no effect on vulvar dysesthesia later in life. Women who experienced pain and discomfort at the time of first tampon use were those most prone to developing a major depression antecedent to a chronic vulvar pain disorder.

There appeared to be only a 20 percent increase in the risk of vulvar pain disorder among those women using oral contraceptives for non-contraceptive reasons, such as for control of menstrual irregularities. Interestingly, women who had experienced pain at the time of first tampon use were not at increased risk for the development of vulvar pain as a consequence of oral contraceptive use. However, women who reported little or no pain with their first tampon use were at a substantially greater risk of developing vulvar dysesthesia as a consequence of oral contraceptive use.

These findings suggested that there may be two etiological pathways leading to chronic vulvar pain: one pathway heralded by childhood abuse or pain and difficulty with first tampon use, as well as major depression; another through oral contraceptive use in women with no childhood victimization or problems with tampon use shortly after menarche.

Dr. Mary Margaret Chren, University of California, San Francisco

Dr. Chren, a discussant, brought out that fact that Dr. Harlow had presented two studies. The first was a population-based, cross-sectional survey of 4,800 women to examine the prevalence of current or past vulvar discomfort, defined as burning or sharp pain on contact, and the second was a case-controlled study to examine exposure associated with vulvodynia, when the outcome was presumed vulvodynia. The cases had current discomfort, and the controls were women of the same age, race, and education, but without discomfort. Exposures were defined as occurring before the onset of discomfort and included: family support in childhood, physical and sexual abuse in childhood, and oral contraception use. In the prevalence study, 12 percent of women

overall reported current or past vulvar pain on contact, and 3 percent reported current or past vulvar burning or knife-like pain lasting at least three months, for a total of around 16 percent. The prevalence of current or past discomfort was consistent among ethnic subgroups, and there was a strong association between current or past discomfort and great pain on first tampon use. She added that Dr. Harlow was to be commended for this study.

She felt, however, that it was essential to note that these were preliminary data and that the studies were ongoing. The 16 percent included women who had dermatitis, infection, or pain on contact from a myriad of causes in addition to vulvodynia. The measures used in these studies were excellent because Dr. Harlow and colleagues took into consideration the temporal relationship of the exposure to the onset of the symptoms. They made certain that the exposure was prior to the onset of the risk for the development of the vulvar pain. In measuring the variables, depression was defined by valid instruments.

Despite this attention to measures, questions remained. Although the survey instruments were valid among less common ethnic groups, were these women as likely to respond as others in more common groups? Was the definition of the cases valid? Were non-responders different from those who responded, and if so, how? Either they were women without vulvar pain and, thus, did not take the time to answer the questionnaire, or they were women with extreme vulvar pain who were so upset that they were offended by the survey. There was also recall bias in exposure ascertainment. Cases may be more motivated to remember exposures. Interviewers may have probed more carefully with cases than with controls.

There was an existing framework for this study based on previous data regarding childhood victimization and oral contraception, and these a priori hypotheses need to be carefully examined. This study was the beginning of establishing the syndrome of vulvodynia more fully. Additional studies were needed to address etiology and pathophysiology, natural history, and course of treatments. Rigorous outcomes and disease statuses were also needed. This situation was problematic for pain syndromes and for vulvodynia in particular because the condition was defined by patient experience. Because there were no specific laboratory tests, accurate measurements of pain and how pain affects the patient were needed.

Dr. Chren reported results from a study of the experiences and perceptions of 280 women with vulvar conditions. In this study, precise definitions for vulvar diagnoses were used, and quality of life was measured using Skindex (-29), a valid dermatologic quality-of-life measure. Of the 280 women, 36 percent had either generalized or localized vulvodynia. In addition, 11 percent had lichen sclerosis, 11 percent had lichen simplex chronicus, 6 percent had lichen planus, and 6 percent had candidiasis, while the remainder had less common vulvar conditions, such as papillomatosis. The quality of life of women with all vulvar conditions was worse than that of patients with eczematous dermatitis (a highly symptomatic disorder) or acne vulgaris (a condition that often affects appearance). Thus, these women's vulvar disorders significantly affected their quality of life. Compared to women with other vulvar disorders, women with vulvodynia were more likely to be Caucasian, more likely to report a history of depression or anxiety, and had worse quality of life.

In the study, two-thirds of those with vulvodynia said that the disorder prevented them from having sexual intercourse. It was determined that vulvodynia affected women's quality of life in unique ways that were not measured by standard psychometric instruments.

In summary, the research agenda for vulvodynia must include: agreement on a precise clinical definition for research purposes to facilitate communication; basic and epidemiologic studies of which Dr. Harlow's was an excellent example; patient-based studies of natural history and course of treatment; measurement of burden of disease; and specific measures of the effects of vulvodynia on the quality of life.

Dr. Elizabeth G. Stewart, Harvard University

Dr. Stewart discussed current therapies by first stating that, until there was a clear etiology of vulvar pain, treatment would be varied. The field was hampered by poor definition and poor description of what constituted improvement. Treatments would only be empirical until a clear etiology was discovered. Currently, there were 26 different modalities for vulvar vestibulitis reported in the medical literature, with little evidence from well-designed studies to support them. The treatment of generalized dysesthesia, or vulvodynia, and localized dysesthesia, or vestibulitis, was challenging. There was a clinical overlap in these pain syndromes, which made their treatment even more challenging. Those who treat pain recognized that pain syndromes require a multimodal approach.

In general, treatment began with an accurate diagnosis; patient education was also important. By first giving a concrete label for the pain problem, the physician reassured the patient. Pain theories, pathophysiology, and proposed treatment plans should all be explained. Referral to counseling and support groups was vital, as was psychosocial help to overcome the learned response to pain. Psychosexual help for women who have pain with intercourse was also essential. In addition, the treatment of depression was a mainstay of any pain program.

Triggers to pain needed to be treated as an adjunct to therapy. The elimination of candida infection was important, however, antifungals alone were insufficient treatment of vulvodynia. Physicians must recognize that most women with vulvar pain disorders stayed sexually active despite lack of arousal, poor lubrication, and incredible pain. Many women treated themselves and often engaged in deleterious practices. Open communication between a patient and her partner was also important and should include a discussion of alternatives to vaginal intercourse.

Specific treatment modalities included topical estrogens, delivered as a cream applied to the vulva or vestibule, or by intravaginal rings or tablets. Estrogen used in this manner was completely unstudied, even though it was often used on a daily basis until pain was significantly improved. It may work through its effect on epithelial maturation, but it also inhibited the production of inflammatory cytokines. Estrogens were a component of oral contraceptives implicated in the etiology of vestibulitis.

Tricyclic antidepressants were widely used in pain therapy, in general, and were effective separately from their antidepressant action. They were the treatment of choice for women with generalized dysesthesia, although only one small, published study documented this phenomenon.

Amitriptyline was the prototype tricyclic and was given at 10 mg to 100 mg doses. Side effects made the tricyclic antidepressants difficult for patients to take, thus switching to different members of the family could be helpful. Localized dysesthesia had shown improvement with up to 75 mg of amitriptyline in one small study. Many physicians prescribed up to 150 to 200 mg of this drug for three months. Serotonin reuptake drugs were very limited in their ability to alleviate pain, although currently utilized dosages may not be high enough to do so. The side effects of these drugs limited their use significantly, however.

Venlafaxine was reported to be effective in three patients with generalized dysesthesia. It was used in the treatment of localized dysesthesia in a dose of 200 mg. Gabapentin, an anticonvulsant drug approved for post-herpetic neuralgia and diabetic neuropathy was widely used in dosages of 1,000 mg for both generalized and localized dysesthesia. Some physicians prescribed 3,600 mg for three months before they declared a treatment failure. Other antiepileptic drugs, such as valproic acid, had been studied as therapy for vulvar pain as well.

Topical 0.2-percent nitroglycerin cream, a cytokine antagonist, used in a pilot study had been reported to show some improvement in vulvar pain. Anecdotal evidence suggested that antihistamines are not effective, and cromolyn had also been shown to be ineffective. Capsaicin and imiquimod may be helpful, but they had irritant properties. Medium- and high-potency steroids were reported to be helpful in some cases; however, evidence for this response was limited. Most clinicians stated that treatment with corticosteroids was disappointing, unless there was an inflammatory dermatosis present.

The current focus of therapeutics was in the combination of a centrally acting drug with a topical anesthetic, as in the case of topical lidocaine combined with tricyclic antidepressants. An open-label study had shown significant improvement, and a placebo-controlled study will be started soon.

In 1999, at the ISSVD meeting, reports from a single center suggested that interferon was just a little bit better than placebo in treating pain, but it was still widely used. One recent study suggested that the inability to produce endogenous interferon might be a factor in vulvar pain.

Women who practiced biofeedback for 16 weeks to diminish hypertonicity in their pelvic floor muscles had decreased pain on follow up. Many women liked this treatment because there were very few side effects. Physical therapy showed modest improvement in symptoms of both generalized and localized vulvar dysesthesia. The physical therapy aimed to alleviate the vulvar pain referred from the ligaments and joints in the spine and pelvis. One pilot study also showed that acupuncture improved the quality of life in women with vulvodynia.

There were no reported controlled studies of low oxalate diet on vulvar pain disorders. (Editors' note: a study is currently being conducted at the University of Medicine and Dentistry of New Jersey, in New Brunswick, New Jersey.) Oxalate may be an irritant to the vulvar vestibule in the same way that acidic foods are an irritant to the bladder in patients with interstitial cystitis.

By far the most controversial therapy for vulvar vestibulitis was surgical vestibulectomy. Surgery was criticized because of a lack of a clear pathophysiology for vulvar pain. Other types

of pain were not treated surgically. In addition, concerns were expressed regarding cosmetic results and the effect on body image. However, neural hyperplasia occurred in vestibulitis, and excision of neural hyperplasia was effective in relieving pain. Careful selection of surgical patients was essential; the most important rationale for surgery should be the failure of other treatment regimens.

Although it was difficult to compare surgery that utilized different techniques, three published case series revealed a success rate of 80 percent to 89 percent. Predictors of surgical failure included primary vestibulitis, diffuse vulvar pain, urinary symptoms, and muscle hypertonicity. Nevertheless, surgical treatment had a successful record for improvement of symptomatology. Reportedly, pain was significantly improved by surgery in combination with behavior treatment techniques. Several pain centers in the United States and Europe have performed decompression of the pudendal nerve, as well as nerve blocks.

In summary, there was a need for careful controlled trials of various therapeutic modalities. Local cytokine antagonists showed promise, as did modulation of central sensitization of neuro-inflammatory pain in localized dysesthesia. There was recognition that pain itself needed to be treated. The meaning of pain to an individual woman and its impact on relationships should also be fully understood.

Dr. Daniel Handel, NIH Clinical Center

Dr. Handel, a discussant, noted that vulvodynia was a burning or combination of a dysesthetic sensation anywhere from the mons to the anus that caused physical, sexual, and psychological distress. There were commonly mixed components and the true incidence was unknown, although it was more common than previously thought. Dr. Stewart clearly stated that there were multiple etiologies. Thus, there was no unifying concept in terms of management. A recent concept was that neuropathic pain was a primary causation. The pain can be one-sided or bilateral, sporadic or constant. The patient often reported a swelling sensation, and paraesthesias were noted. Sometimes there was a mix of paraesthesia with other sensations. Often there were periods of time that were symptom free. The history of a patient may reveal surgery or injury, especially sports injury. The physical history should include information regarding possible back injury or surgery to treat such problems, such as herniated disc, low lumbar disorder, possible arthritis of the hips or pelvis, and spinal stenosis. The type of physical stresses the patient endured at work or leisure must be explored.

The diagnosis was one of exclusion and, as had been mentioned, the patient often saw multiple physicians. During the physical examination, a work up for vaginal infection and irritation must be done. Culposcopy was indicated to evaluate any local lesions and appropriate biopsies should be obtained. Urine culture and spinal imaging studies should also be performed when indicated.

Many women with vulvodynia or vestibulitis experienced loss of hope, which led to psychological, emotional, and spiritual issues. The sexual sense of self provides another dimension to the issues faced by these patients. The fear of loss of self, the feeling that “I don’t know who I used to be,” and the issue of pain without physical findings cannot be underplayed.

There were few people dedicated to this disorder, but they needed to be the professionals consulted by patients. They knew the literature and were aware of the treatments that might be helpful.

Helpful treatments included physical therapy, although it was not known if this treated the disorder, or if the individual's abnormal response to it treated the disorder. Surface electromyographic (EMG) assisted pelvic floor rehabilitation was one therapeutic approach. The elimination of contact irritants was also important and included panty hose, synthetic underwear, jeans, swimsuits, scented soap, bubble baths, wash clothes, feminine sprays, deodorants, and powders.

Multiple studies showed tricyclic antidepressants to be helpful. There were several different tricyclics that offered help, but it was important to avoid their side effects. Anticonvulsants had a mixed history in terms of treating pain. The older ones were often difficult for patients to tolerate, but newer ones had far fewer side effects; however, the efficacy of these agents for vulvodynia needed further research.

It was often helpful to combine a therapeutic central approach with a peripheral approach. At the NIH Clinical Center, the approach was to think about a patient's primary symptoms and let these symptoms guide therapy. Nitroglycerin cream prior to intercourse was one such approach, and there was a large study under way to confirm its usefulness. Opioids should be considered for pain, but they should not be given until a thorough evaluation was done and the diagnosis made. The physician should know why the opioid was chosen and should have specific and measurable goals for therapy.

Pain clinics willing to offer multimodal sex therapy, in addition to providing relief of pain, were very helpful in the treatment of vulvar pain. Vulvodynia was a disorder more common than thought previously; however, its true incidence was still unknown. It was most likely that research would reveal subpopulations in which various types of symptoms existed, but these subpopulations needed to be identified. Until there was a unifying concept of etiology, at least for subpopulations, therapy should be guided by determining the most troublesome symptom for the patient, and treating that symptom first.

Dr. Hope Haefner, University of Michigan

Dr. Haefner, a discussant, brought out alternative therapies for vulvar pain, many of which needed to be studied as to their efficacy. In 1997, there were more than 600 million visits to alternative therapeutic practitioners, a number higher than those to primary care physicians. More than \$35 billion per year was spent on alternative treatments. Patients with vulvar pain often sought alternative therapies when they did not respond to conventional treatment. One study demonstrated that 96 percent of 26 patients with vulvar pain used at least one form of complementary health care product. Many of these products were found to contain steroids. The definition of alternative therapy was problematic because health care providers counsel their patients on a daily basis regarding diet, alcohol consumption, smoking, and physical activity, yet others considered these topics as complimentary or alternative medicine. Physical therapy and biofeedback for vulvodynia were also helpful. Are these alternative therapies?

There were many therapies that could be studied for the treatment of vulvodynia, including hydrotherapy and acupuncture. Hydrotherapy was used in vulvar care, and ice packs were prescribed as well; patients indicated that these were helpful. Although acupuncture was 4,700 years old, its use for the treatment of vulvodynia was new. A biological theory as to how acupuncture works was that it involved opioid peptides. A study of acupuncture points for vulvodynia treatment demonstrated that the points on the sacrum tend to be lateral, and that those on the front of the body tend to be more central than those for other diseases. One study conducted by the National Vulvodynia Association showed that 35 percent of those treated with acupuncture had a positive therapeutic response. Other studies have not confirmed this finding.

The use of pressure points in therapy should also be studied. Other alternative therapies that might be useful and, therefore, should be studied were: aromatherapy, guided imagery, healing touch, hypnosis, herbal medications, vitamin E (i.e., in promoting tissue regeneration and wound healing), massage therapy, meditation, yoga, and tai chi. Health care providers needed to be concerned about the quality and efficacy of alternative medicine. Because natural was not necessarily safer than synthetic chemicals, randomized trials were very much needed to evaluate alternative treatments for vulvar pain.

Dr. Jacob Bornstein, Nahariya Hospital, Israel

Dr. Bornstein, a discussant, mentioned that he had been involved in the treatment of vulvodynia since 1985. In his mind, the real question was: why did surgical treatment for vulvar vestibulitis have such a poor reputation? Not all surgery was successful, even though women were told that surgery was the end of the road. Some surgical techniques were inadequate or inappropriate. When it failed, there was a great disappointment for all. Why did surgery fail? One possible reason was because, in most patients, the sensitivity was localized to the base of the hymeneal ring, not the vestibule.

To be done correctly, the recommended procedure should be done under general or regional anesthesia. The incision was made below and lateral to the urethral meatus and extended along the perineum to half the distance between the fourchette and the anus. The amount of tissue removed varied according to the surgeon, and there was currently little research on the appropriate amount of tissue to be excised. But, typically, about 5mm of mucosa and underlying stroma was removed. The vestibule was excised to the hymeneal ring and the tissue surrounding the meatus. If needed, the anterior vestibule was also excised. The vaginal mucosa was undermined for 1-2 cm, and then advanced and sutured to the skin. The vaginal epithelium was exteriorized, which created a result whereby the anterior vulva looked the same as prior to the surgery. The vaginal tissue was advanced to the perineum so that the mucosal skin scar was not at the introitus, thus avoiding future sensitivity in this area. The patient was hospitalized overnight and treated with ice packs, sitz baths, and analgesics for 10 days.

In most cases, complications were minimal. One more common long-term complication was Bartholin Duct Cysts. Persistent vestibular tenderness or even increased tenderness occurred in 5 percent of patients and posed a serious problem.

Did surgery work? A prospective randomized comparison of vestibuloplasty—that is, undercutting the vestibule without excising it—did not demonstrate relief of symptoms. However, perineoplasty worked. Analysis of the data was limited by a few problems: often, the criteria for success or resolution of symptoms were not mentioned, nor was there good follow-up information; further, surgical terminology and pain measurement were not standardized, nor was patient selection clear.

Reported studies have demonstrated a 20 percent failure rate, in general. Studies with higher failure rates had methodological flaws. In a study conducted by his group, Dr. Bornstein reported that there were two groups of patients who failed to be helped by surgery: one group included women with primary pain on intercourse, and the second group included women who had constant pain in addition to dyspareunia. This latter subset should not undergo surgery because this treatment failed to help the pain in 50 percent of the patients.

Surgery was not recommended as first line therapy, but, rather, as part of a treatment program. In Dr. Bornstein's program, pelvic floor biofeedback, low oxalate diet, calcium citrate supplementation, and estrogen cream application for oral contraceptive users was prescribed initially.

A new direction in the surgical treatment of vestibulitis may come from a different approach. Five women operated on by Professor Peter Petros of Perth, Australia, for enterocele also had vulvar vestibulitis. The correction of the posterior wall defect in these cases cured the vestibulitis, which raises the possibility that releasing stress on the pudendal nerve relieved the pain.

Session II: Areas for Discovery

Dr. Libby Edwards, Southeast Vulvar Clinic, Charlotte, North Carolina

Dr. Edwards addressed *Questions that Need Answers*, which included: epidemiology; the division of vulvodynia into and the characterization of subsets; the possibility of multiple etiologies causing vulvar pain; and the controversy about the existence of inflammation in vestibulitis. Questions regarding the role of neuropathic pain, and the abnormalities of central or peripheral processing and the function of the pelvic floor (muscle and fascia support) were also discussed. And of course, the very major question of what treatments are effective was presented.

Some current epidemiologic studies revealed understandings about vulvodynia that were different from the accepted understandings of the past 10 to 15 years. Past ideas, perhaps, were wrong. For instance, past studies did not show an association with childhood sexual and physical abuse and vulvodynia, but today evidence of such an association was presented. Thus, additional studies were needed. Vulvodynia among African American women needed to be studied further, as well.

The accurate definition of the disease was important, not only for epidemiological studies, but also for treatment protocols. The term *vulvodynia* was a description only. The issues of population subsets also needed to be addressed, especially in determining those individuals for whom vestibulectomy was appropriate. The strictest definition of vestibulitis was that the pain was only located in the vestibule, and that it was only elicited by touch. Dysesthetic vulvodynia, on the other hand, was generalized pain, migratory pain, or pain that sometimes extended beyond the vestibule. Touch, in this case, did not produce pain. While these strict definitions were ones that many physicians and scientists subscribed to, they did not always hold up in the clinical setting. Thus, the question remained—were these distinct, easily separable subset definitions that could be clearly divided in clinical settings, or did vulvar pain exist on a spectrum between the two? Individuals may have aspects of both vestibulitis and dysesthetic vulvodynia, and perhaps, these conditions existed on a spectrum rather than occurring as easily and distinctly separate conditions. Also, perhaps other characteristics distinguished the subsets, such as age, estrogen status, or concomitant conditions, including fibromyalgia, irritable bowel syndrome, interstitial cystitis, back pain, or others, suggesting a central-processing abnormality. Could it be that longstanding vestibulitis tended to worsen and generalize into dysesthetic vulvodynia?

Another important question was whether or not vulvodynia was associated with inflammation. What was the meaning of increased numbers of white blood cells in vaginal secretions, as some noted, and increased inflammatory mediators in tissue of women with vestibulitis? Were these signs of inflammation due to tissue damage, or were they *neurogenic inflammation*? The pain of vulvodynia resembled neuropathic pain, and it often responded to therapy used for neuropathic pain in other areas of the body. If the vulvar pain was due to neuropathic pain, it needed to be characterized. For instance, was it pudendal neuralgia? Was it a complex pain syndrome? Was it due to altered central or peripheral processing, or could it be due to either? What was the role of pelvic floor dysfunction? Women with pelvic floor dysfunction had increased resting muscle tension, as shown by surface EMG measurements; they also had poor muscle strength. If abnormal pelvic floor anatomy was associated with vulvodynia, was it due to weakness of the increased tension? Why did strengthening exercises relieve pain?

Finally, the most important question was how to treat vulvodynia effectively. How can the pain be controlled? Could this condition be cured, as gynecologists often expected, or controlled, as dermatologists often expected? Placebo-controlled clinical trials were needed to determine what treatment(s) was successful.

Those treating vulvodynia could begin to find answers to these questions if they had better definitions, if they really tracked their patients in their offices, and if multicenter, standardized protocols were developed.

Discussion

Various participants raised a number of questions regarding the role of pelvic floor disorders in vulvar pain. Could laxity of the pelvic floor cause nerve stretching and, thus, cause pain? One participant pointed out that constant irritation would increase the area of pain through convergence and divergence of sensation. There was also discussion of the fact that there may be a continuum of pain and not two distinct entities of disease because symptoms overlapped.

Those present noted that there was a wide variety of treatments not related to underlying mechanisms of disease, even though there were solid concepts of the cause of chronic pain reported in the literature. In dealing with pain, it was essential to know where the pain was coming from. A simple diagnostic test, using infiltration of skin with a local anesthetic, would determine if there were sensitized nociceptors in vulvar skin. Participants mentioned two studies that did use local anesthetics, and these substances did seem to be helpful in relieving pain. Surgery was also based on the concept of eliminating abnormalities of the sensory nerve endings, and it did appear to be successful in many situations.

Session III: Basic Science Updates on Related Topics

Dr. Karl B. Thor, Dynogen Pharmaceuticals and Duke University

Dr. Thor discussed a paper titled *Neuroanatomy of the Pelvic Floor in Human, Monkey, and Rat*. His collaborators in these studies were: Matthew Barber of the Cleveland Clinic; Kim Coates, Lisa Pierce, and Michelle Reyes of Scott & White Clinic; and Paul Dolber and Ron Bremer at Duke University. In vulvodynia, there was obviously nerve involvement in pain. Dr. Glazer indicated that vulvar pain could produce spasm of the levator ani muscle, which raised the following questions: could levator ani nerve damage produce spasm and, thus, pain in the vestibule and vulva, and might pelvic organ prolapse possibly be involved in vulvodynia? A hypothesis of the etiology of pelvic floor prolapse was that childbirth produced a crush injury of the levator ani nerve and, thus, eventually caused pelvic floor prolapse.

Classically, textbooks stated that the vulva (external genitalia and perineal skin) was innervated by the pudendal nerve, while the pelvic nerve innervated the vagina and the cervix. However, the pelvic floor innervation was poorly studied. Present studies were conducted to find the innervation of the pubococcygeus and ileococcygeus muscles. Prior to these studies, this innervation was described in the textbooks as originating from “sacral spinal roots,” which was nebulous, and the pudendal nerve. Glazer’s studies demonstrated this was not the case; instead, the pudendal nerve only innervated the rhabdosphincter muscle.

The pelvic floor consisted of the coccygeus, iliococcygeus, and pubococcygeus muscles as the primary muscles, while the smaller puborectalis muscle lied most medially. Dr. Thor’s group conducted a large number of nerve dissections in 13 human cadavers. Nerve biopsies were evaluated to ascertain that nerves were studied and not connective tissue. The nerve that they traced into the levator ani muscles, which they called the levator ani nerve, received contributions from the S3, S4, and/or S5 roots. In some cadaver specimens, the S5 root had its own pathway to innervate the puborectalis muscle. Importantly, the path of the levator ani nerve passed within 5 mm of the ischial spine. Thus, the levator ani nerve was susceptible to damage during suspension surgery for pelvic organ prolapse, or by a fetal head passing through the birth canal.

Because the levator ani nerve was an intrapelvic nerve, it was in a position to be preferentially stimulated when a St. Mark’s electrode was inserted into the rectum. Furthermore, because the

levator ani nerve was so close to the ischial spine, it was likely that this nerve was also preferentially anesthetized at the time of pudendal nerve block during childbirth.

In contrast, the pudendal nerve was an extrapelvic nerve. It quickly exited the pelvis, wrapped around ischial rectal fossa, entered the pudendal canal, and provided innervation to the external genitalia, the urethral sphincter, the anal sphincter, and the external genitalia. The branch that innervated the vulva and vestibule was very superficial, while the branch that innervated the clitoris was deeper. Thus, there was the possibility of trauma to the vulvar branch during bicycling and horseback riding, which could lead to neuropathy.

The levator ani nerve was also studied in rats and squirrel monkeys as model animals. The nerve in these species was composed of large motor axons and IA primary afferent fibers 10 microns or larger in diameter. These large fibers were not seen in the pudendal nerve. Cholinesterase staining revealed a single motor endplate at the midpoint of both the iliococcygeus and the pubodoccygeus muscles. Selective neurectomies showed that cutting the levator ani nerves produced significantly decreased levator ani muscle weight and myofiber diameter, while cutting the pudendal nerve did not.

Tracing studies with cholera toxin B (CTB) injected into the levator ani muscles demonstrated about 400-500 motor neurons, mostly in the S1-S2 segments. Their size distribution was bimodal—large, presumably alpha neurons and small gamma motor neurons. The neurons were diffusely organized and had a dendritic arbor that connected the longitudinal cell column in the ventral horn.

CTB-labeled primary afferent terminals were found just medial to the central canal in an area called medial lamina VI. Special stains (RT97 for neurofilaments found only in large primary afferent fibers, and IB4 and CGRP staining for small nociceptors) were used to determine that putative nociceptor neurons in the dorsal root ganglia innervated the levator ani muscles. About 40 percent of the primary afferent neurons labeled by CTB were small peptidergic neurons, which should project terminals to the superficial dorsal horn. However, there was little CTB terminal staining in this area, attributed to a problem with technique.

In summary, the levator ani muscles were innervated by the levator ani nerve, while no evidence of innervation by the pudendal nerve could be found. The levator ani motor neurons were diffusely distributed in the sacral ventral horn, while the pudendal motor neurons were concentrated in Onuf's nucleus. However, there was a great deal of overlap between the dendrites of levator ani motor neurons and pudendal motor neurons, and both nerves contained primary afferent fibers that projected into the sacral spinal cord. Thus, there was great potential for interactions between both the sensory and motor nerve fibers that controlled the levator ani muscle, the rhabdosphincters, the vulva, and the vestibule. These interactions could be physiological and/or pathological.

Dr. Michael Pezzone, University of Pittsburgh

Dr. Pezzone discussed the function and innervation of the pelvic viscera and their striated sphincters, the pelvic organ reflexes, and the cross-talk between them. There was an overlap of chronic pain disorders, just as there was an overlap in vulvodynia.

The most important function of the pelvic floor structures was support of the pelvic viscera, important to humans because of erect posture. The pelvic floor structures also functioned in urination and defecation. Other functions of the pelvic floor were related to sexual functioning and childbirth.

There were two components of innervation: autonomic innervation, with sympathetic and parasympathetic components; and somatic innervation.

Sympathetic nerves originated in the thoracolumbar segments of the spinal cord, while the parasympathetic came from the sacral components of the spinal cord. The pudendal nerve originated in the sacral area. There was great potential for neural cross-talk because these nerves converged in similar areas in the spinal cord. Local reflexes occurred in the sacral spinal cord and were termed segmental reflexes because they were localized to the cord in the segments where afferent and efferent inputs converged. These reflexes were important for postural control of the pelvic floor. A polysynaptic pathway was activated by muscle stretch due to gravity and position, and this produced a reflex contraction, keeping the organs in place. This reflex was mediated by slow twitch muscles, which were continuously active. Fast-twitch muscles were activated during a cough or sneeze and, thus, further strengthened the pelvic floor muscles to prevent incontinence in a supra-segmental reflex arc involving the brainstem.

Neural cross-talk occurred when afferent activation of one organ influenced efferent output to another. (Afferent signals were conducted away from an organ; efferent signals were conducted from a central area to an organ.)

The pelvic and pudendal afferents overlapped in the spinal cord allowing integration of parasympathetic and somatic motor activity. The cross-talk occurred under normal conditions and was necessary for sexual, bladder, and bowel function. They were mediated by a convergence of sensory pathways in the spinal cord and could be altered by disease or injury. Some of these reflexes included: bulbocavernosus, vesical-anorectal, anal, anal-vesical, and vesicovaginal.

Chronic pelvic pain disorders included those affecting the pelvis (i.e., irritable bowel syndrome and interstitial cystitis) and those affecting the pelvic floor (i.e., vulvodynia, urethral syndrome, prostatodynia, and orchialgia). Between 40 percent and 60 percent of persons with irritable bowel syndrome had interstitial cystitis, 38 percent of patients with interstitial cystitis had irritable bowel syndrome, and 26 percent of those with interstitial cystitis had vulvodynia, which suggested a common predisposition, a shared etiologic factor, or possible cross-sensitization via neural cross talk. Some mechanisms for pelvic organ cross sensitization were dichotomizing or shared C-fibers, afferent-afferent interactions, sympathetic reflexes, extra spinal reflexes via pre-vertebral ganglia and sphincteric interactions.

Studies in rats showed that, when the bladder emptied during micturition, distal bowel contractions were inhibited. When an animal urinated, phasic firing of the urethral and anal sphincters occurred simultaneously, along with tonic contraction of the abdominal muscles. Conversely, when the colon was distended, the bladder and urethral sphincter were inhibited.

In a series of experiments using a rat model, prior injury to the bladder epithelium caused bladder irritation (e.g., acute cystitis) when physiological doses of potassium were infused. During acute cystitis, colorectal distension produced lowered sensory thresholds or bladder-to-bowel cross-sensitization. Likewise, when the colon was irritated acutely to cause colitis, the bladder contracted more frequently and exhibited signs of acute irritation.

When chronic colitis was induced in these animals, bladder capacity decreased, while bladder contraction frequency increased. Microscopic studies of these animals demonstrated evidence of mast cell hyperplasia, suggesting the development of neurogenic cystitis. These studies also indicated the presence of a chronic cross-sensitization process. Further experiments suggested that the chronic irritation led to the production of neurotrophic factors that caused nerve growth, mast cell recruitment, and neurogenic inflammation.

The frequent overlap of irritable bowel syndrome, interstitial cystitis, vulvodynia, and other chronic pelvic pain disorders may be indicative of aberrant neuronal interactions or reflexes, such that the irritation of one organ led to co-sensitization of others. With continued irritation, neurotrophic factors produced by both smooth muscles and sensory neurons may influence neurite outgrowth and axonal sprouting, which could lead to motor and sensory changes in target organs.

Dr. James Baraniuk, Georgetown University

Dr. Baraniuk asked: What is neurogenic inflammation? He noted that this concept needed revision because stimulation of nociceptive nerves did not cause immediate cellular infiltration (i.e., inflammation).

Different types of injury stimulated specific types of non-myelinated Type-C fibers. These neurons released neurotransmitters that caused tissue reactions, such as swelling, redness, or glandular secretion. Previously, it was thought that there was only one type of C neuron, and that vasodilation was the epitome of the axon response. Now scientists recognized a number of functional subtypes. About 5 percent were histamine H1 receptor “itch” nerves, 14 percent were heat sensitive and may express capsaicin/vanilloid receptors, 9 percent were heat plus histamine sensitive, 51 percent were mechanothermal nerves, while the remaining 22 percent had undiscovered sensitivities.

In rats, mice, and most likely humans, there were two distinct sets of Type-C neurons based on neurotransmitters. The first group contained neuropeptides, such as Substance P, calcitonin gene-related peptide (CGRP), and probably others. The second was non-peptidergic and contained the IB4-lectin. These nerves synapsed in spinal cord laminae 1 and 2, and some in lamina 6. The secondary relay interneurons crossed to the opposite spinothalamic tract to synapse in the thalamus. From there, stimuli were sent to various other systems to generate the

perceptions of itch, dull, aching pain, heat, and mechanical stimulation. There were significant links to the limbic system where these inputs affected emotion and memory responses, as well as brainstem autonomic centers to recruit reflexes in response to the nociceptive stimuli.

The itch nerves were the slowest conducting nerves in the body and innervated large areas of skin. They produced large “flare” (redness) or vasodilatation reactions. The erythema was caused by CGRP. Itch neurons were unique because they had distinct dorsal horn, lateral spinalthalamic, and thalamic pathways separate from those for pain and temperature. The itch pathway was stimulated by mast cell degranulation and release of histamine that acted on H1 receptors of the itch neurons. The peripheral axon response for this neural population was the flare.

CGRP caused slow onset, but long-lasting erythema. This peptide, 37 amino acids long, had two forms, α and β , which differed by three amino acids. CGRP, amylin, and adrenomedullin were in the same peptide family. CGRP Type 1 and Type 2 receptors stimulated adenyl cyclase in endothelial and vascular smooth muscle cells to cause potent vasodilation. CGRP accentuated the effects of other mediations that increased blood flow and endothelium-mediated plasma extravasation.

Vanilloid receptor-bearing neurons responded to heat, acid, and capsaicin. Capsaicin, the spicy essence of chili peppers, stimulated such extensive neurogenic inflammation that blisters like third-degree burns formed. Fortunately, most individuals were not exposed to this type of nociceptive stimulation. The neuron receptors were found in small diameter, non-myelinated Type-C neurons and responded to mechanical, moderate thermal stimuli, capsaicin, and related polyunsaturated fats, and to a pH less than six.

The nature of the axon response generated when Type-C neurons were depolarized and released their neurotransmitters depended upon combination of transmitters, distributions of their receptors on cells in the immediate area, the presence of proteases and other degrading enzymes, and the volume within which the neurotransmitters can diffuse. CGRP vasodilatory responses depended on the presence of both blood vessels and CGRP receptors in the region of the neurotransmitter’s release. Substance P release may generate many effects, including glandular secretion, vascular permeability, and, in inflammation, activation of inflammatory cells and increased chemoattraction of these cells.

Substance P was reported to degranulate mast cells, which appeared to occur in some rodent models, but had not been physiologically demonstrated in humans. One problem was that high doses of Substance P were often used to induce degranulation. This may occur by Substance P-specific neurokinin-1 receptors. However, a Substance P mimetic synthesized with the highly positively charged N-terminal attached to a long chain hydrophobic alkane was equally potent at degranulating mast cells. This suggested that, in high concentrations, Substance P nonspecifically activated mast cell membranes or some membrane protein, to lead to anaphylactoid (anaphylaxis-like) mast cell mediator release.

Populations of thinly myelinated A δ fibers rapidly transmitted short-lived intense pain (i.e., first pain) sensations, pin-prick (i.e., sharp/dull), and cold sensations. Large A β and other neurons

innervated specialized cutaneous sensory receptors to convey fine touch, proprioception, vibration, and other sensations.

Neurogenic mechanisms likely contributed to chronic multisymptom illnesses, such as vulvodynia, chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, chronic low back pain, chronic non-migraine headache, and irritant rhinitis. The overlap was demonstrated by examining data from 81 healthy controls (C), seven otherwise healthy women with dyspareunia (C+D), 92 women with chronic fatigue syndrome (CFS), and 21 women with both chronic fatigue syndrome and dyspareunia (CFS+D). The prevalence of dyspareunia was 3 percent versus 19 percent in CFS. These women were in their 40s. Muscle spasms were reported in 7 percent of C, 57 percent of C+D, 52 percent of CFS, and 95 percent of CFS+D. A similar distribution was found for “dry eyes” with 6 percent of C, 57 percent of C+D, 42 percent of CFS, and 71 percent of CFS+D. A different pattern was found for “fever” with 1 percent of C, 0 percent of C+D, 16 percent of CFS, and 52 percent of CFS+D, and for irritable bowel syndrome (Rome I criteria) with 7 percent of C, 0 percent of C+D, 36 percent of CFS, and 81 percent CFS+D. Subjects in the CFS+D group had general tenderness as tested by dolorimetry: 2.21 kg/cm² for C, 1.71 for C+D, 1.49 for CFS, and 0.97 for CFS+D. The differences in prevalences between groups for each variable were highly statistically significant, suggesting potential causal relationships between the mechanisms responsible for each complaint.

Neurogenic responses were protective responses. Unfortunately, prolonged or severe mechanical, thermal or chemical irritation caused excessive local axon responses with the consequence that there was a dysregulation of pain processing in the dorsal horn of the spinal cord. One hypothesis being examined was that this regulated pain processing led to an organ-specific axon-response mediated syndrome, and total body central nervous system mediated syndromes of chronic pain and hyperalgesia.

Dr. Nina Bohm-Starke, Karolinska Institutet, Sweden

Dr. Bohm-Starke described studies of Vulvar Vestibulitis Syndrome (VVS) conducted by her research group. These focused on the pathophysiology of the vestibular mucosa and its innervation and relationship to inflammation. The results of the studies showed indications of an ongoing neurogenic inflammation in the vestibular mucosa in women with VVS.

The vestibular mucosa was by definition visceral tissue deriving from the urogenital sinus endoderm, but it was considered to have a non-visceral innervation and thus, the sensations of touch, temperature, and pain were similar to sensations evoked in the skin. The very peripheral nerves were traveling beneath the basal membrane, penetrating the epithelium at various sites, usually ending with a knob. These were sensory nerves, primary afferent neurons. Further down in lamina propria there was a predominance of autonomic nerve fibers, mainly around blood vessels.

The peripheral nerves in the mucosa were first surveyed by immunohistochemical methods using PGP 9.5, a general marker of neural tissue. Biopsies from the area around the ductal openings of the Bartholin's glands, the most sensitive vestibular area in most patients, showed significantly

more intraepithelial free nerve endings than in healthy control subjects. These intraepithelial nerves were mainly immuno-positive for CGRP, the most abundant neuropeptide in primary afferent nerves in skin. Holtzer considered CGRP to be the major mediator of vasodilatation in neurogenic inflammation.

The observed erythema in the posterior vestibule in the patients could represent vasodilatation and increased blood flow. Therefore, Laser Doppler Perfusion Imaging (LDPI) was performed to evaluate the microcirculation in the tissue (Bohm-Starke et al, 2001). An area of 5x6 cm was scanned, including the whole vestibular mucosa in patients, and in healthy control subjects. The blood flow in a total number of 3,000 sites was measured. After perfusion analysis, the results showed that, in the patients, a gradual increase in blood flow was registered from the anterior to the posterior part of the vestibule. In healthy women, however, no differences in the regional blood flow was registered. Several researchers have shown that this increased blood flow could be consistent with a release of CGRP from normally mechano-insensitive C-nociceptors, which could cause vasodilatation and axon reflex flare even at very low level of activity, thereby indicating a neurogenic inflammatory response.

The somatosensory function of the vestibular mucosa was also investigated using standardized quantitative sensory tests. Perceptual and pain thresholds to mechanical and thermal stimuli were investigated in patients, and in healthy controls. The patients demonstrated a significant hypersensitivity to mechanical and thermal stimulation in the entire mucosa, compared to the control group. The difference was most pronounced in the posterior vestibule, which was in accordance with clinical findings. The somatosensory abnormalities found in women with VVS were psychophysical evidence of sensitization and/or increased innervation of thermo-receptors and nociceptors in the vestibular mucosa.

In summary, the clinical findings and results from several studies indicated ongoing neurogenic inflammation in the vestibular mucosa in women with VVS.

Discussion

It was pointed out that pain thresholds were related to the pain threshold of the most sensitive nerve fibers. Thus, the number of nociceptors did not necessarily make a tissue any more sensitive if the threshold was still the same. A participant stated that there was a sharp demarcation or border in evocable pain from vestibule to vagina. There was a place where pain, tenderness, and the allodynia stopped, suggesting a nerve boundary between the pudendal nerve and the vestibular nerve. This finding could mean that nociceptors, rather than neurogenic inflammation, as inflammation would spread throughout tissue without regard to nerve boundaries. However, because the pain was bilaterally symmetrical, it suggested that the pain was not due to nerve injury. Thus, there was uncertainty in terms of the interpretation of the findings.

There was also an inquiry as to whether or not sensory nerve fibers crossed the midline. The answer was that studies showed the pudendal nerve was one of the few nerves that crossed the midline within the spinal cord. However, this was not shown to occur in the periphery. At the conclusion of this part of the discussion, participants agreed that it was important not to try to

explain the data too much, but to continue with further research. An additional question was raised as to whether a biopsy should be used as a diagnostic standard. Dr. Bohm-Starke indicated that this was not an appropriate topic for this point of the workshop.

Dr. David Foster, University of Rochester

Dr. Foster summarized a series of studies of surgical pathology reports in regard to the presence of inflammation in VVS. He pointed out that inflammatory infiltrate was not specific and varied from mild to severe, which was one reason many professionals thought the terminology should change from vulvar vestibulitis to vestibulodynia or vulvar dysesthesia. The pain of VVS was highly localized in front of the hymenal ring (the vulvar vestibule) and was absent to minimal in degree in the vagina.

Dr. Foster proposed that the differences observed in the vulvar vestibule contrasted to the tissues in the vagina and the external vulva tissues were related to their embryological origin. The external vulva had stratified squamous epithelium. The vestibule was derived from the urogenital sinus and showed remnants of the original transitional epithelium. There was close cell-to-cell communication between surface epithelium and the underlying connective tissue.

Then, Dr. Foster reported on a study conducted by his research team in which biopsies from the vulvar vestibule, obtained at vestibulectomy, and biopsies from subjects who needed the procedure for problems other than pain were matched by race and age. In the area just in front of the hymen, $IL-1\beta$ and $TNF\alpha$, factors central to inflammation, were increased in VVS subjects. A second study tested the idea that fibroblasts in the vestibule might have different immune responsiveness compared to fibroblasts in other areas of the genital tract. Biopsies of the vestibule and the external vulva were obtained, and fibroblast explants derived from these tissues. These biopsies were taken from areas anatomically about 1.5 cm apart. The explants were grown to confluence and were vimentin-positive (a marker for fibroblasts), while cytokeratin, α smooth muscle antibody, CD34, and CD45 were negative, indicating that they were fibroblasts. Compared to stimulated vulvar-derived fibroblasts, stimulated vestibular-derived fibroblasts had a significantly higher density of CD40, a member of the $TNF\alpha$ receptor superfamily. The vestibular fibroblasts differentially produced elevated PGE_2 following stimulation with gamma interferon and CD40 ligand compared to vulvar fibroblasts. PGE_2 was involved in the phosphorylation of certain nerve channels and made them “leakier,” which suggested that the vestibular fibroblasts responded differently to inflammatory stimuli and may facilitate enhanced activity in neural pain fibers. Interestingly, in the control vestibular fibroblasts more PGE_2 was produced compared to control external vulva fibroblasts, although to a lesser degree than VVS-associated fibroblasts. An enhanced PGE_2 production in the vulvar vestibule may, in part, be constitutive.

Dr. Foster then presented studies describing the role of genetics in VVS. Homozygosity of $IL-1RA$ (allele 2) was associated with a number of chronic inflammatory conditions, such as rheumatoid arthritis and ulcerative colitis. There appeared to be a higher level of $IL-1\beta$ with respect to the interleukin 1 receptor antagonist in individuals with homozygosity to $IL-1RA$ (allele 2). This imbalance was thought to increase the risk for inflammation. It was also felt that $NF\kappa B$ signal transduction was responsible for the elevated $IL-1\beta$ levels in these individuals. In addition, six Melanocortin-1 Receptor ($MC1R$) single-nucleotide polymorphisms (SNPs) were

found to exist in significantly higher proportion in women with VVS compared to controls. MC1R was the skin and mucosal receptor for melanocyte-stimulating hormone (α MSH), a 13 amino acid polypeptide, and has significant anti-inflammatory and analgesic properties. A normally increased cyclic AMP activity is inhibited in individuals with the six MC1R SNPs. To test the hypothesis that these MC1R SNPs were associated with reduced α MSH activity and, thus, with increased risk for vestibulitis in conjunction with IL-1 receptor antagonist polymorphisms, 36 cases and 61 controls were studied. DNA was amplified by polymerase chain reaction (PCR) and analyzed by both gel electrophoresis and by direct sequencing. Supporting previous research, significant homozygosity for IL-1RA (allele 2) was found in VVS-afflicted cases compared to pain-free controls. The six SNPs of MC1R of interest were also more commonly found in VVS cases than in controls. IL-1RA (allele 2) homozygosity combined with at least one of the MC1R SNPs of interest was associated with an additive risk for VVS.

The vestibule responded to inflammatory stimulation in a different manner than other areas of the female genital tract, and there may be both constitutive and genetic susceptibility risk for chronic pain in the vulvar vestibule.

Session IV: The Science of Pain

Dr. Ursula Wesselmann, Johns Hopkins University

Dr. Wesselmann discussed connections between visceral pain and vulvodynia using chronic non-malignant pelvic pain as a model. Visceral pain affected about 15 percent of women of reproductive age and cost the U.S. health care system an estimated \$881 million per year. The differential diagnosis of chronic pelvic pain included gynecologic, urologic, gastrointestinal, musculoskeletal, neurologic, and other causes. Dr. Wesselmann noted that, while there were a few better-defined pelvic pain syndromes, such as interstitial cystitis and dysmenorrhea, her interest was chronic pelvic pain that did not fit a defined pattern and for which conventional diagnostic tests were unrevealing. Thus, like in vulvodynia, it was difficult to set up clinical studies for lack of a case definition. The current working definition, put forth by the American College of Obstetricians and Gynecologists, defined chronic pelvic pain as “pelvic pain in the same location for at least six months.”

Dr. Wesselmann gave two experimental models to show the interplay between vulvodynia and visceral pain. One example was from basic science studies in an animal model in her lab; the other was from psychophysical studies in interstitial cystitis patients. In her study, Dr. Wesselmann inflamed the uteri of anesthetized rats and quantified their pain. Once they recovered from this pain, she inflamed their vaginas. She was able to demonstrate C fos expression in the spinal cord of rats that were sacrificed two hours later. C fos was expressed by sensory neurons as a response to painful stimuli. Inflaming the vagina alone caused C fos expression in the spinal cord segments L5 to S1. However, in rats in which the vagina was inflamed after recovery from previous inflammation of the uterus, C fos was expressed all the way up to T10, a much wider field, which demonstrated sensitization by the previous uterine

inflammation. Although unproven, this may explain why, after recovering from a pelvic inflammation, a subsequent vaginal infection could trigger a chronic pelvic pain syndrome.

The other study was a human study on interstitial cystitis, which was often associated with dyspareunia in women. The bladder received innervation from the lower thoracic and upper lumbar spinal cord, and it also received innervation from the sacral spinal cord. Dr. Wesselmann noted that she was interested in studying the areas of referred pain in these patients with interstitial cystitis. Visceral pain typically presented with two components: one was a deep visceral pain component, localized to the organ where the pain was generated; and the other was visceral pain referred to somatic and other visceral structures along dermatomes, which corresponded to the spinal cord segments where innervation of the viscera was derived from. In interstitial cystitis, pain was initiated in the bladder. One would expect referred pain over the lower back and also in the urogenital area. Using quantitative sensory testing, she assessed different nerve fiber properties in the urogenital area of patients with interstitial cystitis and in normal controls. She assessed temperature thresholds, as well as temperature-induced pain thresholds. Control women had very little sensation in the vagina to start with. Their thresholds for both heat and heat pain detection were between 49 and 50 degrees Celsius. These controls usually did not sense cold pain even at 8 degrees Celsius. In contrast, the patients with interstitial cystitis had altered sensation to temperature stimuli and, in several patients, the point at which they sensed the sensation and when it became painful were rather similar. Only one of these patients had a diagnosis of vulvodynia. Some of the others had dyspareunia, without quite fulfilling the criteria for vulvar vestibulitis or vulvar dysesthesia. Dr. Wesselmann added that it would be interesting to see if these patients, later on, developed full-blown vulvodynia. There was no generalized alteration in temperature sensation. There was also no difference between patients' and controls' thresholds for heat and cold sensation, nor for heat and cold pain thresholds at a distant site, along the C5 dermatome of the arm. Thus, the abnormalities were localized to the pelvic and urogenital area and were not a generalized alteration in sensation.

She proposed a similar concept for visceral pain as currently used for neuropathic pain, which was that there was a spectrum of different insults that could lead to chronic visceral pain. Different pathogenetic mechanisms may require different pain treatment strategies. It was also possible that patients with visceral pain may have multiple co-existing pathogenetic mechanisms. Therefore, one would need one or several modalities to really reduce the pain in these patients to a significant extent.

Discussion

One participant commented that ongoing pain in one viscera could turn on central sensitization, to sensitize a second viscera. This was secondary hyperalgesia. Dr. Wesselmann indicated that the insertion of the probe was painful.

Dr. Marshall Devor, Harvard University

Dr. Devor was asked to address two different subjects: neuropathy and heritability. Normal pain presumed that the pain detection system was intact, and that it was detecting a true injury, whereas, in neuropathy, the pain-detection system itself was damaged. If a nerve was injured,

there ought to be less sensation. Paradoxically, neuropathy was often associated with positive symptoms, including spontaneous burning pain, allodynia, itch, pain on movement or deep palpation, hyperalgesia, hyperpathia, and paroxysmal pains. These symptoms were seen following experimental sectioning of a peripheral nerve and occurred after amputation, as in the “phantom limb” phenomenon. Ongoing activity generated by nerve injury could be eliminated by local anesthetics. Post-exotomy, the cell bodies in the dorsal ganglia also developed a large amount of ongoing electrical spike activity. Following a nerve cut, the ectopic activity that originated in the ganglion versus from the peripheral neuroma could be as high as 75 percent and 25 percent, respectively.

Following injury, the cell body changed its behavior and phenotype. The sensitive sodium channel was up-regulated at about the same time that neuropathic pain behavior came on. Sodium channels, which were key molecules for electrical excitability, accumulated at the proximal part of the nerve injury site. To explain allodynia, Dr. Devor used the Chung model of surgical denervation. Normal animals hardly responded to forces up to 30 grams, while the operated side of the test animals quickly responded to 1-2 grams. The hypothesis thus far was that ectopic firing generated in the injured nerve or the dorsal ganglion entered the spinal cord and set up a central hyperexcitability state known as central sensitization. Thus, input in the remaining basically intact fibers was amplified and felt as pain. At the same time, the fast conducting, myelinated A beta fibers, which were low-threshold mechanoreceptors, were recruited such that a light touch caused a pain sensation. This was called A beta pain.

Applying an anesthetic to the surface of the dorsal root ganglion or to a peripheral neuroma did away with tactile allodynia for the duration of the block. Dr. Devor mentioned that Richard Gracely had demonstrated that injecting a small peripheral neuroma ablated pain over an entire extremity. Thus, in the neuropathic hypothesis of vulvodynia, damage to the pudendal nerve from any cause, at any site, could lead to ongoing burning pain and neurogenic inflammation. In this scenario, the vulvar tissue was completely normal and the problem was due to the neuropathy.

Ectopic firing was very sensitive to membrane-stabilizing drugs at concentrations much lower than needed to stop nerve conduction. Infusion of very low doses of local anesthetics, such as lidocaine, into the bloodstream was such an example. Almost all of the drugs used today to treat neuropathic pain, such as the anticonvulsant carbamazepine, were of this sort. Amitriptyline, which had always been considered an antidepressant or a centrally acting drug, demonstrated a peripheral effect, as did gabapentin. In small studies, these drugs were reported to have a positive effect in vulvodynia. Larger placebo-controlled trials were needed.

Although variability in pain response was traditionally thought of in terms of socialization, etc., recent animal work showed that more than 50 percent of this variability was accounted for by a heritable component. Dr. Devor’s research team was able to breed a group of rats that were protected from neuropathic pain, and another group that was susceptible. Crossbreeding revealed that pain susceptibility was caused by a single autosomal-recessive gene, while protection from pain was dominant. Zed Seltzer identified an important quantitative-trait loci in mid-chromosome 15. Animals that expressed this high-pain phenotype also had high ectopic excitability in their injured nerve fibers. Susceptibility to one type of neuropathic pain did not

necessarily cause susceptibility to another, thus, it may not be global. Sex differences were variable from strain to strain. Several genetic mutations and polymorphisms that affected pain in humans were described, the most exciting of which was the discovery of the *CACNA1* genetic mutation, a major cause of familial hemiplegic migraine.

Dr. Devor ended by saying that, whether vulvodynia was a neuropathy, a normal pain, or an inflammatory pain remained an open question, and that it was time to ask for the facts and get evidence from the patient populations.

Dr. Jay Shah, NIH Clinical Center

Dr. Shah outlined the goals of his presentation as: presenting the specific diagnostic criteria for myofascial pain; pointing out the commonly overlooked myofascial components of pelvic pain; discussing the dynamic role of muscle nociceptors; and introducing a novel microanalytical technique for quantitative analysis of the biochemical milieu associated with muscle pain.

Dr. Shah defined myofascial pain as referred regional pain, which may be local or distant, from a “trigger point” within muscle and fascia. Myofascial trigger points were two to five hard, extremely tender areas in palpable taut bands along a skeletal muscle that may be active or latent, depending on whether they were associated with reproducible pain or not. Myofascial pain was characterized as cramping or aching and may be accompanied by autonomic dysfunction.

Dr. Shah explained that a nociceptor was a receptor that was preferentially sensitive to noxious stimuli, and whose activation may contribute to a sensation of pain. The nociceptor for skin and mucosa were the thin, lightly myelinated to non-myelinated, high-threshold C fibers, which could be sensitized to noxious stimuli. The analogous structures in muscles were the group IV fibers, which constituted more than 50 percent of the axons in a muscle nerve. Nociceptors not only encoded noxious stimuli, which *could* lead to pain, but they also released neuropeptides, such as Substance P and CGRP, which caused vasodilatation and plasma extravasation, meaning they were intimately involved with inflammation and healing. He pointed out that muscle pain was more difficult to localize because of a lower density of muscle sensory afferents compared to skin, and because there was a convergence of sensory input at the dorsal horn of the spinal cord from skin, bone, viscera, and periosteum.

He emphasized that treatment for myofascial pain required a multi-faceted approach, including: manual techniques, dry needling, trigger-point injections, physical modalities, and approaches to the psycho-emotional components of pain. He added that his preferred technique for dry-needling was to use an acupuncture needle (which has a rounded tip that pushes cells aside compared to a bevel-edge hypodermic that is designed to tear cells) for treating myofascial trigger points. After careful examination and palpation, he inserted the needle directly into the active trigger point of a muscle, obtaining local muscle twitch responses, and continued the procedure until the twitch responses diminished and the muscle softened and became less tender.

To better understand the local mechanisms of muscle pain, he described an ongoing protocol at the NIH clinical center using microdialysis. This technique was being used to study the chemical activators of muscle pain, and to measure and differentiate a variety of biochemicals among three

clinically distinct groups at a standard anatomical location in the upper trapezius muscle: *normal* (without neck pain and without trigger points); *latent* (patients with a trigger point but no neck pain); *active* (patients with a trigger point and neck pain of less than three months' duration).

Substance P, CGRP, bradykinin, norepinephrine, serotonin, TNF- α , and interleukin-1 (IL-1 β) were significantly higher in the active group than in the other two groups. The pH was significantly lower in the active group than the other two groups. At five minutes, Substance P and CGRP differed in all three groups, with active being more than latent, which was more than normal. Furthermore, eliciting a local twitch response in the active group caused a significant drop in Substance P and CGRP, suggesting a change in the local milieu with the local twitch response. This technique showed promise in determining the biochemical milieu and mechanisms of myofascial pain and, eventually, in determining the local effects and mechanisms of different treatment modalities.

Dr. Richard Gracely, University of Michigan

Dr. Gracely divided his presentation into three parts: 1) a capsule history of pain research over the past 30 years; 2) some background information on altered central processing as it pertained to vulvodynia; and 3) some new data, including the results of functional magnetic resonance imaging (fMRI) in fibromyalgia and other chronic pain conditions.

He pointed out different significant milestones since the 1960s. The first was Melzack's proposed gate theory of pain, which stated that input from small fibers produced pain and input from larger fibers caused inhibition at the spinal level, while messages from the brain descended causing attenuation of pain. The second was the presence of endogenous substances, including opioids, endorphins, and enkephalins, that mimicked the action of morphine. Thirdly, there was central sensitization from persistent peripheral stimulation, a mechanism that increased the magnitude of pain. He described the observations that he and Gary Bennett made showing that constant firing from microneuromas could cause central sensitization such that stimulation of large A-beta touch fibers, which would normally not provoke pain, would evoke pain. He could produce the same phenomenon by injecting capsaicin into the skin. Capsaicin may cause some nerve damage, but it also activated focal nociceptors. In his opinion, central sensitization could be evoked by true nociception, such as by inflammation, by neuropathy (i.e., neuromas), or by sympathetic nervous system sensitivity to adrenal receptors expressed in injured nerves.

Dr. Gracely described experiments on patients with vulvodynia that he did with Maria Turner. In a series of patients with constant vulvar pain that extended way beyond the vulvar vestibule to the inner thighs and perianal area, they found that local injections of minute amounts of 1% lidocaine without epinephrine, just underneath the openings of exquisitely tender Skene's and Bartholin's glands, resulted in abolition of the pain over the injection sites, with decreased pain and allodynia over the rest of the vestibule and surrounding glabrous skin. He provided diagrams and highlighted overlapping areas of secondary hyperalgesia along the midline of the vulva. He ascribed this result to nociceptive, possibly inflammatory stimuli coming from the ostia of Bartholin's and Skene's glands. Dr. Gracely then theorized that the beneficial effects of vestibulectomy may be the result of removing the source of the generator of this dynamic input, and suggested that therapies should be directed at these superficial sites.

Dr. Gracely devoted the last part of his talk to current studies involving a different kind of sensitization, such as that seen in fibromyalgia. Inducing thumbnail dysesthetic pain with a dolorimeter, he had been mapping sites of brain activity with fMRI. The thumbnail was not a tender point for fibromyalgia, nor was it a myofascial trigger point. Thumbnail pain caused a response in the thalamus, primary and secondary somatosensory cortex. In a study of non-depressed patients with fibromyalgia and with controls, he demonstrated that patients experienced intense pain with 2 kilograms of pressure, while controls barely felt any pain. He noted that this finding may not be a marker for fibromyalgia because patients with low-back pain responded similarly. Using an equal pain comparison, patients had increased activation in five of seven areas identified in pain processing, while controls showed activation only in one. His current studies on vulvodynia, using a calibrated vulvodolorimeter, suggested that a mild stimulus to a densely innervated area could produce a focal discharge of the spinal cord and mechanical allodynia.

Session V: Evolving Therapies

Dr. Anthony Visco, University of North Carolina

Dr. Visco spoke on the hormonal effects of estrogen and progesterone on muscle and collagen integrity. He covered the following topics: the extracellular matrix (ECM), including collagen elastin, proteoglycans, and glycoproteins and the substances that affect it, such as metalloproteinases (MMP) and their inhibitors (TIMP); muscle, including actin, myosin, and their regulatory proteins; and hormonal influences on ECM and muscle. He explained some of the current techniques, such as using high-performance liquid chromatography (HPLC) to determine the amount of hydroxyproline, which, in turn, was a measure of the amount of collagen present. He found that the greatest difficulty in evaluating the literature rested in the fact that biopsies were taken from different sites, and from women at different ages.

With these caveats in mind, he indicated that, as far as prolapse was concerned, weak connective tissue could result from either decreased collagen overall, or from decreased collagen cross-linking, which may be influenced by increased amounts of MMP activity, a phenomenon negatively regulated by TIMP. MMP activity appeared to be stimulated by estrogen and inhibited by progesterone.

He described his experiments in which he plated RNA extracts from pubococcygeus muscle cells that were obtained from patients with uterine prolapse and from normal controls. Using gene chip technology, he found that smooth muscle actin and myosin, as well as myosin light chain kinase were 11 to 12 times overexpressed in patients. Tenascin C, involved in adult tissue remodeling was also overexpressed. Myosin-binding protein, myosin skeletal muscle, and C-fos were markedly underexpressed. Using reverse transcription PCR (rtPCR) for confirmation, he found a three-fold reduction in myosin heavy polypeptide three in patients with prolapse.

On the question of estrogen and progesterone receptors, a MEDLINE/PubMed search revealed several studies. One found a significant increase of estrogen, progesterone, and androgen

receptors in vaginal smooth muscle. No estrogen receptors, a moderate number of progesterone, and just a few androgen receptors were found in the levator ani muscle.

Dr. Visco concluded that, so far, weakness of the pelvic floor might be due to decreased amounts of actin or myosin, and may also have to do with regulatory proteins. The effects of estrogen and progesterone were yet unknown, while the effects of MMP and TIMP needed more study.

Dr. Peter Smith, University of Kansas

Dr. Smith concentrated on the effects of estrogen on the peripheral nervous system pathways, emphasizing those that pertained to pain. He started out by saying that a review of the literature revealed a somewhat common idea that there was a relationship between estrogen and sensation, and that a decrease in threshold occurred with increased estrogen levels, such as during the menstrual cycle, during pregnancy, and estrogen replacement. However, dysesthetic vulvodynia was more common during menopause, when estrogen levels were decreasing.

He pointed out that there were a lot of potential estrogen responsive sites in the nervous system. Utilizing estrogen receptor protein as a sign of estrogen responsiveness, several studies showed that this was present at all levels of the nervous system, from the cerebral cortex to the dorsal root ganglion. There were two types of estrogen receptor proteins, ER α and ER β , which may occur singly or may co-localize. These receptors were thought to possibly have antagonistic effects. Estrogen bound to the receptor and was internalized in the nucleus, where it affected transcription to change proteins levels. Estrogen modulated sensory pathways by changing the levels of neurotrophic proteins, especially nerve growth factor and brain-derived neurotrophic factor. These factors could initiate axonal sprouting, promote neuronal survival, and modulate the functional properties of neurons. The situation was complicated because estrogen also affected the expression of receptors that detected these neurotrophic proteins.

To assess effects of estrogen on peripheral nerves of the reproductive tract, he used PGP 9.5 as a panneuronal marker to label all intact axons and dopamine beta hydroxylase, a sympathetic marker, and CGRP to identify sensory nociceptor nerves. In experiments on rat myometria, during diestrus, he found numerous nerve fibers, while two-and-a-half days later, during estrus, he found relatively few fibers. He demonstrated that estrogen caused sympathetic nerve terminals to degenerate transiently, while sensory nerves in the uterus were not obviously affected by the estrus cycle.

In contrast, following estrogen stimulation of the mammary gland, he demonstrated a robust increase of sensory nerve fibers, most of which were associated with blood vessels. There was no significant increase in sympathetic fibers.

Dr. Smith outlined an ongoing study of the innervation of the vagina. After giving ovariectomized animals estrogen for seven days, he removed the vagina and quantified innervation density by immunofluorescence. Using the panneuronal marker PGP 9.5, the distal vagina showed many submucosal nerves, with an obvious decrease following estrogen therapy, much like the uterus. Also in common with the uterus, vaginal sympathetic innervation was decreased by estrogen. However, using vasoactive intestinal polypeptide as a marker for

parasympathetic nerves, these nerves also decreased in estrogen-treated rats. There was also a marked decrease in sensory nerves as a result of estrogen administration. Thus, estrogen appeared to suppress innervation density in regions of the rodent reproductive tract.

Did increased nerve fiber density contribute to pain, as was proposed by some other speakers? Extrapolating results from ovariectomized animals to the menopausal state, one could hypothesize that increased nerve density may contribute to these pain syndromes. Did estrogen affect innervation density by altering production of trophic factors, and what cells were involved? Knowledge of the cellular and molecular mechanisms by which estrogen mediated peripheral neuroplasticity may give rise to novel therapeutic strategies aimed at alleviating vulvodynia pain.

Discussion

It was mentioned that progesterone was an excellent antagonist of estrogen. One participant shared his observation that taking away progestational oral contraceptives from young women with vulvar vestibulitis and adding topical estrogen was generally helpful. Another participant mentioned that healthy women on oral contraceptives, without a history of dyspareunia had a significant decrease in pain thresholds using Von Frey hairs, with no change in thermal sensitivity.

Session VI: Therapeutics

Dr. Gerald Gebhart, University of Iowa

Dr. Gebhart spoke about his group's work on animal models to study the mechanisms by which nociceptor activation from the viscera could be modulated. He started out by reviewing some aspects of visceral hypersensitivity (e.g., interstitial cystitis and functional gut disorders), noting that these were characterized by pain and discomfort in response to physiologic stimuli, in the absence of pathology. He illustrated how neurons in the spinal cord received convergent input from a variety of sources, including skin, muscle, and other viscera. Thus, he indicated that he would prefer to interfere with input arising from the periphery of an organ, as Dr. Gracely demonstrated was possible in an earlier presentation. He also reviewed the concept that sensitization, such as that from previous inflammation, decreased response threshold and increased response magnitude and spontaneous activity leading to a tonic low level of input into the central nervous system.

With respect to opioid modulation of visceral nociception, he focused on peripheral kappa receptor (κ R) agonists. He explained that there are three opioid receptors, Δ , μ , and κ . Only the κ subtype were able to attenuate responses to distention of hollow organ and "visceral" motor responses to distension, when they were restricted to the periphery. These visceral κ opioid effects were not reliably blocked by κ R agonists, nor were these effects blocked by knocking down the receptors with anti-sense oligo nucleotides, suggesting that κ R agonists acted at a non- κ opioid site, a novel site, or by a different mechanism.

These κ or κ -like agonists reduced the activity of calcium channels causing a decrease in neurotransmitter release in the second-order neuron in the spinal cord, hence, an analgesic effect. These agonists also decreased sodium currents, which were important for neuron excitability. Using recordings from single afferent fibers in the pelvic nerve, Dr. Gebhart noted that there was no decrease in response magnitude to colon distension using a ΔR agonist (i.e., DPDPE) and a μR agonist (i.e., morphine), whereas a selective κR agonist (i.e., U5488) caused a precipitous dose-dependent decrease in the response of that afferent fiber. This was true using other afferent fibers. The potency of these κ compounds increased when the organ was inflamed, suggesting that, in conditions such as interstitial cystitis and vulvodynia, there may be up-regulation of some of these receptors, including arylacetamides, which were very effective at blocking sodium currents as shown by *in vitro* experiments using single sensory-cell recordings. Dynorphin, an endogenous κR agonist, did not produce this reduction, but it does not have an arylacetamide structure. Thus, arylacetamides were peripherally restricted κR agonists that acted at calcium and sodium channels. These compounds were being tested in clinical trials.

Another mediator of nociception was Substance P. With dye-labeling, colon-distension experiments, Dr. Gebhart showed that Substance P receptors (SPRs) or neurokinin1 (NK1) localized in the commissure dorsal to the central canal of the spinal cord, and that SPRs were on the cell surface until the colon was inflamed or distended, upon which time they internalized (a measure of activation). Viscera were innervated by more than one nerve. Thus, pelvic viscera had innervation that went to the lumbosacral and thoracolumbar spinal cord. Distension of normal colon showed an increase in internalization of these receptors in the sacral and thoracic, but not the lumbar spinal cord. However, when the colon was inflamed prior to distension, there was not only a much greater receptor internalization in the sacral and thoracic spinal cord, but lumbar 2-5 also responded, which resulted in a spread of excitability. Dr. Gebhart then looked at different neurokinin (NK) receptor antagonists and found that NK1 and NK2 receptor antagonists, singly, did not decrease response magnitude to colon distension, whereas they were effective when used in combination. NK1 receptor antagonist may not have worked because the spinal cord released not only Substance P, but also NKA and other substances, which likely have effects at NK2 receptors. NK3 receptor antagonists worked as a single agent. This and a combination of NK1 and NK2 receptor antagonists had potential utility as analgesic drugs, particularly with respect to visceral analgesia.

Another promising group of drugs were the N-methyl-d-aspartate (NMDA) receptor antagonists, which, in animal models, were the most clearly efficacious antihyperalgesic when instilled in the spinal cord, with rapid reversal that lasted as long as the drug was present. Its disadvantage was that NMDA receptors were ubiquitous in the central nervous system, such that dysphoria and hallucinations were common side effects. The pharmaceutical industry was looking at other sites of action for this group of drugs to avoid the side effects. In answer to a question from the audience, Dr. Gebhart suggested that pentazocine, a κ -opioid receptor agonist that has μ receptor antagonist properties, might be a good agent to try for visceral pain.

Dr. Don Manning, University of Virginia and Celgene Corporation

Dr. Manning explained the hurdles to the introduction of new drugs to the U.S. market, but added, and on a brighter note, that there were at least 70 different compounds in clinical

development for the treatment of pain. Dr. Manning traced the evolution of pain management, starting with anesthetics, which were able to totally wipe out all sensory input, to analgesics (i.e., opioids), which were more specific for pain states, to the current development of antihyperalgesics, where one took the patient's pain state and brought it back to normal without reducing it below the baseline pain sensitivity. He emphasized the importance of recognizing the difference between analgesics and antihyperalgesics because the animal models, targets, regulatory processes, and marketing material were different for each. He felt that some questions needed to be asked, including: did clinicians want anti-inflammatory activity? Did clinicians really want no sedation? Did clinicians want continuous or intermittent use? Were clinicians looking for a once-a-year dosing? What if an adverse reaction developed to such a long-lasting drug? Were clinicians only interested in drugs with rapid onset, considering that these drugs would be used for chronic pain? He pointed out that none of the current guidelines for the U.S. Food and Drug Administration or the European equivalents adequately dealt with chronic pain, and certainly not with visceral hyperalgesia, such as in vulvodynia.

He emphasized the lack of animal models available for pain research, except those for induced pain. Other than those presented at this workshop, there were no models for chronic visceral pain. Another neglected area of research, for which there were no models, was for dysesthesias, such as itching, and other altered sensory states, which could sometimes be much worse for the patient than pain. There was also a disconnect between animal models and the human condition. For instance, NK1 antagonists worked extremely well in animal models, but failed miserably in humans. It had been suggested that once the toxicology of a new drug was worked out, a limited two-week human trial should be undertaken. He encouraged organizations to develop well-validated, well worked-out epidemiologic models of chronic pain, clarifying not only the disease state, but also the number of patients affected (market share). The size of the market was not as important as it being definable.

Genomics and molecular pharmacology had identified many targets, although their clinical relevance was not clear. Industry utilized a reverse process, using drugs with known efficacy to identify molecular targets. Gamma-aminobutyric acid B (GABA-B) receptors had been fully identified using baclofen; the $\alpha 2\Delta$ component of the calcium channel had been identified as the site where gabapentin and pregabalin would bind. Work continued on the mechanism by which tricyclics worked on the sodium channels.

Dr. Manning spoke about membrane stabilizers. Drugs such as lidocaine, antiepileptic drugs, and tricyclics blocked sodium channels so that sodium entry into the cell was prevented, which decreased cell excitability. Similarly, blocking calcium entry into the cell prevented release of neurotransmitters and the development of second level sensitization within the nervous system. Ziconotide, a snail toxin, quieted these calcium channels. Potassium channel stimulators, such as tricyclics and antiepileptic drugs, which increased potassium efflux from the cell, also stabilized the membrane. Experimentally, combining potassium channel stimulators with new opioid agents greatly enhanced their activities. The ubiquity of these ion channels necessitated identification of the specific channels involved in pain transmission to minimize adverse effects.

There were also receptor-targeted drugs. Capsaicin was a vanilloid receptor (VR) agonist, and a VR antagonist had been found to be very effective in animal models. Work was in progress

toward developing agents that would work on cannabinoid receptors CB1 and/or CB2 in the periphery. These compounds showed effectiveness in both the nociceptive and neuropathic models, making the debate over whether vulvodynia was one or the other, moot. Other classes of drugs in development included glutamate, nicotinic, muscarinic and acetylcholine receptor antagonists, adenosine receptor modulators, and glycine receptor agonists.

Dr. Manning touched on inflammatory modulators such as TNF α inhibitors, NF- κ B modulators, and regulation of gene transcription. Finally, he described proteomics, which was looking at the proteins produced from the genes. These proteins were orders of magnitude more broad and varied than the genes themselves. Thus, pharmaceutical agents could be targeted toward specific abnormal proteins.

In closing, Dr. Manning appealed to the group to develop better and validated ways to study and diagnose clinical pain syndromes, so that tens of patients could be enrolled in registration quality trials of new drugs.

Dr. Keith Bley, NeurogesX, Inc.

Dr. Bley indicated that he found the current conceptual framework to be inadequate and limiting. As an example, the concept of “irritable nociceptor” was used in both inflammatory and neuropathic pain conditions. He considered vulvodynia to be a chronic inflammatory pain condition, not a neuropathic one. In vulvodynia, there was evidence for sensitization, as well as for an increase in nerve-fiber density, whereas in neuropathic pain syndromes, there was a decrease in nerve-fiber density. Dr. Bley said that there was evidence to show that, when immune cells infiltrated into tissues or were in proximity to nerve fibers, there was hyperactivation of the sensory nervous system. For instance, superfusion of TNF α or interleukin-1 β over nerve trunks or cultured sensory neurons *in vitro* resulted in robust excitatory responses. Moreover, cytokines could induce the Langerhans cells to proliferate in the skin; Langerhans cells, in turn, could increase their production of neurotrophins like nerve-growth factor, which was known to excite directly sensory neurons.

The work of Dr. Bley focused on the peripheral drivers for pain and on therapies directed at these drivers. He cited strong evidence that there were peripheral drivers to vulvodynia. First was the efficacy of surgical skin removal (e.g., vestibulectomy). Interestingly, similar observations had been made in postherpetic neuralgia. Second was efficacy of local anesthetics for vulvodynia, for which anecdotal evidence had been cited and there was apparently a forthcoming publication on this topic. In another parallel with postherpetic neuralgia, he pointed out that topical anesthetic patches were thought effective against this pain syndrome.

He concentrated a large portion of his talk on capsaicin, a highly selective agonist for VR1, which was a ligand-gated ion channel, preferentially expressed in nociceptive sensory fibers, which were mostly a subpopulation of C- and some Ad-fibers. These fibers had the ability to integrate temperature, acidification, and endogenous agonists, such as metabolites of arachidonic acid. One could postulate that, in chronic pain conditions, especially inflammatory pain, VR1 was tonically activated, resulting in excessive afferent input. VR1 antagonists were promising medicines, and several were in development. Dr. Bley described the conditions for which

existing topical capsaicin had been helpful, but pointed out their limitations, including irritation, burning, and short duration of action. In experiments with low-concentration capsaicin creams, after one week of application, there was decreased PGP 9.5 staining of epidermal (and possibly dermal) nerve fibers. In order to demonstrate that comparable efficacy could be attained quickly, his company conducted a Phase 1 trial using a 60-minute application of an 8% capsaicin patch; an equivalent degree of nerve density reduction was noted. He considered high-concentration capsaicin to be a promising therapy for pain conditions affecting the urogenital sinus because the nerve fibers there express high levels of VR1. He cited the use of 30-minute capsaicin bladder infusions for interstitial cystitis and overactive bladder. To decrease irritancy, lidocaine infusions could be given prior to capsaicin instillation. He described a Phase II clinical study in postherpetic neuralgia using the high concentration, 60-minute patch protocol. The skin was pretreated with topical anesthetic, and a rescue opioid was made available. Evaluation at two to four weeks showed a 33 percent pain reduction from this one application, similar to that achieved after weeks of high-dose gabapentin therapy. High-concentration capsaicin did not produce adverse effects on the skin, either in the 44 postherpetic neuralgia patients, or in animal tests. The presumed mechanism of action was superactivation of VR1-expressing nociceptive fibers. These fibers were quickly pushed to a state of depolarization block, with a robust influx of calcium activating all calcium-dependent enzymes, which caused the fibers to become non-functional. Accompanying all the positive charge entering the nerve fibers was a lot of water, giving rise to osmotic shock as well. Because of the lack of capsaicin effects on non-VR1-expressing fibers, it was anticipated that this situation represented a specific antihyperalgesic that could abolish peripheral ectopic drivers, while preserving normal sensations. Dr. Bley mentioned that in the postherpetic neuralgia study, patients were pretreated with an effective topical anesthetic, and this alone caused a robust and rapid reduction in pain, a phenomenon also noted in trials involving the sole use of topical anesthetics for postherpetic neuralgia. Again, this finding proved that much more could be done to alleviate pain by targeting the periphery.

Another approach to inhibit drivers of peripheral ectopic activity was to go after cytokines or the VR1 itself with an antagonist. This approach was limited by redundancy and parallel signaling pathways within the nervous system.

Dr. Bley ended his talk by saying that VR1 agonist therapies, given intermittently, could chronically attain a level of pain relief that could then be topped off with additional local anesthetic therapy, or with centrally acting therapies. He added that he saw the major challenge as the development of a convenient, rapidly acting, and low-pungency formulation.

Dr. Sophie Bergeron, Université du Québec à Montréal

Dr. Bergeron reported the long-term follow-up results from the first prospective, randomized treatment outcome study comparing vestibulectomy, EMG biofeedback, and cognitive behavioral sex therapy with pain management. The outcome measures were pain reduction, improvement of sexual functioning, and psychological adjustment. She also hoped to delineate some predictors of outcome.

Dr. Bergeron reported that 78 women met the very specific study criteria. They were neither perimenopausal, nor menopausal. They had moderate to severe entry dyspareunia of at least six

months duration, without chronic pelvic pain, vulvar dysesthesia, or vaginismus. The patients had a mean age of 27, with the equivalent of a university undergraduate degree, and had had vulvar vestibulitis for about five years. Most were cohabiting or married. They were divided into three treatments groups: total vestibulectomy with vaginal advancement; biofeedback, including muscular contraction and relaxation exercises done for 20 minutes, twice a day; and cognitive behavioral therapy (CBT), including half pain management and half sex therapy. Of these three treatments groups, only the CBT group did not experience any dropouts, despite the fact that the patients had significantly less trust than members of other groups that this treatment would help their pain.

At the six-month follow-up, women who had vestibulectomy had significantly greater improvement of pain on intercourse than women in the other two groups, although all participants significantly improved when compared to pre-treatment levels. The vestibulectomy group averaged 52 percent pain reduction, while biofeedback had a 35 percent pain reduction, and CBT had a 38 percent reduction. Across the board, improvement was better at six months than at three months and was maintained at the two-and-a-half year mark. Despite this improvement, there was no change in frequency of sexual intercourse, nor was there an increase in sexual desire in any of the groups. They did not have intercourse more often than they used to, which was four times per month, half the frequency of unaffected women their age. Looking at predictors of pain, in all three groups, pretreatment pain severity predicted and explained 9 percent to 21 percent of the variance in outcomes, depending on the treatment condition. The lower their trust in the treatment, the more pain they had at the end of treatment. For the vestibulectomy group, negative sexual attitudes explained 55 percent of the variance in the negative outcome of the surgery.

Dr. Bergeron considered the results of their study as supporting a biopsychosocial model of pain, in which sensory, cognitive, affective, and behavioral factors all played a role in the development and maintenance of pain. The improvement from vestibulectomy supported the theory of nociceptor sensitization. The findings also highlighted the importance of sex therapy to improve the quality of sexual functioning and to reduce the negative attitudes toward sexuality.

She concluded by saying that multimodal treatment approaches may be necessary to achieve significant improvement in all aspects of the disorder, and that there was a need for randomized treatment outcome studies to compare multimodal and unimodal approaches.

Discussion

A participant pointed out that a difference in post-operative care might explain Dr. Bergeron's finding of a 68 percent improvement in subjects undergoing vestibulectomy in contrast with an 80 percent to 85 percent improvement reported by other investigators.

Final Discussion

There was general agreement that, while much has been learned since the last workshop in 1997, there was much that needed to be studied in the future. The fact that the disorder was more prevalent than previously thought should stimulate the medical profession to educate medical students, residents, and practitioners about vulvodynia diagnosis and current treatments. The conference clearly highlighted the fact that vulvodynia was a disorder that would benefit from research into pain and its etiologies. Pain as a symptom occurred in many disorders and, thus, sufferers of vulvar pain had much to gain from studies on pain in general. It was hoped that the conference would stimulate basic scientists to expand the information on basic mechanisms of this disorder, and to encourage further studies on the etiology. Randomized trials of therapies based on underlying mechanisms of disease were essential, and one goal of this conference was to stimulate public- and private-sector physicians and scientists to carry out these needed clinical trials, with the ultimate goal being to eliminate the suffering of countless women and their families.

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